Effects of Cholinesterase Inhibition on Brain Function

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A thesis submitted to University College London for the degree of Doctor of Philosophy

Dedicated to my parents, Annie, Mia, Tess and Noah

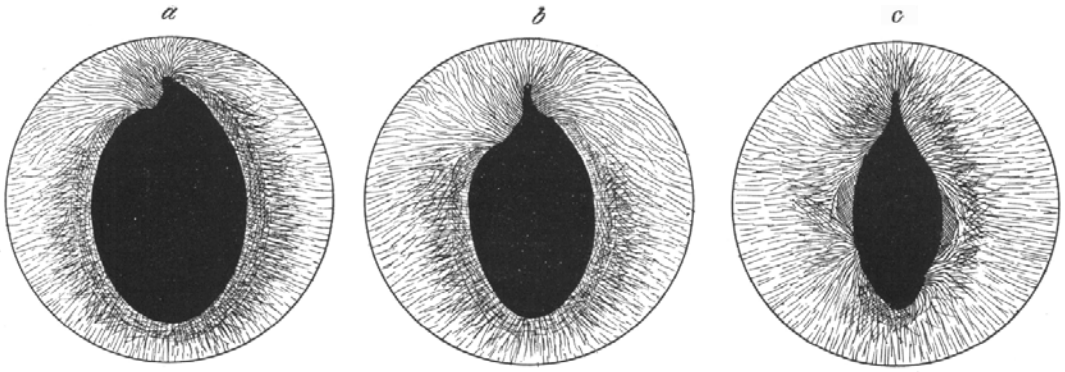
Acknowledgements

This thesis would not have been possible without the pivotal help of my two supervisors Ray Dolan and Jon Driver – the former for launching me off the starting blocks, uncompromising coaching, and ensuring that I complete; the latter for steering me around some tight methodological and theoretical turns en route. My other collaborators are Christian Thiele – who was patient enough to teach me 'the ropes', Patrik Vuilleumier, and Masud Husain. I would also like to thank Joel Winston and Bryan Strange for their valuable pearls along the way, as well as Martin Rosser who facilitated patient recruitment from the Dementia Research Group, UCL. Finally, pursuing an academic career and seeing this through, at least to PhD, would have been most unlikely without the guiding wisdom from my father Harvey Isenberg PhD for whom I am quite indebted.

Abstract

Pharmacological-functional imaging provides a non-invasive method by which the actions of neurotropic drugs on the human brain can be explored. Simply put, it assesses how neural activity patterns associated with cognitive functions of interest are modified by a drug challenge. Since one of the most widely-used cognitive-enhancing drugs in clinical practice are cholinesterase inhibitors, this thesis applies pharmacological functional imaging to the question of understanding how such drugs work - both in healthy people and dementia. The experiments in this thesis describe how brain activations – as revealed by functional magnetic resonance imaging (fMRI) – are modulated by the cholinesterase inhibitor physostigmine, during tasks designed to isolate sensory, attentional, and memory processes. While non-human and human psychophysical studies suggest that all three of these cognitive functions are under the control of the endogenous cortical cholinergic system, understanding how neurobiological models of cholinergic function translate into human brain activation modulations is unclear. One main question that is particularly relevant in this regard, that recurs through all the experiments, is how physostigmine-induced neuromodulations differ between sensory-driven 'bottom-up', and task-driven 'top-down', brain activations. The results are discussed with reference both to non-human physiological data and to existing human cholinergic-functional imaging studies (fifty studies published to date), which are themselves reviewed at the outset. Furthermore, assumptions based upon the physical and physiological principles of pharmacological functional imaging, being critical to interpretation, are discussed in detail within a general methods section.

Contents



Early cholinergic functional imaging: the cholinesterase inhibitor physostigmine constricts the pupillary sphincter in a pinch-like manner (Anderson, 1905)

1. Introduction

Purpose

This thesis is about how one neuromodulatory system – the cortical cholinergic system – interacts with brain activity during normal and disease states. The question of course implies that there are, in very broad terms, two types of brain system that themselves are interconnected. On the one hand, we have the main platform for information processing – the cerebral cortex with its rapid and specific synaptic connections mediated by transmitters such as glutamate and GABA. On the other hand, we have neuromodulatory systems that influence information processing relatively uniformly, over broad spatial and temporal ranges. Instead of signalling 'there is now a light stimulus present at 37 degrees East, 42 degrees North' as a glutamatergic synapse might, neuromodulators might convey the request that *all* receptors should be 'on guard' for any new incoming input.

Put like this, neuromodulation seems quite ancillary to cortical processing, and perhaps therefore plays only a minor role in brain function. Its actions, in being broad and non-specific, make its relationship with cortical function analogous to the volume knob's role in relation to a sophisticated music recording. However, there are at least two reasons why we shouldn't relegate neuromodulation just yet. Firstly, as we shall see in the next chapter, neuromodulators are able to orchestrate processing over wide regions and so are ideally placed to change the mode of cortical function to suit an animal's currently required behavioural set (e.g. to be vigilant, bored, restful, etc). Rather than acting merely as a volume knob, neuromodulators may act more as the overall control panel switching between radio and CD functions! Neurophysiologists, moreover, can work backwards from this viewpoint by identifying all the actions of one neuromodulator as together

serving a common functional purpose (cf. the usual neurophysiological approach of correlating isolated neural responses with behavioural or environmental events). Furthermore, the conception of neuromodulators, and especially acetylcholine, as acting non-specifically over wide swathes of the brain is increasingly challenged (Sarter et al, 2009) on the basis of mounting data showing ACh release patterns more temporally and spatially precise than previously believed.

The second reason for being interested in neuromodulation is clinical. Following brain injury due to whatever cause, the potential for recovery is slow and usually incomplete. Rehabilitation therapies may extend or hasten the capacity for repair but still fall far short of being able to provide a return to normal function universally. One reason for this is that correction of brain injury, where this involves damage to specific, one-to-one connections of cortical circuits, would require insertion of a replica circuit – a challenge as good as impossible by today's technology. Rehabilitation therapies may work by re-creating copies, albeit imprecisely, of damaged circuitry in unaffected parts of the brain. But to be more successful rehab-based strategies will require a considerable advance in our understanding of compensatory neural mechanisms, and technological ability to manipulate these.

A short cut to brain recovery may be provided by neuromodulation. For a start, if any component of brain injury includes a neuromodulatory deficit this could be conveniently restored through a systemically-acting drug given the properties of neuromodulators of acting over a distance, en masse etc. For example, Alzheimer's disease, like many

neurodegenerative diseases, involves degeneration of multiple systems simultaneously, but of these, it is only the cholinergic deficiency that can be easily reversed (Mesulam, 2004). Furthermore, given the usual location of diseases such as stroke and multiple sclerosis (Selden et al, 1998), it is likely that their associated lesions often interrupt cortical cholinergic pathways, again laying open the opportunity for pro-cholinergic therapies.

Aims

The first aim of this thesis is to explore how one of the most well-known class of neuromodulator drugs – cholinesterase inhibitors (ChEIs) – modifies cerebral processing, and related cognitive performance, in its principle application in dementia. ChEIs were first used medicinally for glaucoma after it was observed that they had a pupillary-constricting effect (see Figure, page 9). However, it was only after the cholinergic basis of ChEIs actions were discovered that it became apparent that they may be of use in diseases characterised by impaired cholinergic neurotransmission, viz. myasthenia gravis and Alzheimer's disease.

Ironically, the contrast between ChEI use in these two diseases couldn't be greater. Current knowledge of myasthenia gravis - an immune disorder in which autoantibodies are directed against neuromuscular-junction cholinergic transmission - allows for a comprehensive understanding of its associated peripheral cholinergic transmission dysfunction. The centrality of acetylcholine to its pathophysiology predictably accounts

for the high efficacy and universality of ChEI benefit in this patient group. By contrast, the same drug type in Alzheimer's disease - a neurodegenerative disease characterized by early cerebral cholinergic fibre loss amongst other findings - is associated with only a mild benefit, on only certain cognitive and behavioural measures, and then only in certain patients (Giacobini, 2000). Hence, at least within the dementia community, there is a clinical need for an understanding into how ChEIs exert their cognitive effects. It would be useful to know which brain systems or neural processes can be influenced by ChEIs; whether these drug-induced modulations effectively reverse deficits seen in dementia, and whether these effects on brain activity can provide a sensitive indicator of therapeutic responsiveness.

A second aim of the thesis is to question the effects of ChEIs in healthy subjects. On the one hand, a limited number of experiments have shown that such drugs may improve cognitive performance (Davis et al, 1978), albeit by only a small effect size and with wide variance (as we see with AD). On the other hand, evidence from diverse sources suggests that a central hypercholinergic state may be detrimental, for example by inducing a hypervigilant state, and may partly explain the pathogenesis of disorders such as anxiety and schizophrenia (Berntson et al, 2003). Only through examining brain activity, its relation to behavioural responses, and the effects of cholinergic modulation on both, might we begin to formulate models that could explain these prima facie conflicting sets of observations.

From an evolutionary perspective, we would not expect a normal-functioning biosystem – such as the mammalian cortical cholinergic system - to be significantly lacking along any dimension that could readily be corrected by a simple biochemical alteration. We would especially not expect any such fault to be present in a cognitive system residing in an organism that has excelled in its behavioural capacities. Hence it is highly likely that, as with virtually all other physiological parameters, the normal range of brain levels of acetylcholine are tightly regulated to ensure optimal functioning. Deviation from the norm, in either direction, is likely to entail net performance deterioration, i.e. over the average of behavioural states that an animal typically finds itself in.

One possible explanation for the paradoxical finding of both positive and negative effects of a hypercholinergic state is that while a limited number of performance measures may improve, this is at a greater cost in the long run due to impairments on other measures. We might predict therefore that a low-cholinergic state may also be useful given that it intermittently occurs in normal people, even if when induced pharmacologically, low cholinergic states induce deleterious effects such as sedation and impaired attention. Interestingly, something similar to this has recently been found: if the muscarinic blocker scopolamine is administered shortly *after* an object-to-be-remembered is presented (i.e. after encoding) subsequent memory of it increases; but if scopolamine is given immediately *before* encoding then memory is decreased (Winters et al, 2007). Of course, real life does not resemble a controlled psychology experiment. So the problem facing an animal is that it does not know following a significant experience whether it should decrease its acetylcholine levels so as to improve subsequent recall of the event just

passed, or whether it should heighten acetylcholine levels so as to enhance memory of any subsequent, and possibly more important, experiences. Consequently, we might expect animals to have the facility to adjust their acetylcholine levels in line with a running internal estimate of environmental predictability: a prediction borne out across diverse animal and psychophysical studies (see Yu & Dayan, 2005).

The final aim of this thesis, but perhaps one that needs to be addressed before tackling the first two, is to question the role and capabilities of functional imaging in psycho-pharmacology. Until recently many of the explanations behind ChEIs' actions have been circumstantial, involving extrapolations from animal models or human drug effects to human disease. For example, it is assumed that ChEIs produce effects in opposite directions to those induced by selective cholinergic lesions in non-human animals, or by administration of scopolamine to healthy individuals. Given that Alzheimer's disease shows a loss of cholinergic cell markers it is assumed that ChEIs, in raising acetylcholine levels, can help to restore cholinergic control of cortical processing. However, such leaps of faith – e.g. from monkey lesion to human disease, or from drug model to disease model – do not always hold. Given the extent of cholinergic damage seen in AD, and the importance of acetylcholine to a wide number of cognitive actions, is it not surprising to observe only small benefits when ChEIs are actually used in AD? Why also do some patients respond well to ChEIs, while others not at all? Hence there is a calling for technologies that are able to go beyond mere behavioural testing, that actually probe brain function, and subsequently to observe how drugs modulate both neurophysiological processes and performance in one.

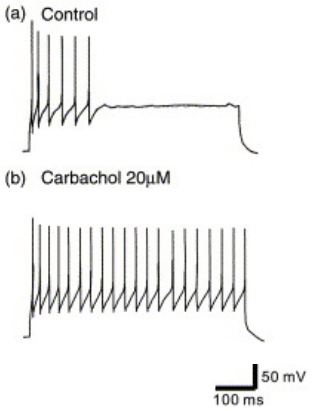
The advent of new non-invasive imaging technologies – fMRI, MEG, EEG, and radioligand-based techniques - as well as invasive recording in patients needing this for clinical reasons (e.g. as part of a perioperative protocol for severe epilepsy or Parkinson's disease), make it increasingly possible, firstly, to examine brain function directly in patients themselves, and secondly to observe the effects of psychoactive drugs on these measures. The results of such studies will need to be complemented by the findings of animal studies which, in so far as they enable recording of single units in any part of the brain, still provide by far the best resolution of neurophysiological measurement. Yet animal studies have limitations: there are clear phylogenetic differences with humans in brain anatomy, organisation, cognitive capacities, and diseases. It is only to be expected that animal models of Alzheimer's disease that recreate the biochemical and pathological characteristics of the human equivalent are difficult to compare at the level of cognitive impact. So our best understanding will most likely come from a synthesis of human-based imaging techniques with animal-derived data.

A good example of how measurements of neural responses can be compared across different instruments, at different scales, in different organisms is shown in the following example (Figure 1.1; for Details see pages 45 - 47 and 85). The effects of increasing acetylcholine levels, either directly or through ChEI administration, on occipital cortex responses to visual stimulation have been looked at using: 1) single cell recording; 2); voltage-sensitive dye optical imaging 3) event-related potentials; and 4) fMRI (from the current thesis), in cats (1,2,3) and humans (4), respectively. The animal studies seem to

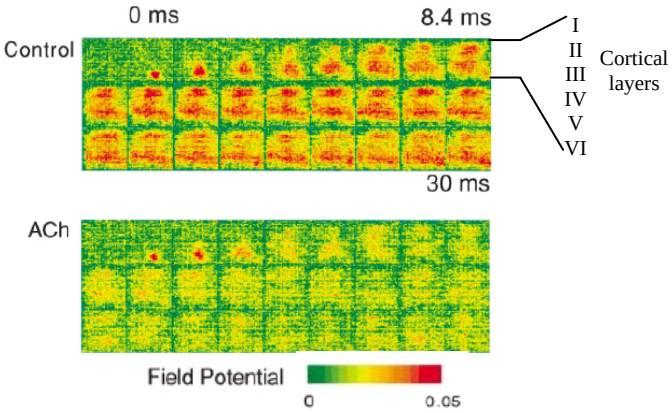
converge on showing that increasing acetylcholine concentrations decreases the overall level of neural activity, due to an increase in the ratio of signal (afferent input) to noise (including feedback or intrinsic input). The latter is demonstrated by comparing activation levels from layer IV (afferent input) with that of other layers that receive intrinsic input, which is illustrated succinctly by example (2). In humans, the blood oxygen level dependent (BOLD) – fMRI response in primary visual cortex, but not other cortical areas, was also found to be reduced by physostigmine (that increases extracellular acetylcholine levels) suggesting that what is seen by BOLD in humans is equivalent to that seen with voltage dyes and single unit recordings in animals. For certainty of this claim, however, it would be necessary to perform pharmacological investigations in animals that are simultaneously probed with electrophysiological and BOLD measures, as performed in monkey visual cortex (Logothetis et al, 2001). For our example, it would be crucial to see if cholinergic-induced changes in neural activity paralleled that observed with BOLD, since it is likely that factors underlying monkey and human BOLD signal generation are very similar – certainly more similar than the link between recordings of animal single units or columns and human BOLD or cerebral blood flow.

Figure 1.1: Different scales and methods by which cholinergic function can be measured.

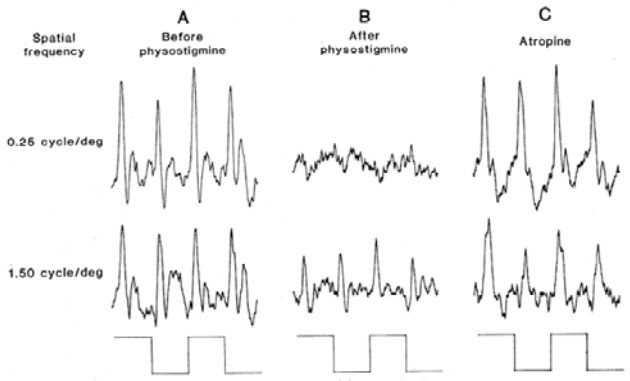
1) Single Unit (Tatano et al, 2005)



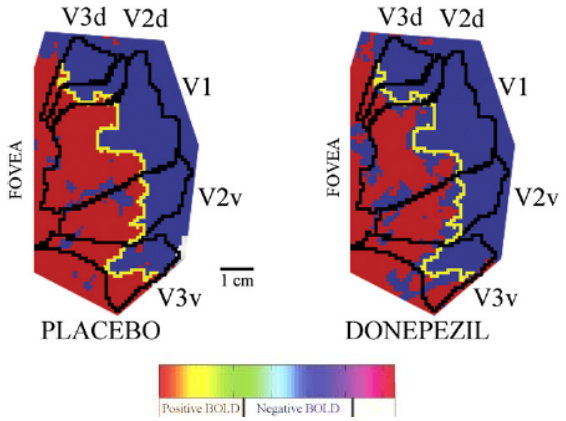
2) Cell Column (Optical Imaging) (Kimura et al, 1999)



3) Evoked Responses (Harding et al, 1983)



2) BOLD Response (Silver et al, 2008)



Layout

Cholinesterase inhibitors work primarily by boosting the brain's cholinergic system, and so the thesis **Background** begins with a survey of cholinergic neuroanatomy, neurophysiology and neuropsychology. Three broad cognitive systems can be identified with which endogenous and exogenous cholinergic manipulations interact: sensory, attention and memory. A description of cholinergic influences on these systems using traditional neurophysiological methods such as single-unit recordings, micro-iontophoresis, and lesion studies is first described, before the most well-established models of brain cholinergic function are expounded. This section is followed by a brief account of cholinergic pharmacology, including a summary of physiological and psychological effects cholinesterase inhibitors. Finally, since the last two experiments in this thesis investigate effects of cholinesterase inhibitors in Alzheimer's disease, this section evaluates evidence for and against a central cholinergic deficiency as a cause of this disease's clinical manifestations.

The experiments presented in this thesis contribute to approximately fifty cholinergic functional imaging studies reported in healthy human adults. These previous functional imaging studies are described in the **Human Cholinergic Functional Imaging Review** section, by first dividing up effects into sensory cortex, frontoparietal and memory-associated modulations – in line with current models of cholinergic function. For each study is listed: the imaging methodology and paradigm; cholinergic drug employed; the resultant neuromodulation, and the nature of any behavioural effects concomitantly observed. A summary of the most consistent cholinergic functional imaging results is

then presented, together with an attempt to link these findings with current neurobiological and computational models of cholinergic function.

The **Methods** section next explains the theory and practice of functional magnetic resonance imaging (fMRI), both in terms of its physical basis, and in terms of the statistical analysis of its data. This section then questions the assumptions, criticisms and counter-criticisms to the application of fMRI to neuropharmacology, upon which all experiments in this thesis are based.

Corresponding to the three divisions of brain function with which acetylcholine interacts, the **Experiments** of this thesis encompass the question: how does cholinesterase inhibition – using the centrally-acting drug physostigmine - modulate neural activity associated with sensory stimulation, attention, and memory? Since contemporary accounts of cholinergic function postulate a role for acetylcholine in modulating processes that are both bottom-up (i.e. stimulus-dependent) and top-down (i.e. dependent upon task or internal-set), the experiments in this thesis are designed to tease apart the differential effects of cholinesterase inhibition on both types of process. Thus Experiments 1 and 4 probe effects of physostigmine on brain activity associated with **attentional** and **sensory** factors, manipulated orthogonally. Similarly, Experiment 3 contrasts effects of physostigmine on visual stimulation with that on attention and working memory, while also questioning cholinergic interactions with spatial-directed attention. Interweaving with cholinergic influences on sensory and attentional functions, are its effects on memory – especially since pharmacological studies show that

cholinergic blockade interferes primarily with stimulus encoding. Correspondingly, Experiments 2 and 5 question how physostigmine modulates **memory**-associated neural activity, using the functional imaging memory signatures of repetition decreases and subsequent memory effects, respectively. It should be noted that Experiments 2 and 5, involve the same general behavioural paradigm as Experiments 1 and 4, respectively (thus explaining their ordering), yet are analysed in ways that specifically focus on their memory-related factors. Finally, given evidence for differences in cholinergic status between healthy people and those with Alzheimer's disease, and given the clinical role of pro-cholinergic drugs, Experiments 4 and 5 question whether cholinesterase inhibition exerts different neuromodulatory effects between **Alzheimer's disease** patients and age-matched **healthy** controls.

The main results of the five experiments are summarised in the **General Discussion**, before a more general interpretation of these experiments is presented in the form of six key findings. The thesis concludes by questioning the role and potential future applications of pharmacological-functional imaging.

2. Background

Cholinergic Neuroanatomy

A helpful starting point in understanding the functionality of any neural system is a detail of its anatomy, and more specifically, what other structures it connects with. Unlike neuromodulators such as dopamine and serotonin which are confined to well-localised brain circuits (e.g. dopamine with premotor and reward pathways), neurotransmission involving acetylcholine occurs in virtually all parts of the brain, reflecting its purported involvement in nearly all cognitive domains.

The localisation of cholinergic neurons in the post-mortem brain has been achieved most specifically through immunohistochemical identification of choline acetyltransferase (ChAT), the synthetic enzyme for ACh (Fig. 2.1). This has shown in the human CNS that cholinergic neurons occur in the following locations, some of which are assembled in cell groups, or nuclei, designated 'Ch' (Figs. 2.2, 2.3):

1. Basal forebrain (Ch1-4):
    - substantia innominata = magnocellular nucleus basalis of Meynert = Ch4
    - medial septum = Ch1
    - vertical limb nucleus of the diagonal band = Ch2
    - horizontal limb nucleus of the diagonal band = Ch3
2. Striatum: interneurons
3. Cerebral cortex: interneurons
4. Brainstem / reticular formation:
    - pedunculopontine tegmental nucleus (Ch5): connects diffusely to thalamus
    - lateral dorsal pontine tegmental nucleus (Ch6): connects to specific

thalamic nuclei

- parabrachial nuclei (pons): connects to thalamus

5. Habenula, medial nucleus (Ch7) (posterior thalamus)
6. Parabigeminal nucleus (Ch8): interacts with superior colliculus
7. Motor nuclei and parasympathetic branches of cranial nerves
8. Motor neurons of spinal cord

Animal lesion studies have shown that it is the basal forebrain, rather than other sources of ACh, that is most critical for higher cognitive processes such as attention and memory. The wide range of cognitive processes that appear to be under cholinergic influence is suggested by the broad connections of basal forebrain cholinergic cells with all parts of cerebral cortex, as well as to thalamus and brainstem. A minor input to cingulate and medial prefrontal cortices is also provided by pontine tegmental ACh fibres (Satoh & Fibiger, 1986). Cholinergic structures other than basal forebrain, e.g. in brainstem and thalamus, are concerned with more basic performance aspects, such as arousal.

The major functional divisions of cholinergic basal forebrain are as follows (Fig. 2.2):

1. Nucleus basalis (caudal basal forebrain; ventromedial pallidum) – Ch4
   - supplies whole of neocortex, and has broad sensory, attentional and memory functions
   - supplies amygdala via ventral amygdalofugal pathway and stria terminalis
   - divisible into medial and lateral cholinergic pathways (Selden et al, 1998) (Fig. 2.3). The medial pathway supplies the gyrus rectus, medial orbitofrontal, cingulate, retrosplenial and medial occipital cortices. The lateral pathway divides into a capsular division that courses in the white matter of the external

capsule and uncinate fasciculus, and a perisylvian division that travels with the claustrum.

2. Medial Septum – Ch1
   – supplies hippocampus via fornix-fimbria (along with GABAergic and neuropeptide fibres), and has selective effects on episodic memory
3. Vertical Limb of the Diagonal Band – Ch2
   - supplies cingulate cortex, as well as hippocampus, and performs a specific role in types of learning e.g. conditional discrimination
4. Horizontal Limb of the Diagonal Band – Ch3
   - supplies olfactory bulb

At a finer level of structural organisation, it appears that basal forebrain – corticopetal fibre system is clustered into longitudinal bands that separately innervate different parts of the cortical mantle (Zaborszky, 2002). This observation together with the fact that cholinergic projections to the cortex are only to a very limited degree, collateralised (Semba, 2000), suggests that the cholinergic basal forebrain may be divisible into functional modules with each modulating separate, parallel cortico-subcortical circuits. Spatially precise modes of cholinergic modulation within neocortex may also arise from two further anatomical arrangements: 1) cortico-cortical glutamatergic interactions at the *termini*, rather than (nucleus basalis) cell bodies of cholinergic fibres (Parikh et al, 2008), and 2) cortical cholinergic interneurons that are confined to neocortical columns (von Engelhardt et al, 2007).

For the purposes of the current thesis it cannot be underemphasised that any intravenously-administered cholinergic-acting drug e.g. physostigmine, which crosses the blood-brain

barrier, would be expected to act on all of these cholinergic structures. The aim of this thesis is to describe how cholinergic manipulation using such a globally-acting drug – as occurs in real-life clinical scenarios - can alter function-related activity within specific brain regions. Hence the anatomical specificity provided by *functional* imaging, as presented in this thesis, applies to neural consequences that are downstream to the sites at which cholinergic neurotransmission occurs, and not to the exact areas at which cholinergic pathways are modified. This lies in contrast to various *neurochemical* imaging techniques that employ radioligands to target chemically-defined structures such as receptors or transporters, and which delineate the sites of neurotransmission of the very neurochemical in question.

Figure 2.1: Schematic of cholinergic neurotransmission

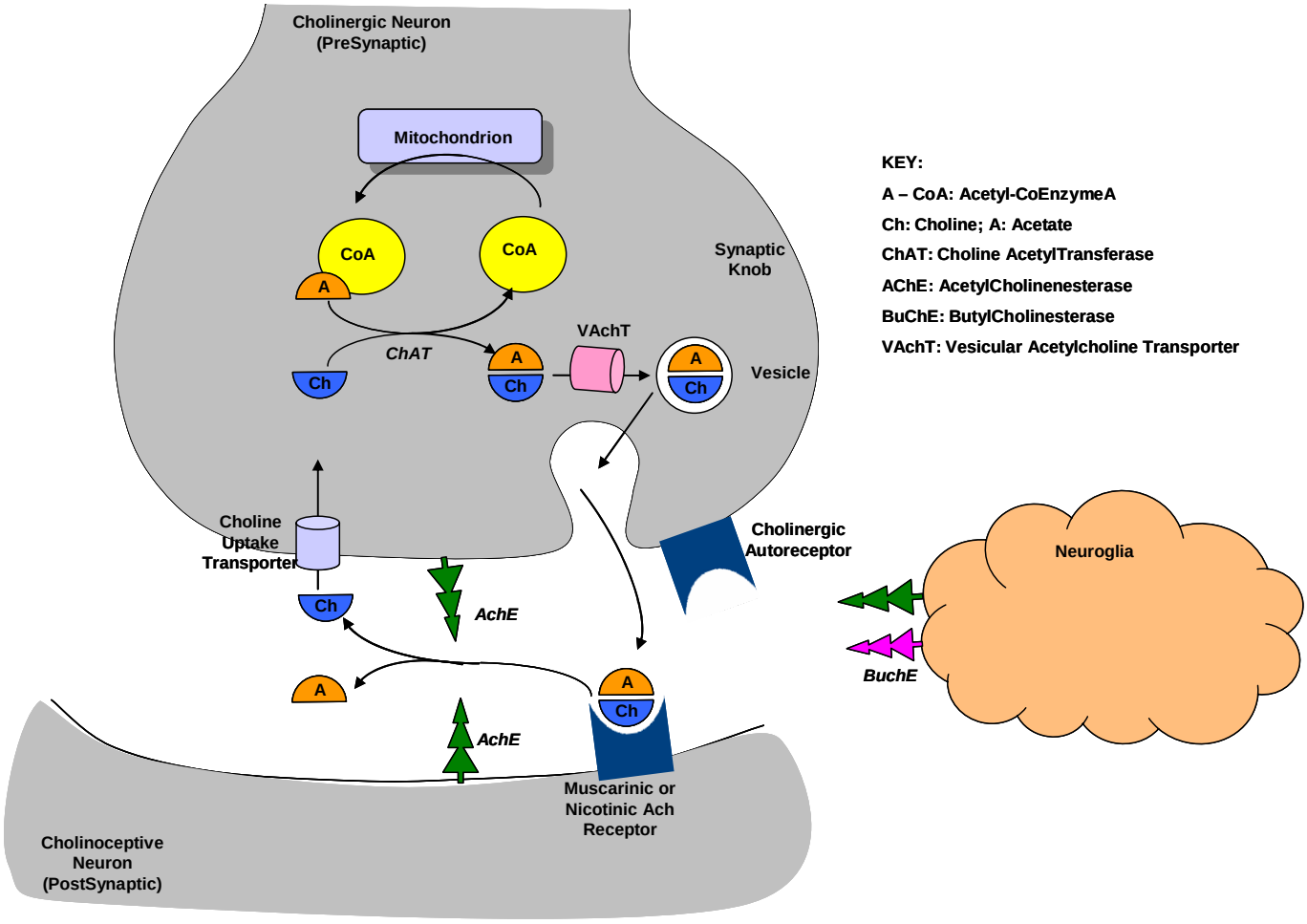
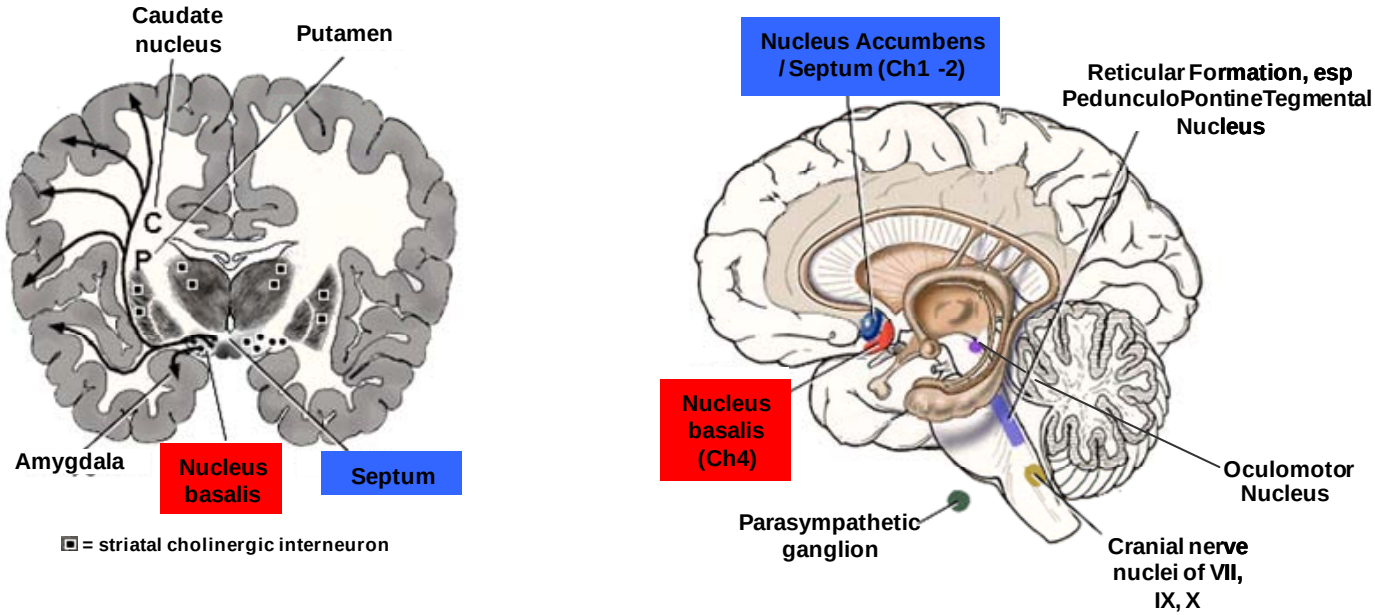


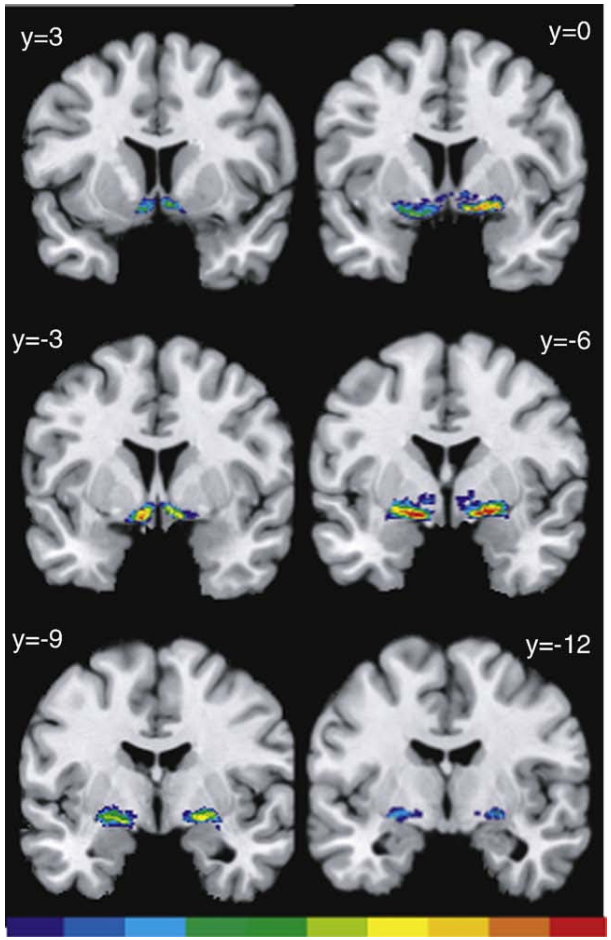
Figure 2.2: Principle cholinergic pathways of the human brain



adapted from from www.lib.mcg.edu

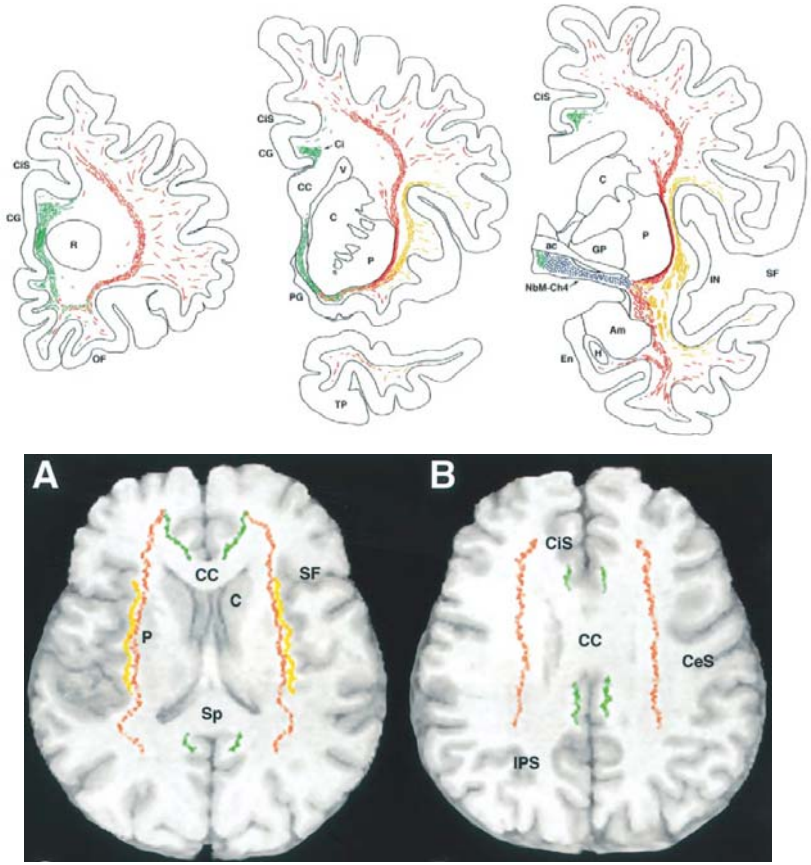
Figure 2.3: Disposition of cholinergic cell-bodies within nucleus basalis (A), and main cholinergic corticopetal trajectories (B) in human brain

A. Probabilistic maps of the Ch4 cell groups at six rostro-caudal levels



Zaborsky et al, 2008

B. AchE-rich (cholinergic) fibre bundles in hemispheric white matter.



Green=medial pathway; Red=lateral pathway (capsule division);
Orange=lateral pathway (perisylvian division).
Blue=Cholinergic Ch4 neurons of the nucleus basalis of Meynert.

Selden et al, 1998

Cholinergic Neurophysiology – General vs Specific Neuromodulation

Diffuse, tonic effects

The cortical cholinergic system has traditionally been thought of as a general activator of cerebral activity, with the nucleus basalis being conceived as a rostral extension of the brainstem ascending reticular activating system. During tonic states such as wakefulness (Kametani & Kawamura, 1990) and REM sleep (Jasper & Tessier, 1971), there are increases in basal forebrain activity; cortical acetylcholine release, and cortical EEG arousal patterns; while during inactivity or slow-wave sleep all these measures decrease (Detari et al, 1999). Cholinergic antagonists, or lesions of nucleus basalis, also induce large slow waves in the cortex similar to those seen during non-REM sleep (Buzsaki et al, 1988). If only a partial basal forebrain lesion is made then only the cortical region corresponding to the denervated cholinergic fibres shows EEG slowing. Activating fibres from mesencephalic reticular formation to nucleus basalis explain cholinergic dependency of arousal-state cortical EEG patterns (Wainer & Mesulam, 1990), while cholinergic projections within pedunculopontine tegmental nucleus can induce arousal patterns of thalamocortical activation (Ye et al, 2010).

Corresponding to the behavioural-EEG data are structural and physiological properties of cortical cholinergic cells that suggest ACh acts non-specifically to increase cortical responsiveness. Cholinergic fibres project to all parts of the cerebral cortex and access all layers (Mechawar et al, 2000). The number of cholinergic basal forebrain neurons is far less (e.g. ~ 7000 in the rat nucleus basalis) than the number of target neocortical neurons (Miettinen et al, 2002). Correspondingly, cholinergic cells that innervate the neocortex have extensive terminal fields implying co-activation of

widely divergent cortical regions (Adams et al, 1986). Furthermore, any one cortical region receives input from disparate nucleus basalis regions (Baskerville et al, 1997). Early studies suggested that the predominant response to acetylcholine is excitation (Krnjevic & Phillis, 1963; Sillito & Kemp et al, 1983). However, more recent studies, employing ACh concentrations and temporal durations matched to physiological modes of release, suggest that neural inhibition may be a more widespread neocortical response to ACh (Gulledge et al, 2007).

Activation of the basal forebrain cholinergic neurons occurs in a diffuse, all-or-none fashion. Input connections to the basal forebrain from disparate cortical, subcortical and brainstem areas suggest a broad, unselective activation pattern (Zaborszky et al, 1997). Furthermore, sampling of acetylcholine concentrations in widely separate parts of cortex shows no significant inter-regional difference in ACh release following sensory stimulation (Phillis & Chong, 1965; Collier & Mitchell, 1966), or following bidirectional pharmacological manipulation of basal forebrain activity (e.g. Casamenti et al, 1986; Moore et al, 1995).

At an ultrastructural scale, it is found that acetylcholine vesicles are mostly disposed within axonal varicosities that, in ~90% of cases, do not make immediate contact with synapses (Umbriaco et al, 1994; Umbriaco et al, 1995; Descarries et al, 1997). This layout suggests that activation of such cells sprays ACh over a wide cortical area, producing postsynaptic effects over a large spatial scale (so-called 'volume-transmission'). Furthermore, this structural arrangement accounts for post-synaptic effects of acetylcholine occurring over significantly longer time periods (~10-20

seconds) than classical glutamatergic or GABAergic synapses (< 1 second) (Krjnevic et al, 1971; Hasselmo & Fehlau, 2001).

Focal, phasic effects

In spite of many characteristics that lend favour to the traditional model of the cholinergic system as a mere arousal-heightening or 'gain increase' mechanism, a growing body of evidence disputes this. Anatomically, this has been suggested by noting that although cholinergic innervation is pervasive (see above), the regional and laminar arrangement of both cholinergic fibres, and cholinergic receptors, is heterogeneous (Lidow et al, 1989). For example, most cholinergic varicosities are concentrated in layers I and V, accounting for ACh's ability to modulate columnar output (Mechawar et al, 2000). Furthermore, contrary to some groups showing a predominance of non-synaptic cholinergic varicosities in rodents (Umbriaco et al, 1994), other groups have found in monkeys (Mrzljak et al. 1995) and humans (Smiley et al. 1997), as well as rodents (Turrini et al. 2001; Casu et al. 2002), that that the majority of corticopetal cholinergic fibres make specialised synaptic connections with cortical neurons (Fig. 2.4).

The relative importance of volume transmission (i.e. non-synaptic and tonic) relative to wired transmission (i.e. synaptic and phasic) in understanding neocortical cholinergic modulation has also been questioned by noting that non-synaptic ACh concentration changes are an order of magnitude of less than that required to depolarise pyramidal neurons (Pepeu & Giovannini, 2004). Furthermore, basal forebrain cholinergic neurons can be divided into whether they modulate cortical activity tonically or phasically (Detari et al, 1999).

More recently, a sensitive electrochemical method of measuring extracellular ACh concentrations at a second-scale temporal resolution suggests that apparent tonic changes in ACh concentration, as recorded by traditional microdialysis methods recording at a minute-scale resolution, can in fact be at least partially accounted for by recurrent, but phasic, release of ACh (Parikh et al, 2007) (Fig. 2.5). Critically, these results show that the dependency of performance, e.g. cue detection, on acetycholine occurs via such phasic transmission, correlating as it does with trial-by-trial accuracy. If volume transmission was a major mode by which ACh acted, then bursts of cholinergic neuron activity would be expected to increase extracellular ACh levels – which in fact is not found (Giuliano et al, 2008).

Increasingly, evidence is also emerging for differential activation of specific sectors of nucleus basalis, allowing for variation of acetylcholine release between cortical regions. Anatomically this is suggested by segregated topographical (Carey & Rieck, 1987; Semba, 2000) and laminar (Rieck & Carey, 1984) organisations of basal forebrain cells. The significance of this arrangement is that different parts of basal forebrain can be selectively activated depending on task demands. For example, rats presented with either visual or somatosensory stimulation are found to release relatively more ACh in the correspondingly stimulated, than unstimulated, sensory cortex (Fournier et al, 2004) (Fig. 2.5). Furthermore, passive sensory stimulation induces ACh release in the appropriate sensory cortex without prefrontal cholinergic release (Laplante et al, 2005); but if the animal attends to the stimulus then frontoparietal ACh release also occurs (Arnold et al, 2002). Differential activation of nucleus basalis may itself originate from selective activation of sensory cortices,

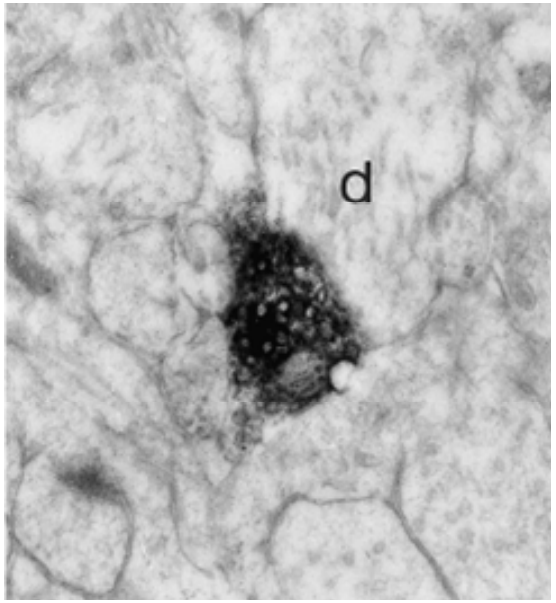
possibly via indirect pathways involving prefrontal cortex (Golmayo et al, 2003; Rasmusson et al, 2007).

The traditional model by which nucleus basalis cholinergic projections modulate cortical glutamatergic signalling appears in fact to be too simplistic, with evidence for the reverse interaction also co-existing. Specifically, phasic ACh release within neocortex may depend upon local activity within glutamatergic thalamocortical afferents, some of whose termini signal with cholinergic termini (Parikh et al, 2008). This raises the intriguing possibility that the cholinergic system has 'evolved' from being a general activator of cortical activity (e.g. for arousal) – as suggested anatomically – to being placed under more specific control by cortical inputs themselves (Sarter et al, 2009). Furthermore, while nucleus basalis activation may serve to deliver ACh to large and widespread areas of neocortex, the arrangement of *cholinergic cortical interneurons* allows for cortical modulation to occur in much more spatially-circumscribed postsynaptic fields (von Engelhardt et al, 2007).

In summary, the capability of the cholinergic system to be selectively activated and, in turn, to direct its output towards specific neocortical targets, helps in explaining its modulation of functions characterised by spatially segregated patterns of activity, e.g. attention and learning.

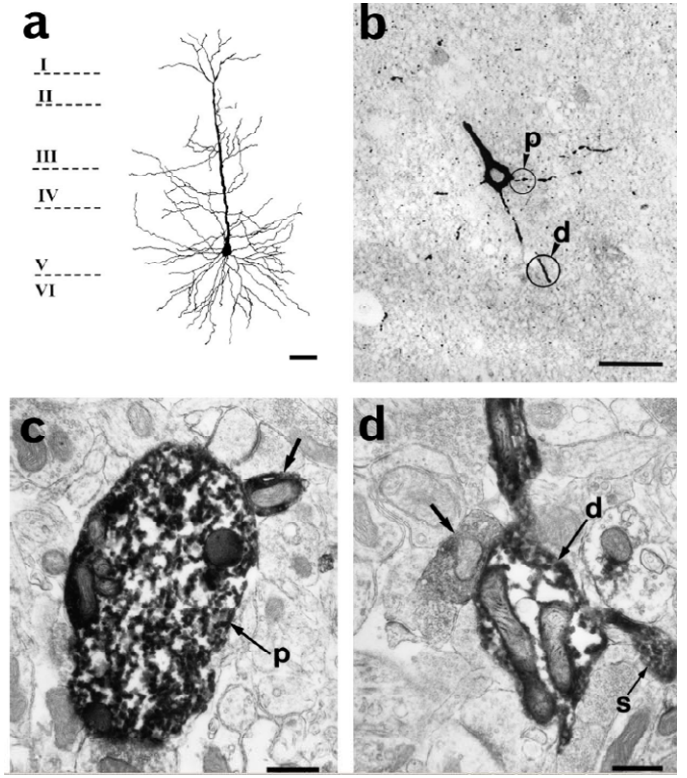
Figure 2.4: Cholinergic neurotransmission can occur diffusely (A), or via specific synapses (B), with the latter mode predominating in humans. (A) Rat cortex showing a non-synaptic cholinergic varicosity (staining positive for ChAT) abutting with, but not forming synapses with, cortical dendrites (d). (B) Rat cortical section showing how both proximal (p) and distal (d) dendrites of a pyramidal cell (silver stained) form specialised synaptic contacts with cholinergic axonal varicosities (i.e. VAchT-positive; unlabelled arrows). (s) = dendrite spine.

A.



(Mechawar et al, 2002;
see also Descarries et al, 1997)

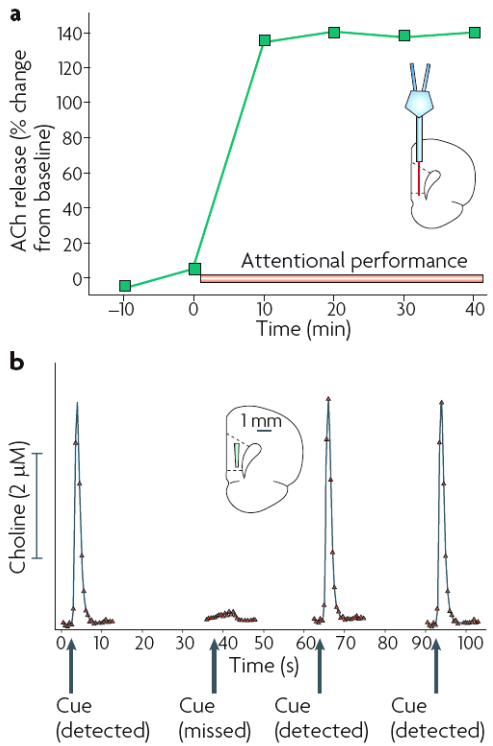
B.



(Turrini et al, 2001;
see also Smiley et al, 1997)

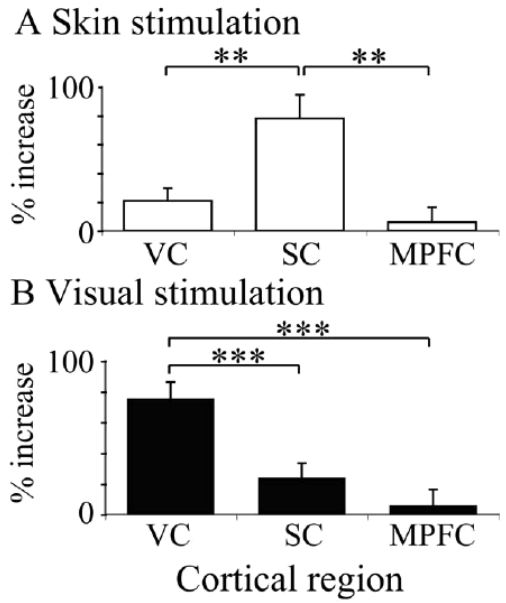
Figure 2.5: (A) Apparently tonic modes of ACh release in cerebral cortex, as measured by microdialysis (a) may in fact be accounted for by recurrent phasic release of ACh as resolved with enzyme-plated electrodes (b). Moreover, the phasic release of Ach into prefrontal cortex correlates strongly with accuracy on a trial-by-trial basis. (B) Region-specific release (% increase) of ACh is found in a paradigm in which rats are presented with either skin or visual stimulation; ACh was measured simultaneously in visual cortex (VC), somatosensory cortex (SC), and medial prefrontal cortex (MPFC).

A.



(Sarter et al, 2009)

B.



(Fournier et al, 2004)

Cholinergic Neurophysiology – Cellular Actions

One of the earliest characterisations of electrophysiological responsiveness to acetylcholine was that it heightened cortical responsiveness to synaptic inputs, but did so in a manner quite different to the direct and fast actions of glutamate, the main cortical excitatory neurotransmitter. Specifically, ACh tends only to increase firing in cells in which there is all ready an excitatory input, but does not increase baseline activity (Krnjevic & Phillis, 1963; Fig. 2.6). Excitatory patterns of ACh modulation in neocortex include: 1) an initial temporary decrease in spontaneous activity, before spike frequency slowly increases for ~10-20 seconds after ACh activation has stopped (McCormick & Prince, 1985); 2) a reduction of spike adaptation seen in cortical cells following prolonged input (McCormick & Prince, 1986; Zinke et al, 2006), and 3) potentiation of sustained-spiking following a single depolarising event, seen especially in perirhinal-entorhinal cortex (Klink & Alonso, 1997; Egorov et al, 2002). The prolongation of input-driven activity may be of functional importance, as we shall see later, both in attention – by increasing the likelihood of response to brief stimuli (Sarter et al, 2005a), and in memory – by facilitating long-term potentiation and other mechanisms of synaptic strength modification (Rasmusson, 2000).

More recently, groups using transient ACh stimulation (20-40 ms) to simulate a physiological mode of ACh release have shown net inhibitory effects of ACh in neocortex, especially in layer V pyramidal cells – the main output of cortical columns (Xiang et al, 1997; Gulledge et al, 2007; Fig. 2.6). Suppressive effects due to ACh are partly mediated by NMDA receptor-dependent pathways that may explain why cholinergic modulation of sensory processing is activity-dependent (Levy et al, 2006).

In order to reconcile these disparate effects of ACh on neural responsivity, it has been suggested that the initial, phasic onset of cholinergic activation may initially 'reset' populations of output cells by forcing a common hyperpolarisation potential. Subsequently, after a period of high-frequency and long-duration ACh stimulation, the net effect may be voltage-dependent excitation (Andrade, 1991; Haj-Dahmane & Andrade,1996; McCormick & Prince, 1985). These two serial effects may help to explain the well-established cholinergic property of enhancing cortical signal-to-noise ratio: by first suppressing background activity, and then favouring pyramidal cells that have the strongest synaptic input drive (Gulledge et al, 2007).

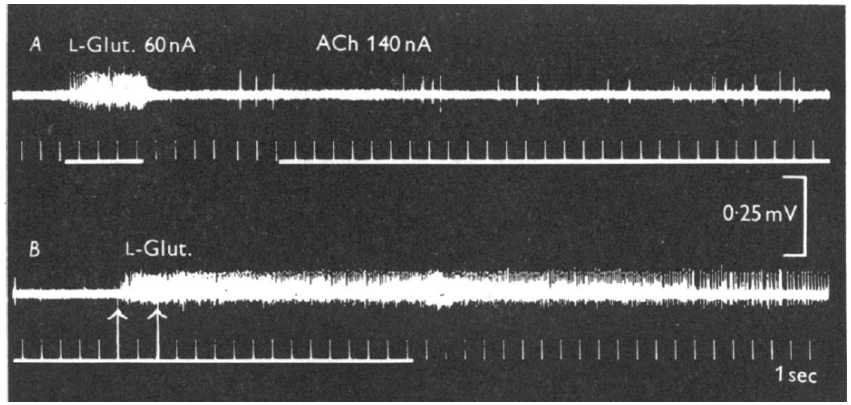
A similar conclusion has also been suggested by computational models that attempt to account for bi-directional effects of ACh on inhibitory interneurons in hippocampus and sensory cortices (Behrends & ten Bruggencate, 1993). Under weak afferent input, ACh enhances *spontaneous* release of GABAergic-inhibitory neurotransmitter, thereby dampening background pyramidal cell activity. Conversely, under strong afferent input, ACh decreases pyramidal cell-driven GABA release, which suppresses local negative feedback, and effectively turns an asymptotic input-output function into an exponentially-rising one (Patil & Hasselmo, 1999).

There are two further patterns by which heterogeneous cellular effects of ACh may converge to serve common microcircuitry functions. Firstly, opposite effects of ACh on two classes of cortical inhibitory interneuron produces the net effect of decreasing intracolumnar signalling, while facilitating intercolumnar signalling (Xiang et al, 1997). In sensory cortex, this will tend to result in heightened ACh levels reducing

surround inhibition, while facilitating learning-associated enlargement of relevant stimulus representations (Fig. 2.7) (Froemke et al, 2007). Secondly, spatially-segregated effects of ACh on pyramidal cell inputs has the net effect of potentiating cortical inputs (in cortical layer IV), whilst inhibiting intracortical neurotransmission (Hasselmo & Bower, 1992; Linster et al, 1999; Hsieh et al, 2000). In this way, the net effect of ACh can be to suppress intracortical feedback or lateral connectivity, while sparing, or even enhancing, thalamocortical-originating signals (Hasselmo & Cekic, 1996; Fig. 2.8). Such patterning of information-flow by ACh has been surmised to be responsible for numerous functional consequences of ACh modulation depending on which part of neocortex is modulated, including: 1) enhancing stimulus detection and thereby attention (Sarter et al, 2005a); 2) favouring memory encoding over consolidation (Hasselmo & McGaughy, 2004), and 3) shifting perceptual inference towards an 'uncertain' mode in which bottom-up inputs outweigh top-down influences (Yu & Dayan, 2002).

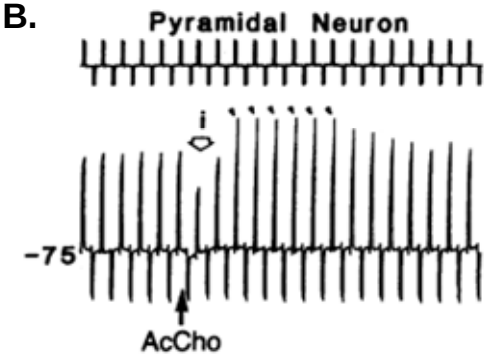
Figure 2.6: (A) Cholinergic stimulation does not increase baseline firing rate of cortical neurons but does increases their response to excitatory input (here provided by glutamate application). (B) Biphasic response to cholinergic stimulation in cortical pyramidal cell showing initial depression and later potentiation of depolarising inputs. (C) Transient cholinergic stimulation induces widespread cortical inhibition in layer 5 pyramidal neurons of rat cortex.

A.



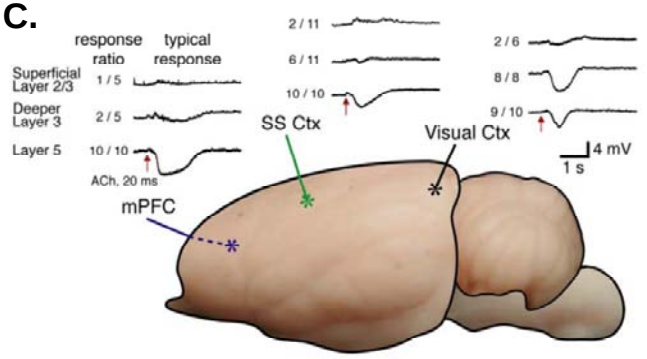
(Krnjevic & Phillips, 1963)

B.



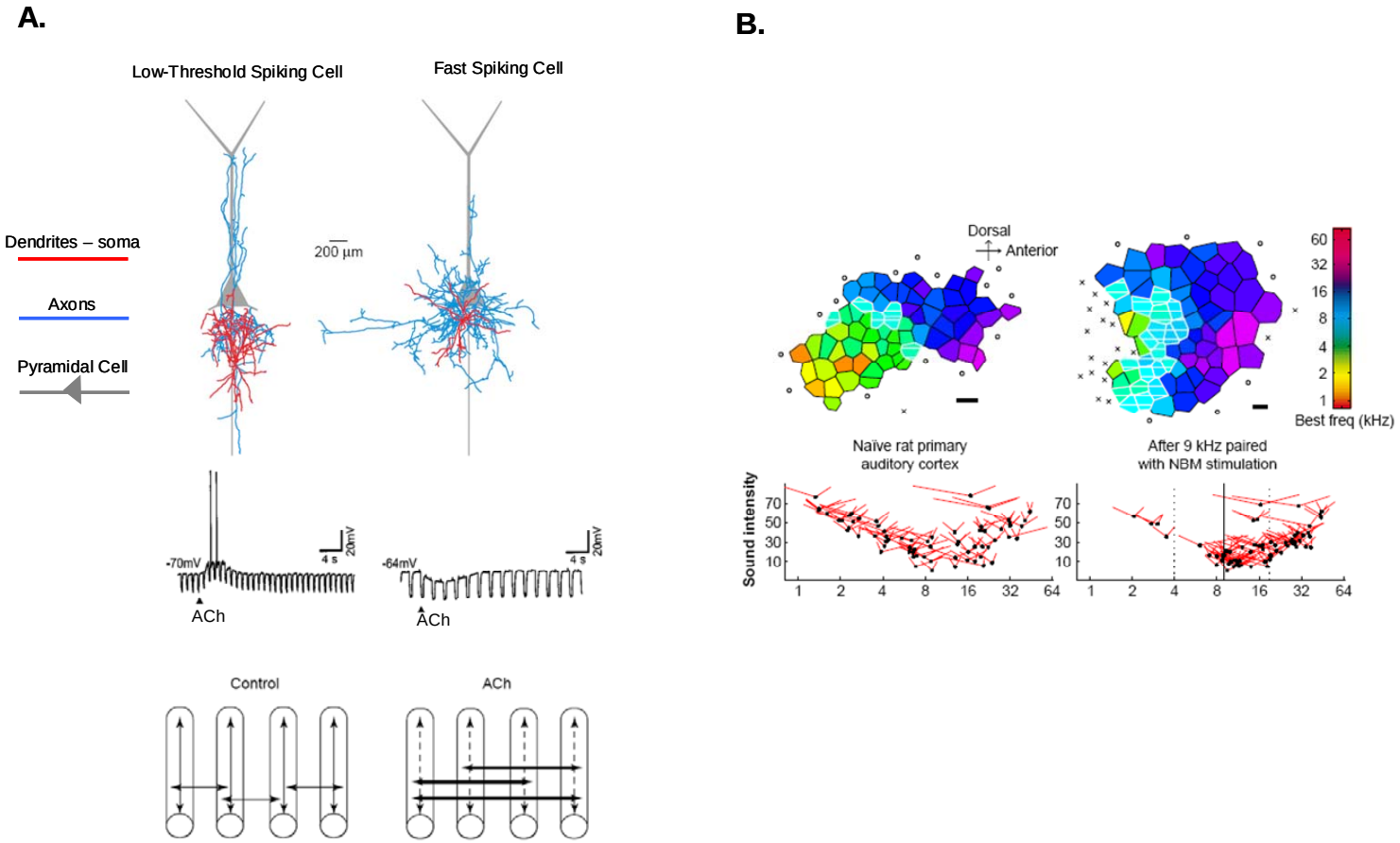
(McCormick & Prince, 1985)

C.



(Gulledge et al, 2007)

Figure 2.7: (A): Response to acetylcholine of cortical inhibitory interneurons (red - blue), and their differential disposition relative to pyramidal cells (grey) and cortical columns (bottom), results in high ACh states favouring intercolumnar over intracolumnar signalling. (B) Pairing of tone-specific auditory input (e.g. 9kHz) with cholinergic stimulation increases lateral extent of neurons whose tuning curves peak at the input auditory frequency (x-axis): note that the cellular modulations of ACh observed in (A) suggest one mechanism by which this could be achieved.

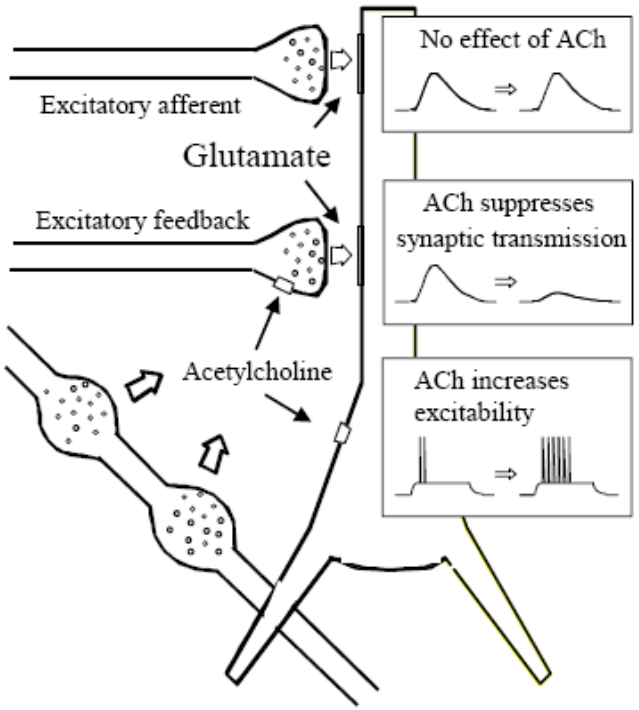


(Xiang et al, 1997)

(Kilgard & Merzenich, 1998)

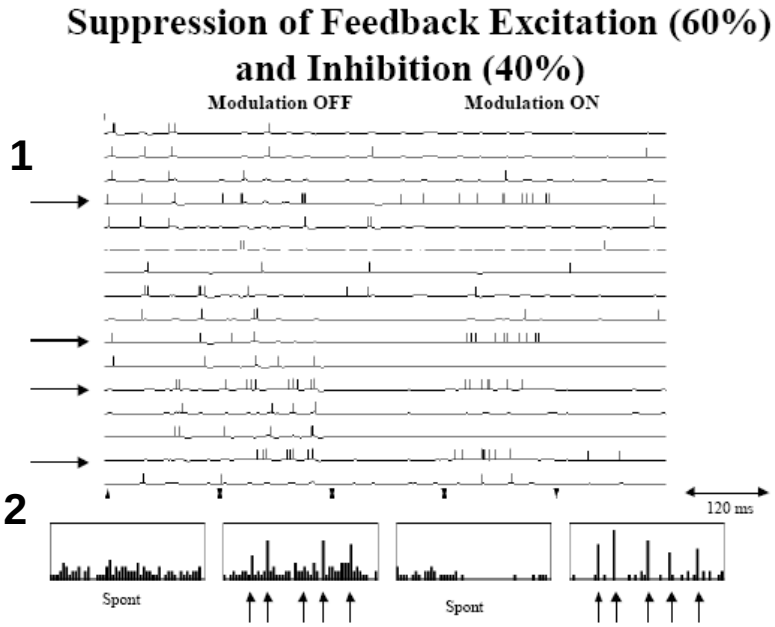
Figure 2.8: (A) Segregated effects of ACh on cortical pyramidal cells can account for pattern of enhancing columnar inputs (e.g. from thalamus) while decreasing feedback. (B) Modelling of single-fibre activity in piriform cortex. In the absence of ACh (Modulation OFF), pyramidal cell output is a combination of both afferent input (ARROWS) and feedback signals. On activating ACh modulation (Modulation ON), afferent inputs are favoured, while background activity is suppressed. Afferent-driven pyramidal cells are also more likely to synchronise thereby potentiating associative firing. 1: individual unit rasters; 2: spike histograms during spontaneous firing and afferent input activity periods.

A.



(Hasselmo & McGaughy, 2004)

B.



(Hasselmo & McGaughy, 2004)

Cholinergic Neurophysiology - Systems

One of the greatest neuroscientific challenges is to bridge the gulf between cellular, or cell-network, processes on the one hand, and cognitive or behavioural phenomena, on the other. While the range of psychological constructs that acetylcholine has been shown to influence is wide, within these categories its effects are specific and well-defined. For example, cortical cholinergic integrity is essential for **sensory** signal enhancement, but only for signals that are deemed task-relevant (Sarter et al, 2001); is required for **attentional** enhancements to novel stimulus contingencies, but not attentional reductions to repetitive contingencies (Chiba et al, 1995; Baxter et al, 1997), and underlies certain aspects of **memory** encoding and retrieval (Everitt & Robbins, 1997). Reiterating the pattern of neuronal effects produced by acetylcholine, it is clear that the particular behavioural profile associated with cholinergic modulation is far from being easily captured by a simplistic, umbrella-type property such as 'increasing overall efficiency' or 'processing speed'.

In attempting to explain behavioural influences of acetylcholine it is tempting to peer over the 'explanatory divide' to cellular and network impacts of equivalent cholinergic manipulations, in the hope that links can be made between the two disciplines. One example that is commonly made in this regard is the neurophysiological finding, described above, that ACh enhances input-driven neuronal activity, whilst suppressing background noise. For behavioural phenomena, such as that an intact cortical cholinergic system is required for stimulus detection, especially of briefly-occurring events, the relationship with the neuronal observation seems fairly clear. Ideally, both neuronal firing and performance would need to be

measured in the same animal, and shown to be closely correlated, for this explanation to be consolidated.

Extending this idea, it has been speculated that other ACh-dependent performance effects –such as attention-driven target detection, conditioned stimulus acquisition and memory encoding and retrieval - may also follow from what we know about cholinergic enhancement of neuronal signal-to-noise ratio (Everitt & Robbins, 1997; Furey et al, 2000; Gu, 2002). By this account, competition between multiple signals, whether originating from sensory events or stored memory traces, can be facilitated by processes that exaggerate differences in signal strength. The attraction of this account is that it helps to unite diverse behavioural phenomena by interpreting them as different applications of the same fundamental neural processes. In a similar fashion, neuronal-level, or network-level, effects of ACh, such as neuronal synchronisation and sustained-spiking, and regulation of cortical-laminar flow have been adduced as explanatory crutches for diverse effects of ACh on sensory, attentional and memory processing.

There is another angle by which the multiple, seemingly disparate behavioural effects of acetylcholine can be seen to converge through common physiological processes. This can be appreciated by noting the types of naturalistic conditions that increase neocortical ACh levels (Himmelheber et al, 2000). In general the cortical cholinergic system becomes activated during novel or uncertain circumstances; or in response to emotionally salient stimuli, when attention to certain aspects of the environment has to be maximised, even after controlling for variables such as motor activity and reward (Giovannini et al, 2001). During these behavioural ‘states’, it makes

evolutionary sense for the animal to enhance both: 1) sensory processing and orienting, and 2) encoding of the current situation, so as to reduce future uncertainty and to enable learning of appropriate behavioural responses. The nucleus basalis - by virtue of its cortex-wide connectivity - is able to orchestrate this facilitation of sensory, attentional and memory processes. Furthermore, effects of ACh on sensory and perirhinal-entorhinal cortex information flow – namely, enhancing input processing and feedforward associativity, while suppressing feedback – are argued to be supportive of both of these neuromodulatory roles (Hasselmo & McGaughy, 2004).

Sensory

Cholinergic fibres from nucleus basalis provide rich innervation to all layers of primary sensory cortices (Zilles et al, 1990). Following stimulus presentation, acetylcholine is released into sensory cortices (Laplante et al, 2005; Fournier et al, 2004), that increases under circumstances when active sensory processing is favoured, i.e. – with attention (Sarter et al, 2005). About 90% of visual cortical neurons are responsive to acetylcholine, of which 60 – 75% show a facilitatory response (Sillito & Kemp, 1983; Sato et al, 1987a; Zinke et al, 2006). This facilitatory response often manifests itself as a reduction of usual adaptation to sustained sensory input, rather than as heightening of early peak activity (McCormick & Prince, 1996; Zinke et al, 2006). Additionally, removing cholinergic input to visual cortex causes a profound impairment in sensitivity to visual stimuli (Sato et al, 1987b). However, cholinergic facilitation of cortical activity only occurs in cells in which there is all ready a significant synaptic input (Krjnevic & Phillis, 1963). In other cortical cells, ACh has the effect of decreasing baseline activity (Sato et al, 1987b).

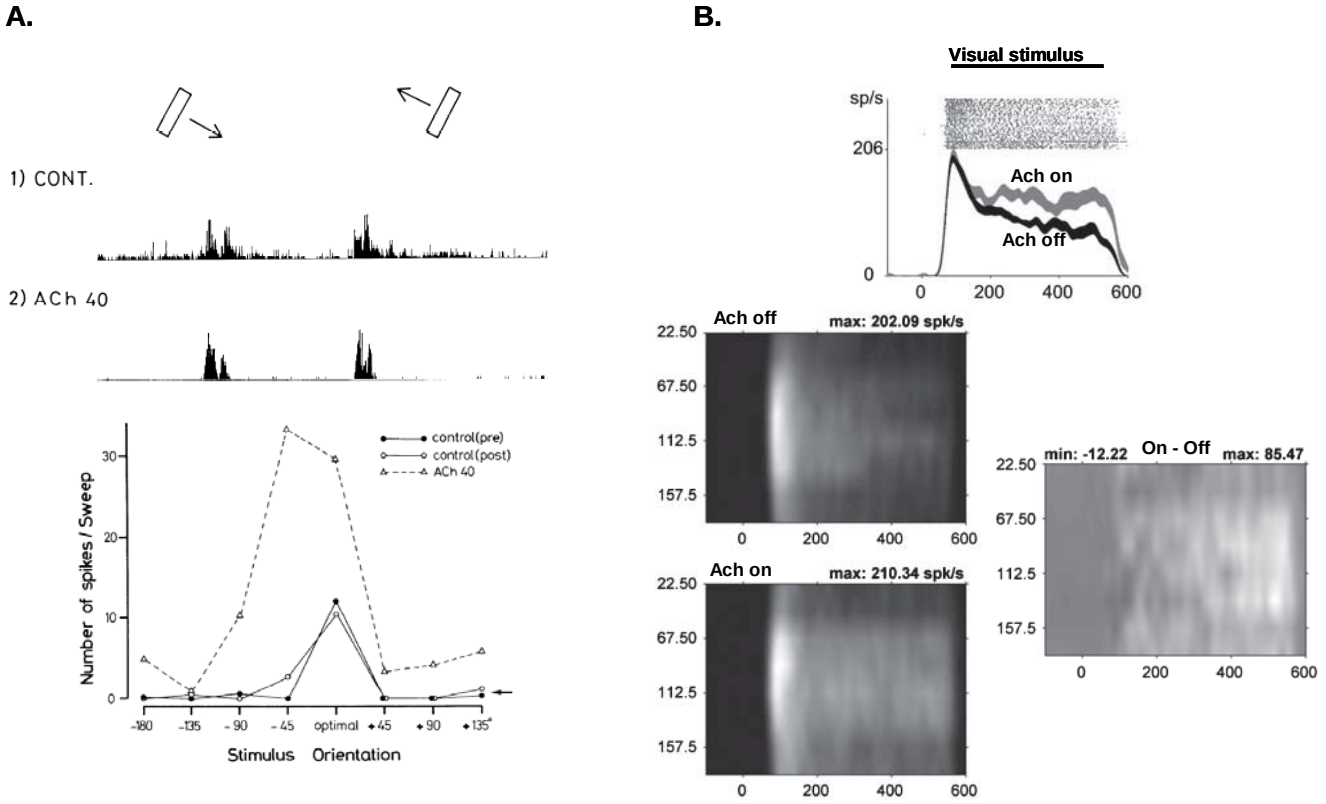
By heightening stimulus-driven input while dampening background firing, acetylcholine is able to increase gain, or signal-to-noise ratio, rather than just increasing cortical activity in general (Fig. 2.9). However, signal-to-noise ratio is also dependent on input timing: the more synchronised inputs are, the stronger and more stable are the resultant postsynaptic potentials, and consequently output firing frequencies. Hence the finding that ACh drives synchronisation between sensory cortical neurons (Rodriguez et al, 2004), which itself is associated with more efficient signal detection (Womelsdorf et al, 2006) reveals another aspect by which ACh facilitates sensory responses. Cholinergic-driven cortical synchronisation occurs at high frequency (gamma-band: ~40Hz) which maximises the effect of concerted inputs on target cells over unit time. The same frequency of cortical synchronisation also occurs during periods of high-attention (Fries et al, 2001) suggesting that cholinergic actions on cortical rhythmicity may underlie sensory attention.

A separate, and orthogonal, issue relates to the effects of ACh on stimulus selectivity. Studies examining visual cortical responses have shown that ACh increases input-driven activity more for stimuli at non-optimal, than at optimal, orientations (Sato et al, 1987a; Zinke et al, 2006; Fig. 2.9), thereby broadening tuning curves (however, see also Sillito & Kemp, 1983; Murphy & Sillito, 1991). This has been explained with reference to the well-established circuitry effects of ACh noted earlier - in which the net cortical response to ACh is general inhibition of all layers, except in input layer IV, for which inputs are facilitated (e.g. Gil et al, 1997; Kimura et al, 1999). Since selectivity arises from lateral or top-down, intracortical connections to layers other

than layer IV, their suppression by ACh would be expected to broaden sensory tuning curves (Zinke et al, 2006).

The ability of ACh to reduce intracortical communication is also able to account for the finding that ACh decreases spatial summation of neighbouring receptive fields, thereby reducing contextual or integration effects (Roberts et al, 2005). Given that ACh also enhances thalamocortical input within each neuron's own classical receptive field, these properties help to explain facilitatory effects of ACh on selective attention - when small-targets need to be detected and adjacent distractors need to be suppressed. Furthermore, in reducing contextual influences, while selectively enhancing input, ACh encourages a 'pure, unadulterated' mode of sensory processing, advantageous during periods of uncertainty (Yu & Dayan, 2002). Indeed in certain situations, basal forebrain activation may even potentiate weak, indirect sensory cortex inputs, e.g. from receptors peripheral to a sensory unit's classical receptive field, more than it potentiates direct inputs that are all ready strong (Kuo et al, 2009) – possibly encouraging detection and encoding of unexpected input patterns.

Figure 2.9: (A) Response of direction-orientation specific cat visual cortical neurons in absence and presence of 40 nA ACh, showing ACh reduces background activity, and hence increases signal-to-noise ratio. Lower graph shows that signal also increases at non-optimal orientations so broadening tuning curve. (B) Response of orientation specific marmoset visual cortical neurons in absence and presence of ACh, showing ACh increases visual response at later time periods, especially at non-preferred angular orientations (y-axis on grey-scale plots), thereby broadening tuning curve.



(Sato et al, 1987a)

(Zinke et al, 2006)

Attention

Neocortical cholinergic lesions impair the ability to detect, identify, or localise brief stimuli, especially in the presence of attention-demanding challenges such as distractors, while not affecting overall motivational state, response rate, rule memory, or directional bias (Robbins et al, 1989; Moore et al, 1995; Muir et al, 1994). The fact that such lesions result in performance impairments that are proportionate to the degree to which sensory/attentional processing is taxed (Himmelheber et al, 2001), suggests that the cortical cholinergic system may play an important role in interactions of attention with sensory processing, rather than influencing either process in isolation (McGaughy et al, 2002). An influential model that has emerged from such observations relates neocortical cholinergic release with the degree of mismatch between motivation-driven goals and actual performance, i.e. 'attentional effort' (Sarter et al, 2006). For example, cortical ACh levels increase following challenges that degrade reward-driven performance, which itself is instrumental in reversing the initiating behavioral impairment (Himmelheber et al, 2000; Kozak et al, 2006) (Fig. 2.10). This may account for correlations between ACh release and either sensory demands or motor response (Richardson & DeLong, 1990; Passetti et al, 2000).

Cholinergic interactions with attention are also manifest during learning paradigms. For example, attention shifts that normally occur in associative-conditioning, when a previously learnt contingency is suddenly violated, are critically dependent upon cholinergic inputs from nucleus basalis to posterior parietal cortex (Chiba et al, 1995; Bucci et al, 1998). Furthermore, learning, but not retrieval, of new feature conjunctions can be selectively disrupted by cortical cholinergic lesions (Botly & De

Rosa, 2009). To the extent that feature-binding is believed to require frontoparietal control (e.g. Esterman et al, 2007), and that learning of single features is found not to be impaired, this cholinergic-dependency is considered to be attentional, rather than sensory or learning per se.

The functional anatomy (and effective connectivity) by which the cortical cholinergic system supports attention involves interactions between prefrontal, parietal and sensory regions (Golmayo et al, 2003; Nelson et al, 2005) (Fig. 2.11). Performance monitoring information from prefrontal regions combines with arousal and motivational information from reticular and limbic regions, as well as contingency-violation or fear-conditioned signals from amygdala, in providing the main input to basal forebrain, and subsequent cortical acetylcholine release (Holland & Gallagher, 1999; Sarter et al, 2006; Gozzi et al, 2010). In turn, cholinergic inputs to prefrontal and parietal regions are thought to modulate processes such as distractor suppression (Gill et al, 2000), or attentional shifting (Davidson & Marrocco, 2000) and disengagement (Bushnell et al, 1998) between spatial locations or features (Bucci et al, 1998). Following repeated training with an attention-taxing task, cellular mediators of cholinergic neurotransmission are upregulated in prefrontal regions, and correlate with enhanced signal detection (Apparsundaram et al, 2005). Cholinergic inputs to prefrontal cortex may also serve to inhibit impulsive responses via subcortical structures (Bushnell et al, 1998; McGaughy et al, 2002).

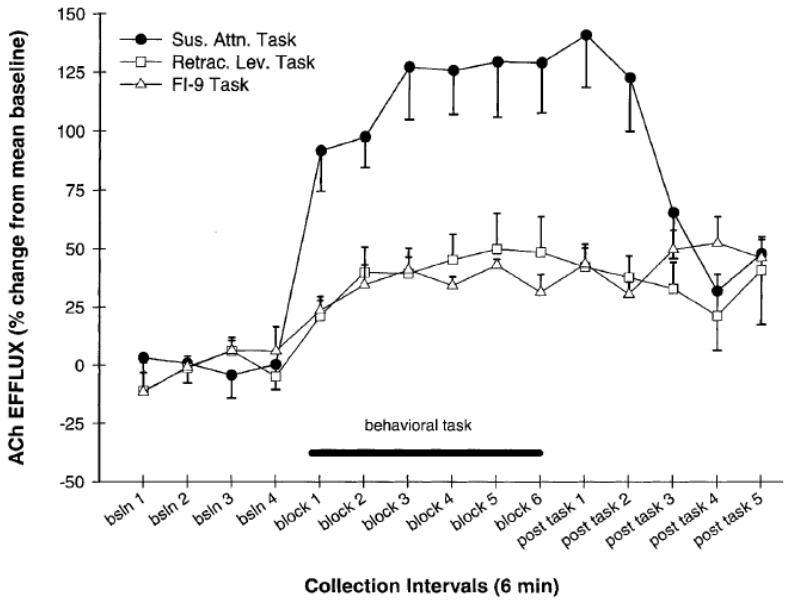
It seems likely that that cholinergic influences on *bottom-up* sensory processing (discussed in preceding section) may complement the effects of ACh on *top-down* attentional shifting and focusing (Sarter et al, 2001), given evidence for pan-cortical

covariations in ACh efflux (Phillis & Chong, 1965). This appears supported by the fact that selective attention-related activity (e.g. cue versus distractor associated neural activity) is found to be dependent upon local ACh concentrations in both frontoparietal (Gill et al, 2000; Broussard et al, 2009) and sensory cortices (Herrero et al, 2008), in a congruent fashion.

A further account for cortical acetylcholine release is that it correlates with 'uncertainty' regarding established stimulus-stimulus or stimulus-response contingencies (Bucci et al, 1998; Dalley et al, 2001). On this view, high acetylcholine levels favour bottom-up, over top-down, processes, and in so doing appropriately reduce cortical inference in times of uncertainty (Yu & Dayan, 2005). Importantly, this model accords with ACh efflux being related both to 'attentional effort' in the face of performance challenges (Sarter et al, 2006) and to novelty (Acquas et al, 1996). It also fits cortical slice data demonstrating that ACh promotes feedforward over feedback signalling (Hasselmo & McGaughy, 2004). The model successfully predicts that cholinergic levels are inversely correlated with cue validity in a Posner spatial-attention paradigm, and that as ACh levels increase, the degree to which a cue focuses attention - i.e. the cue validity effect - decreases (Phillips et al, 2000). Furthermore, prefrontal ACh innervation mediates cognitive flexibility during serial contingency reversals, but not initial acquisition of contingency (Cabrera et al, 2006), consistent with ACh communicating expected, rather than unexpected, uncertainty (Yu & Dayan, 2005).

Figure 2.10: (A) ACh efflux in prefrontal cortex increases most intensely during a sustained attention task (i.e. requiring stimulus processing and response selection) rather than during tasks that control for sensorimotor and reward components. (B) ACh efflux in frontoparietal cortex increases yet further following presentation of a distractor. Initially, performance worsens (as indicated by an inappropriate response bias in block 3), but then recovers synchronously with the peak in ACh release.

A.



(Arnold et al, 2002)

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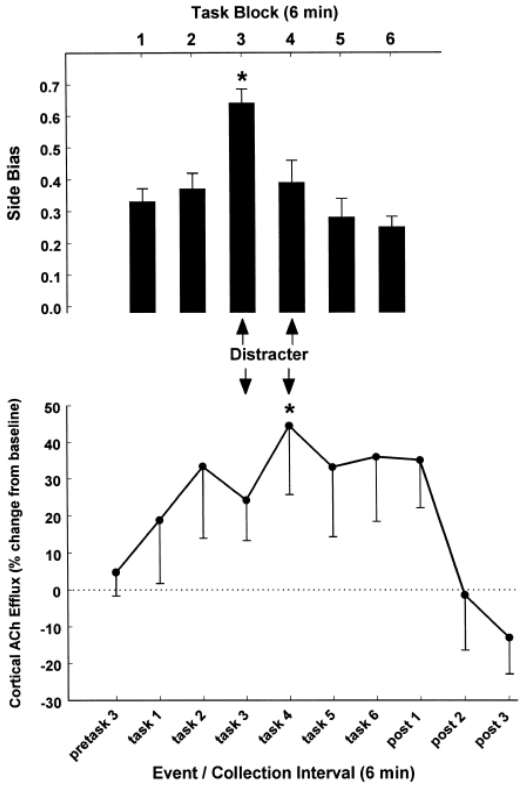
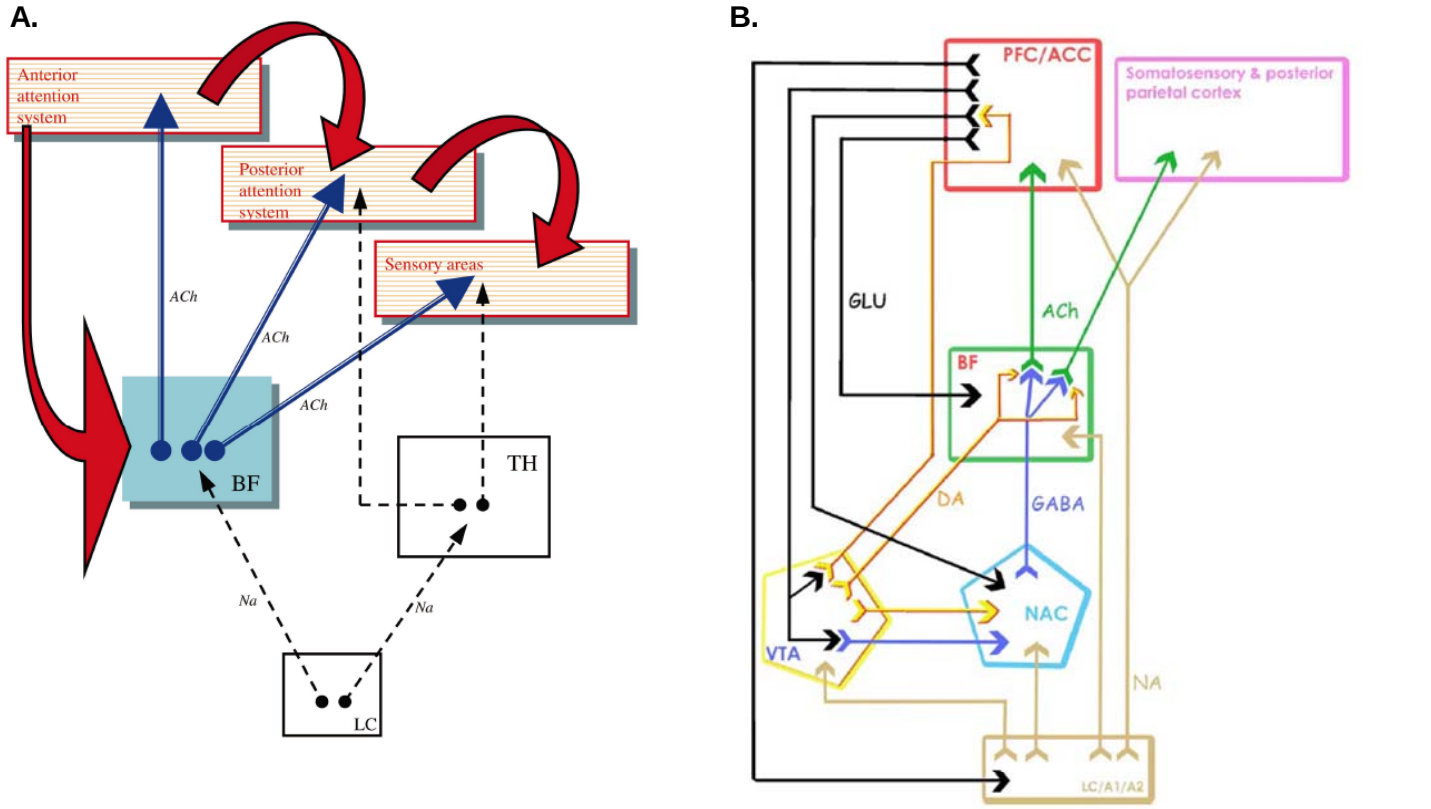


(Himmelheber et al, 2000)

Figure 2.11: (A) Model of corticopetal cholinergic interactions with both 'top-down' and 'bottom-up' cortical processes. Basal forebrain (BF) may itself be activated either by top-down prefrontal glutamatergic inputs, or by bottom-up norepinephric (NA) inputs from locus cereleus (LC), as well as nucleus accumbens and amygdala (not shown). TH: thalamus. (B) Drivers of basal forebrain (BF) activity can be divided into those from prefrontal, including anterior cingulate cortices (PFC/ACC), which may convey error signals, and those from limbic structures (nucleus accumbens, NAC; ventral tegmental area, VTA; locus cereleus, LC; and amygdala, not shown) which communicate motivational signals.



(Sarter et al, 2001)

(Sarter et al, 2006)

Memory and Learning

The link between acetylcholine and memory has traditionally been asserted on the back of two sets of robust findings (Bartus et al, 2000). Firstly, the archetypal memory disorder Alzheimer's disease is characterised by early and profound losses of cholinergic neurons (e.g. Bowen et al, 1976). Secondly, administration of anticholinergic drugs, are well-known to induce memory and learning deficits (e.g. Plakke et al, 2008), while cholinergic enhancing drugs tend to improve memory (e.g. Davis et al, 1978; Stratton & Petrinovich, 1963). In support of this are anatomical considerations: the main structure responsible for episodic memory in the brain - the hippocampus – has two inputs: one from entorhinal cortex, and the other from fornix-fimbriae, that carries predominantly cholinergic fibres from the medial septum division of the basal forebrain.

One of the most important characteristics of the memory impairment induced by cholinergic blockade is that it is specific for the *encoding* of new information, while having little effect on the *maintenance* or *retrieval* on previously stored information (Sherman et al, 2003). For example, exploring rats forget which earlier paths they had visited if injected with scoplamine before the task, but not if injected at half-time (Buresova et al, 1986). The significance of this principle is that it directs our enquiry towards cholinergic interactions with systems believed to be responsible for memory *encoding* – especially sensory, prefrontal and hippocampal cortices. Given that the cortical cholinergic system can be divided into: 1) nucleus-basalis – neocortical, and 2) septohippocampal components, cholinergic interactions with memory within each of these systems are reviewed separately, before looking at more general cholinergic mechanisms.

Nucleus basalis – Neocortical Cholinergic System

Successful encoding requires sufficient resource allocation to sensory processing, accounting for the well-established dependency between attention and memory (Baddeley, 1990; Chun & Turk-Browne, 2007). Thus the fact that memory deficits following cholinergic blockade are specific for the acquisition, rather than retrieval, of new sensory information, is in keeping with the pro-attentional role of the nucleus basalis cholinergic system (Hasselmo & McGaughy, 2004). This point is made by the findings that pro- or anti-mnemonic effects of cholinergic drugs interact with'depth of processing' during encoding, with a greater drug effect observed under conditions where stimuli are most deeply processed (e.g. Warburton *et al*, 2001; Fitzgerald *et al*, 2008). Furthermore, effects of cholinergic manipulation on working memory interact with information-processing load, e.g. span length, suggesting influences on attention (Turchi & Sarter, 1997). In this sense, many actions of ACh on memory may be derivative to its primary effects on sensory-attentional processing.

A further question is whether ACh can influence memory directly, independent from its attentional effects, and if so, in which neural systems does this take place? In answer to this, a number of lesion studies in rats and monkeys have suggested that while lesions to the nucleus basalis-neocortical cholinergic system cause clear, robust deficts in attention, they do not in general affect memory performance (see Everitt & Robbins, 1997). This profile has also been used to explain why performance improvements in Alzheimer's disease patients taking pro-cholinergic therapies are seen more consistently with attention than memory tasks (Sahakian & Coull, 1993).

Yet, certain paradigms, particularly in studies using highly precise cholinergic lesions, have suggested ACh interactions specifically with neocortically-implemented memory or learning systems. These are:

1) Reversal Learning - Prefrontal Cortices

Lesions to prefrontal cholinergic inputs result in a specific deficit in reversal learning - in which a double-option, stimulus-response contingency switches between the two contingencies after unpredictable intervals (Robbins & Roberts, 2007).

2) Working Memory - Prefrontal Cortices

Prefrontal cholinergic inputs are necessary in many paradigms where there is a relatively short (< ~1 minute) delay between exemplar exposure and response (e.g. Chudasama et al, 2004). Not all working memory paradigms though are cholinergic-dependent: for example, while scopolamine impairs episodic memory formation, it spares aspects of short-term memory such as the recency effect of serially-ordered items (Crow & Grove-White, 1973), and digit-span (Beatty et al, 1986).

3) Discrimination Learning – Extrastriate, Perirhinal and Cingulate Cortices

Cholinergic inputs to inferotemporal cortex are required for acquisition, of perceptually-demanding, but not simple, visual discriminations (Fine et al, 1997). One mechanism by which this may occur is though ACh-induced enhancement of protein synthesis in visual cortex input layer (Dotigny et al, 2008). Conditional discrimination learning, in which both a sensory discrimination and a rule are learnt, depends specifically upon cholinergic innervation of the cingulate cortex (Marston et al, 1994).

4) Recognition Memory - Perirhinal and Sensory Cortices

Recognition memory depends upon cholinergic inputs to medial temporal regions, especially perirhinal cortex, during the encoding, rather than test, phase (Tang et al, 1997). This effect is not seen with spatial memory (Winters & Bussey, 2005).

5) Conditioning and Receptive Field Plasticity - Sensory Cortices

The remapping of sensory cortex so as to favour responses to behaviourally-relevant stimuli properties is critically dependent on basal forebrain – cholinergic inputs (Edeline, 1999). This is seen most readily with associative conditioning paradigms, where, for example, tonotopic auditory cortex neurons become increasingly 'tuned' to a specific tone, when paired with an electrical shock, whilst unpaired tones become progresssively less represented (Weinberger, 2007). Similar ACh-dependent effects have been observed in motor cortex (Conner et al, 2003)

SeptoHippocampal Cholinergic System

Cholinergic input to the hippocampus via the medial septum-fornix-fimbriae pathway provides a likely anatomical basis for cholinergic interactions with long-term memory. However, while multiple studies have demonstrated consistent *correlations* between hippocampal cholinergic activity and memory, it has been more difficult to prove that the cholinergic septohippocampal projection is *necessary* for memory or learning (Parent & Baxter, 2004). Learning paradigms that have been shown to be dependent upon this pathway include conditioning, social discrimination, and non-match-to-sample spatial memory (e.g. McAlonan et al, 1995).

Noting that cholinergic activation of hippocampus has a net inhibitory effect (Buzsaki et al, 1988), and that cholinergic lesions of the medial septum disrupt processes such as latent inhibition or blocking in which attention is reduced to stimuli in familiar contexts (Baxter et al, 1997), it has been suggestd that the function of the septohippocampal cholinergic system is to suppress processing of inputs regarded as irrelevant e.g. due to overexposure, thereby enabling the hippocampus to concentrate

its resources on a small number of relevant or novel stimuli (Everitt & Robbins, 1997). This is supported by the finding that cholinergic lesions of the medial septum impair trace conditioning – where there is a delay between a conditioned and unconditioned stimulus, and for which stimuli generally need to be attended to be remembered, whereas the same lesion actually causes a paradoxical *improvement* in contextual conditioning – i.e. memory for unattended features of the environment.

A further mechanism by which ACh influences memory within hippocampus is through its interactions with theta rhythm (4-8 Hz) that occurs selectively during alertness, and enhances learning (Lee et al, 1994; Allen et al, 2002). Theta rhythm is thought to be required for new synaptic modifications, through phase-locking of stimulus features. In this regard, effects of ACh on hippocampal rhythmicity may be analagous to ACh influences on stimulus-binding, and attention-associated gamma rhythm of sensory cortices (Rodriguez et al, 2004). Recent data suggests that phasic hippocampal ACh release actually begins slightly after the onset of theta suggesting a synergistic interaction rather than a causal relationship (Zhang et al, 2010).

General Memory Mechanisms of Acetylcholine in Neocortex and Hippocampus

An important insight made by Hasslemo and colleagues is that neurophysiological characteristics of ACh modulation are strikingly similar between sensory, perirhinal, hippocampal and frontal fields. Indeed there are three main cellular-network effects of ACh that are observed repeatedly across these cortical regions, and which are all likely to facilitate memory. These mechanisms are: cortical layer feedforward-versus-feedback regulation; sustained-spiking, and long-term potentiation.

The first of these cholinergic-dependent mechanisms - cortical layer feedforward-versus-feedback regulation - relates to a general effect of ACh on columnar circuitry noted under sensory mechanisms – but which equally is found in perirhinal and rhinal regions (Hasselmo et al, 1995) (Fig. 2.12). Specifically, the usual configuration of cholinergic inputs in relation to cortical pyramidal cells is such that stimulus-driven input activity to layer IV is preserved, or enhanced, while inputs to other layers, conveying feedback, are suppressed (Hasselmo & Cekic, 1996). By favouring a bottom-up rather then top-down (or 'model-driven') mode of sensory processing, high ACh levels, seen for example with arousal or REM sleep, enhance new ('feedforward') memory associations, while suppressing activation of previously stored representations (Sarter et al, 2005a). The advantage of feedback inhibition lies in the suppression of previously encoded associations, which can 'proactively interfere' with acquisition of new associations (Atri et al, 2004).

The fact that ACh levels vary with behavioural state implies an advantage to cortical processing in the presence of low ambient ACh levels – typically found during rest or slow-wave sleep. In the foregoing model, low ACh favours a backwards shift in information flow from a limited-capacity hippocampus to high-capacity perirhinal and sensory neocortices. This fact combined with the finding that oscillatory patterns found during low-ACh states are conducive to the strengthening of modifiable synapses, suggest that the function of low-ACh states is in consolidation of memory traces (Ellenbogen et al, 2006).

The second general cellular mechanism by which ACh may support memory is sustained-spiking. Sustained-spiking is found in a subset of entorhinal - perirhinal

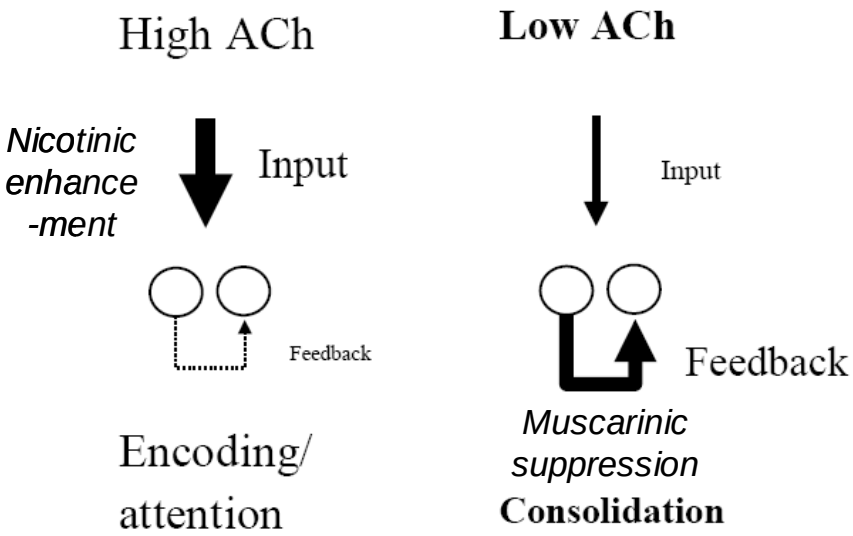
neurons between the study and test phases of working-memory tasks (Young et al, 1997; Suzuki et al, 1997), and is stimulus-specific, conferring upon it the ability to hold stimulus representations active following stimulus removal. Since this phenomenon occurs in entorhinal neurons that input hippocampus, it is likely that sustained-spiking is relevant for both short-term and long-term memory encoding. The non-specific cation current, I_{NCM} that gives rise to sustained-spiking (Fransen et al, 2002) is sensitive to muscarinic receptor activation (Klink & Alonso, 1997), and so may account for a performance susceptibility to scopolamine in tasks such as delayed matching, spatial working memory and fear-conditioning (Buresova et al, 1986).

Finally, the most well-known cellular mechanism of long-term memory – long-term potentiation (LTP) – is a further target of cholinergic control in both hippocampus and sensory neocortex (Huerta & Lisman, 1993; Brocher et al, 1992). Since LTP requires repeated synchronous activity between pre- and postsynaptic membranes, ACh facilitates this by: 1) potentiating spontaneous oscillations, such as exploratory-associated hippocampal theta rhythm (Anagnostaras et al, 2003); 2) enhancing the duration of sensory-driven inputs (McCormick & Prince, 1986); and 3) potentiating sustained-spiking in perirhinal-entorhinal cortices (Klink & Alonso, 1997).

It is noteworthy that many of the physiological mechanisms by which ACh appears to influence memory – enhancing feedforward laminar flow, prolonging input-driven responses, and modulating rhythmicity – are either the same, or directly analogous to mechanisms by which ACh appears to influence sensory - attention processing.

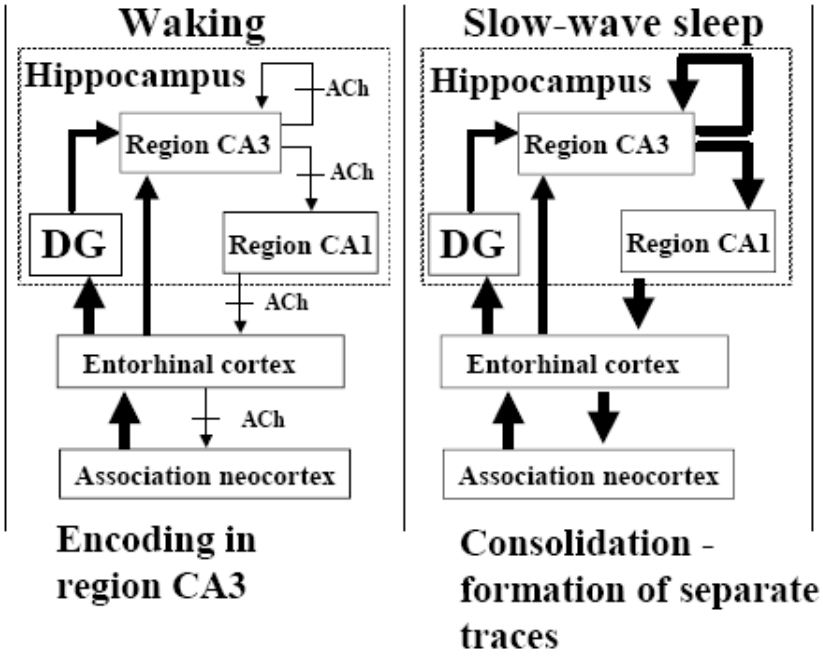
Figure 2.12: (A) Cholinergic effects on cortical laminar flow support different components of memory depending on ambient ACh levels. High-ACh states favour encoding by enhancing signal-to-noise, synchronisation and self-association between afferent-driven inputs (see also Figure 2.9). Low-ACh states favour feedback whereby representations can be shifted between cortical sites and weak synaptic connections strengthened. (B) Feedforward versus feedback model of ACh accounts for different patterns of neocortical and hippocampal dynamics in different behavioral states. During high-ACh states activity is favoured in pathways from sensory to hippocampal cortices, whereas in low-ACh states, flow of activity reverses.

A.



(Hasselmo & McGaughy, 2004)

B.



(Hasselmo & McGaughy, 2004)

Cholinergic Receptor Pharmacology

The cognitive influences of drugs acting at cholinergic receptors can be seen through manipulation of either muscarinic or nicotinic receptors. Antagonism of muscarinic M1 receptors, with drugs such as scopolamine, produces the most consistent memory (Rusted & Warburton, 1988); attentional (Wesnes et al, 1988) and sensory (Erskine et al, 2004) impairments. Nicotinic receptor antagonism, e.g. using mecamylamine, may also impair memory, especially during working memory tasks (Levin et al, 1997), although this is less consistent than with muscarinic blockade (Green et al, 2005). Nicotinic antagonism also results in attentional impairments (Rezvani et al, 2002), yet, again, this is not as robust a finding as seen with scopolamine (McQuail & Burk, 2006), and does not extend to sensory (Erskine et al, 2004) dysfunction. Combining muscarinic and nicotinic receptor antagonism worsens the memory impairment of either alone suggesting a synergism (Green et al, 2005). However, this same combination can also result in a lesser impairment than either alone, implying that blockade of one pathway can overstimulate the alternative pathway that is itself dysfunctional (Maviel & Durkin, 2003). Nicotinic receptor stimulation, e.g. with nicotine, can enhance memory – an effect that can be reversed with scopolamine (Terry et al, 1993). Furthermore, nicotine can improve both arousal and attention (Newhouse et al, 2004).

Both muscarinic and nicotinic-mediated drug potentiation of memory occur only if the drug is given just before, but not after, the first presentation of study items (Atri et al, 2004), suggesting that cholinergic activation, acting via both sets of receptors, facilitates encoding selectively. By contrast, as discussed under the memory

mechanisms of ACh earlier, muscarinic blockade can enhance memory if administered soon after the study phase (Young et al, 2005; Winters et al, 2006).

Cholinergic stimulation of receptors may exert opposite effects on related receptors. For example, blockade of M2 receptors has been shown to improve memory, possibly because of presynaptic disinhibition of ACh release (Baratti et al, 1993). Additionally, chronic pharmacological modulation of the nicotinic receptor may exert long-lasting changes in memory that persist even after drug withdrawal (Levin & Simon, 1998). Positive effects of chronically-exposed nicotine on memory may arise via neurotrophic effects acting via nerve growth factor receptors (Hernandez & Terry, 2005).

Brain Cholinesterases

Once acetylcholine has been released into the synaptic cleft, the only significant way its action can be terminated is through rapid hydrolysis mediated by the catalytic site of the enzyme cholinesterase (ChE). ChEs also have plasticity-related effects, including cell differentiation, process extension, and dendritic modelling. These latter types of actions are mediated through the enzyme's peripheral anionic, rather than catalytic, site.

The human brain contains two cholinesterases (ChEs): acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE is by far the more predominant of the two, and is associated with cholinergic pathways (Mesulam, 2000). AChE is manufactured and expressed in all neurons that express cholinergic receptors. Since all cholinergic neurons (i.e. those containing ACh-synthesising enzyme ChAT) bear cholinergic

autoreceptors, it is the case that all neurons that synthesise acetylcholine also express AChE. AChE is synthesised in the perikaryon of cholinergic neurons, and is then transported to dendrites and axons where it becomes inserted into membranes as an ectoenzyme. The localisation of AChE enzyme activity within cholinergic cells is found on synaptic boutons, proximal dendrites and perikarya (reflecting the presence of cholinergic autoreceptors at these sites), and axonal varicosities which make contact with cholinoceptive neurons (Mesulam & Geula, 1991).

Neurons that are cholinoceptive (i.e. bear cholinergic receptors), but non-cholinergic (i.e. do not express the ACh-synthetic enzyme ChAT) express most of the brain AChE. However, unlike cholinergic neurons, AChE in non-cholinergic neurons is confined to the dendrites and perikarya, and not axons, reflecting the location of cholinergic receptors in the two types of neurons (Mesulam & Geula, 1992). In the cerebral cortex, AChE is localised predominantly to the glutamatergic pyramidal cells of (output) layers III and V (Mesulam & Geula, 1991). These neurons respond to ACh through M1 and nicotinic receptors, which differs from AChE-containing neurons in layer VI and subcortical zone that respond via M2. Expression of ChE may also occur in glia, where as well as serving to mop up extraneous ACh, it may regulate cell growth, plasticity and blood-brain barrier regulation (Mesulam, 2000).

Cholinesterase Inhibitors – Behavioural Effects

Cholinesterase inhibitors (ChEIs), in their role as cognition-enhancing drugs, were first trialled in healthy young humans. First experiences were unfavourable with subjects becoming withdrawn, sedated and even depressed (Davis et al, 1976). Furthermore, short-term memory capacity as assessed by digit span tasks was

worsened in the presence of physostigmine, whilst long-term memory was unaffected. These adverse effects were felt to be due to excessive dosage (1.5 – 3mg), as a subsequent trial employing a lower dose (1mg given over 1 hour) showed improved long-term memory on a word-list task (Davis et al, 1978).

Further support for a cognition-enhancing function of ChEIs comes from studies in aged monkeys who, in manifesting memory deficits, act as an animal model of Alzheimer's disease (Bartus et al, 1982). In order to magnify the cognitive impairments of aged monkeys, and in order to emulate the presumed hypo-cholinergic basis for memory decline seen with natural ageing and Alzheimer's disease, monkeys are pre-treated with the muscarinic antagonist scopolamine. Monkeys impaired in this way show reversibility of recall and recognition deficits following treatment with either physostigmine or tacrine (Rupniak et al, 1992). Subsequent studies showed that the performance of both aged and young monkeys in memory tasks could be improved by ChEIs even in the absence of scopolamine pre-treatment (O'Neill et al, 1998), mirroring the small memory improvements observed in humans. However, the beneficial effects of physostigmine, e.g. on visual recognition, were found to be curtailed by inflicting selective lesions to the nucleus basalis (Aigner et al, 1987), suggesting that the benefits of ChEIs in clinical scenarios are diminished if cholinergic deficiency has progressed beyond a certain point.

Several psychophysical experiments have tried to tease apart the processes modulated by ChEIs. In one of the first human studies (Davis et al, 1978), physostigmine improved storage of items on each of six learning trials presenting the same material, without interacting with trial order. This suggests that the drug interacted with initial

stimulus encoding, maintenance or retrieval, rather than with repetition or familiarity-based processes. However, physostigmine was also found to benefit recall of items presented both *before* and after the drug was infused, suggesting a specific effect on maintenance or recall. This result has since been challenged by experimental and computational modelling studies suggesting that pharmacological elevation of ACh is more likely to interfere with retrieval (Hasselmo & McGaughy, 2006).

A separate set of studies have emphasised the sensory or attentional enhancing properties of ChEIs. Thus, while physostigmine shows no effect on recall accuracy or overall speed in short-term memory tasks, the drug has been found to speed responses to low-contrast, as opposed to high-contrast, probe stimuli (Wetherell, 1992), suggesting a specific perceptual-attention benefit. Physostigmine also speeds responses more during selective attention than simple perceptual tasks (Furey et al, 2008). Given that ChEIs improve memory more for items deeply-processed, than superficially-processed, during encoding (Fitzgerald et al, 2008), it is possible that a further mechanism by which ChEIs improve memory is through effects on sensory or attentional processing. This would be compatible with computational simulations of ACh influences on encoding (Hasselmo & McGaughy, 2006), and resembles the profile of promnemonic actions seen with nicotine (Warburton et al, 2001).

Pre-clinical studies in monkeys and humans show a wide-degree of variability in responses to ChEIs. One of the reasons for this is that individual monkeys (Bartus et al, 1983), or humans (Davis et al, 1979), show different optimum drug doses with steep inverted U-shaped performance curves either side of the optimum. This can

have the effect of providing a negative result when individuals are averaged over for specified doses, even across the entire range of administered doses (Bartus, 2000).

A further reason for inter-subject pharmacodynamic variability relates to the observation that cognitive improvements in healthy subjects with ChEIs or nicotine are more apparent in elderly than young individuals, or in subjects with relatively low baseline performance (Davis et al, 1979; Drachman & Sahakian, 1980; Newhouse et al, 2004). It is possible that this type of inverted-U shaped response arises from variability in subjects' cholinergic systems - with the benefits of ChEIs being realised more in patients, or elderly subjects, with relative cholinergic deficiencies, than in health. For example, working or short-term memory capacity is unaffected by ChEIs in healthy subjects (Davis et al, 1978; Wetherell, 1992), yet the same drugs can reverse memory deficits induced by scopolamine given before encoding (Mewaldt & Ghoneim, 1979). As well as disease and ageing, subjects may vary in the efficiency of their cholinergic neurotransmission, and response to ChEIs, by virtue of genetic polymorphisms in ApoE (Bizzarro et al, 2005), ChAT (Harold et al, 2006), and AChE (Scacchi et al, 2009) genes, as well as in enzymes that metabolize such drugs (Varsaldi et al, 2006).

Cholinesterase Inhibitors – Chemical Effects

A key assumption regarding cholinesterase inhibitors made in this thesis is that they increase ACh levels in the brain. The first studies to consider this were in rats, and showed that toxic levels of ChEIs, such as physostigmine 1mg/kg, could increase brain ACh levels by 50-100% (Giarman & Pepeu, 1962; Pazzagli & Pepeu, 1965). Microdialysis studies in rats show that ACh concentrations increase by 300-400%

from baseline in neocortex (Kosasa et al, 1999a) and hippocampus (Kosasa et al, 1999b) following either direct or oral administration of a range of cholinesterase inhibitors. More recently using combined PET imaging and in vivo microdialysis in monkeys, it has been confirmed in the same animals that the ChEI donepezil: 1) inhibits AChE activity; 2) increases prefrontal cortex ACh levels; 3) increases muscarinic ACh receptor binding, and 4) improves performance in a working memory task (Tsukada et al, 2004). Having established relationships between these variables it has been possible to use PET imaging non-invasively to quantify the degree of ACh release, e.g. by measuring the competitive uptake of the radiolabelled nicotinic receptor agonist (18)F-nifene (Easwaramoorthy et al, 2007).

Estimates of the level of cholinesterase inhibition required to achieve a given rise in brain ACh vary widely depending on drug. For example, the organophosphate DFP causes inhibition of 70% cholinesterase activity that is followed by a six-fold rise in ACh levels (van der Staay et al, 1996), whereas an 83% cholinesterase inhibition by paraoxon causes only a doubling of brain ACh (Wecker et al, 1977). The extent of ACh rise may also differ between regions with, for example, metrifonate causing a 3-fold ACh rise in neocortex but a 4-fold ACh rise in hippocampus (Giovannini et al, 1998). These two regions also differed in the ACh response to chronic ACh treatment with only neocortex showing a sustained elevation in ACh levels.

The extent of ACh rise secondary to ChEI is likely to depend upon factors that alter cholinergic fibre density such as age. In one study, the same level of cholinesterase inhibition by metrifonate caused higher neocortical ACh levels in aged than young rats, that was associated with aged rats only showing an improvement in object

recognition; conversely hippocampal ACh levels were pushed up by ChEI more in young than aged rats (Scali et al, 1997a). The same group found that a different ChEI, tacrine, this time increased hippocampal ACh levels by significantly more in aged than young rats (sixfold versus twofold) while increasing neocortical ACh levels equally by age (Scali et al, 1997b). Aged rats showed improvements following tacrine treatment in both passive-avoidance and object recognition tasks that conceivably are related to the increases in ACh in hippocampal and neocortical regions respectively.

Finally, it should be appreciated that ACh release may also be increase following cholinergic receptor blockade; for example, systemic scopolamine increases ACh release within hippocampus (e.g. Mishima et al, 2000), suggesting local autoregulation, and presumably resulting in nicotinic receptor overstimulation.

Alzheimer's Disease – 'The Cholinergic Hypothesis'

Evidence for Cholinergic Hypofunction in Alzheimer's Disease

Degeneration of cerebral cholinergic innervation is a well-established pathological hallmark of Alzheimer's disease (Geula & Mesulam, 1989), and provides the basis of the 'cholinergic hypothesis': that a significant component of the cognitive deficits of Alzheimer's disease (AD) arises from deficiency of cortical cholinergic input (Bartus et al, 1982). Cell loss secondary to neurofibrillary tangle accumulation occurs primarily in the Ch4–nucleus basalis complex (Geula & Mesulam 1999), resulting in degeneration of cholinergic axons that innervate wide areas of diffuse cerebral cortex. Since animal studies have shown that targeted lesioning of the cortical cholinergic system results in a profile of attentional and memory impairments similar to that seen

with Alzheimer's disease, the argument is made for cholinergic deficiency being instrumental to, at least components, of the clinical syndrome of this disease.

Neuropathological studies show that choline acetyl transferase (ChAT) activity is reduced by ~60-70% in AD (Perry et al, 1981) but not other dementias (Bowen et al, 1982). Moreover, the loss of this cholinergic neural marker correlates with cognitive decline (Davis et al, 1999), although this relationship cannot be fully unconfounded from degeneration of non-cholinergic parts of the brain. A further problem with ChAT-measuring studies, is that it is high-affinity choline uptake, and not ChAT enzymatic activity, that is the rate-limiting step for ACh synthesis (Jenden et al, 1976). Moreover, adequate ACh synthesis can occur in the face of losses of upto 90% of enzyme activity (Haubrich & Chippendale, 1977). Hence in itself the reduction in ChAT levels in AD brains neither explains the cognitive symptomatology, nor proves that the cholinergic system is dysfunctional, in AD. However, further pathological studies have shown that the density of basal forebrain cholinergic neurons is reduced in AD (Whitehouse et al, 1981), which might be sufficient to reduce cortical cholinergic input, and so account for the similarity in cognitive profile of AD to animals with selective cortical cholinergic lesions.

The cholinergic hypothesis of AD is supported by the degree of similarity, and specificity, of cognitive deficits comparing AD patients to animals with precise lesions of the nucleus basalis or cortical cholinergic projections. For example, one of the attentional impairments observed in AD - the ability to disengage from invalid spatial cues in a spatial orientation task (Parasuraman et al., 1992) – is also seen as a selective deficit following restricted lesioning of the nucleus basalis and its

cholinergic projections (Voytko et al, 1994). Furthermore, to the extent that cholinesterase inhibitors reduce the deterioration of (certain) cognitive functions in (some) Alzheimer's disease patients, and to the extent that such drugs act by enhancing endogenous acetylcholine levels, the cholinergic hypothesis receives independent support. For example, attentional deficits in AD can be partially reversed with cholinergic agonists (Sahakian et al, 1989; Sahakian et al, 1993). Conversely, it is well established that cholinergic antagonists administered to healthy humans and adults result in memory and attentional impairments (Drachmann & Leavitt, 1974).

Interestingly for the cholinergic hypothesis, it is found that cortical cholinergic deafferentation varies consistently between regions (Geula & Mesulam, 1996; Figure 2.21), with cortical regions subserving memory and associational processing being most affected. The greatest depletion of cholinergic axons occurs in the temporal lobes, including the hippocampus and entorhinal cortex, in which upto 80% of cholinergic neurons can be lost. However, neurons within these temporal areas are also affected by Alzheimer's-specific plaques and tangles directly, and so it is difficult to know the extent that correlations between region-specific pathology and clinical severity (Pappas et al, 2000) are cholinergic dependent. The regional heterogeneity of cortical cholinergic deficiency mirrors sectorial involvement of the nucleus basalis, e.g. the severely affected posterior sector supplying the temporal lobes. Cortical areas depleted of cholinergic input also show downregulation of cholinergic receptors, especially M2 and nicotinic receptors (London et al. 1989).

Challenges to the Cholinergic Hypothesis

In contrast to the dopaminergic deficiency of Parkinson's disease, the cholinergic deficiency of AD is not the pre-eminent lesion, and appears neither necessary nor sufficient to account for the clinical syndrome (Mesulam et al, 2004). Degeneration of non-cholinergic neurons in associational neocortex is at least equal if not more, important in accounting for the clinical deficits of AD. Indeed, the relative contributions of neuromodulator deficiency in accounting for the symptomatology of Parkinson's and Alzheimer's diseases can be appreciated by the significantly greater efficacy of levodopa, relative to cholinesterase inhibitors, in the amelioration of symptoms of each disease, respectively.

A particular challenge to the cholinergic hypothesis is that whilst loss of cholinergic cells undoubtedly occurs in *advanced* AD, evidence for cholinergic deficiency in *early* Alzheimer's disease, as well as its precursor stage of mild cognitive impairment (MCI), is not as clear-cut. For example, in the study of Davis et al, 1999, that showed a correlation between densities of the cholinergic cell markers ChAT and AChE and clinical dementia severity, there was no significant difference in the level of such cholinergic markers in early AD compared to unaffected age-matched controls. Even more surprisingly, DeKosky et al (2002) showed that ChAT levels are not only not decreased in mild AD, but may even be raised in MCI, specifically in prefrontal and hippocampal cortices. This appears to argue against cholinergic lesions being causative of the cognitive deficits of AD, at least in early disease, and may even suggest that the cholinergic system is capable of a compensatory hyperactivation - as observed with restricted animal cholinergic lesions (McGaughy et al, 2002).

However, while the results of DeKosky et al and Davis et al suggest that cholinergic cell *numbers* may not alter in early AD, they are unable to inform about the possibility of impaired cholinergic cell *function* (Sarter et al, 2002; Mesulam, 2004). This might explain why alternative methods of evaluating cholinergic cell function – e.g. cell volume (Mesulam et al, 1987), synapse number (Geula & Mesulam, 1989), nerve growth factor receptor expression (Mufson et al, 2003) or ACh vesicle release (Efrange et al, 1997) have shown impairments in early AD. The distinction between cell number and function is also relevant given previous findings that nucleus basalis Ch4 neurons may suffer from neurofibrillary tangle degeneration, with secondary impaired cholinergic innervation, without nucleus basalis cell counts being affected (Mesulam 2004). In fact the use of ChAT as a reliable marker of cholinergic status has been questioned for several reasons, including that it does not represent a rate-limiting step for ACh transmission (Jenden et al, 1976), its measurement can be confounded by changes in background protein levels, and it may be upregulated in response to cholinergic cell dysfunction manifest in other ways e.g. following a loss of cholinergic synapses in cerebral cortex (Mesulam, 2004).

In common with many neurodegenerative diseases, including Parkinson's disease, AD brains exhibit lesions in more than one of the brainstem-basal forebrain neuromodulatory systems. A loss of epinephric cells in locus ceruleus may be even more profound than the loss of cholinergic cells in the basal forebrain (Zarow et al, 2003). However, comparisons of multiple neurochemical markers with progression of clinical disease suggest the strongest correlation with cortical acetylcholine levels (Perry et al ,1981; Franceis et al, 1985; Minger et al, 2000).

Cholinesterase inhibitors in Alzheimer's Disease

The use of cholinergic enhancement in AD followed rationally from: 1) histochemical evidence for cholinergic defiency in post-mortem AD specimens, and 2) psychopharmacological data showing that disruption of cholinergic neurotransmission in humans, or lesioning cholinergic nuclei in animals, leads to cognitive impairments similar to those observed in AD. In the first blinded trial of 17 AD patients with moderate-advanced disease, significant improvements in a wide array of cognitive scores was obtained following treatment with the cholinesterase inhibitor oral tetrohydroaminoacridine (tacrine) (Summers et al, 1986). Whilst, subsequent larger trials confirmed a beneficial effect for cholinesterase inhibitors it became clear that the effect sizes were small, and rarely would there be complete clinical reversibility. Pooling the effects of randomised-controlled trials over eight types of ChEIs, in over 10,000 patients, shows that mild-to-moderate AD patients improve by ~ 5 points relative to both baseline, and placebo, on the 70-point cognitive and behavioral ADAS-Cog scale (Giacobini, 2000). By comparison, the mean annual change in ADAS-Cog scores in untreated AD patients is ~ 8-9 per year. Beneficial effects are sustained for at least 1 year (Homma et al, 2009), and may still be present in excess of 5 years from treatment initiation (Bullock & Dengiz, 2005). There is also limited evidence for a disease-modifying effect of ChEIs, with a slightly better outcome for patients started on ChEI treatment early in their disease course, as compared to later on (Winblad et al, 2006). Similarly, ChEIs may delay the rate at which mild cognitive impairment progresses to AD (Diniz et al, 2009).

One of the reasons for the relatively mild efficacy on average of ChEIs in AD is a high degree of inter-patient variability. Thus the ADAS-Cog response to ChEIs

includes 10-15% patients showing no difference relative to placebo, 35% showing a significant improvement and 5% showing an ADAS-Cog improvement of significantly more than 5 points (Giacobini, 2000). Reasons for this variability include patients' baseline performance (Calabria et al, 2009) with greater benefit in patients with worse baselines (Wattmo et al, 2008); integrity of cholinergic fibres as measured by MRI (Behl et al, 2007), and genetic profile (see above). The long-term response to cholinesterase inhibitors may also be predictable on the basis of psychometric response following a single-dose challenge of the same drug. AD patients who improved on attentional and executive (but not memory) tests after a single tacrine dose, were also those who showed an improvement (defined as MMSE increase of ≥3) after 4 weeks of tacrine treatment (Alhainen et al, 1993).

The benefits of pro-cholinergic therapies in Alzheimer's disease tend to be manifest more for attentional than memory deficits (Sahakian & Coull, 1993) – an observation that mirrors the previously discussed findings of performance deficits following induced basal forebrain cholinergic lesions. Additionally, administration of nicotine to Alzheimer's disease resulted in a significant improvement in tests of sustained visual attention and perception, while not improving auditory or visual short-term memory (Jones et al, 1992). Where ChEIs do improve recognition performance in Alzheimer's disease, this has been found from signal-detection analysis to be due to a combination of improved old verus new discriminability (i.e. memory storage), and a greater likelihood for saying that an item was old (i.e. change in the criterion) (Mohs & Davis, 1982).

One of the reasons why ChEIs may not be that effective is because they act through tonic stimulation rather than though enhancing normal release patterns (as levodopa is able to). Hence pharmacologically suppressing GABA-mediated inhibition of nucleus basalis, e.g. with the benzodiazepine antagonist beta-carboline, might be better than a ChEI strategy (Sarter et al, 1990). Other theoretical problems with ChEIs are that they depend upon a minimum of baseline cortical ACh transmission, as well as a minimum of ChE activity. In AD, axonal cholinesterase activity decreases early in the disease course (Davies et al, 1976) since axonally-located AChE occurs exclusively in cholinergic neurons. However, total AChE levels do not decrease until AD is advanced since the enzyme is found not only axonally but also in cholinoceptive perikarya, neuroglia, and the plaques and tangles of AD.

It is likely that neurological diseases other than Alzheimer's disease involve deficiencies in central cholinergic neurotransmission, and so might be amenable to pro-cholinergic therapies including ChEIs. There are a multitude of case-reports or case-series showing positive effects of physostigmine on memory in various neurological scenarios, including: herpes simplex encephalitis (Catsman-Berrevoets et al, 1986), Huntington's disease (Nutt, 1983; Davis et al, 1979), traumatic brain injury (Poole & Agrawal, 2008), and delirium (Rose & Moulthrop, 1986). Furthermore, based upon neuropathological evidence for lesions in nucleus basalis and cortical cholinergic fibre disruptions in cortical Lewy Body disease, Parkinson's disease-dementia and vascular dementia, ChEIs have been trialled in these diseases with modest therapeutic gains (Liepelt et al, 2007; Ballard et al, 2008; Dichgans et al, 2008).

3. Human Cholinergic

Functional Neuroimaging Review

Role of Pharmacological - Functional Imaging

Functional neuroimaging has increasingly established itself as a valid and informative tool for studying activation patterns across the whole brain in different cognitive and/or pharmacological contexts, to complement more invasive methodologies such as single-unit or lesion-based techniques. Multiple examples exist where functional neuroimaging activations provide reliable 'signatures' of processes previously characterised at (and thus cross-validated by) other levels of neural recording, including invasive animal work. Such examples include: retinotopic (e.g. DeYoe et al, 1994) and category-specific (e.g. Kanwisher et al, 1997) mappings of visual cortex; attentional influences on sensory cortices (e.g. Martinez et al, 2001); attentional control signals in frontoparietal regions (e.g. Hopfinger et al, 2000); learning-related plasticity of sensory cortex (e.g. Morris et al, 1998); repetition suppression (e.g. Henson & Rugg, 2003); working memory-delay activity (e.g. Courtney et al, 1997); and subsequent-memory effects in medial temporal cortex (e.g. Wagner et al, 1999).

Such convergence has arisen even though most functional neuroimaging measures primarily reflect regional metabolic or vascular responses, as indirect indices of associated neural activity (Logothetis, 2002); and despite the restricted spatial resolution of existing functional neuroimaging approaches (on the order of sub centimetre) compared with others. Assumptions inherent in the interpretation of functional imaging recordings, in relation to the vascular - metabolic origin of its datum; and interactions of this with systemic pharmacological interventions are discussed subsequently in Methods.

From the standpoint of this thesis, it is notable that many such functional imaging paradigms probe neural mechanisms that non-human studies have shown to be under cholinergic control. Consequently, it becomes meaningful to ask whether or not cholinergic manipulations alter functional neuroimaging activation patterns in directions consistent with existing accounts; and furthermore whether human neuroimaging can provide new data to help refine existing models of cholinergic function. Pharmacological functional imaging provides a methodological strategy of asking whether patterns of brain function observed under well-characterised cognitive paradigms interact with a pharmacological manipulation of interest.

Pharmacological functional imaging has the potential to complement more traditional physiological and psychological approaches for the following reasons: 1) It may provide independent corroboration of existing neuropharmacological theoretical models, firstly by measurement of a neural activity at a grosser spatiotemporal scale than that achieved by single-unit studies, and secondly by opening up neurophysiological enquiry to healthy humans. 2) If offers testing of predictions of existing neuropharmacological models that are not easily testable given the constraints of traditional neurophysiological techniques. For example, functional imaging provides the advantage of sampling neural activity over the entire brain in order to explore interactions, and connectivity relationships, between sensory, attentional and memory systems. Furthermore, testing in humans can provide more naturalistic contexts, with short-duration testing intervals, than many animal paradigms in which animals often need to be trained for many weeks before achieving a behavioural criterion. 3) It may help explain the psychotropic effects of a multitude of

drugs, by filling an explanatory gap between neurophysiology-based models of endogenous neuromodulator function and psychophysics. 4) It may explain some of the inter-individual variance in behavioural responsiveness to drugs either in terms of the brain response to a single drug challenge, or in terms of the pattern of brain activation prior to drug exposure (e.g. Giessing et al, 2007). 5) It can provide models of neuropathological states characterised by hypo- or hyperfunctioning neuromodulatory systems, e.g. as found with dopamine antagonists inducing activation patterns similar to those observed in Parkinson's disease (Honey et al, 2003), or with amphetamine in its capacity to model features of mania (Willson et al, 2004) and drug-addiction (Vollm et al, 2004).

Types of Pharmacological Functional Imaging

The type of pharmacological functional imaging studies reviewed here need to be distinguished from two other types of physiological study probing neuromodulatory systems in humans.

The first distinction lies between measuring brain activity during a set of precisely-controlled tasks versus imaging the resting state. Resting state studies have the advantage of not being confounded by performance, as may affect patient studies (Geaney et al, 1990), and may still be related to function through correlations with performance (Ebmeier et al 1992), or other physiological variables, e.g. EEG (Gustafson et al, 1987). This is supported by studies showing changes in baseline cerebral metabolism after prolonged

treatment with a drug, typically a cholinesterase inhibitor (Staff et al, 2000), which itself correlates with clinical response to that drug (Venneri et al, 2002).

Certain functional imaging data collected with subjects at rest suggest mechanisms of action. For example, the fact that nicotine enhances activation of thalamic nuclei and reticular formation, but not neocortex (McNamara et al, 1990), while scopolamine decreases thalamic activity (Blin et al, 1994), specifically during rest, suggests that many instances of cholinergic interactions with performance could be subcortically mediated. Differences between nicotinic and muscarinic contributions to memory have also been suggested by reductions in temporoparietal and prefrontal resting-state blood flow following mecamylamine and scopolamine, respectively (Gitelman & Prohovnik, 1992). Moreoever, correlations between nicotine-induced “rush” or “high” feelings and activations enhancements in frontal and limbic structures supports models implicating these regions in mediating drug reinforcement and dependency (Stein et al, 1998).

Cholinergic modulation of resting-state activity may also been analysed in terms of fractal complexity, and inter-regional correlations, rather than merely amplitude, thereby providing information on cholinergic modulation of functional connectivity (Suckling et al, 2008). For example, scopolamine is found to decrease the fractal complexity of resting-state activity (which can be thought of as similar to the number of superimposed wavelets that make up a time-series), while increasing the low-frequency coherence between hippocampus and frontotemporal regions, both of which may characterise age-related memory impairment (Wink et al, 2006).

A second important distinction of pharmacological functional imaging is between metabolism-related functional imaging and neurotransmitter-related imaging. The first sort of study explores *downstream* consequences of cholinergic manipulation – including effects of cholinergic drugs on other neuromodulatory systems such as dopamine (Svensson & Giacobini, 2000). By contrast, neurotransmitter-related imaging informs about the particular *upstream* cholinergic pathway at which the drug is acting upon. For the latter type of inquiry, use is made of radioligand methods that measure aspects of cholinergic function, such as receptor occupancy (Easwaramoorthy et al, 2007), high-affinity choline uptake (Zheng et al, 2007), vesicular ACh transporter uptake (Mazere et al, 2008), and acetylcholinesterase (Kikuchi et al, 2007). The anatomical disposition of cholinergic receptors or AChE may account for regional variations in functional modulation secondary to drugs. For example, a concentration of nicotinic receptors within thalamus and basal ganglia (Paterson & Nordberg, 2000) is congruent with strong nicotinic influences on fMRI or PET responses in these same regions (e.g. Jacobsen et al, 2004).

Literature Review of Cholinergic Functional Imaging

As a background to the human cholinergic-functional neuroimaging experiments of this thesis, a comprehensive listing of existing studies employing similar methodology is presented (Tables 1 -3), and then interpreted. An exhaustive search for human cholinergic functional imaging studies was performed using the PubMed database with combinations of the search terms [*cholinergic* OR *acetylcholine* OR *nicotine* OR *scopolamine* OR

cholinesterase] AND [*functional imaging* OR *fMRI* OR *PET*] up to July 2010. PubMed-suggested 'Related Articles', references and citations of relevant articles were also interrogated. Selected studies were those in which: 1) functional neuroimaging measures were obtained in healthy humans during a stimulus-driven and/or behavioural activation paradigm; and 2) the effects of a systemic cholinergic manipulation on brain activation patterns were examined. The vast majority of such studies actually scanned subjects over at least two behavioural conditions, sometimes including a resting state. Hence the results of such studies often take the form of interactions between drug and task- (or stimulus-) determined conditions in determining regional brain activations.

Cholinergic functional neuroimaging studies in patient groups (for which the vast majority have been in Alzheimer's disease or mild cognitive impairment) are not included because of differences in the general methodology of such studies. The majority of such clinical studies observe changes in neural activation over a *long course* of treatment (typically many months), rather than using placebo-controlled, single drug challenges, as employed by the experiments in this thesis. Moreover many published clinical cholinergic functional imaging studies measured only resting-state metabolic profiles, rather than task and/or stimulation-related activations which are of main interest here. Two more clinically-focused, cholinergic functional neuroimaging reviews can be found elsewhere (see Dickerson, 2006; Nordberg, 2004). Resting-state studies are also omitted from the list, having been discussed above.

In order to assist exposition, and in line with the various functional conceptualisations of acetylcholine summarised in the Background chapter (viz. sensory, attention and memory functions), studies are categorised according to whether the critical effects primarily arose in sensory, frontoparietal or medial temporal cortical regions. Activations in other brain regions (e.g. lateral temporal cortex and subcortical structures) are listed alongside frontoparietal effects for convenience. Furthermore, within each anatomical division, effects are secondarily classified according to the broad cognitive construct putatively tested (e.g. passive viewing, attention-demanding or memory task). Then for each study and anatomical region we tabulate: a more accurate description of the behavioural paradigm; the drug administered; the imaging modality; plus the critical functional neuroimaging and behavioural results. Studies are duplicated across tables where, for example, both sensory and frontoparietal regions were studied.

The general format of all studies is that of a randomised-controlled trial in which subjects receive drug or placebo, sometimes as part of a within-subject design, other times as a between-subject design. The predominant cholinergic drugs used were the muscarinic receptor antagonist scopolamine; the nicotinic receptor stimulant nicotine; the cholinesterase inhibitor physostigmine (that is given intravenously and has a well-documented pharmacokinetic time-course); or the cholinesterase inhibitor donepezil. Mecamylamine has also been used as a nicotinic receptor antagonist. In all the non-clinical studies reviewed here, the manipulation involved a single-challenge of drug, with most studies adopting a placebo-controlled within-subject cross-over design.

Table 1: Cholinergic functional imaging studies – sensory cortices

A: No task / irrelevant task	*Scanning task*	*Drug*	*Effect of drug on functional activations*	*Effect of drug on performance*
Cohen (94)* PET-FDG	Auditory discrimination	Scopolamine	↓es primary visual, parieto-occipital cx (i.e. *irrelevant* sensory cx); N.B. no control task	Poorer target discrimination. Performance inversely correlated with parieto-occipital cx activity
Grasby (95) PET-rCBF	Auditory word: 5- & 15-spans	Scopolamine	↑es bilateral lateral occipital cx (i.e. *irrelevant* sensory cx), in sub- and suprascan tasks	Memory impairment on supraspan task only
Bahro (99) PET-rCBF	Auditory – eyeblink conditioning	Scopolamine	↑es occipital-temporal, especially lateral, cx (i.e. *irrelevant* sensory cx); N.B. no direct comparison with placebo group	Not measured
Thiel (01)	Word-stem completion	Scopolamine	No effect in primary visual cortex	No effect on performance independent of repetition
Sperling (02)	Face-name pairs	Scopolamine	No effect in primary visual cortex	Memory impaired
Jacobsen (02)	Chequerboard	Nicotine	No effect in sensory cortices	Not measured
Hahn (07)	Chequerboard	Nicotine	No effect in sensory cortices	Not measured
Hahn (09)	Chequerboard	Nicotine	No effect in sensory cortices	Not measured
Mentis (01) PET-rCBF	Alternating eye light flash	Physostigmine ± Scopolamine	Physostigmine ↓es middle occipital, Physo + Scop ↑es middle occipital; No effect of physo in primary visual cx Physo + Scop ↓es primary visual cx	Not measured
Furey (00a)	Face WM	Physostigmine	No effect on control stimuli in extrastriate cx	Not measured
Silver (08)	Chequerboard	Donepezil	↓es primary visual cortex extent & magnitude	Not measured

* All studies are fMRI except where indicated under study first author

Table 1: Continued – sensory cortices

B. Attention-demanding task				
Thienel (09a)	Attention Network Task	Mecamylamine	↓es superior occipital cx; ↑es anterior fusiform cx. - orienting; ↓es calcarine cx – conflict	Slowing across all trial types; no interactions
Thienel (09b)	ANT	Scopolamine	↑es middle occipital cx – alerting; ↓es lingual gyrus, inf temporal cx - conflict	Slowing of responses
Ghatan (98) PET-rCBF	Visual Maze	Nicotine	↑es occipital-temporal-parietal cx more during difficult than control task	No effect
Thiel (05)	Alerting / Spatial cues	Nicotine	↓es L lateral occipito-temporal, R medial occipital cx - alerting ↓es post occip, post fusiform cx, but ↑es ant occip, ant fusiform cx - orienting	Speeding of invalidly-cued trials; alerting numerically but insignificantly speeded
Hahn (07)	Spatial cues	Nicotine (smokers)	↑es cuneus in valid precise-cueing trials, but ↓es cuneus in valid imprecise-cueing trials; ↑es lingual gyrus in invalid low-intensity target trials, but ↓es lingual gyrus in invalid high-intensity target trials	Speeding in precise-cueing trials
Thiel (08)	Spatial cues	Nicotine	No effect in occipital cx	Less slowing in invalidly cued trials
Vossel (08)	Spatial cues	Nicotine	↓es anterior lingual gyrus to invalid vs valid cues in high versus low-predictability blocks	Reduced invalidity effect
Hahn (09)	Visual angle; colour; signal-detection	Nicotine (smokers)	↓es occipital-temporal cx across all tasks (i.e. high- and low-attention)	Speeding in selective-attention and signal-detection tasks, but not divided attention
C. Memory (Encoding)				
Rosier (99) PET-rCBF	Shape recognition	Scopolamine (at encoding); scan 3 days later	↓es bilateral fusiform cx., esp. L (both tasks), and middle occipital cx. (during sensory-challenge rather than standard conditions)	Impaired recognition accuracy. Fusiform activity correlates with memory accuracy.
Sperling (02)	Face-name pairs	Scopolamine	↓es fusiform cx	Activity correlates with subsequent memory

Table 1: Continued – sensory cortices

C. Memory (Encoding)				
Bullmore (03)	Object-location	Scopolamine	↓ lateral occipital; inferior temporal; cuneus during task independent of memory load	No effect
Schon (05)	Delayed match-to-sample	Scopolamine	↓es bilat mid-fusiform, parahippocampus (delay-period of WM);↓es R fusiform, (delay-period of subsequently remembered trials)	Impairs performance on control task, WM task and subsequent memory
Lawrence (02)	Visual number WM (RVIP)	Nicotine	↑es middle occipital, fusiform cx. in RVIP and visuomotor control task	Improved accuracy on RVIP task (dependent on treatment order)
Hong (09)	RVIP	Nicotine	↑es cuneus, fusiform, parahippocampal cx	Improved accuracy on RVIP task
Jacobsen (04)	Auditory n-back; dichotic vs binaural	Nicotine	↑es posterior sup. Temporal cx during 2-back, not 1-back; ↓es medial occipital (i.e. *irrelevant* sensory cx) during dichotic presentation	Accuracy worsened in hardest condition (2-back, dichotic)
Jacobsen (06)	Auditory n-back	Nicotine	↓es sup. temporal cx during 2-back, dichotic; ↓es occip., fusiform (i.e. *irrelevant* sensory cx)	Accuracy worsened in hardest condition (2-back, dichotic)
Furey (97) PET-rCBF	Face WM	Physostigmine	↓es lateral temporo-occipital cx. in WM vs control tasks	Speeded responses
Furey (00b) PET-rCBF	Face WM	Physostigmine	↑es medial occipital correlates with RT decreases	Speeded responses, and correlation with activation increases
Furey (00a)	Face WM	Physostigmine	↑es amplitude in fusiform, occipital, parietal cx. (encoding phase); ↑es activation volume in occipital, inf temporal cx. (encoding and delay)	Trend to speeded responses
Freo (05) PET-rCBF	Face WM	Physostigmine	↑es medial occipital (in elderly); ↓es lateral occipital, ventral temporal cx. (esp in young)	Speeded responses
Furey (08) PET-rCBF	Face WM	Physostigmine	↓es lateral occipital cx. (1, 6, 16s delays) ↑es medial occipital cx. (6 – 16s delays)	Speeded responses independent of delay
Chuah (08) (Sleep-deprived)	Visual color WM	Donepezil	↑es visual extrastriate cx. in sleep-deprived, independent of item number	Improved performance; correlated with activation enhancements
Ricciardi (09) PET-rCBF (Young & Old)	Face WM	Physostigmine	↓es lateral occipital, ventral temporal cx. (young) for long delays; ↑es lateral occipital (elderly) for long delays ↑es medial occipital cx. (all) for long delays	Speeded responses independent of delay

Table 1: Continued – sensory cortices

Memory (Conditioning)				
Thiel (02a)	Auditory fear conditioning	Scopolamine	↓es auditory cx plasticity due to ↓ response to CS+ or ↑ response to CS-	Reduced speeding of responses to CS+ (paired) relative to CS- (unpaired tone)
Thiel (02b)	Auditory fear conditioning	Physostigmine	↓es auditory cx plasticity due to ↑ CS- response (unpaired tone)	No effect
Memory (Priming)				
Thiel (01)	Word stem-cell completion	Scopolamine	↓es L lateral occipital repetition decrease due to ↑ed response to repeated stimulus. No effect in primary visual cortex.	Reduced priming (accuracy) for previously presented words
Thiel (02c)	Faces – judging famousness	Scopolamine	↓es R fusiform cx repetition decrease to famous face repetition, mostly due to higher signal with repeated face; also overall ↓ in response to all face types in L fusiform cx.	Reduced priming (RT) for repeated famous faces; no effect if drug given *after* study phase

Abbreviations: WM: working memory; ANT: attention network task; RVIP: rapid visual information processing task; cx: cortex; PFC : prefrontal cortex; RT: reaction time; sup.: superior; occip.: occipital

Table 2: Cholinergic functional imaging studies – task-related activations in fronto-parietal-temporal cortices, and subcortical, regions

A. Sensory Judgement	*Scanning task*	*Drug*	*Effect of drug on functional activations*	*Effect of drug on performance*
Cohen (94) PET-FDG	Auditory Discrimination	Scopolamine	↓es thalamus, R PFC, cingulate, inf parietal cx; ↑ L anterior prefrontal, superior parietal cx	Poorer discrimination of targets. Correlation between R PFC and score
Thienel (09a)	Attention Network Task	Mecamylamine	↑es orbitofrontal cx during alerting; ↓es bilat superior frontal during orientation; (and ↑es during no-orientation trials); ↓es precuneus, sup parietal during conflict; ↑es L inf parietal during conflict	Slowing across all trial types; no interactions
Thienel (09b)	Attention Network Task	Scopolamine	↑es R middle temporal during alerting; ↓es L superior prefrontal during orientation (and ↑es during alerting trials); ↓es ant cing, OFC, R superior frontal, precuneus, during conflict (and ↑es during no-conflict trials); ↑es L inf parietal during conflict	Slowing across all trial types; greater slowing for incongruent (conflict) trials; also reduced interaction of alerting with conflict
Ghatan (98) PET-rCBF	Visual maze	Nicotine	↓es ant cing, basal ganglia, thalamus, cbllm	No effect
Mentis (01) PET-rCBF	Alternating eye light flash	Physostigmine ± Scopolamine	Physo ↓es inf parietal; ↑es thalamus; Scopolamine did not affect this	Not measured
Thiel (05)	Alerting / Spatial Cues	Nicotine	↑es rt angular gyrus and rt prefrontal during alerting trials (and ↓es during no-cue trials) ↓es lt lateral occipito-temporal during alerting ↓es left parietal, precuneus during invalid-cue	Speeding of invalidly-cued trials, esp in subjects with large validity effect at baseline; alerting and false-alarm rate not affected
Giessing (06)	Visual spatial cues	Nicotine	↓es right posterior parietal during invalidly cued trials, esp when cues highly reliable; ↑es right posterior parietal during validly cued trials when cues poorly reliable	No effect
Thiel (07)	Auditory / visual alerting	Nicotine	↓es cued trials; ↑uncued trials in R parieto-occipital, frontal, sup temporal, ant cingulate; ↑es cue trials in R angular gyrus (visual trials); ↓es uncued trials (both modalities)	Trend to speeding for cued visual trials and uncued auditory trials

Table 2: Continued – task-related activations in fronto-parietal-temporal cortices, and subcortical regions

Thiel (08)	Spatial cues	Nicotine	↓es right parietal, left inferior frontal, bilat middle temporal during invalid trials	Speeding of invalidly cued trials
Vossell (08)	Spatial cues	Nicotine	↓es R parietal, temporal, parietal, ant cing in invalid trials (and ↑es in valid trial) in 90% valid block, but ↑es R parietal during invalid trials (and ↓es in valid trial) in 60% valid block	Speeding of invalidly cued trials in 90%-valid block, but slight slowing in 60%-valid block
Hahn (07)	Spatial cues	Nicotine (smokers; but no difference with non-smokers)	Enhances deactivations in medial PFC - parietal, L angular gyrus. ↓es target-related activity in thalamus (valid), precuneus (invalid); but ↑es R PFC. ↓es invalid trials in R PFC and L parietal for high-intensity targets, but ↑es in these regions for low-intensity targets.	Speeding in precise-cue, high-intensity target trials only, and in invalid trials. Improved accuracy with high-intensity targets. Correlation between RT ↓ and nicotine-induced BOLD deactivations
Hahn (09)	Visual angle; colour sequence; signal-detection	Nicotine (smokers; no difference with non-smokers)	↓es dorsal prefrontal during low-attention, but ↑es during high-attention; also main-effect ↓es (enhances deactivation) in ant cing, medial PFC, parahippocampal cx	Speeding of high and low-attention tasks. Correlations of RT ↓ with thalamus, PFC deactivations in signal-detection task
Ettinger (09)	Pro- and Anti-Saccades	Nicotine	↓es dorsal prefrontal during anti-saccades; ↓es posterior cingulate, precuneus, R sup temp gyrus during pro-saccades	Speeding of anti-saccades
Azizian (09)	Color-word Stroop task	Nicotine vs smoking withdrawal	↓es anterior cingulate; ↑es middle frontal	Speeding independent of congruency
B. Working memory				
Grasby (95) PET-rCBF	Auditory word lists: 5- & 15-words	Scopolamine	↓es bilat PFC, ant cing. (supraspan task); ↓es premotor, R thalamus, precuneus, and ↑es OFC in supra- and subspan tasks	Memory impairment on supraspan task only
Dumas(08)	Visual verbal n-back WM	Scopolamine / Mecamylamine	↓es R prefrontal (either drug); ↓es precuneus (scopolamine)	No effect

Table 2: Continued – task-related activations in fronto-parietal-temporal cortices, and subcortical regions

Ernst (01) PET-rCBF	Visual letter 2-back WM	Nicotine	↑es L lateral PFC; bilat parietal cx; ↓es ant. cingulate (in ex-smokers); ↓es frontoparietal, ant. cingulated (smokers)	Improves accuracy in smokers; accuracy correlates positively with PFC, cingulate activity under nicotine
Lawrence (02)	RVIP and target detection	Nicotine	↑es bilat. parietal, post. cingulate, caudate, thalamus (RVIP); enhances insula deactivations	Improved accuracy on RVIP task (dependent on treatment order)
Hong (09)	RVIP	Nicotine	↑es bilat. prefrontal, cingulate, parietal, cx; insula, thalamus; striatum; midbrain, cbllm.	Improved accuracy on RVIP task; correlated with activity in prefrontal, parietal, striatal, cingulate, brainstem
Kumari (03)	n-back WM	Nicotine	↑es dorsofronto-parietal, ant cingulate, esp at 1-back; ↓es R dorsal parietal for 3-back	Increased accuracy. Cing, parietal effects covary with performance. Speeding in 3-back
Jacobsen (04)	Auditory 1- or 2-back	Nicotine	↓es R frontal, pallidum and thalamus during dichotic (high-attention) or 2-back conditions	Impaired accuracy during dichotic, 2-back condition
Jacobsen (06)	Auditory 1- or 2-back	Nicotine	↓es L prefrontal, posterior cingulate during dichotic 2-back condition	Impaired accuracy during dichotic, 2-back (more so in 957T carriers)
Furey (97) PET-rCBF	Face WM	Physostigmine	↓es R prefrontal cx.	Speeded responses and correlation with prefrontal reductions
Furey (00b) PET-rCBF	Face WM	Physostigmine	↓es in R prefrontal cx. ant cingulate, L lateral temporal cx. correlates with RT decreases	Speeded responses and correlations with activation decreases
Furey (00c) PET-rCBF	Face WM	Physostigmine	↓es in R prefrontal cx.	Speeded responses
Furey (00a)	Face WM	Physostigmine	↓es anterior dorsal prefrontal cx,; ↑es inferior PFC, to all phases of task	Speeded responses
Freo (05) PET-rCBF (Young & Old)	Face WM	Physostigmine	↓es dorsal (young) and anterior, inferior (elderly) PFC,; trend to ↑ in ant. cingulate cx.; greater deactivations in insula, medial frontal	Speeded responses in both young and elderly
Furey (08) PET-rCBF	Face WM	Physostigmine	↓es anterior, inferior prefrontal cx., esp. at longer WM delays; ↓es sup. PFC at all delays	Speeded responses independent of delay
Ricciardi (09) PET-rCBF	Face WM	Physostigmine	↓es anterior prefrontal cx	Speeded responses independent of delay
Chuah (08) (Sleep-deprived)	Visual color WM	Donepezil	↑es R intraparietal sulcus. L prefrontal in sleep-deprived	Improved performance; correlated with activation enhancements

Table 2: Continued – task-related activations in fronto-parietal-temporal cortices, and subcortical regions

C. Short-Term Memory				
Rosier (99) PET-rCBF	Shape recognition	Scopolamine (drug given during encoding); scan 3 days later	↑es posterior thalamus, bilat parietal	Impaired recognition accuracy. No effect on stimulus discrimination or detection control tasks (at time drug given)
Thiel (01)	Word stem-cell completion	Scopolamine	↓es inferior and middle PFC repetition decrease due to ↓ed response to new items	Reduced priming for previously presented words
Sperling (02)	Face-name pairs	Scopolamine	↓ inferior, dorsolateral, orbital PFC; deactivations in lateral parietal, precuneus, lateral temporal cx.	Slowed responses to gender judgement. Impaired subsequent memory.
Bullmore (03)	Object-location learning	Scopolamine	↓ bilateral dorsolateral PFC, ant. cingulate, striatum for high memory loads; ↓ parietal for high and low memory loads	No effect
Bozzali (06)	Word retrieval	Scopolamine	↓ bilateral PFC in exclusion condition (i.e. source not familiarity memory) for New but not Old items	No overall effect. Correlation of ↓ in left PFC activity with score on New items
Craig (09)	Subsequent memory effect for written words	Scopolamine	↓es L inferior frontal in subgroup treated with GnRH (reduces estrogen release)	Impaired recognition

Abbreviations: WM: working memory; ANT: attention network task; RVIP: rapid visual information processing task; cx: cortex; PFC : prefrontal cortex; RT: reaction time; cbllm: cerebellum

Table 3: Cholinergic functional imaging studies – Medial temporal areas

A. Memory	*Scanning task*	*Drug*	*Effect of drug on functional activations*	*Effect of drug on performance*
Sperling (02)	Face-name pairs	Scopolamine	↓es fusiform cx, anterior hippocampus	Correlates with memory impairment
Bullmore (03)	Object-locations	Scopolamine	↓ hippocampal, parahippocampal cx. For higher memory load	No effect
Schon (05)	Scenes: delayed match-to-sample	Scopolamine	↓es bilat mid-fusiform, parahippocampus during delay-period of WM, not control. ↓es right-fusiform, bilat parahippocampus, hippocampus in delay-period of subsequently remembered items presented once; ↑es bilat hippocampus subsequent memory effect for stimuli previously presented twice	Impairs accuracy and speed on visual control task and WM task. Impairs subsequent confident memory
Bozzali (06)	Word retrieval	Scopolamine	↓ left perirhinal cx in exclusion condition (i.e. source not familiarity memory) for New but not Old items	No overall effect. Correlation of ↓ in left perirhinal cx activity with score on New items
Kukolja (09)	Item & Spatial Source Memory	Physostigmine	↑es R hippocampal for successful spatial source *encoding*; ↓es R amygdala during item *encoding* regardless of subsequent source memory ↓es R amygdala for successful spatial source *retrieval*	Trend for reduction in spatial source memory accuracy. Baseline item memory accuracy negatively correlated with effect of cholinergic stimulation on item memory accuracy.
B. Other				
Dumas(08)	Visual verbal n-back WM	Scopolamine / Mecamylamine	↑es R parahippocampal cx (mecamylamine)	No effect
Thienel (09a)	ANT	Mecamylamine	↑es L parahippocampal cx during orienting	Slowing of responses
Thienel (09b)	ANT	Scopolamine	↓es L hippocampus during alerting	Slowing of responses
Lawrence (02)	RVIP	Nicotine	Enhances L parahippocampal, amygdala deactivations	Improved performance

Table 5: Continued - Medial temporal areas

Hong (09)	RVIP	Nicotine	↑es parahippocampal cx	Improved accuracy
Vossel (08)	Spatial cues	Nicotine	↓es R hippocampus to invalid vs valid cues	Reduced invalidity effect
Hahn (09)	Several attention tasks	Nicotine	Enhances L parahippocampal deactivations	Speeding of responses
Furey (00a)	Face WM	Physostigmine	↓es L hippocampus correlates with RT ↓	Speeding of responses

Abbreviations: WM: working memory; ANT: attention network task; RVIP: rapid visual information processing task; cx: cortex; PFC : prefrontal cortex

Interpretation

Sensory cortex modulations

Directionality of cholinergic modulation of sensory cortex is task-dependent

One pattern that emerges on comparing Table 1A with that of Table 1B and 1C is that the direction of modulation of cholinergic drugs on sensory cortex activity depends upon whether or not subjects attend to the stimulus. When stimuli are observed passively, or are irrelevant to task, cholinergic stimulation (with nicotine or cholinesterase inhibition) generally either elicits no effect (e.g. Jacobsen et al, 2002), or else suppresses sensory cortex activity, both in higher (e.g. Furey et al, 2000b) and early processing areas (e.g. Silver et al, 2008). By contrast, the muscarinic receptor antagonist scopolamine results either in enhanced activations (e.g. Mentis et al, 2001) or in no influence (e.g. Thiel et al, 2001) within early sensory cortices during similar similar low-attention conditions. Resting-state studies support this general pattern with scopolamine tending to increase, but physostigmine tending to decrease, sensory cortical regional glucose consumption (Blin et al, 1994; Blin et al, 1997). Together, these findings suggest that stimulation of cholinergic receptors, especially muscarinic-type, can lead to net *suppression* of activity within sensory cortical regions, for stimuli that are task-irrelevant.

In contrast, in situations where the stimulus is relevant to the task – either because of an instructed sensory judgement (Table 1B) or encoding for later memory (1C) - the opposite pattern is typically found. Thus, stimulus-evoked visual cortex activity is enhanced by physotigmine (e.g. Furey et al, 2000a), but suppressed by scopolamine or

mecamylamine (e.g. Sperling et al, 2002; Thienel et al, 2009a; Thienel et al, 2009b), selectively in tasks requiring stimulus processing. Nicotine too induces higher visual or auditory cortex activity during demanding spatial orienting, or working memory tasks, but not in sensorimotor control tasks (Hahn et al, 2007; Ghatan et al, 1998; Lawrence et al, 2002; Jacobsen et al, 2004).

A related observation is that subjects who show attentional impairments – e.g. through sleep-deprivation (Chuah & Chee, 2008), age (Freo et al, 2005; Ricciardi et al, 2009) or disease (Kumari et al, 2006; Goekoop et al, 2006) - can exhibit a greater enhancement of (task-dependent) sensory cortex activity with cholinergic stimulation than for unimpaired subjects. This dependency upon state/trait also appears to be reflected in a greater performance response to cholinergic stimulation among poorly performing subjects (Kukolja et al, 2009). Presumably less able subjects will experience greater difficulty than more typical healthy subjects for a given task, so that their characteristic response to cholinergic stimulation can be thought of as reflecting increased task demands.

From the perspective of existing accounts of cholinergic impacts on sensory processing that recognise separable influences for bottom-up and top-down processes (Sarter et al, 2001), the results of cholinergic functional imaging can be summarized as: 1) cholinergic stimulation typically suppresses (or cholinergic blockade enhances) net sensory activations under conditions in which bottom-up processing predominates – e.g. with passive or task-irrelevant sensory stimulation, or alerting but non-orienting cues; while 2) cholinergic stimulation instead typically enhances (or cholinergic blockade decreases)

net sensory cortical activations under conditions in which top-down influences are strong, e.g. with demanding perceptual discrimination, higher attentional load, or deeper encoding for later memory. Furthermore, cholinergic modulation of task-dependent sensory cortex activity correlates with drug effects on working memory (Furey et al, 2000b; Chuah & Chee, 2008) or short-term memory performance (Sperling et al, 2002; Schon et al, 2005). This apparently supports models in which cholinergic influences on sensory cortices also go on to influence attentional and memory functions (Hasselmo & McGaughy, 2004; Sarter et al, 2005).

Can we relate the profile of cholinergic modulation of sensory cortex activations, as found with PET or fMRI, to electrophysiological studies? As discussed in the Background chapter, the effects of ACh stimulation in sensory cortices as studied electrophysiologically are varied, with some potentiation of activity restricted to stimulus-driven units in layer IV, but the predominant modulation among other cortical layers, subserving feedback or lateral interactions, being suppressive instead (Gil et al, 1997; Roberts et al, 2005). The net effect from such combined modulation is suggested by voltage-sensitive optical imaging, which demonstrates that ACh generally suppresses overall strength and propagation of afferent-driven electrical activity across and between columns within cat visual cortex (Kimura et al, 1999). From a functional perspective, widespread neural suppression may 'reset' sensory processing (Gulledge et al, 2007), thereby heightening signal-to-noise ratio specifically for sensory, i.e. thalamocortical inputs (Sato et al, 1987b), while reducing lateral or feedback influences (Roberts et al, 2005). On comparison with the above functional imaging review, it is apparent that pro-

cholinergic drugs can also be associated with reduced sensory activations (or vice versa for anti-cholinergics), albeit specifically when attention to the stimulus is absent or low. Following the schema of Sarter et al (2001) (Fig. 2.11A), and bearing in mind that most electrophysiological studies measure stimulus-evoked responses divorced from top-down inputs, the functional neuroimaging findings of pro-cholinergic sensory suppression may correspond to the electrophysiological findings of ACh-induced *net* suppression in sensory cortex - that itself is thought to reflect enhanced bottom-up (relative to top-down or lateral) processing.

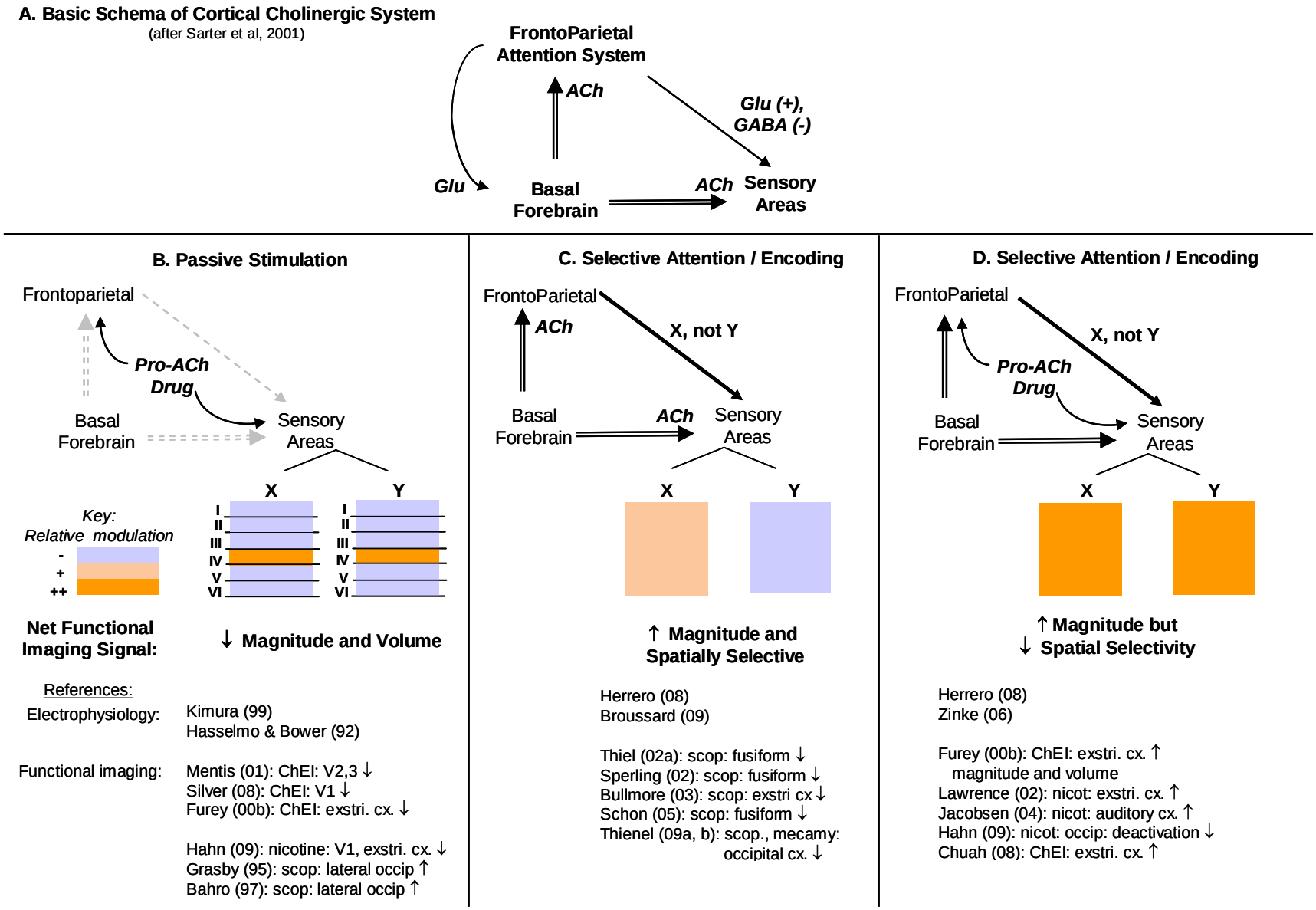
If *decreases* in sensory cortex activation induced by cholinergic stimulatory drugs reflect net neural suppression as seen following ACh application to cortical slices, then what neurophysiological events do pro-cholinergic drug-induced *increases* in sensory activation relate to, as we have generally found in high-attention conditions within neuroimaging paradigms? To recap, a critical role for the cholinergic system is to maintain sensory processing in the face of performance challenges such as distractors (Sarter et al, 2006). Thus we would expect ACh to potentiate neural correlates of selective attention, in which sensory processing is biased towards task-relevant stimulus features, and away from task-irrelevant ones. In keeping with this, two recent studies in awake monkeys and rats respectively, indicate that cholinergic input to sensory (Herrero et al, 2008) and parietal (Broussard et al, 2009) cortices can potentiate neural correlates of selective attention by disproportionately increasing weighting of task-relevant versus task-irrelevant inputs. However, of relevance here, is that acetylcholine application also increased the *overall* level of visual neural activity, both in cells coding for task-relevant

and task-irrelevant locations, specifically during target detection. Accordingly during attention-demanding relative to baseline conditions, as listed in Tables 1B and 1C, we might expect pro-cholinergic treatments to enhance stimulus-evoked responses at the spatial scale of fMRI or PET, that integrate activity over thousands of such units (potentially including both task-relevant and task-irrelevant).

Combining neurophysiological accounts of cholinergic modulation on bottom-up (e.g. Hasselmo & McGaughy, 2004) and top-down (e.g. Herrero et al, 2008) processes within sensory cortices, one can propose an account that accommodates the task-dependent profile of cholinergic impact on sensory activations studied with neuroimaging (see Figure 10.1). Whenever a stimulus is presented, regardless of task, cholinergic stimulation facilitates bottom-up circuitry, whilst reducing feedback and horizontal influences - the net metabolic signature of which seems to be a decrease in sensory cortex activation (e.g. Kimura et al, 1999). Conversely, in a subset of sensory paradigms, in which attention is focused towards the stimulus, top-down glutamatergic-mediated signals will enhance activity in selected, task-relevant sensory regions. Given that ACh potentiates neural activity of task-relevant, as well as (to a lesser extent) task-irrelevant regions (Herrero et al, 2008) during active sensory processing, the functional imaging correlate of this would be an increase in sensory cortex activations following pro-cholinergic drug administration. This would also fit with findings that pro-cholinergic enhancement of sensory activations is more apparent in subjects with poorer baseline performance - for in these subjects it is plausible that a greater top-down 'attentional effort' is operative in order to sustain error-free performance (Sarter et al, 2006).

Figure 3.1. Model that links effects of acetylcholine on attentional and sensory systems based upon animal studies, with effects of systemic cholinergic stimulation or antagonism on functional imaging in three scenarios: A) pro-cholinergic drug modulation of sensory stimulation in passive / low-attention paradigms, relative to no drug ; B) selective attention paradigm (X versus Y) under normal cholinergic tone, relative to cholinergic inhibition or deficiency; C) selective attention under pro-cholinergic drug modulation versus no drug. Effects of ACh on the basic cortical circuit are known for ex vivo slices and so are more relevant for passive-stimulation functional imaging paradigms (A). Conversely, effects of ACh on attention have been determined from in vivo recordings, and do not accurately delineate laminar-specific effects. Effects of endogenous ACh on attention (B) are suggested by functional imaging studies employing cholinergic antagonists, or comparing untreated with treated Alzheimer's disease patients.

Figure 3.1



Abbreviations: ACh: acetylcholine; Glu: glutamate; GABA: gamma-amino butyric acid; ChEI: cholinesterase inhibitor; scop: scopolamine (i.e. anti-ACh receptor drug) ; nicot: nicotine; Mec: mecamylamine; ExStri Cx: visual extrastriate cortex; V1, 2,, 3: early visual cortices; occip.: occipital cortex; AD: Alzheimer's disease

Frontoparietal cortex modulations

Hypercholinergic or hypocholinergic states reduce neural markers of top-down orienting

A consistent pattern of parietal modulations by nicotine is observed in a series of studies employing a spatial cueing (Posner) task, in which detection of lateralised targets is enhanced by prior cues that signal the likely location of an upcoming target. Nicotine decreases inferior parietal cortex activations specifically during invalidly-cued trials - i.e. in a minority of 'catch' trials when the cue incorrectly predicts the subsequent target location, and for which there is a need for reorienting away from a cued location (Thiel et al, 2005; Thiel et al, 2007; Thiel et al, 2008). Since nicotine also decreases the performance-cost of invalid cues (Phillips et al, 2000), this decreased parietal activation during invalid trials seems not to reflect impaired reorienting, but rather implies a processing benefit. Further variations of this paradigm reveal that nicotine-induced decreases in parietal responses to invalid cues are diminished when cue-derived expectation is reduced (Vossel et al, 2008; Giessing et al, 2006). This suggests that nicotine decreases cue-elicited spatial biasing - which secondarily reduces parietal-mediated reorientation. Moreover, the fact that performance may be enhanced by nicotine on invalid trials suggests that it broadens attention: i.e. that it favours bottom-up over top-down processing, in keeping with a key prediction of a computational model, that foresees ACh as signalling expected uncertainty (Yu & Dayan, 2005).

Other patterns of frontoparietal modulation by cholinergic drugs support an account of reduced top-down orienting. First, nicotine reduces anterior cingulate, as well as parietal

cortex, activity during invalid trials, coincident with speeding and reduced response variability (Vossel et al, 2008). Given that anterior cingulate reflects a source of attentional control (Sarter et al, 2006), a nicotinic-induced reduction in its activity might reflect reduced 'attentional effort' and/or error detection, on invalidly cued trials, due to less of a preceding top-down bias towards the cued location, as proposed above. Second, nicotine powerfully decreases right angular gyrus (parietal) activations during uncued relative to cued (i.e. 'alerting') trials (Thiel et al, 2005; Thiel et al, 2007). Since this region appears to mediate reorienting to unattended stimuli (Yantis et al, 2002), this suggests that nicotine reduces the 'surprise' element of uncued stimuli, possibly by heightening vigilance (Wesnes & Warburton, 1984), and thus reducing the subsequent need to reorient. Third, activity in supramodal superior temporal gyrus – thought to be a node within a stimulus-driven, bottom-up 'interrupt' system (Corbetta et al, 2000) – is increased by nicotine in uncued trials but decreased in cued trials, effectively resulting in a 'levelling out' of responses (Thiel et al, 2007). This too indicates that cholinergic stimulation can enhance processing for less attended stimuli (e.g. as for uncued/unexpected targets), to the detriment of top-down influences (e.g. as for cued/expected targets).

Mirroring pro-cholinergic reductions in parietal activity, studies exploring attentional effects of cholinergic *antagonists* (with either anti-muscarinic scopolamine or anti-nicotinic mecamylamine) have demonstrated increases in parietal activity, with associated impaired performance (Thienel et al, 2009a; Thienel et al, 2009b). Since these drug-induced hyperactivations occurred selectively with target-distractor conflict, when

parietal activity might reflect attentional refocusing (Corbetta et al, 2000), this can be interpreted in terms of the anticholinergics having decreased selective attention to the cued target location prior to target appearance. Thus both ACh-deficient and hypercholinergic states can be associated with parietal modulations and behavioral accompaniments that suggest impairment in top-down processing.

Hypercholinergic reductions in activity may reflect enhanced processing efficiency

When a drug reduces task-related cerebral activity, and yet is accompanied by improved performance, one parsimonious account is that the drug enhances cortical processing efficiency. Across a wide range of many (non-pharmacological) functional imaging paradigms, better performance often correlates with reductions in prefrontal activations (Rypma et al, 2006), arguably because of reduced processing times and/or metabolic demands. Regional hypoactivation may reflect improved processing efficiency within the region itself; in remote region(s); or in the interconnections between them.

Numerous examples exist where pro-cholinergic drugs improve performance while decreasing frontoparietal activations (Table 2). For example, physostigmine-induced reductions in dorsal prefrontal cortex activity, during encoding and maintenance-phases of a working memory task (Furey et al, 2000a), have been interpreted in terms of reduced task effort, on account of a correlation of this with drug-induced speeded responses (Furey et al, 1997). One explanation for this is that physostigmine produces a more robust neural representation of studied stimuli - indexed by enhanced responses in visual extrastriate regions during encoding (Furey et al, 2000a) - thereby necessitating less prefrontal-mediated activity during a subsequent working memory delay period. Since

such drug effects on BOLD responses and performance are more marked at longer memory delays (Furey et al, 2008a; Ricciardi et al, 2009), the benefit appears to be specific for memory processes, perhaps reflecting efficiency of encoding into memory, and/or stimulus-specific sustained-spiking in higher sensory cortices (Schon et al, 2005). The idea that drug-induced facilitation of memory-related sensory processing may secondarily decrease task-related prefrontal activations seems complementary to other findings that more taxing sensory conditions increase prefrontal activations during encoding (Grady et al, 1996). Furthermore, scopolamine consistently decreases fusiform cortex activations, at the same time as increasing thalamic and parietal activations, during the recollection stage of a visual pattern memory task (Rosier et al, 1999). In this case, enhancements of thalamus and parietal cortex were interpreted as 'effortful' compensatory strategies secondary to non-specific, drug-induced impairments in stimulus encoding, found as they were with both scopolamine and diazepam.

Diminutions in prefrontal activity, in association with improved performance, have also been found following nicotine (Hahn et al, 2009; Ettinger et al, 2009). Similar to the profile for physostigmine, nicotine-induced reductions in prefrontal activity during a perceptual task are associated with increased posterior cortical activations (Ghatan et al, 1998), suggesting that nicotine may primarily be enhancing sensory processing efficiency. Alternatively, such findings may reflect increased efficiency within prefrontal cortex itself, by, for example, facilitating presynaptic neurotransmitter release, without increasing presynaptic electrical activity (Lambe et al, 2003; Wonnacott et al, 2006).

Deactivations in frontoparietal cortex secondary to pro-cholinergic drugs, and their associated performance enhancements, occur with varying levels of functional specificity across paradigms. On the one hand, nicotine-induced deactivations of frontoparietal cortices or thalamus correlate with response speeding, yet do not interact with cue accuracy in a spatial attention task (Hahn et al, 2007; Hahn et al, 2009). This suggests that in certain situations, nicotine exerts a general preparatory or alerting effect in frontoparietal regions, rather than interacting with spatial orienting per se. However, in numerous other cases, pro-cholinergic treatments induce modulations that are specific to task or stimulus properties. Examples of this include parietal deactivations to invalid trials during sessions of high, but not low, cue-predictability (Vossell et al, 2008), or deactivations of medial prefrontal cortex seen only with imprecise, but not precise, cued trials (Hahn et al, 2007). Hence to the extent that nicotine or cholinesterase inhibition enhances cortical processing efficiency, this may be manifest only under certain functional states (e.g. during invalidly cued trials), and is not simply proportionate to the degree of task-induced cortical activation in the absence of drug. It should be noted, from a methodological point of view, that both the specificity of drug-induced deactivations, and correlations of these with corresponding behavioural effects in these studies strongly argue against a purely vascular 'epiphenomenon' account for these results.

Challenges to an 'increased efficiency' account for pro-cholinergic drug effects in frontoparietal areas come from further data showing that nicotine or cholinesterase inhibition can sometimes reduce prefrontal activity while accompanied by *performance impairment* (Ernst et al, 2001; Jacobsen et al, 2004); or improve performance in the

absence of frontoparietal effects (Ettinger et al, 2009); or be associated with performance improvements plus prefrontal activation *increases* (Chuah & Chee, 2008). Furthermore, cholinergic *blockade* can also decreases prefrontal activations during rest (Honer et al, 1988), attention (Thienel et al, 2009a; Thienel et al, 2009b), and memory tasks (e.g. Sperling et al, 2002; Bullmore et al, 2003; Craig et al, 2009), often while impairing performance, suggesting that prefrontal cholinergic stimulation is required for normal functioning. Taken together, the overall pattern of results indicates an inverted U-shaped function response profile, such that both hyper- and hypo-cholinergic stimulation can impair performance, and can reduce task-related frontal-parietal activiations.

An alternative to efficiency-based accounts has been considered in the context of recently-withdrawn smokers. In these cases, nicotine can induce reductions in prefrontal activity while improving performance (Ernst et al, 2001; Azizian et al, 2009). Since nicotine reduces withdrawal symptoms, that aspect alone may improve performance while correspondingly reducing prefrontal activations that signify 'attentional effort'. More generally, performance improvements due to a drug may alter the level of attentional demand for a given task, which may itself cause a secondary modification of cerebral activations. It therefore becomes important to consider the relation between drug-induced brain activations and changes in performance wherever possible, regressing out behavioural change when appropriate.

Hypercholinergic-induced deactivations of the 'default network' may reflect a shift from internal to external processing

Many of the fronto-parietal-temporal regions whose activity is suppressed by pro-cholinergic treatments are either medially-located (e.g. cingulate, precuneus, and parahippocampal gyri), or involve superior–middle temporal, and angular gyri (Ghatan et al, 1998; Hahn et al, 2007; Hahn et al, 2009; Ettinger et al, 2009; Azizian et al, 2009). These regions overlap with the so-called 'default' or 'resting-state' network (Raichle & Snyder, 2007), and as such suggest another mechanism by which the cholinergic system and cholinergic drugs may act. Cholinergic stimulation typically exaggerates deactivations within these regions, seen without drug during attention-demanding tasks, while not affecting activity at rest. At the same time, many of these studies also show that cholinergic stimulation increases task-related activity in dorsolateral frontoparietal or posterior regions, suggesting a reciprocal shift in the balance of processing or activation between 'resting-state' and 'attentional-sensory' cortices. Conversely, in the resting state, or for low-attention tasks, nicotine can increase activations in medial frontoparietal regions (Stein et al, 1998; Lawrence et al, 2002; Kumari et al, 2003).

Given the similarity between locations showing nicotinic-mediated, task-related hypoactivations and the 'resting-state' network, this pattern of cholinergic modulation may represent a switch in processing from an internally-focused state to one where sensory processing is required (Hahn et al, 2007) (Fig. 3.2C). The fact that such drug-induced hypoactivations are independent of the level or type of attention (Hahn et al, 2009) implies that cholinergic modulation may act generally to focus attention towards any externally-specified task. Furthermore, positive correlations of nicotine-induced deactivations with performance are in keeping with the idea that performance depends

upon the efficiency with which the resting-state network can be deactivated, possibly because of a reciprocal enhancement of task-relevant processing (Polli et al, 2005).

Such cholinergic-mediated transition from a resting-state, internally-focused network to one favouring processing of external stimuli appears analogous at the cortical column level to the well-recognised tendency for acetylcholine to switch cortical dynamics from a cortico-cortical, or feedback state, to one that favours thalamocortical, or input-driven, signaling (Gil et al, 1997). Hence to extend our earlier discussion of sensory cortex effects, the neuroimaging signature of cholinergic-driven biasing of sensory over internal processing may include both deactivations of sensory regions (Silver et al, 2008), and enhanced deactivations of a default network.

As a caveat on the above account, it should be considered whether nicotine-induced response speeding may itself have led to some of the relevant deactivations, (e.g. Herath et al, 2002), especially where BOLD-behavioural correlations were found, including within thalalmus (Hahn et al, 2007; Hahn et al, 2009). Furthermore, nicotine-induced *hype*ractivations of anterior cingulate can be associated with positive performance effects (Ernst et al, 2001; Kumari et al, 2003), while cholinergic blockade is associated both with hypoactivations in similar regions and with performance impairment (Grasby et al, 1995; Thienel et al, 2009a; Thienel et al, 2009b), indicating that not all medial cortical regions respond homogeneously. Furthermore, nicotine-induced hypoactivations of medial prefrontal regions may occur specifically in conflict scenarios (Hahn et al, 2007; Vossel

et al, 2008), while speeding responses (Hasenfratz & Battig, 1992), suggesting a more specific interpretation than a 'default network' account alone would suggest.

Hypercholinergic-mediated increases in frontoparietal activity may reflect recruitment of cortical processes

Certain studies clearly show that pro-cholinergic drugs can *increase* activations in frontoparietal regions (Ernst et al, 2001; Lawrence et al, 2002; Kumari et al, 2003; Thiel et al, 2005; Chuah & Chee, 2009; Hahn et al, 2009), in contrast to nicotine or physostigmine-induced deactivations discussed in the preceding sections. Many of these drug-induced increases correlate positively with performance improvements. Consistently, multiple studies demonstrate that cholinergic blockade can engender task-related, frontoparietal hypoactivations, concomitant with performance decrements (Cohen et al, 1994; Sperling et al, 2002; Grasby et al, 1995; Bullmore et al, 2003; Bozzali et al, 2006; Thienel et al, 2009a; Thienel et al, 2009b). One of the factors that may resolve the apparent discrepancy of these 'activating' results versus the 'deactivating' patterns described earlier is anatomical. Pro-cholinergic *deactivations* tend to occur predominantly in *medial* prefrontal-parietal locations; whereas increased *activations* induced by cholinergic stimulants are often in *dorsolateral* frontoparietal cortices (Fig. 3.2C). This supports the suggestion that ACh facilitates the reciprocal balance of resting-state/default versus task-engaged processes (Hahn et al, 2007).

But a different sort of explanation is required to account for situations in which the *same* frontoparietal regions can show either cholinergic-dependent activation increases or

decreases, depending upon condition. A notable pattern is that pro-cholinergic increases often occur specifically during the most challenging stimulus or task conditions, with accompanying performance improvements (e.g. Lawrence et al, 2002; Hahn et al, 2007; Hahn et al, 2009); while anti-cholinergics typically reduce activations in these regions and impair performance during the most challenging conditions (Bullmore et al, 2003; Bozzali et al, 2006; Thienel et al, 2009a). One interpretation of these findings is that ACh mediates recruitment of frontoparietal processes when resources are pushed to near-maximum use (Fig. 3.2D), or with 'attentional effort' (Sarter et al, 2006). This is consistent with single-unit studies in rats showing that prefrontal cholinergic inputs are essential for both increases in prefrontal activity, and maintenance of performance in the face of distracters (Gill et al, 2000). Furthermore, rat studies showing that co-activation of a prefrontal – cholinergic basal forebrain loop is essential for sensory cortex potentiation and performance (Golmayo et al, 2003), finds similarity in human neuroimaging studies showing positive correlations between cholinergic-mediated enhancement (or depression) of frontoparietal cortices, visual cortices and accuracy (Chuah & Chee, 2008; Thienel et al, 2009a; Thienel et al, 2009b).

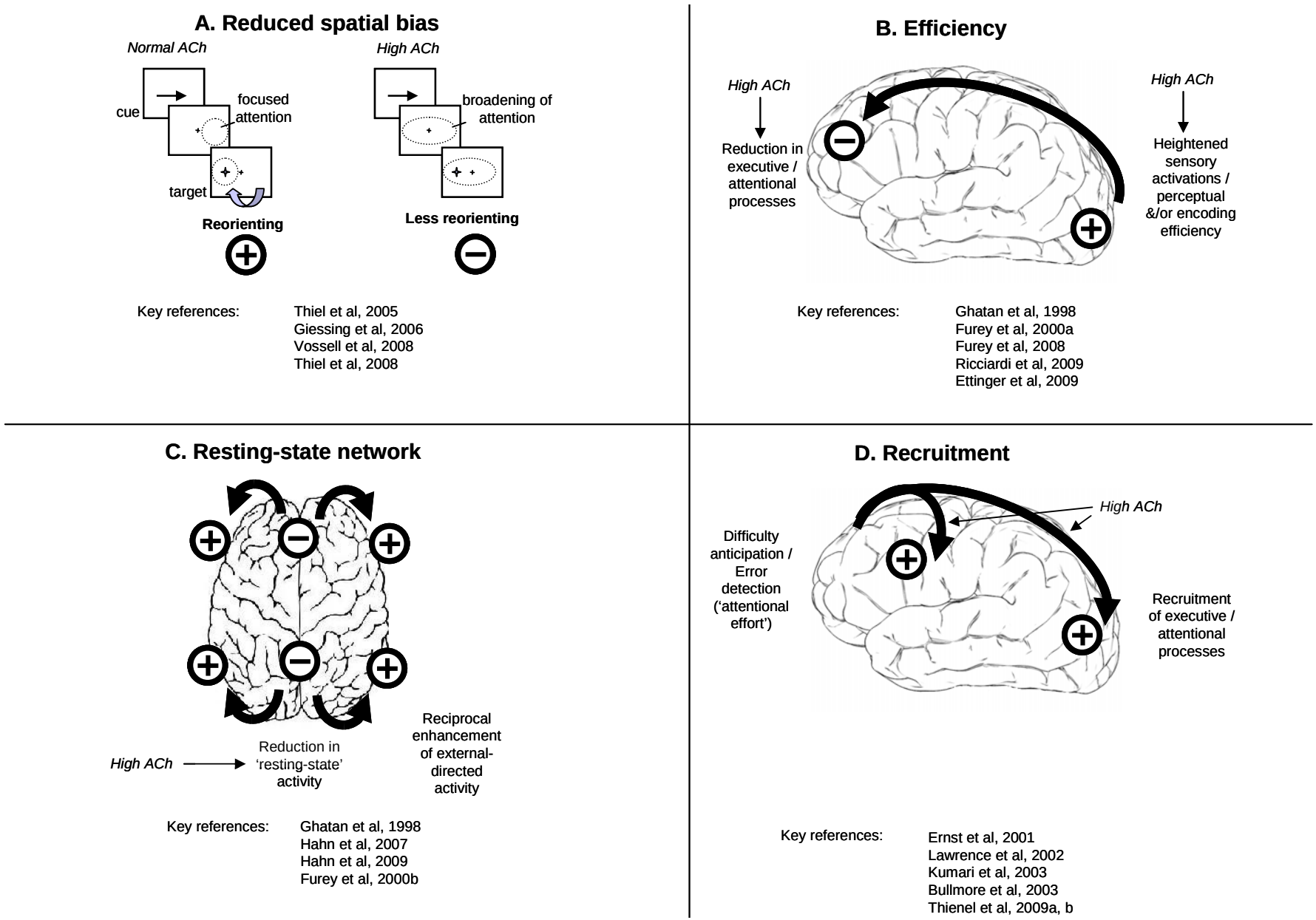
Frontoparietal hyperactivations due to cholinergic stimulation are also found to interact with the relative contingency of a cue-target relationship, with nicotine tending to increase trial-related frontoparietal activations selectively during periods of low cue reliability (Giessing et al, 2006; Vossel et al, 2008), or when no cue occurs. These effects complement findings discussed above of nicotine-induced parietal hypoactivations during periods of high cue reliability (on invalid trials), and may be interpreted in terms of high-

ACh states favouring bottom-up processing (that predominate when cues are poorly informative or absent). The fact that nicotine-induced hyperactivation of dorsolateral prefrontal cortex during a high cue reliability condition correlates *negatively* with performance (Hahn et al, 2007) further indicates that nicotine does not benefit top-down processing.

Finally, the fact that cholinergic stimulants induce frontoparietal hyperactivations during a highly circumscribed set of task parameters argues against explanations in terms of the cholinergic system's proposed role in general arousal (see Section 1). This assertion is supported by the facts that frontoparietal hyperactivations due to donepezil do not correlate with arousal (Chuah & Chee, 2009), in contrast to activations induced by nicotine specifically within the midbrain (Kumari et al, 2003).

Figure 3.2: Explanations for modulations of frontoparietal activations in cholinergic - functional imaging studies.

Figure 3.2


A. Reduced spatial bias
Normal ACh
High ACh
cue
focused attention
target
broadening of attention
Reorienting
Less reorienting
Key references:
Thiel et al, 2005
Giessing et al, 2006
Vossell et al, 2008
Thiel et al, 2008
B. Efficiency
High ACh
Reduction in executive / attentional processes
High ACh
Heightened sensory activations / perceptual &/or encoding efficiency
Key references:
Ghatan et al, 1998
Furey et al, 2000a
Furey et al, 2008
Ricciardi et al, 2009
Ettinger et al, 2009
C. Resting-state network
High ACh
Reduction in 'resting-state' activity
Reciprocal enhancement of external-directed activity
Key references:
Ghatan et al, 1998
Hahn et al, 2007
Hahn et al, 2009
Furey et al, 2000b
D. Recruitment
Difficulty anticipation / Error detection ('attentional effort')
High ACh
Recruitment of executive / attentional processes
Key references:
Ernst et al, 2001
Lawrence et al, 2002
Kumari et al, 2003
Bullmore et al, 2003
Thienel et al, 2009a, b

Memory-associated modulations

Medial temporal regions

Given a strong evidence base for cholinergic influences on memory performance (e.g. Kopelman, 1986), plus anatomical considerations regarding cholinergic innervation of hippocampus and surrounding structures (see Background), it is reassuring that numerous neuroimaging studies demonstrate direct associations between cholinergic modulation of medial temporal structures and memory encoding (Fig. 3.3). Hence scopolamine reduces activations of hippocampal and parahippocampal cortices, specifically during working memory delay periods, while decreasing subsequent memory success (Sperling et al, 2002; Schon et al, 2005). This has been interpreted in terms of sustained-spiking multi-unit activity, that is observed in perirhinal and entorhinal cortex neurons during, and after, encoding (Young et al, 1997); is stimulus-specific (Egorov et al, 2002) and cholinergic-dependent (Fransen et al, 2002). Importantly, cholinergic-dependent, delay-period BOLD activity predicts not only working memory success, but also subsequent confident memory on a later surprise recognition test (Schon et al, 2005), thereby suggesting a role for sustained-spiking activity in encoding of long-term, recollection-based memory, as predicted by computational models (Koene et al, 2003). Consistently, cholinesterase inhibition increases hippocampal responses to stimuli subsequently remembered versus forgotten (Kukolja et al, 2009).

Complementary findings are found with scopolamine, that reduces perirhinal activity specifically for new, rather than old, words (Bozzali et al, 2006). In Alzheimer's disease or mild cognitive impairment, the pro-cholinergic enhancements of memory-related hippocampal activity are even more apparent (Potkin et al, 2001; Goekoop et al, 2004; Gron

et al, 2006; Teipel et al, 2006), implying that cholinesterase inhibitors are able to partially reverse hippocampal dysfunction due specifically to a cholinergic deficiency.

Not all examples of cholinergic neuromodulation of memory accord to this simple pattern, however. As well as *increasing* right hippocampal responses to stimuli at *encoding*, physostigmine *decreases* activity in adjacent right amygdala at *retrieval* (Kukolja et al, 2009). Moreover, in the same study, physostigmine caused a trend for worse memory accuracy relative to placebo. Donepezil has also been found to decrease hippocampal activity at rest, but to induce the opposite pattern during stimulus presentation (Teipel et al, 2006).

This dependency of cholinergic-functional imaging data on the phase of memory testing is important since it mirrors behavioral and neurophysiological studies (see Background). For instance, scopolamine impairs memory when administered prior to, but not after, encoding (Rasch et al, 2006); whereas cholinesterase inhibition enhances encoding but impairs retrieval (Gais & Born, 2004). Moreover, cortical-layer studies (Hasselmo & McGaughy, 2004) indicate that elevated ACh levels increase feedforward, encoding-associated activity, but decrease feedback, retrieval-associated activity in medial temporal cortices. On this account, physostigmine would be expected to enhance novel stimulus-driven responses, but suppress responses to retrieval prompts of the same stimuli – as was indeed found in human hippocampus and amygdala (Kukolja et al, 2009).

In the earlier discussion of frontoparietal cholinergic-functional imaging modulations, it was noted that pro-cholinergic drugs may *suppress* task-related activity in a 'resting-state network' that includes also medial temporal regions (e.g. Furey et al, 2000a; Lawrence et al,

2002; Hahn et al, 2007). However, unlike paradigms such as those just described where the same drug types *enhanced* hippocampal activity (e.g. Schon et al, 2005; Kukolja et al, 2009) - when memory was a task requirement - those studies showing pro-cholinergic hippocampal decreases typically did not make memory demands, and used simple, abstract stimuli that are less likely to engage parahippocampal regions than scene or face stimuli used in memory paradigms. Hence the pattern of cholinergic modulation in medial temporal regions seems likely to depend upon task (e.g. whether or not memory is an explicit aim); phase (e.g. encoding or retrieval); and the specific contrasts performed (e.g. whether as a function of subsequent memory, or task type).

Sensory cortex

In one influential model of memory, cholinergic influences within sensory cortices complement similar modulations within hippocampal - perirhinal cortices in supporting encoding and retrieval (Hasselmo & McGaughy, 2004). Functional neuroimaging studies support this by demonstrating that scopolamine suppresses hippocampal and fusiform cortex conjointly, specifically during visual memory-delay periods (Sperling et al, 2002; Bullmore et al, 2003; Schon et al, 2005); and impairs long-term fusiform cortex plasticity (Rosier et al, 1999); in both cases matched by impaired subsequent recognition. Conversely, physostigmine increases extrastriate visual activations during visual working memory delay-periods (Furey et al, 2000a), with greater modulation for longer delays (Furey et al, 2008a; Ricciardi et al, 2009), suggesting a cholinergic interaction with a memory, rather than merely sensory, process. Presumably, recognised influences of ACh on neural processes such as feedforward associativity, long-term potentiation, and sustained-spiking, found within sensory as well as perirhinal - entorhinal cortices, may underlie many of these effects (Gu, 2003).

Accounts of cholinergic influences on memory processes within sensory cortices need to dovetail with models of cholinergic impacts on attentional processing in similar regions (Sarter et al, 2005). In this regard, modelling has suggested that cholinergic influnces on sensory cortex circuits - viz. enhancing feedforward relative to feedback connectivity (Gil et al, 1997) - serve both to enhance signal detection (and therefore certain aspects of attentional performance) and formation of novel input associations, likely to be critical for memory encoding (Hasselmo & McGaughy, 2004). One prediction that stems from this, is that cholinergic modulations of memory will be greater during high- relative to low-attention conditions. Psychopharmacological studies (Warburton et al, 2001; Fitzgerald et al, 2008), employing depth-of-processing paradigms, appear to support this, with nicotine or cholinesterase inhibition boosting memory selectively for deeply, relative to superficially, encoded items.

Cholinergic drugs are also found to interact with two well-recognised functional imaging signatures of *implicit* memory within sensory cortices – viz. conditioning-associated sensory remapping, and repetition priming - often with congruent effects on behaviour (Table 3C). The finding that scopolamine impairs conditioning-associated remapping of tonotopic auditory cortex in a human functional imaging paradigm (Thiel et al, 2002a), represents a neat translation of investigations in rats showing cholinergic dependency of a very similar sensory cortex plasticity mechanism (Weinberger, 2007). Perhaps unexpectedly though was the additional finding in humans that physostigmine also impairs conditioning-related sensory remapping (Thiel et al, 2002b). There were subtle differences in the manner by which scopolamine and physostigmine disrupted sensory cortex remapping, since scopolamine reduced differential sensory responses by suppressing responses to *relevant*

conditioned stimuli (CS+) (Thiel et al, 2002a) – while physostigmine heightens responses specifically to *irrelevant* non-conditioned stimuli (CS-). One possible explanation for this is that physostigmine reduced differential activations in sensory cortex because of this drug's tendency to increase ACh levels tonically, rather than phasically - which might then encourage pairing of both CS+ and CS- stimuli with the unconditioned (i.e. noxious) stimulus, in the presence of high ACh levels (Thiel et al, 2002a). However, another possible interpretation is that, in line with effects of nicotine on spatial orienting (see above), and computational models of ACh emphasising its enhancement of bottom-up processing (Yu & Dayan, 2005), physostigmine induced a hypervigilant state in which processing of stimuli were enhanced regardless of top-down attention (which in a conditioning paradigm is based upon previous experience of CS+ relevance).

Repetition suppression and priming are also found to be disrupted by scopolamine (Thiel et al, 2001; Thiel et al, 2000a). This manifests itself through visual extrastriate cortex activation being *increased* selectively to old items under scopolamine, in contrast to the normal reduction of activation with repetition under placebo. Given that concomitant effects on behaviour are also selective for old items, this suggests that scopolamine reduces memory *storage* (i.e. maintenance of a particular representation), or *reactivation*, within sensory cortices. However, the additional findings of *reduced* new-item activity in prefrontal cortex (Thiel et al, 2001), and an absence of drug effect on priming if given after the item-study phase (Thiel et al, 2002c), suggests that *encoding* too may be disrupted, as is more generally recognised (Hasselmo & McGaughy, 2004). While repetition suppression recorded electrically amongst monkey inferior temporal cortex neurons has not been found to be cholinergic-dependent (Miller & Desimone, 1993), the discrepancy of this with

pharmacological-neuroimaging results may have arisen because of restricted neural sampling, or shorter lag times, in the electrophysiological study.

Prefrontal cortex

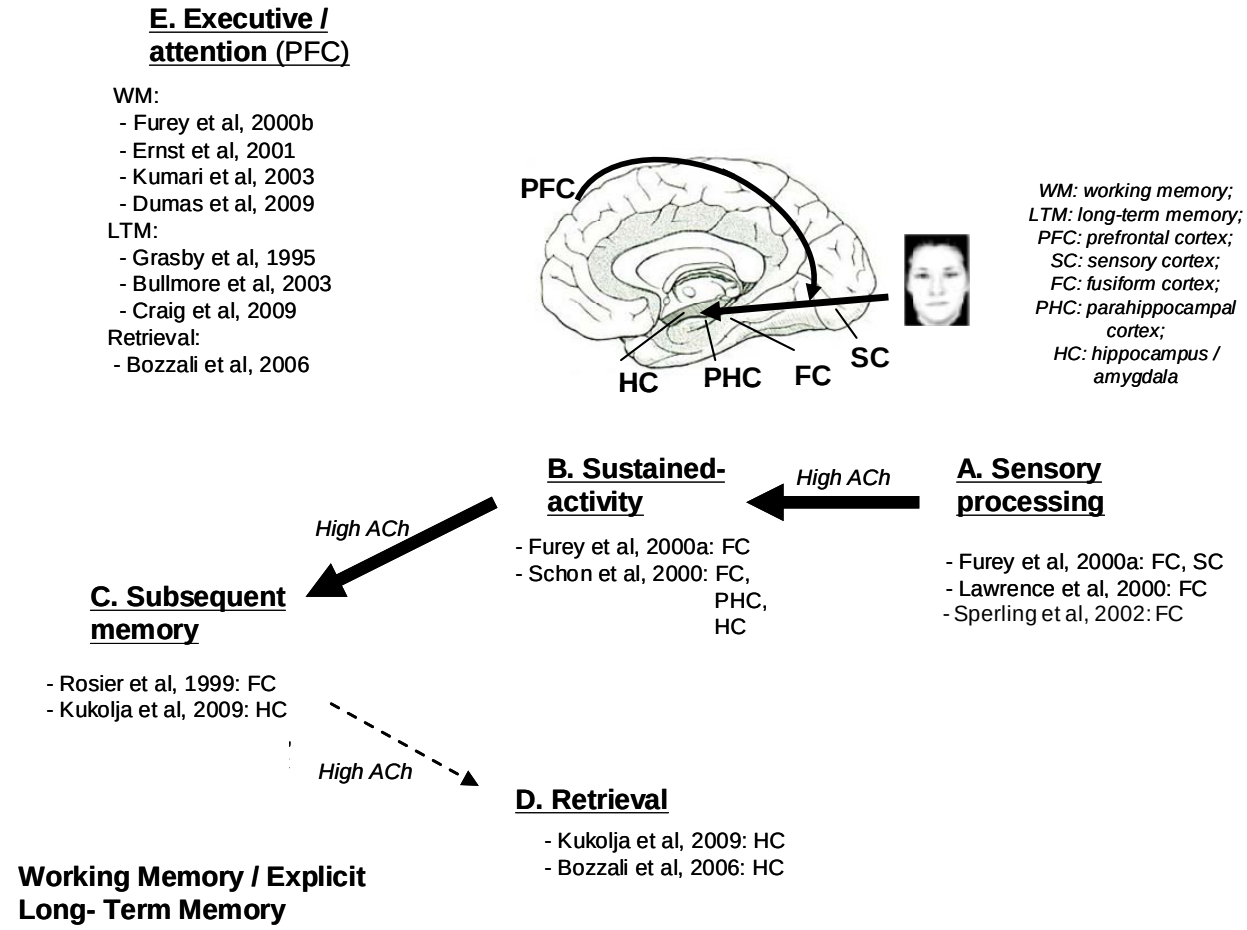
Cholinergic modulations of prefrontal activity during working memory tasks have been discussed earlier in the context of attentional effects (Sections 6.2 and 6.4), where it was noted that both cholinergic blockade and stimulation may decrease activity, albeit with different performance accompaniments. Prefrontal modulations related to long-term memory have also been observed, although in an analogous pattern to cholinergic neuromodulation of medial temporal regions (Kukolja et al, 2009), effects may vary depending on task and phase. Thus scopolamine-induced suppression of prefrontal cortex is associated with *impaired* performance when given *prior* to encoding (Sperling et al, 2002; Craig et al, 2009); but with *improved* performance when given *afterwards* (Bozzali et al, 2006). Prefrontal modulations may reflect both direct actions, e.g. due to scopolamine disrupting semantic processing of encoded words (Craig et al, 2009); and/or indirect actions, e.g. secondary to cholinergic potentiation of sensory or perirhinal cortices (Furey et al, 2008a).

Scopolamine-induced reductions of memory-related frontoparietal (and sensory) cortex activity, as well as of performance, resemble those induced by benzodiazepines within the same experimental paradigm (Thiel et al, 2001; Rosier et al, 1999; Sperling et al, 2002), thereby implying a non-specific sedation effect. Arguing against this though is that these studies found no correlations between drug-induced modulations of memory-associated activations and vigilance scores (Thiel et al, 2001; Sperling et al, 2002). Strong interdependencies between cholinergic and GABAergic neurotransmission in many brain

regions, including critically the septohippocampal pathway (Parent & Baxter, 2004), may account for such overlap in neuromodulatory responses between benzodiazepines and anti-cholinergics. Furthermore, the profile of behavioural and neural responses in a priming paradigm (Thiel et al, 2001) suggested a tendency for a greater relative effect of scopolamine on item storage, but for lorazepam on initial item encoding.

Figure 3.3: Overview of memory-related activations modulated by cholinergic drugs. Note that functional imaging studies support the general proposal that high ACh levels facilitate encoding (A, B, C) while suppressing retrieval (D), as previously modeled computationally, based upon slice-recording data (Hasselmo & McGaughy, 2004).

Figure 3.3


E. Executive / attention (PFC)
WM:
- Furey et al, 2000b
- Ernst et al, 2001
- Kumari et al, 2003
- Dumas et al, 2009
LTM:
- Grasby et al, 1995
- Bullmore et al, 2003
- Craig et al, 2009
Retrieval:
- Bozzali et al, 2006
PFC
HC
PHC
FC
SC
WM: working memory;
LTM: long-term memory;
PFC: prefrontal cortex;
SC: sensory cortex;
FC: fusiform cortex;
PHC: parahippocampal cortex;
HC: hippocampus / amygdala
B. Sustained-activity
- Furey et al, 2000a: FC
- Schon et al, 2000: FC, PHC, HC
High ACh
A. Sensory processing
- Furey et al, 2000a: FC, SC
- Lawrence et al, 2000: FC
- Sperling et al, 2002: FC
High ACh
C. Subsequent memory
- Rosier et al, 1999: FC
- Kukolja et al, 2009: HC
High ACh
D. Retrieval
- Kukolja et al, 2009: HC
- Bozzali et al, 2006: HC
Working Memory / Explicit Long- Term Memory
Implicit Memory
F. Sensory priming
- Thiel et al, 2001: SC, PFC
- Thiel et al, 2002a: FC
G. Conditioning-induced sensory remapping
- Thiel et al, 2002b: SC
- Thiel et al, 2003c: SC

Questions Addressed by the Experiments of this Thesis

From the growing number of human cholinergic functional imaging studies certain common patterns emerge that are categorizable, and moreover, are interpretable within the theoretical frameworks of cholinergic function derived mostly from non-human physiological or human psychophysical studies. The experiments described in this thesis aim to consolidate further bridges between human and non-human data, by testing important hypotheses that arise from contemporary neurobiological models of cholinergic function. More specifically, the experiments test the effects of inducing a hypercholinergic state in humans with the cholinesterase inhibitor physostigmine, in both healthy subjects and Alzheimer's disease. Several of the questions are addressed in more than one experiment so that the degree to which findings can be replicated is also assessed. These questions are:

1. How does the hypercholinergic state influence both sensory-driven (bottom-up) and attention –driven (top-down) modulation of visual cortices? (Experiment 1, 3, 4)
2. Does the hypercholinergic state increase or decrease attention-driven selectivity of sensory cortices e.g. as observed in retinotopic visual cortex by spatial cueing? (Experiments 1, 3, 4)
3. Does the hypercholinergic state influence neural reponses to emotional stimuli? (Experiment 1)
4. Are there differences in how the hypercholinergic state modulates spatial attention and spatial working memory? (Experiment 3)
5. Is repetition suppression - one mechanism by which priming may occur - increased or decreased by a hypercholinergic state? (Experiment 2)

6. Do effects of a hypercholinergic state on sensory activations have knock-on effects on subsequent recognition memory? (Experiment 5)
7. Do effects of a hypercholinergic state on activations related to sensory, attentional and memory function differ between healthy elderly subjects and Alzheimer's disease? (Experiments 4, 5)

Prior to describing the specific experiments, and their results, a general account of the methodology of pharmacological-functional MRI, with specific reference to using the cholinesterase inhibitor physostigmine, is described.

4. Methods

Introduction

Functional magnetic resonance imaging (fMRI) is the methodology used in this thesis. In brief, fMRI enables measurement of a surrogate marker of population neural activity in conscious humans, whose only imposition for subjects is the need to lie horizontally with the head kept immobile. The method rests upon the biophysical principle that there is a relatively consistent relationship between brain neural activity and a secondary haemodynamic response. The spatiotemporal resolution of the technique is therefore related to the anatomical and dynamic properties of the brain's microvasculature.

Given a relatively tight spatial coupling between neural activity and arteriolar dilatation, the spatial resolution of fMRI can be in the order of millimeters, or ~10^5 neurons. This is superior to any other contemporary human neurophysiological technique with only the exception of clinically-justified deep-brain recordings. The temporal resolution of fMRI, paralleling dynamic properties of microvascular patency and blood redistribution, is in the order of seconds. Compared to techniques such as electroencephalography (EEG) or magnetoencephalography (MEG), whose temporal resolution is ~1 – 20 milliseconds, this is poor, yet by modeling the hamodynamic response function it is still possible to resolve activity between different behavioral trials (so called, event-related fMRI). It is important that we appreciate the temporal range of the measuring technique employed given what was discussed in the Background: namely, that cholinergic modulation occurs at different time-scales, with both tonic, or session-related, effects, and phasic, or trial-related, influences (Sarter et al, 2009).

Unlike potentiometric or ammeteric measurements of neural electrical activity, the physical processes proposed to underlie fMRI measurements are far from straightforward. An account of what is actually measured with fMRI starts with the magnetic properties of atomic nuclei; relates this to interactions with local electron and applied magnetic fields; which is fortuitously relevant to different chemical states of haemoglobin, which itself is found to be an accurate reflection of regional neural activity. Yet, whilst only achieveing an indirect measure, combined fMRI-electophysiological recordings demonstrate that the fMRI signal is in general tightly coupled to neural activity (Logothetis et al, 2001; although see Ekstrom, 2010, for counterexamples). Moreover, similarities of fMRI results with those from more classical neurophysiological approaches, e.g. spatiotopic mapping of sensory cortices (Tootell et al, 1995; Rees et al, 2000), engender confidence that the technique is a valid and reliable measure of neural activity. The development of increasingly sophisticated methods and statistical analyses enables fMRI to dissect cognitive factors of interest (Friston et al, 1998), as well as to model inter-regional brain activity (Friston et al, 1997; Stephan et al, 2008).

However, an inherent weakness of fMRI lies precisely in the long-windedness of its multiple biophysical assumptions. In particular, if variations occur in the core relationships between neural activity, haemodynamic parameters, and induced MR signal, then it becomes impossible to discern 'interesting' effects on neural activation from 'uninteresting' differences in vascular phenomena. Such variations in neurovascular coupling have been shown to occur between individuals, or between anatomical regions within the same individual. Furthermore, of relevance for this thesis, aging, disease and

drugs may also influence blood flow response, and consequently on the size and distribution of fMRI signal, to a given change in neural activity.

Considerations of the biophysical principles underlying fMRI are essential therefore for increasingly-popular clinical and pharmacological fMRI-studies that ask the generic question: how does disease X, or drug Y, influence neural activity during task T, (or cognitive process P)? The next sections discuss the physics of fMRI; pre-processing analysis and statistical techniques; the relationship of the fMRI signal to hemodynamic and neural physiology; evidence for clinical and pharmacological variation of the critical neurovascular relationship, and, finally, ways in which these problems can be addressed.

Functional Magnetic Resonance Imaging (fMRI)

MRI – Physical Principles

The principle of MRI, including functional MRI, rests upon two fundamental physical properties: 1) a magnetic dipole inherent in all *nucleus*-located protons, from which the MRI signal is derived, and 2) magnetism of *electron* clouds that varies between biological molecules and tissue types, and which crucially can modify the strength of the MRI signal itself (either through a direct magnetic interaction, or indirectly by causing differing amounts of molecular motion). Both electromagnetic properties are subject to a fundamental quantum physical law, the constancy of which all examples of MR signalling are predicated upon: namely, that subatomic particles can exist in one of two states, and a fixed amount of energy is required to be absorbed, or released, moving from

a low to high, or high to low, energy-state, respectively. The two states are referred to as '*spins'* because it is usually electromagnetic radiation, in the form of photons travelling at a specific *frequency*, that constitute the means by which energy is absorbed or released.

Most protons in everyday matter are paired off into complementary high and low energy states, or up and down-pointing magnetic dipole moments, that exert no net external force and so are effectively 'silent'. However, certain biological substances possess protons and/or electrons that are unpaired, and it is here that subatomic electromagnetic processes can be 'tapped' and applied to useful biological measurement. The commonest occurrences of naturally-occurring unpaired protons are hydrogen nuclei, (i.e. single protons) found in water and organic compounds e.g. fat, protein. By being unpaired, their magnetic dipole moment is susceptible to an externally applied magnetic field, and transitions in their energy states can be measured by an electromagnetic receiver coil.

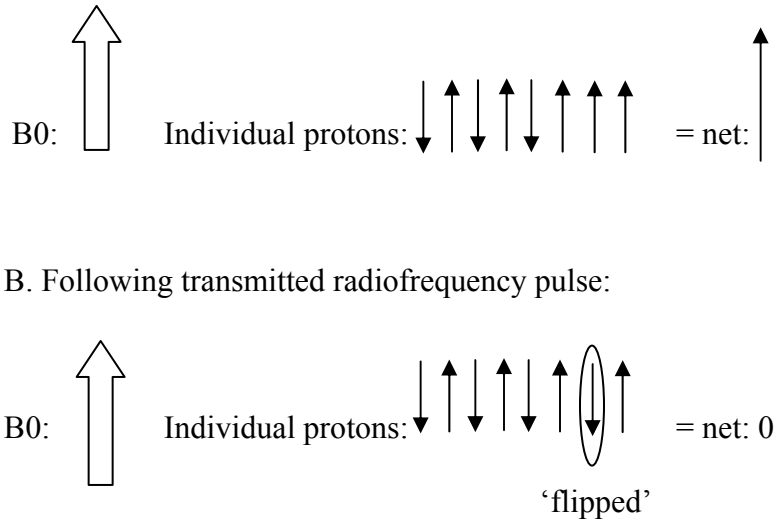
As mentioned, the energy state, or spins, of protons can be flipped from low to high, or vice versa, by the exchange of electromagnetic radiation. The amount of input or output energy associated with such flipping is directly proportional to the frequency of the applied, or received, radiation, as asserted by Planck's law:

$$\text{Energy} = h * \text{frequency} \qquad (h = 6.62 * 10^{-34} \text{ Joules / sec})$$

In MRI, a baseline energy state can be achieved through application of an external magnetic field B0 that is applied along the longitudinal body axis (i.e rostral – caudally).

In this state, all proton-related magnetic dipole moments will align themselves either in the same direction (low-energy states), or in the opposite direction (high-energy states), to the applied field. While approximately similar numbers of protons align themselves into one of these two states, approximately one excess proton in a million will align itself in the same direction as B0, meaning that the net magnetic dipole moment over the entire body is also in the direction of B0. This configuration of protons is considered to be a net low energy state for the system (i.e. of body within magnetic field). With the protons still aligned in B0, one can transmit an electromagnetic pulse into the body that injects sufficient energy to flip half of those one in a million excess protons into the high energy state, so that there is no net longitudinal magnetism.

Figure 4.1: Effects of RF pulse on proton alignment in a magnetic field



The amount of energy required to flip the extra protons so as to achieve a net zero magnetic dipole moment is determined by the strength of B0:

$$\text{Energy} = 2 * \text{magnetic dipole moment} * B0$$

Relating this to Planck's law above:

$$\text{Energy} = 2 * \text{magnetic dipole moment} * B0 = h * \text{frequency}, \quad \text{or :}$$

$$\text{Frequency} = \frac{2 * \text{magnetic dipole moment}}{h} * B0 \qquad \text{(Larmor Equation)}$$

The expression $\frac{2 * \text{magnetic dipole moment}}{h}$ is known as the gyromagnetic ratio, γ.

It is constant for any nucleus type, e.g. for hydrogen, $\gamma = 42.6$ MHz/T. So in a 1.5 Tesla MRI scanner, the frequency required to flip protons is given by:

$$\text{Frequency} = \gamma * B0 = 42.6 * 1.5 = 64 \text{ MHz}.$$

MRI - Signal

So far we have discussed: 1) how the protons of hydrogen nuclei within the body can be aligned by an external magnetic field B0, and 2) how aligned protons can be flipped by a specific radiofrequency so that the net magnetic dipole moment shifts from a longitudinal vector (in the direction of B0) to zero. There are two further electromagnetic properties of protons that can take us from these facts to understanding how a MR signal can be generated, and how such signals can discern different tissue types. These facts are:

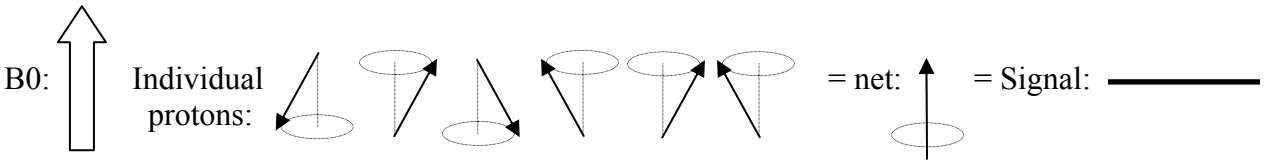
1) In addition to the **longitudinal component** of the magnetic dipole moment of protons, that aligns itself to B0, is constant, and can be theoretically measured along the z-axis (of a 3D-coordinate system), there is also a **transverse component**, which oscillates at the frequency governed by the Larmor equation, and can be measured along either the x or y-axis as a sinusoidal wave. The summed longitudinal and oscillating transverse vectors can be depicted as if the proton traced out the path of a spinning top, and this pattern of movement is referred as 'precession'.

The relevance of the transverse component is that it enables us to measure a MRI signal. Although, at baseline, there is an induced longitudinal net magnetic moment in the z-axis, this cannot be measured practically in the presence of the applied magnetic field B0 as the latter is several orders of magnitude stronger than that which is induced, and acts in the same (z-axis) direction. However, if the induced longitudinal magnetic field can be flipped into an orthogonal axis, this field is potentially measurable by a coil sensitive only to induced magnetic fields acting along the x- (or y-) axis. At baseline, in the longitudinally-applied external magnetic field B0, the transverse components of the magnetic dipole moments of separate protons are all in different phases, but on average cancel each other out, and so there is nothing measurable in the x- (or y-) axis. The transmission of a radiofrequency (RF) pulse enables us to measure something, providing it is at the Larmor frequency for protons given field strength B0, because it: i) flips the net longitudinal magnetic dipole moment into the transverse plane; and ii) synchronizes the transverse component of magnetic dipole moments across all individual protons. Note

that the rotating transverse components of magnetic dipole moments, when synchronized, can be measured along one axis of the x-y plane by a receiver coil as a sinusoidal waveform.

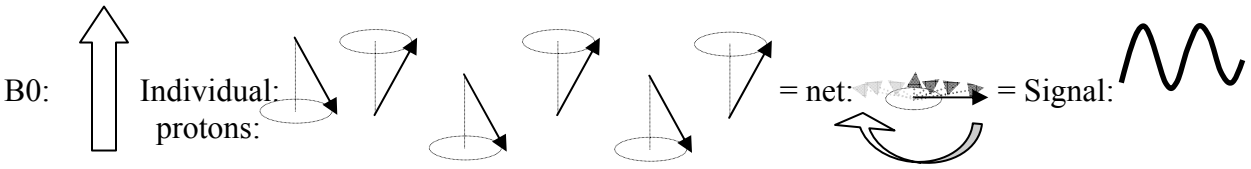
Figure 4.2: Transverse components of proton spins combine to generate signal

A. Baseline:



Note how transverse components of individual precessions cancel each other out, due to protons being in different phases, and so no net measurable MR signal.

B. Following transmitted 90° radiofrequency pulse:



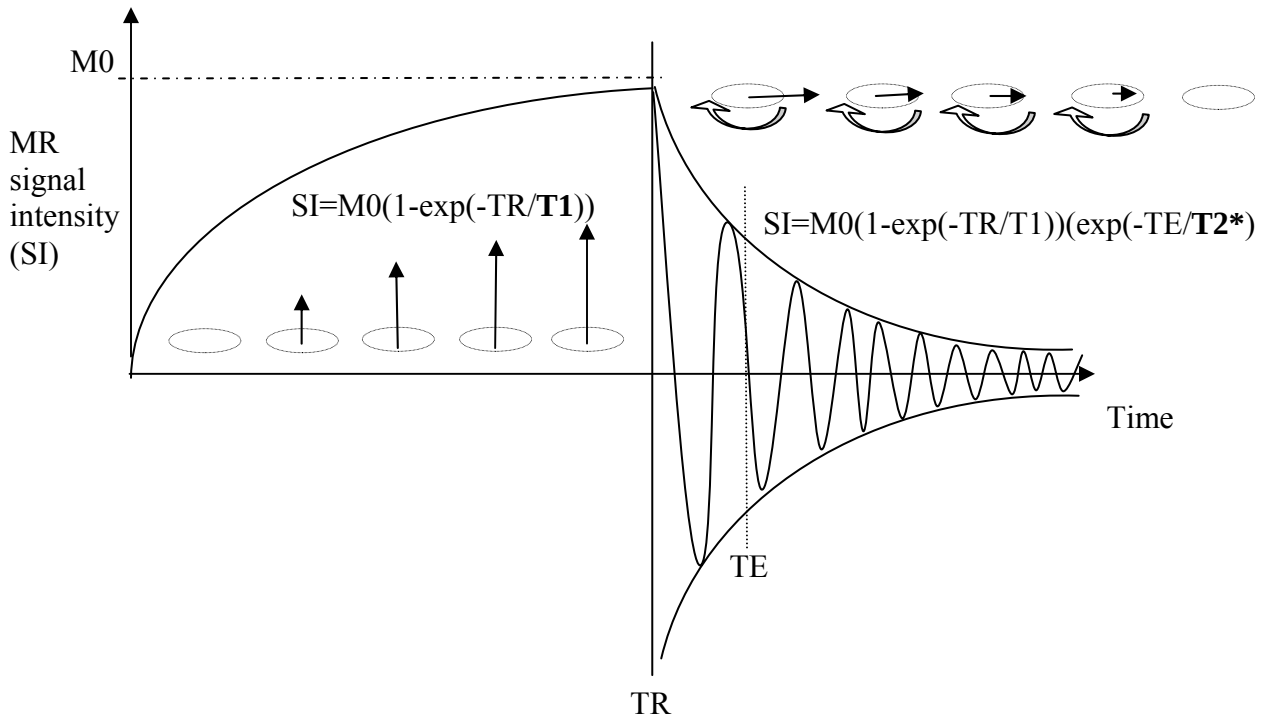
Note how transverse components of individual precessions are now in phase, thus generating net measurable sinusoidal MR signal. In fact, the size of the transverse vector just after the onset of the pulse is the same as that of the net longitudinal vector just before the pulse (thus the net magnetic dipole vector is 'flipped' 90°).

2) As discussed, an RF pulse causes both: i) conversion of the longitudinal magnetic dipole moment into a transverse component, and ii) synchronization of the transverse components of precessing protons. When the RF pulse stops, these two processes reverse, and, importantly, the rates at which each of them reverses, or their **relaxation rates**, depend upon tissue type. The rate at which net longitudinal magnetic component is

restored from zero occurs with an exponentially decreasing rate, described by the relaxation time constant T1. The rate at which net transverse magnetic component decreases, due to cumulative proton-proton dephasing, also occurs at an exponentially decreasing rate, albeit with a time constant T2* that is independent of T1.

Following a single 90° RF pulse, the longitudinal magnetic moment starts from zero and increases gradually to time TR. If then at time TR (repetition time), a second 90° RF pulse is transmitted, the intensity of the transverse vector at that time becomes the intensity of the longitudinal moment at time TR. Thus after two pulses, the net signal intensity received at time TE (time to echo) relates to both T1 and T2* time constants. Furthermore, while the received signal is a cosine wave (maximal at time=0 or TR), the intensity of this wave decreases according to an envelope that decreases exponentially.

Figure 4.3: Relaxation of protons following two 90° RF pulses separated by time TR



As a further refinement of relaxation rates, it should be noted that T2*-characterised relaxation – i.e. proton dephasing – can be divided into dephasing due to 1) *external* magnetic field inhomogeneities, including due to local changes in magnetic susceptibility within the surrounding tissues, and 2) *intrinsic* spin-spin lattice interactions that vary according to physical properties of the tissues itself (e.g. solid / liquid). The first kind of dephasing can be neutralized by following the initial 90° RF pulse with repeated 180° flips of the direction of net induced transverse magnetism (this combination of pulses is called a spin-echo sequence). The resultant relaxation rate, characterized by T2 time constant, is a purer quantification of tissue type being less subject to external magnetic inhomogeneities (i.e. noise) and so is commonly used for structural MRI scans. However, in functional MRI, it is precisely these external magnetic inhomogeneities, acting over a microscopic range to modify local proton T2* values, that need to be measured, reflecting as they do different tissue metabolic states (see below). Thus, for functional MRI, the pulse sequence and measurement times are designed specifically to be sensitive to differences in T2* relaxation rate.

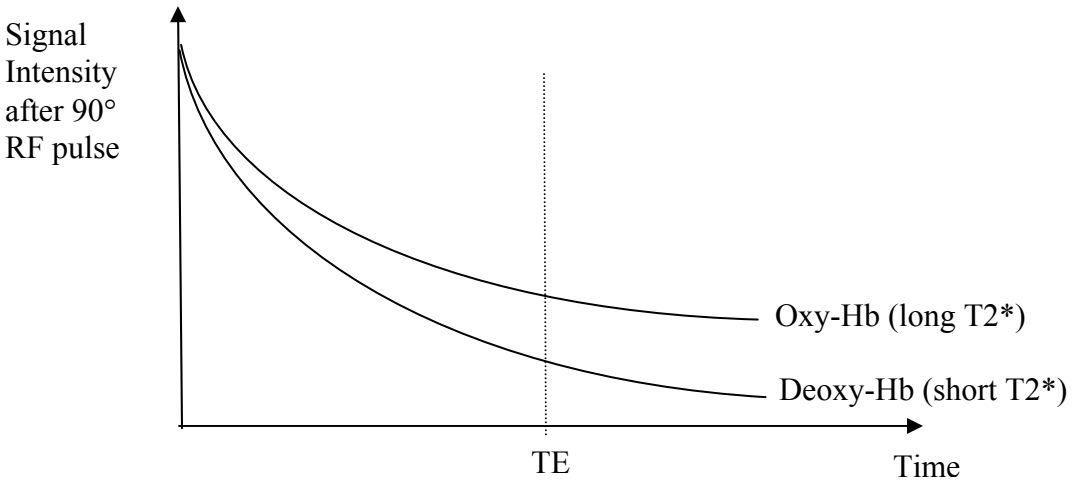
Functional MRI – Signal Contrast

The key objective with MRI is the ability to discriminate different tissue types, or different physicochemical states of a single tissue type. This is achievable because the relaxation time constants of protons (within hydrogen nuclei) differ depending upon the physical properties of the tissue-type they form part of, or because of electromagnetic influences of nearby chemicals. For example, tissue in which protons are packed close

together e.g. fat-containing white matter, show rapid T2 relaxation because the protons easily interfere with one another and so dephase readily. By contrast, in water-based cerebrospinal fluid, protons are far apart, and so dephasing is slow, and T2 is long.

With functional MRI, the T2* contrast of interest reflects interesting differences in physiological activity. Specifically, a natural MR contrast is found between two metabolic states of haemoglobin – oxyHb and deoxyHb - whose local relative concentrations are a surrogate marker of regional neural activity (see page 166). The chemical basis for this is that the iron atom within haemoglobin possesses an unpaired electron when not bound to oxygen (i.e. Fe^{2+}-deoxyHb), which becomes paired off with the binding of oxygen (i.e. Fe^{3+}- oxyHb). The presence of an unpaired electron in Fe^{2+}-deoxyHb enables it to become weakly magnetic in the presence of an external magnetic field, itself causing local magnetic-field inhomogeneities, which facilitates dephasing of adjacent protons. This selective property of deoxy-Hb is referred to as paramagnetism, and is distinct from the magnetically-neutral properties of oxy-Hb that is referred to as diamagnetism. Since deoxyHb dephases more quickly than oxyHb, the T2* time constant is shorter for deoxyHb than for oxyHb. Thus when we measure the signal at time TE after a 90° RF pulse, the intensity of the received signal will be greater from tissue with high, relative to low, oxyHb:deoxyHb ratios. This explains why the signal is also referred to as being blood oxygen level dependent (BOLD) (Ogawa & Tso-Ming, 1990).

Figure 4.4: Signal contrast employed in fMRI



It can be seen from the figure that the difference in signal intensity between oxy-Hb and deoxy-Hb will be increasingly distinguishable as the time at which the signal is measured (TE) is increased. Furthermore, in order to eliminate differences in T1 constants between tissues, it can be seen from Figure 4.3 that a long TR is required to enable all tissues to return to magnetic equilibrium (maximal longitudinal magnetic dipole moment). Combining these observations, we note that in order to weight tissues selectively by differences in their T2* constants, we should select a long TR and long TE.

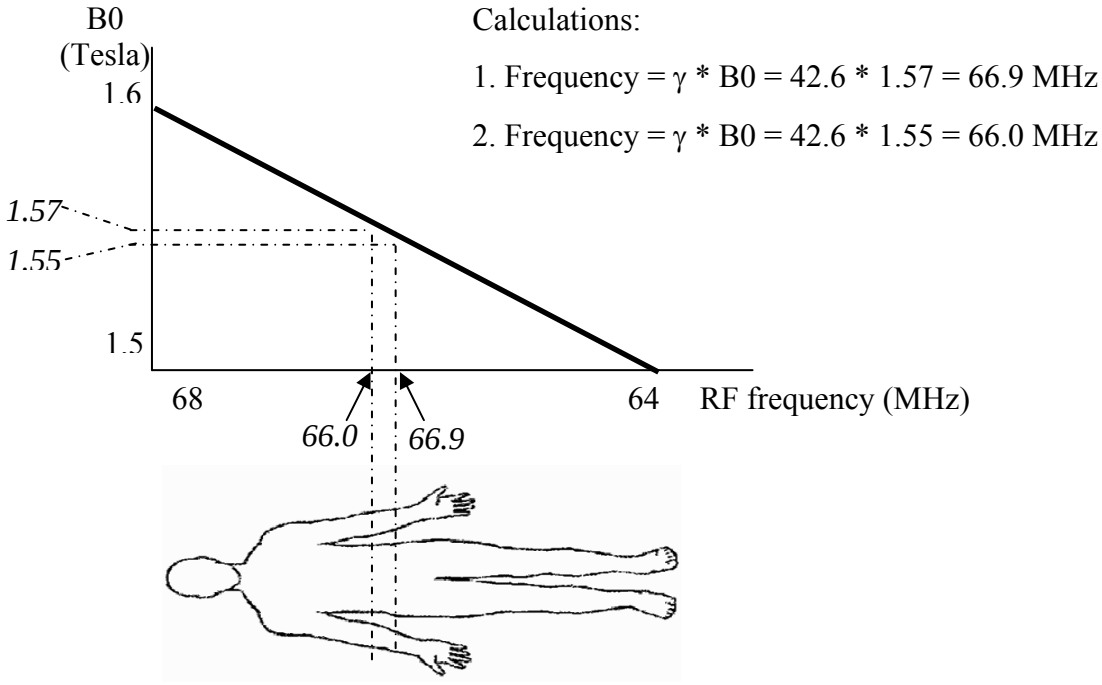
MRI – Localization

The preceding account of MR signal generation and measurement is simplistic because it does not take into account two further MRI parameters – one to do with spatial localization, and the other to do with timing. Both are highly relevant as they determine the technique's spatiotemporal resolution, and thus how accurate fMRI is at measuring neurophysiological activity.

The ability to localize signal within MRI is achieved through application of the Larmor equation, which as discussed earlier, is a derivation of Planck's law. To recap, a precise amount of energy is required to 'flip' protons from a low-to-high energy state, and this amount, expressed as an RF pulse frequency, depends upon the strength of the externally-applied magnetic field, B0: Frequency = γ * B0, where γ is a constant for hydrogen.

1) **Slice-Select Gradient** (z-axis). The baseline magnetic field, B0, is varied linearly along the z-axis (i.e. longitudinally), so that a specific-frequency RF pulse only flips protons in that slice where there is a specific magnetic field strength as defined by the Larmor relationship. In order to sample a volume of protons this slice cannot be infinitely thin, and so the transmitted RF pulse is actually a range of frequencies within a narrow bandwidth, the width of which determines the slice thickness.

Figure 4.5. Slice-Select Gradient

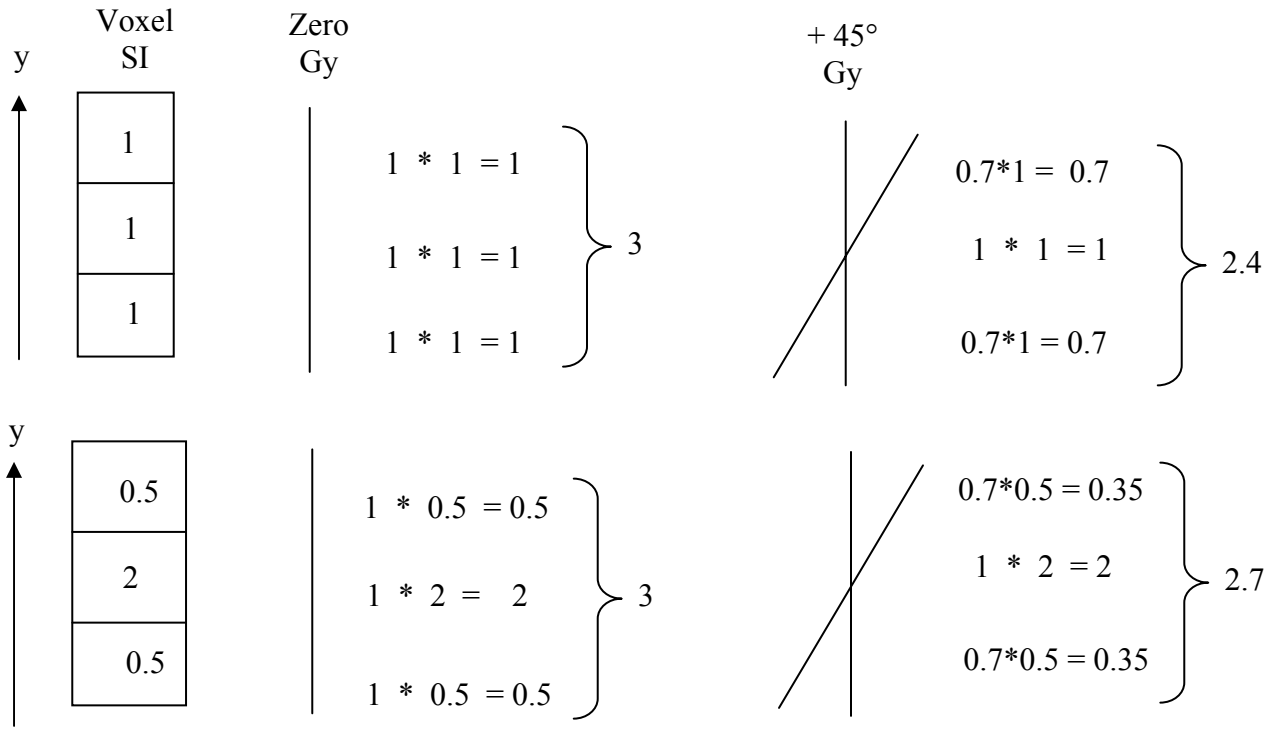


2) **Phase-Encoding Gradient** (y-axis). Immediately after an RF pulse has been transmitted, protons within a slice are 'flipped' and their transverse magnetic dipole moments in that instant are synchronised, i.e. in phase. These protons subsequently dephase due to T2* time constants of the various tissues within this slice. However, dephasing can also be induced by subjecting the slice to an additional magnetic gradient, this time within the slice plane. This gradient is therefore switched on just after the RF pulse and is conventionally applied along the y-axis (antero-posterior direction).

The way in which the Gy gradient is able to dephase protons is again related to the Larmor equation. This time though the applied magnetic field modifies the frequency of the *received* signal, rather than determining what frequency we set the *transmitted* signal at. Protons in those rows of the slice in which the local field strength is relatively high spin faster, whereas those protons in low-field strength rows spin slower. The net effect is that the stronger the Gy gradient, the more dephasing, and the lower is the overall received signal (which is the sum of signal from all rows).

The extent to which the Gy gradient dephases protons will depend upon the degree of spatial variation in T2* relaxation times along the y-axis *before* the Gy gradient is turned on. For example, by using a 45º Gy the intensity of the signal from peripheral, relative to central, protons is cosine(45º) = 0.7, enabling differential weighting of signals by location.

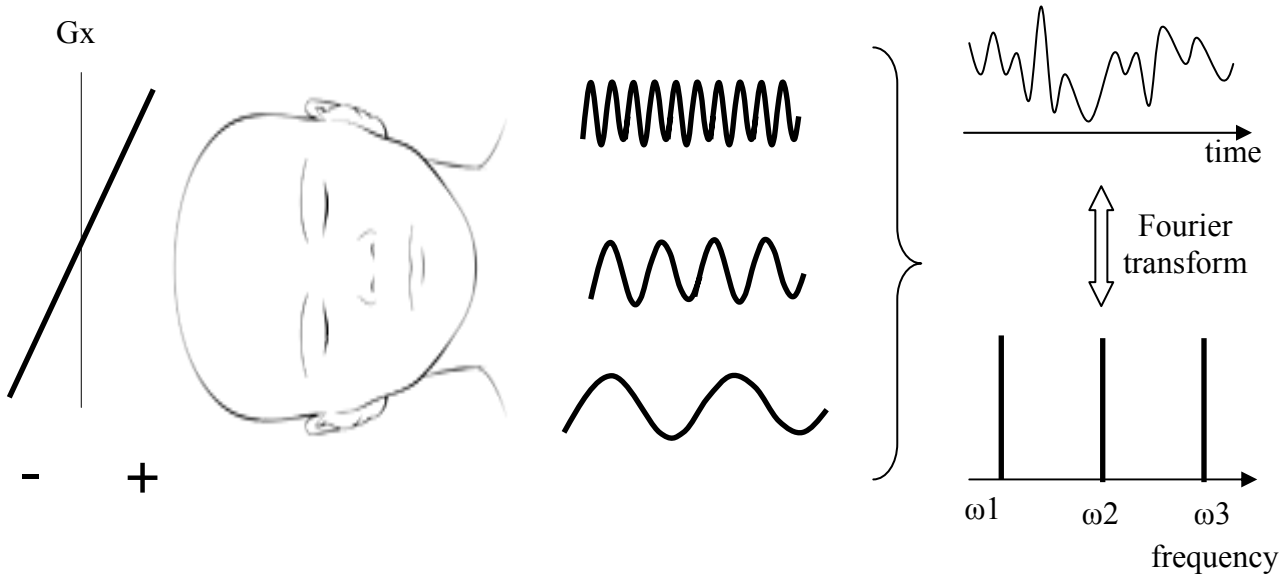
Figure 4.6. Phase-Encoding Gradient: two Gy gradients can distinguish two possible spatial conformations along the the y-axis from differences in the net signal intensity.



In fact, it can be shown that the number of different Gy gradients employed determines the spatial resolution in the Gy direction. In standard MRI sequences each Gy gradient has to be applied to a separate RF pulse, meaning that if a standard spatial resolution of 256 is required, and a typical TR is 1 second, then the entire sequence for just a single slice would be 256 seconds, or about 4 minutes. Thus the biggest time constraint for MRI data collection derives from phase-encoding. Different strategies exist to reduce the overall acquisition time required, and these are of particular importance for fMRI where as narrow a sampling time as possible is required.

3) **Frequency-Encoding Gradient** (x-axis). The phase–encoding gradient, Gy, is turned off some time *before* the signal is measured, meaning that while different protons are in different *phases* as a result of Gy, their *frequency* is constant (and equal to the original transmitted RF pulse frequency). This allows for a further spatial manipulation during the measurement phase itself. This is performed by applying a magnetic gradient in the x-axis (right-left direction) during read-out, which similar to the effect of Gy earlier on, modifies spin frequency according to the Larmor equation. Consequently, the final signal is a mixture of frequencies, and decomposing it into its component frequencies enables spatial decoding in the x-axis direction.

Figure 4.7. Frequency-Encoding gradient



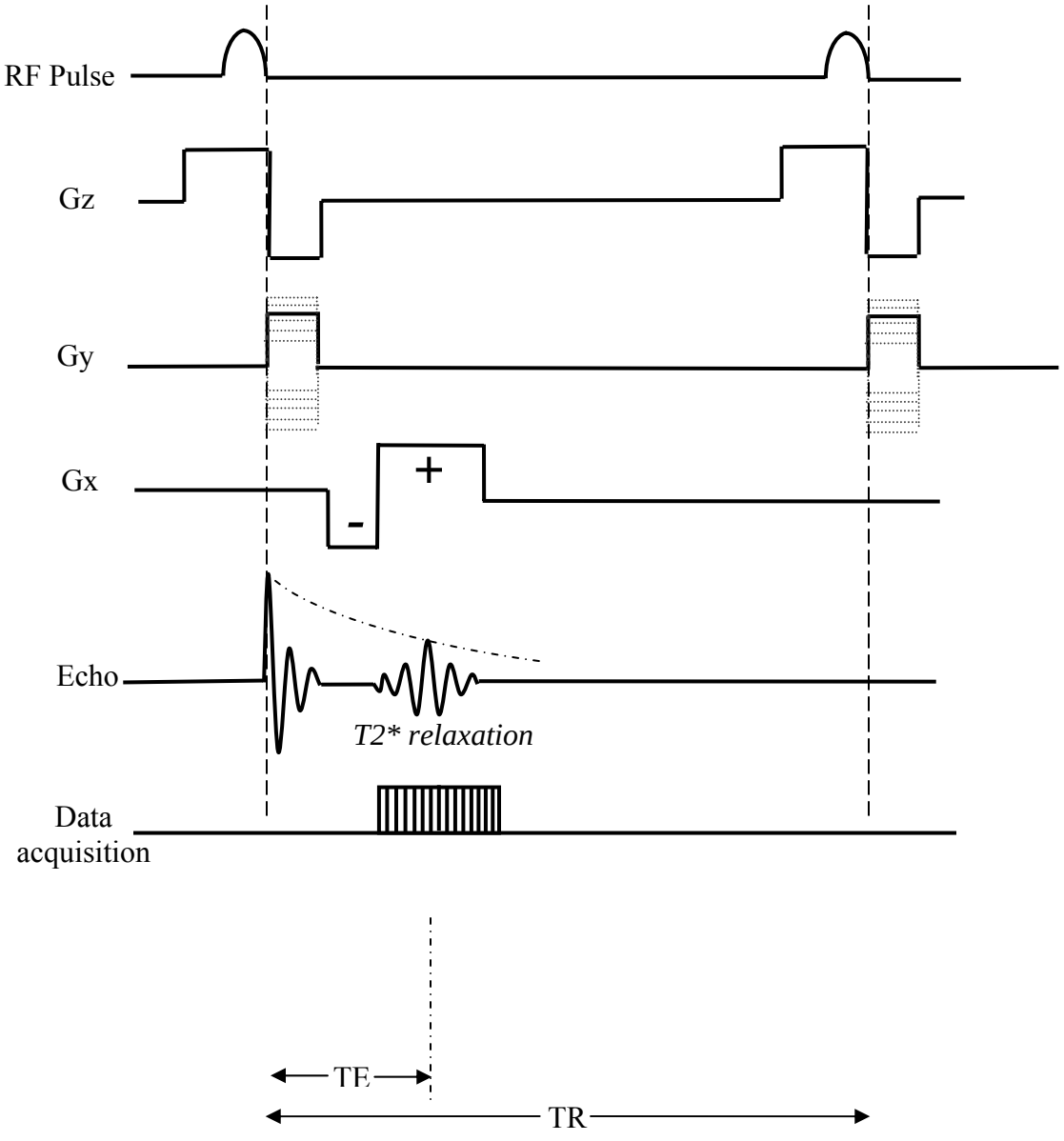
Functional MRI – Pulse Sequence

In functional MRI we wish to create a sequence that is both sensitive to T2* effects (since this discriminates deoxyHb from oxyHb), and enables rapid data acquisition (to gain a temporal resolution similar to that of the time-scale in which neural-driven vascular changes occur). The theoretically most simple, and quickest, way of T2* weighting is to sample the signal emitted soon after a 90° RF pulse – the so-called 'free induction decay' (Figure 3). However, this is impractical as it occurs too soon (< 10ms) after the RF pulse, and allows insufficient time to prepare the phase-encoding and frequency-encoding gradients by which the signal can be localised. One way of inserting a time delay, as described earlier, is to use a spin echo sequence (90° RF followed by one or more subsequent 180° RF pulses) that essentially regenerates the original signal after ~40ms. However, in using 180° refocusing pulses, spin-echo selectively neutralizes dephasing due to external inhomogeneities and so is insensitive to T2* effects.

An alternative method of inserting adequate time delay between the RF pulse and signal measurement is to use a gradient-recalled echo (GRE) sequence. In this technique, the frequency-encoding gradient Gx is applied *just before* and *during* readout, albeit *in opposite directions.* The effect of -Gx just before readout is to induce systematic dephasing in the x-axis direction (just as Gy, applied before -Gx, causes systematic dephasing along the y-axis). However, by then applying +Gx in the reverse direction, this rephases the spins along the x-axis, so that after an amount of time equal to the initial dephasing gradient -Gx, there is a maximal echo.

As well as spacing apart the echo from the RF pulse, the initial negative-phase serves to ensure that protons are maximally refocused half-way through the readout period, which is set as the duration of the positive-phase.

Figure 4.8. Gradient-Recalled Echo (GRE) Pulse-Sequence Diagram



The pulse sequence diagram depicts the order in which the various linear magnetic gradients are activated in relation to the RF pulses and data acquisition. The slice-select gradient Gz is turned on immediately before and during the RF pulse so as to excite only a slice-worth of protons. Within this slice, in thie z direction, there will be dephasing which is not measured, and which acts to reduce the overall signal intensity. To minimize this, a refocusing –Gz gradient is activated immediately after +Gz is turned off.

After the RF pulse is applied, a phase-encoding gradient Gy is applied. The strength of Gy varies with every repetition of the RF pulse (as indicated by the dotted boxcars). Then finally the bi-lobed Gx gradient is activated, with data acquisition occurring during the second, positive-phase.

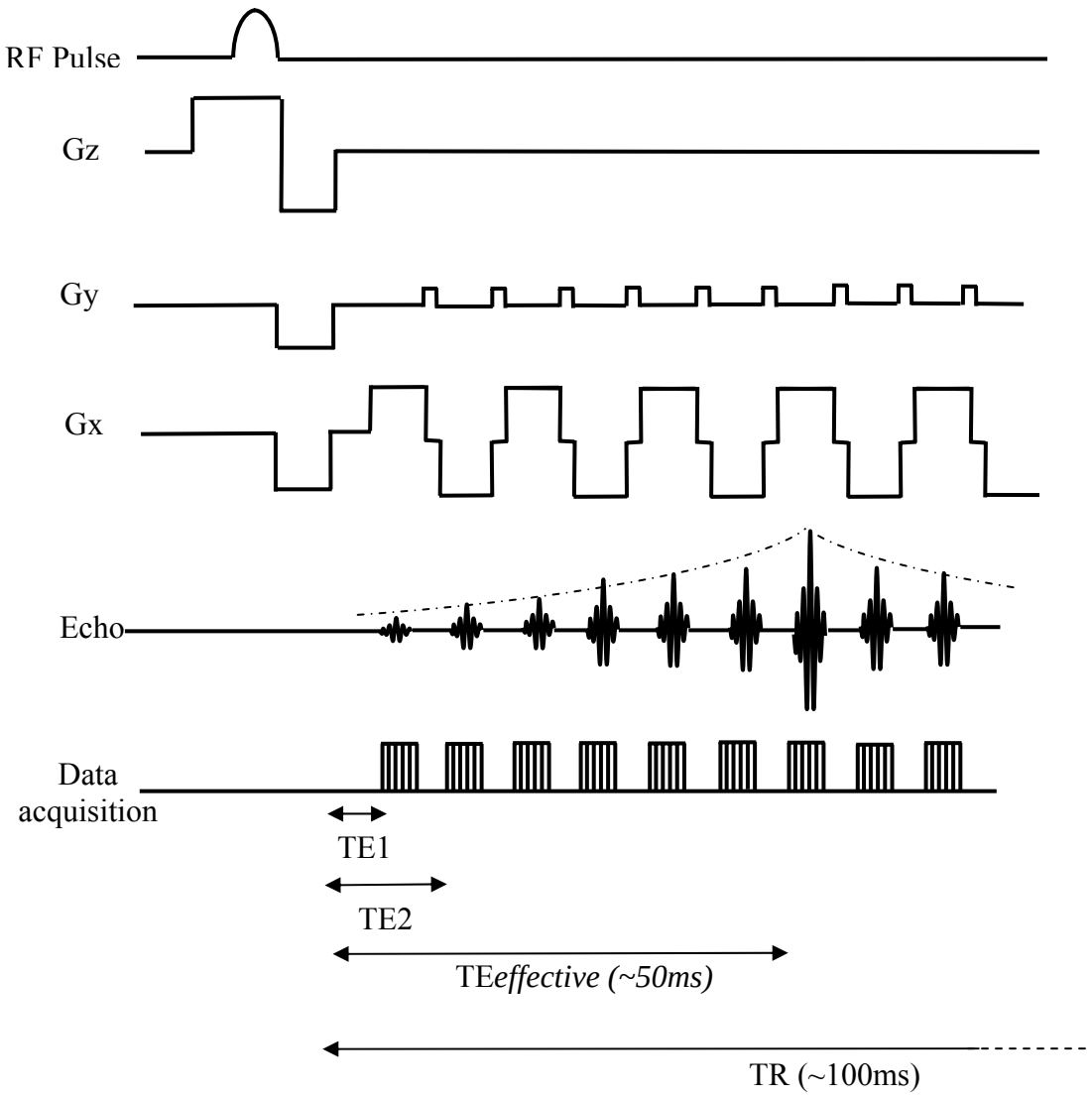
At the time of signal measurement, that dephasing which was induced by -Gx has been neutralized by the new gradient +Gx. But there still has also been dephasing because of both spin-spin interactions and external inhomogeneities. Thus the intensity of the signal at TE in gradient-recalled echo is a reflection of T2*, rather than T2 as it is with spin echo.

A major drawback of the gradient-recall echo sequence, as shown, is that a new RF pulse is required for every phase-encoding step. So for each slice, we need to wait the length of TR, and then repeat this however many times we wish to resolve spatially in the y-direction. A way round this is to perform *multiple phase-encoding and frequency-encoding steps following each RF pulse* so that spatial information from the entire slice is

obtained with a single-shot. This sequence is termed echo-planar imaging (EPI), and by being the fastest means of T2* data acquisition, is the standard fMRI sequence.

There are two further time-saving steps often employed in EPI that enable shorter repetition times. Firstly, instead of performing a separate bi-lobed Gx gradient for each Gy step, the Gx gradient is simply reversed between each Gy step. This way, the readout Gx for one step acts as a primer for the next step. While the Gy gradient is turned momentarily on, the Gx gradient is turned off – this being referred to as 'blipped phase-encoding'. Secondly, the flip angle is reduced to < 90º, so that less time is required for protons to relax to their starting value of longitudinal magnetization, allowing for a shorter TR. By contrast, if a short TR is selected for a 90º pulse, then protons will have only regained a fraction of their starting longitudinal magnetization, such that the induced transverse magnetization following subsequent pulses will be small, and signal-to-noise ratio low. Small flip angles also reduce T1 contrasts more than they do T2* contrasts, rendering it better suited for functional imaging.

Figure 4.9. Echo-Planar Imaging Pulse-Sequence Diagram



The repeated application of a constant, small-amplitude Gy blip serves to increment the net phase-encoding gradient successively with each echo, because unlike the case for Gx, there are no refocusing pulses. In order to weight the image optimally for T2* effects, the baseline and subsequent Gy blips are set so that the smallest net dephasing – i.e. strongest signal - is selected at the time, TE*effective* when the greatest T2* contrast occurs (~30-60ms).

Variations of EPI sequences exist, for example sinusoidally-varying Gx and Gy gradients, but the principle of obtaining all phase-encoding (y-axis) and frequency-encoding (Gz) information for each slice following a single RF pulse is the same for these.

Echo-Planar Imaging – Advantages and Disadvantages

The EPI sequence enables both rapid data acquisition (typically, 100ms per slice) and T2* sensitivity. The first of these benefits also means that movement-related error is less than standard MR sequences.

However, EPI has several shortcomings. Firstly, by limiting the number of echoes to a single-shot, the number of phase-encoding steps, and thus spatial resolution in the y-axis direction, is limited. Typically, the field of view obtained is 64x64 or 128x128, compared to 256x256 for routine MRI. The limitation on this parameter is the speed with which magnetic gradients can be reversed (characterised by the slew-rate = gradient / rise time, where gradient ~ 20mT/m, and rise time ~ 300μs).

A second problem relates to the incremental way in which phase-encoding information is acquired (see above). If an error occurs in one of the early phase-encoding steps, then every subsequent measurement carries forward that error – i.e. artifact propagation.

Thirdly, artifacts are more likely to occur in the first place with EPI, because of i) the use of rapidly alternating magnetic fields, and ii) its high sensitivity to magnetic susceptibility (T2*) effects. The use of a high slew rate makes artifacts of the main image more likely to appear along the phase axis, during either the odd or even echoes (hence called "N/2 ghosts"). They occur because of eddy currents, imperfect gradients or field non-uniformities, and mismatch between the timing of odd and even echoes. Artifacts also occur because of a difference in proton precessional frequency between water and fat. This can be avoided by using a preceding 180° RF pulse, and timing TE so that the signal from fat is approximately zero (i.e. suppressed) at the time of the 90° RF pulse.

fMRI Image Processing

Introduction

In a typical fMRI experiment, a time-series of EPI volumes is acquired and related in a time-corresponding fashion to recorded behavioral events. So far we have noted that EPI images are sensitive to T2* relaxation effects, of which changes in the local ratio of oxyHb:deoxyHb, or blood oxygen level dependent (BOLD) signal - provide one contribution. For functional imaging, we are not interested in anatomical variations in T2*, and aim only to observe dynamic T2* variation that correlates with behavioral (or cognitive) variation. Thus a critical part of fMRI data analysis is *comparison* of images between selected time points, or *covariation* of images with a behavioral measure of interest.

As shall be discussed, the comparison of image signal intensities, across many spatial locations (or 'voxels'), involves the same statistical methods, such as t-tests and correlations, as are employed in any other scientific setting. However, before such comparisons can be made, a number of pre-processing steps need to be undertaken so as to make the assumptions of subsequent statistical tests valid.

One of the biggest issues that needs to be addressed is that of signal specification. The data set we collect is a four-dimensional construct – i.e. the brain volume replicated approximately several 100 times along a continuous time-line – which itself is often repeated in different sessions or subjects. Thus for each data-point it is essential that we are confident of its spatial and temporal 'address', so that grouping or comparison with equivalent data-points can be performed reliably. To achieve this, we perform several steps that regiment the data into a common spatial-temporal framework that allows, not only data pooling and constrasting within the one study, but also permits cross-comparison with other studies employing similar methods (including those using different imaging modalities).

The pre-processing steps performed are:-

1. **Spatial registration and realignment:** this adjusts the 3D coordinates of each EPI volume so that the brain is spatially aligned between images.
2. **Slice-timing correction:** this adjusts for timing of each scan to account for the different times at which each slice (along the Gz axis) is acquired in a single volume.

3. **Spatial normalization:** this adjusts the 3D coordinates of each EPI volume so that the brain is 'fitted' into a standard brain template that permits between-subject or between-study comparison.
4. **Spatial smoothing:** this step takes a Gaussian-shaped, weighted average of signal at each voxel, thereby reducing the relative influence of outlying values, and so increases signal-to-noise. It is also a requirement for subsequent statistical comparisons in 3D space as set by random field theory.
5. **Co-Registration:** this enables EPI data to be cross-compared with an alternative imaging modality e.g. to enable detailed anatomical localization by overlaying activation data on a structural scan.

The pre-processing and subsequent statistical analyses performed were all implemented within the computer package Statistical Parametric Mapping (versions 2 and 5 were used in this thesis; available from www.fil.ion.ucl.ac.uk/spm).

Spatial Registration and Realignment

In spite of securing subjects' heads within a transmitter / receiver head coil, small amounts of head movement inevitably occur during a scanning run that can take upto 30 minutes or so, This problem is often magnified in elderly subjects, especially with dementia. When we come to model the time-series of signal intensities, we need to be confident that changes in BOLD signal reflect experimental manipulation, rather than movement artifact. Movement correction is achieved by two steps.

Firstly, we assume that a rigid-body spatial transformation matrix exists that maps each brain volume in a time series onto the first such volume. This matrix is set to have six unknown 'affine' parameters that correspond to magnitudes of movement in six possible ways: x, y, and z translations, and rotations around each of these axes (or pitch, roll and yaw). An iterative procedure, such as Gauss-Newton optimization, estimates these six parameters by using the principle of least squared differences, applied over all voxels of two scans (Friston et al, 1995a). Once these parameters are estimated, the original volumes can be rewritten (realigned) using signal interpolation where necessary.

Secondly, even having aligned the scans, it is possible that the signal intensity was modified as a result of the movement itself. For example, had the head moved in the z-axis direction, a true activation could be missed by failing to be excited by the RF pulse. Moreover, movement during slice acquisition results in a phase shift that propagates to the remainder rows for that slice. Movement can also alter the timing relationship between the RF pulse and T1 relaxation which can have knock-on effects on the subsequent signal. Certain EPI-related artifacts such as N/2 ghosts do not behave as rigid bodies with head motion and may further confound the realignment stage. So as to reduce the variance of BOLD signal associated with movement, we save the movement parameters generated from the realignment step, and apply these as covariate 'factors of no interest' to our eventual multiregressive statistical model (see below).

Temporal Realignment

In experiments where TR (or strictly speaking, acquisition time) is set to longer than 2 seconds, the separation in time of data acquisition from different slices becomes methodologically significant. In other words a brain volume labeled as occurring at time t, in fact contains slices that were collected at t±1s (or greater). This will result in brain activity being imperfectly modeled with respect to behavioral events.

In order to correct for these timing errors, a sinc-interpolation procedure is conducted. In this step, the central slice of each brain volume is set as the reference time point, and the timing of other slices is calculated from knowledge of the sequence - usually interleaved, and timing, with which slices were acquired. Interpolation can then be performed between temporally-offset voxels and their equivalent voxels on the previous, or subsequent, scan.

Slice-timing correction is avoided where TRs are less than 2 seconds to avoid the introduction of interpolation and re-writing errors. Skipping this step also enables realignment parameters from the first spatial registration stage to be carried forwards to the normalization step.

Spatial Normalization

The principle of normalization is similar to that of spatial registration, only now we are interested in aligning equivalent voxels between, rather than within, subjects or sessions. The reference scan, rather than simply being the first scan of a session, is now based upon an internationally-recognised brain template into which the vast majority of studies now

render their images. This template is known by its origin - the Montreal Neurological Institute (MNI) – and was created by averaging the structural scans of 305 healthy volunteers. The spatial coordinates of the template approximate to those of Talairach space (Talairach & Tournoux, 1988). In SPM, the templates are necessarily MRI scans at different MR weightings (T1, T2, EPI, etc.). The SPM template used within this thesis is an averaged EPI from 13 different subjects, that itself has previously been normalized into MNI space.

In order to incorporate variations in brain size and shape between individuals, the transformation matrix has to be expanded from the six parameters we had used for rigid-body registration, to twelve parameters that now include 3 shear and 3 zoom components. The difference between source image (i.e. that which needs to be fitted) and template image is also approximated by the coefficients of a 3D low spatial frequency cosine basis set. In SPM, the usual approach is to select the mean spatially-realigned EPI as the *source* image, and an EPI template that is inbuilt within the software as the *template* image

Normalization proceeds as an iterative process in which a set of parameters and coefficients are estimated; then applied to the source image, and the mean squared difference between this and the template is calculated. This difference is referred to as the cost function, and the process proceeds until this is minimized. The process is also regularized by incorporating Bayesian model priors. The priors weight different possible transformations by the likelihood that each is found to occur during previous

transformations. This regularizes the procedure by preventing overfitting to local features.

In the experiments described in this thesis, the mean spatially-realigned EPI over all sessions was selected as the source image. Following normalization, the resultant parameter set was then applied to all the coregistered and realigned EPI images for that subject, including over sessions. Furthermore, the same parameter set was also applied to the T1 structural scan for each subject, having previously coregistered it (see below).

Spatial Smoothing

Smoothing entails averaging the intensity of each voxel with those of its neighbours, with greater weightings afforded to nearby than distant voxels. The spatial pattern of weightings assumes a Gaussian profile whose maximum (i.e. 1.0) is centered on the voxel to be smoothed (this then being replicated for all voxels using the original data only). The extent of smoothing can be varied, and this can be characterized by a 'full-width half maximum' (FWHM) kernel parameter that specifies, for 3D space, the width of a sphere of voxels, within which the weighting average for the centre voxel, is at least 0.5.

The main effect of smoothing is to 'iron out' small voxel-to-voxel variations, while enhancing effects that occur systematically over a large number of nearby voxels. Since physiological changes in BOLD occur over a spatial scale (~10mm) greater than that of a single voxel (3-5mm), we would like to take into account only those variations where such a change is observed in a spatial cluster of voxels at least as large as that expected

physiologically. In fact for optimal sensitivity, the matched filter theorem states that the data smoothing kernel should match the smoothing kernel of the thing that you ultimately wish to measure. A similar rationale explains neural processes (regularized by acetylcholine) that optimize signal-to-noise ratio within sensory networks. For fMRI, the FWHM smoothing kernel is usually set to 8 or 12mm given what is known about the spatial scale of haemodynamic responses to regional neural activity. By reducing the influences of non-physiological signal intensity variation, smoothing serves to decrease inter-subject variability.

Smoothing also increases the validity of subsequent statistical analyses. The central limit theorem implies that the distribution of errors from smoothed data will be more normal, which itself is an assumption of parametric statistical methods such as t-tests and regression. Furthermore, inferences based upon Gaussian random field theory (see below) are predicated on the assumption that the error terms conform approximately to a lattice approximation of an underlying smooth Gaussian field.

Co-Registration

Co-registration is used to fit two different types of scan into the same anatomical space. Similar to normalization, its ultimate aim is to estimate the 12-parameter affine transformation matrix required to fit one volume into another, except that these volumes are derived from different imaging modalities. For further optimization of this fitting, after co-registration, spatial normalization is usually performed on both types of co-registered scans. This can be done by applying the normalization parameters for one scan

type to the co-registered image of the second scan type. In this thesis, co-registration was performed so as to enable functional activations (derived from EPI scans) to be superimposed on detailed T1 structural scans for each subject. This enables more precise anatomical localization of group and individual functional effects.

The co-registration process is very different from simple spatial registration since a difference image cannot be used when different scan types are being compared. This is because there is no simple linear relationship between signal intensities of equivalent anatomical regions comparing imaging modalities (e.g. between T1 and T2-weighted scans). The comparison procedure is therefore based upon maximizing mutual information between the two images. This process involves plotting a 2D joint-histogram in which the intensity values for each image are plotted along each axis. Two images that are maximally aligned are defined by a joint histogram in which there is a precise one-to-one relationship – i.e. depicted as a sharp line on the 2D plane - between the intensity values of the two images. Images that are progressively less well aligned are characterized by joint histograms that are progressively more dispersed, or possess higher joint entropies, in their inter-intensities. Co-registration is an iterative process that estimates the 12 affine transformation parameters required to minimize joint-entropy.

Statistical Analysis of fMRI Time Series

Introduction

The standard functional imaging paradigm involves a comparison of neural activation patterns between two behavioral states A and B, say. This is repeated over multiple subjects enabling us to estimate an average difference in neural activation between A and B, as well as to test hypotheses, such as that the activation difference between A and B is greater than zero, or greater than the activation difference between states C and D, etc. In this abstract sense, the statistical analysis required by fMRI (and PET, MEG etc.) is no different from that in other scientific contexts with the usual assumptions required, e.g. for normal distribution of data where parametric tests are used.

However, statistical analyses of functional imaging data are complicated by two features inherent to their structure. Firstly, fMRI data that reflects behavioral state A, say, is actually a spatial array of many individual measurements – voxels (of which there are ~ 100,000 in a standard EPI volume) - each testing something meaningful in itself – namely, anatomical localization. The simplest way of comparing activation states therefore between A and B, say, while preserving anatomical information, is to take each voxel as an independent measure of states A and B, and to perform statistical tests within its own data series, and then to repeat this process for each voxel. However, this 'mass univariate approach' unavoidably presents the problem of multiple comparisons – i.e. that significant effects appear to occur by chance alone given enough things being measured (since significance *is* just the occurrence of things less commonly than five, or one, in a hundred). In order to correct for false-positives, whilst still enabling sensitivity to cognitive effects, is therefore one of the statistical adaptations that functional imaging methodology has had to develop.

The second structural feature of functional imaging data that statistical tests need to take account of is that it reflects a time series. This issue is more relevant in event-related fMRI where each scan is considered one of many separate time points, than in block-related fMRI or PET studies, in which only one data point is obtained per testing session, per voxel, per subject. Consequently, the various questions that naturally arise with time series analysis of any sort become applicable to fMRI. For example if each instance of behavioral state A is measured as 10 consecutive time points, how can we reasonably pool those data points to provide an accurate reflection of brain activity? Furthermore, given that time series are inherently autocorrelated, how should we adjust the degrees of freedom to allow for the fact that these 10 data points are not independent? These considerations are further motives for the particular machinery used to implement fMRI statistical analyses that shall be discussed.

Generalised Linear Model (GLM)

Many common statistical tests are examples of a basic principle: that a dependent variable is related to one or more independent variables according to a simple linear dependency. This can be stated more formally by the terms of a general linear model:

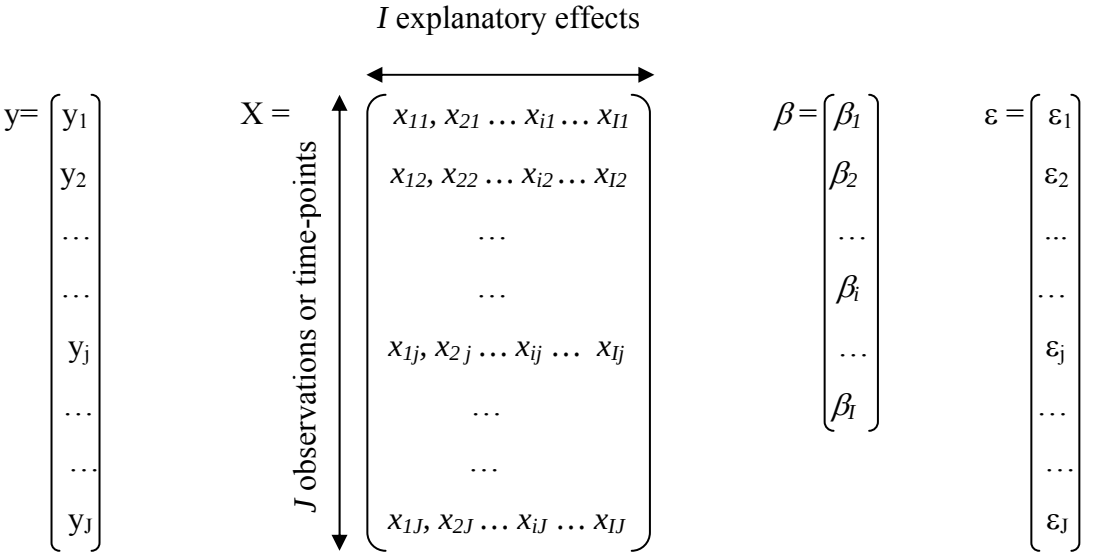
$$y_j = x_{1j}\,\beta_1 + x_{2j}\,\beta_2 + \ldots x_{ij}\,\beta_i \ldots x_{Ij}\beta_I + \varepsilon_j$$

where y_j is the jth observation, of a total of J observations, of the dependent variable y, that can be expressed in terms of I independent variables x_{1j}, x_{2j} … x_{Ij}, also measured at

the *j*th observation, by multiplication of each with a set parameter $\beta_1, \beta_2 \ldots \beta_I$, respectively, plus an error term for the *j*th observation. The system of *J* equations can be rewritten in matrix notation:

$$y = X\beta + \varepsilon$$

where:



X is also referred to as the design matrix specifying as it does a model of the entire data set by decomposing it in terms of explanatory variables. It should be noted that the GLM equation is equivalent to that used in multiple regression, in which the *x* terms represent various explanatory variables or regressors, the β terms the effect sizes, and the dependent variable *y* is a continuous parameter. Statistical tests such as t-tests or ANOVAs represent particular examples of this relationship. For example, a t-test reflects the special case when there is only one explanatory variable and for each case, this is either present or absent (i.e. x = 0 or 1).

Let us return again to one of the main purposes of functional imaging: typically, this can be phrased as a wish to determine whether 1) certain behavioral states, A or B say, are associated significantly with changes in brain activation, and 2) and to see how this relationship changes from region to region. Leaving aside the latter spatial issues for one moment, this purpose can be framed in terms of the GLM by substituting different behavioral states, A,B…, into different explanatory variables x_1, x_2 etc, and by substituting the level of BOLD intensity (for a particular spatial location or voxel) into the dependent variable y. As can now be seen, one of the advantages of the GLM is that multiple explanatory variables, related to discriminable components of the experiment or performance, can be modeled and estimated simultaneously. Furthermore, possible confounding factors, e.g. fatigue, reaction times, head movement, can also be measured and included as separate explanatory variables. This way changes in brain activity due to the behavioural / cognitive effects of interest can be determined, while partialling out possible confounds.

In order to determine the significance of each behavioral state (i.e. explanatory variables x_{1j}, x_{2j} … x_{Ij}) in terms of their contribution to variation in brain activity at each voxel (i.e. dependent variable y) we need to calculate the effect sizes ($\beta_1, \beta_2 \ldots \beta_I$) for each behavioural state, for each voxel. Thus we need to solve a system of simultaneous GLM equations for the unknown β parameters. In order to do this, we must first ensure that there are at least as many observations ($j_1, j_2, \ldots j_J$), as explanatory variables ($\beta_1, \beta_2 \ldots \beta_I$). Usually, in experiments, observations far outnumber explanatory variables. Secondly, the explanatory variables need to be made independent of one another so β that can be

uniquely estimated for each. In a complex design matrix this sometimes requires a mathematical adjustment in which the columns are orthogonalised with respect to one another. Finally, we wish to solve the set of equations in such a way that the error terms ($\varepsilon_1, \varepsilon_2, \ldots \varepsilon_J$) are minimised – this being the condition under which the estimated parameters provide the best prediction of the measured signal ($y_1, y_2, \ldots y_J$).

Solving a system of GLM-type linear equations, while minimizing their error terms, is achieved through the 'ordinary least squares method'. This method can be derived by rearranging the GLM equation so as to express the sum of squared errors $\Sigma\varepsilon^2$ (= E) in terms of β, and subsequently differentiating this with respect to each β. Knowing that the sum of squared errors term is minimal when $dE/d\beta = 0$, we can then directly derive a set of 'normal equations' that relates the estimates of β under this condition (now referred to as the least squares, or maximum likelihood, estimates $\hat{\beta}$) to y and X:

$$\hat{\beta} = (X^T X)^{-1} X^T y$$

Since $X^T X$ is only invertible if X is of full rank - which is rarely ever the case - in practice the pseudoinverse, or pinv, of $X^T X$ is used. Thus:

$\hat{\beta} = pinv(X^T X) X^T y,$ which resolves to:

$$\hat{\beta} = pinv(X) y$$

Having calculated the effect sizes for each effect of interest one can perform statistical tests on these parameters, e.g. comparing them to zero, or comparing between different effect types, or between different sessions. This step is described later on (*Contrasts*). It should also be noted that the *fitted response* is defined as $Y = X\beta$, and the *residual errors* are given by $\varepsilon = y - Y$.

So far, we have modeled the BOLD activity for just one spatial location, or voxel. Our second main aim is to show how this relationship varies between regions. This can be incorporated easily within the GLM equation by making Y a *J* x *N* matrix, where each column reflects the *J* data-points acquired from a separate voxel, for a total of *N* voxels. Correspondingly we need to make β a *I* x *N* matrix in which a different set of $\hat{\beta}$s are specified for each of *N* voxels, and ε a *J* x *N* matrix reflecting error terms for each data-point for each voxel. Note that the design matrix X is not affected by voxel number, since X reflects a constant model applied to every voxel. Also note that an inherent assumption of the GLM, as with all parametric statistical methods, is that all error terms are independent from one another and identically distributed in a normal fashion.

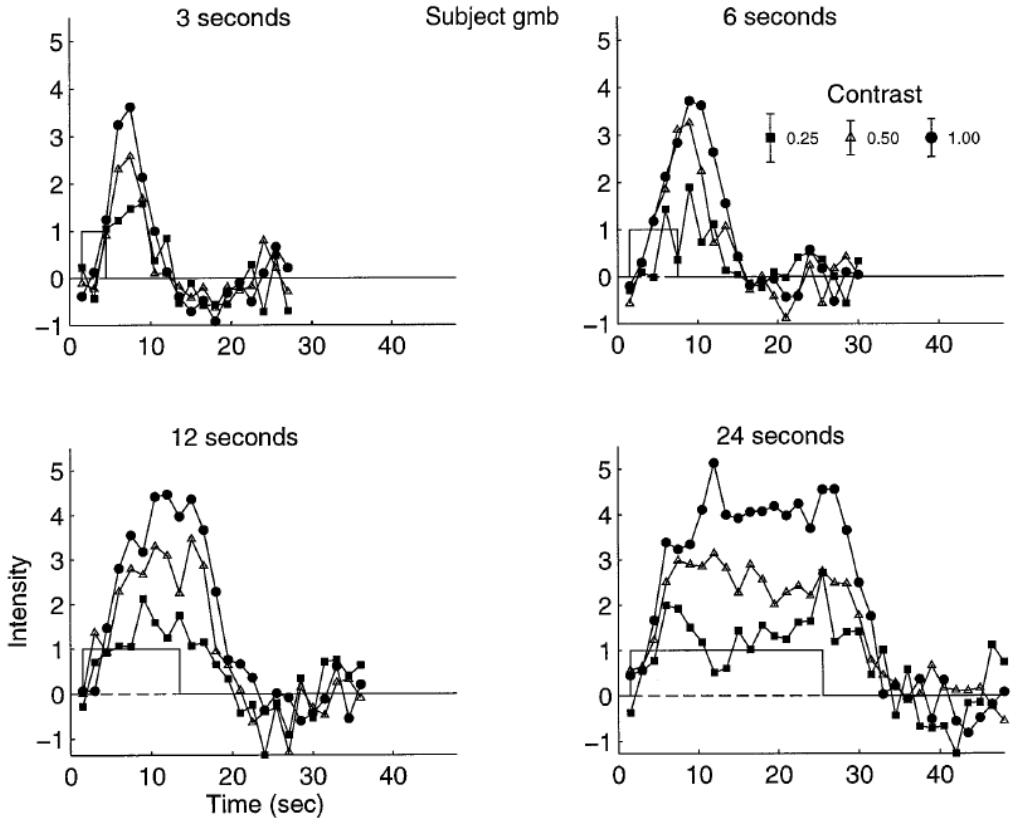
Haemodynamic Response Function and its Neural Equivalent

The design matrix approximates to a model of the underlying brain processes proposed to be activated during an experiment. However, since the data acquired with event-related fMRI is not a direct measure of neural activity we adjust the model to account for factors that intervene between hypothesized neural activity and actual measured signal intensity.

Specifically, each occurrence of a modeled 'neural event' (e.g. stimulus or movement onset) is modeled within the design matrix as a time-locked 'haemodynamic response function' (HRF) that reflects the recognized pattern of BOLD increase following a unit increment in regional neural activity.

The temporal profile of BOLD signal change following neural stimulation reflects its metabolic - vascular origins, with a relatively gradual onset; a peak several seconds later, and an even more gradual decay. The entire duration of the BOLD signal, even following the briefest of evoking stimuli (or movements) lasting tens of milliseconds, is in the order of 10 – 20 seconds. Each behavioral or experimental event that is considered momentary is therefore modeled as a stick (or delta) function convolved with a canonical HRF. As the duration of the modeled event increases, the resultant HRF is assumed to be a convolution of a suitably-lengthed boxcar with the canonical HRF. Similarly, if multiple brief events occur within the time of one canonical HRF (e.g.. if the stimulus-onset asynchrony, SOA, is < 20 s), then the modeled signal is a convolution of multiple stick functions with the HRF. In other words, it is assumed that HRFs can be linearly superposed and scaled, and that the convolution is linear time invariant. This assumption has been found to be approximately true for SOAs as low as 1 – 2 s (Boynton et al, 1996; Friston et al, 1998).

Figure 4.10. BOLD response (dots) to visual stimuli (boxcar) of differing duration and contrast in primary visual cortex (Boynton et al, 1996).



At short event durations the assumption of linearity breaks down, with stimuli of ~500ms resulting in HRFs upto 10x larger than that extrapolated from a linear projection of longer duration stimuli HRFs (Birn et al, 2001). Moreover, events spaces apart by short intertrial intervals (~5s) show ~20% reduction in HRF amplitude relative to those with greater spacing (~20s) (Miezin et al, 2000). A breakdown in linearity is also believed to occur with high stimulus intensities causing saturation (Howseman & Bowtell, 1999).

Differences in HRF profile exist between brain regions, with for example, the peak of activation in anterior prefrontal cortex occurring 4s after that in visual cortex (Schacter et

al, 1997). Whether such variability arises from hemodynamic rather than neural effects is unclear. Furthermore, the dependency between event duration, or neurophysiological intensity (e.g. visual contrast), and HRF also varies between brain regions (Birn et al, 2001). Variations in the HRF to a given level of neural activation are also likely to occur under any circumstance in which changes in vascular, haemodynamic or oxygenation properties occur, including with disease and drugs. Given the importance of this matter to the present thesis, this is discussed later.

One of the mechanisms by which we can adjust for small variations in the onset timing, and temporal dispersion, of the HRF is to model, separately from the HRF, its first and second temporal derivatives, respectively.

Finally, it should be noted that there have been attempts to model the HRF according to the various properties of underlying neural processes. By simultaneous neural and BOLD measurements (Logothetis et al, 2001; Goense & Logothetis, 2008; Rauch et al, 2008) it is found that the BOLD response most closely parallels local field potentials (LFP). This follows from the fact that LFP often reflects the sum of regional synaptic activity, which has its own metabolic footprint (due to transmitter release and uptake). BOLD responses are less related to the rate of regional cell-firing, or mean unit activity (MUA). Given the spatial resolution of fMRI, this indicates that the level of BOLD signal from a single voxel reflects a *summation of inputs* - regardless of whether excitatory or inhibitory in nature – rather than the *output* or rate of cell firing, the latter of which reflects the spatiotemporal integration of neural inputs. Nevertheless, since transmitter release is

directly related to spiking activity, a relationship between neuronal spike frequency and BOLD is also often apparent (Smith et al, 2002). In some circumstances - especially within hippocampus - dissociations between BOLD and LFP, or MUA, can occur that might be due to such regions having similar numbers of inhibitory and excitatory synapses, which when active, result in increased BOLD but no net increase in LFP or MUA. Such dissociations may also occur in regions such as hippocampus that have a sparse and less-well autoregulated blood-supply (Ekstrom, 2010).

Design Matrix Specification in event-related fMRI

In event-related fMRI the time resolution with which we are able to observe changes in neural activity is far superior – in the order of seconds, to that achievable in epoch-related methodologies such as PET, for which the datum reflects neural activity averaged over many minutes. One of the main advantages of this finer temporal resolution is that we can model phasic cognitive processes – that vary trial-to-trial, or even within the same trial – and distinguish these from tonic processes occurring over the course of a session, each of which are likely to have distinct neural bases (e.g. Forster et al, 2000; Marklund et al, 2007). The ability to model separable behavioral / cognitive components, occurring at different times, and on different time-scales, is achievable by virtue of the versatility of design matrix specification as defined above. Furthermore, we are also able to model potential confounds such as reaction time or fatigue as extra terms within the GLM.

A typical design matrix for an event-related fMRI experiment is partitioned into multiple factors, each represented by a separate column in the matrix, that provide the best

characterization of processes underlying variations in the BOLD signal time-series. The other constraint that we impose is that partitions are independent of each other; as we have seen this is required in order for the $\hat{\beta}$ estimates to be solved in the GLM.

The usual components of a design matrix are:

1. **Effects of interest.** These are distinguishable components of the experiment such as stimuli (e.g. houses versus faces); tasks (e.g. passive viewing versus 'look for a target'); response type (e.g. move right versus left hand); response meaning (e.g. correct verus incorrect); and repetition (e.g. first versus second stimulus presentation or movement). Some effects may be categorizable only through testing in sessions before or after the scanning session itself, e.g. priming (e.g. word studied earlier versus novel word); introspective evaluation (e.g. pleasant versus unpleasant); or subsequent memory (e.g. correctly recognized versus incorrectly rejected).

   In general, each event type is modeled as a separate explanatory variable, and each event occurrence is modeled as a canonical HRF. Exceptions to this are where there may be insufficient trial types and where sub-classification of variables is not directly relevant to the research question. By modeling more then one factor the possibility of interactions can be tested.

   The duration of each event type can be modeled by convolving the HRF with a suitably lengthed boxcar. This enables us to distinguish tonic from phasic

processes even where they are occurring concurrently (e.g. Otten et al, 2002). Furthermore, by orthogonally manipulating the duration of trial subcomponents we can distinguish different cognitive processes that may act together within a single trial (Zarahn et al, 1999; Rowe et al, 2001). For example in Experiment 3 in this thesis, stimulus-related activations are distinguished from attention and working memory-related activations by varying the duration of an intervening maintenance period during which sensory and motor requirements are held constant between conditions.

A further way in which effects of interest can be modeled, is to scale the HRF by a physiologically-meaningful variable. In this way a series of events can be contained within the same column of the design matrix, with each being assigned a parametric modulator. The nature of the parametric modulation can be linear, quadratic, logarithmic etc., depending on the model (Buchel et al, 1998). For example, in Experiment 1 of this thesis, different stimuli and task types were modeled both as time-invariant, and time-dependent, factors. The latter were specified as a time series of HRFs convolved with a linearly decreasing function. Responses found to decrease (or increase) over the session may capture time-dependent component of the task such as habituation or learning.

2. **Error Trials.** Trials in which the subject committed an error (whether of commission or omission) are often modeled separately to reduce possible variance within the effects of interest themselves.

3. **Movement Parameters.** The initial spatial registration step generates for each scan an estimate of the rigid-body parameters required to realign the scan to the reference scan (i.e. the first scan in a session). These six parameters are included within the design matrix to account for movement-related activations, or failure of activations.

4. **High-Pass Filter**. A set of time-varying cosine regressors may be added to the model to remove a large component of noise that occurs at low temporal frequencies (Zarahn et al, 1997). Low-frequency noise originates from physiological effects (e.g. respiratory and cardiac high-frequency cycles being sampled slowly, and thus aliasing); slow movements of the head, and scanner B0 magnetic field drift. An alternative correction method used in this thesis is to transform the time series into its frequency-power profile and to remove all components below a threshold (usually set at 1/256 Hz).

5. **Temporal Autocorrelation**. A significant problem with the GLM approach to fMRI time-series analysis is that the error term does not contain independent residuals. This is because each data point (matrix row) is inherently correlated with data before and after it in time – i.e. is temporally autocorrelated. This occurs partly because each event is associated with a long HRF duration (10-20 s) that will be sampled multiple times by an fMRI acquisition run (TR ~ 3 s). One of

the effects of this problem is that the actual number of degrees of freedom is less than the number of scans.

Several strategies have been proposed to correct for this problem. In the "pre-whitening" method, the autoregression between each data-point and its immediate temporal neighbour is used to estimate an AR(1) model which can correct the data (Bullmore et al, 1996). A more robust, but arguably less sensitive, way of rendering residuals independent of each other is to use a Gaussian low-pass filter matched to the HRF, in addition to the previously mentioned, high-pass filter, which together swamps any intrinsic autocorrelation (Friston et al, 1995b).

Notwithstanding the above account of autocorrelation adjustment, it turns out that the problem of temporal autocorrelation is not too problematic if experiments are designed to make inferences at a random-effects level (see below). This is because such analyses compare only the parameter estimates of the first-level analyses, and not the error terms about those estimates. For this reason, Experiments 1 and 2 in this thesis (analysed using SPM99 software) did not perform any autocorrelation correction. In Experiments 3, 4 and 5, residual temporal autocorrelation is estimated using a one-step restricted maximum likelihood (ReML) estimation (Kiebel et al, 2003). In this method, the covariance of residuals (i.e. correlation of each datum with each other data-point, for each voxel) is modeled as a set of variance components, and the solution to the GLM proceeds by estimation of both GLM parameters and variance components'

hyper-parameters. The advantage of this method is that it acts as a general method for non-sphericity correction, rather than being specific for temporal autocorrelation.

6. **Session Effects**. Each session within the scanner is modeled by its own constant, this reflecting the average, or baseline, BOLD signal for each voxel. This factor may or may not be important depending on whether any effects of interest are separated as session-specific. However since it is likely that non-cognitive factors – such as magnetic field, head position, or subject fatigue – are also likely to vary between sessions, then estimation of session effects are likely to be inefficient (including extra noise) or confounded.

Data Scaling

It is possible that from session to session the level of signal received from the brain as a whole varies e.g. because the head is more or less optimally sited in the head coil. This *grand mean* reflects the mean over all voxels, and all scans of a session. This value is automatically removed by grand mean scaling, i.e. multiplying each voxel's value at each acquired time-point by 100/grand mean.

Extending the logic of grand means, it is also possible that the global signal varies from scan to scan (and not just session to session). This may reflect uninteresting variations in head or scanner properties from scan to scan, e.g. head movement, or even physiological effects e.g. arousal or drug effects that may affect the whole brain in one go. The *global*

mean is defined as the mean of voxel signal intensity for any one scan. This can be removed by global scaling in which each voxel for a given scan is multiplied by the 100/global mean for that scan.

In this thesis, both grand and global scaling were applied to eliminate effects of drug or disease on whole-brain mean activation that may have arisen from changes in the neural-BOLD relationship (see below), rather than through changes in neural activity. We could afford to apply this correction because the hypotheses tested were concerned with drug or disease interactions with region-specific activations.

One of the problems with global scaling is that if a large number of voxels become activated then regions which become activated less than the average may appear as deactivations. Furthermore, to the extent that global changes usually reflect gradual changes over the course of a session, global scaling is unnecessary as high-pass filtering (see above) effectively serves the same purpose.

Hypothesis Testing

By reviewing the GLM equation ($Y = X\beta + \varepsilon$), and recalling that the design matrix, X, specifies a series of HRFs for each effect of interest, it can be seen that the size of β, estimated for each explanatory variable, reflects the strength of the HRF that best accounts for the measured BOLD time-series Y. Having *estimated* the size of each explanatory variable's effect ($\beta_1, \beta_2 \ldots \beta_I$), for each voxel, we subsequently wish to make *inferences* regarding them in relation to pre-specified experimental hypotheses. This step

involves comparing effect sizes with the variance of the data set, and assessing the statistical significance of this ratio. Usually these hypotheses takes the form of comparing one condition to another (e.g. β_1 versus β_2), or of comparing several conditions concomitantly (e.g. β_1 versus β_2 versus β_3). Comparing only one effect to baseline (e.g. β_1 versus 0) is not often performed owing to the ambiguity over what the baseline state is, especially since it cannot be assumed that this is synonymous with 'rest'. Thus for each subject tested, we usually perform either two-sample t-tests or F-tests that compare conditions of interest as described by the experimental hypotheses.

T-tests or F-tests are performed on specific combinations of GLM regressors by the conventional method. Mathematically, the contrasts of interest can be specified by a pattern of 1s and 0s within a row-vector *c*, which then multiplies into the β matrix so as to perform a simple linear combination of the estimated parameters. When the effects of interest are replicated in more than one session, this involves adding the β contrast of interest over all relevant sessions.

The t-statistic reflects the size of the effect of interest (β) as a ratio with the standard error of the associated dataset (SE(ε)). In other words, using the c vector to specify a contrast of interest:

$$T = c\,\beta / \text{stdev}(c\,\beta) = c\,\beta / \text{sqrt}(\sigma^2\, c\, \text{pinv}(X^T X)\, c^T)$$

in which the estimated error variance is given by:

$$\sigma^2 = \varepsilon^T\varepsilon / df, \qquad \text{and} \qquad df = J - \text{rank}(X) \qquad \text{(where J = no. of scans)}$$

assuming *iid* (i.e. residual errors are *i*ndependently and *i*dentically *d*istributed).

The t-statistic can then be compared to reference t values at which the null hypothesis (i.e. that c $\beta = 0$) would be falsely rejected with no more than α probability. If the t-statistic is greater than this cut-off then the null hypothesis can be rejected.

An F-statistic can also be estimated in conditions where more than 2 conditions of interest are present. The hypothesis in this case is that any combination of a number of simple contrasts of βs is significant. An F-contrast is formulated as a matrix specifying a multitude of contrasts that together span the experimental space – i.e. all possible linear combinations of contrasts. The F-statistic is derived from the ratio of variance accounted for by the experimental model to the total amount of variance. This too can be compared to an F-distribution of probabilities reflecting the null hypothesis.

The estimation of t or F-statistics proceeds for all voxels resulting in a statistical parametric map (SPM(t) or SPM(F)). These can be thresholded at a level set for rejection of the null hypothesis and then rendered into a 3D image to form an 'activation map'. It should be emphasized that the analysis, as described, reflects a fixed-level, or first-level, analysis performed on individual subjects, and is to be distinguished from a random-effects, or second-level analysis (see below).

Correction for Multiple Comparisons

So far it has been described how a statistic – t or F – that relates modeled effects X to a data-series Y is estimated, *for a single voxel*. With functional imaging we are of course interested in generating such a statistic for as many locations that can be spatially resolved by the particular scanning technique used. This introduces two sorts of problems that act in opposite directions.

Firstly, there is the well-known problem of multiple comparisons by which the probability α of a particular occurrence increases in proportion to the number N of times this occurrence is allowed to happen. Specifically, if α is the probability that we falsely reject the null hypothesis for a single statistical test (i.e. commit a type 1 error), then if we repeat the test N times:

$$P(\textit{no} \text{ type 1 error is committed on any test}) = (1 - \alpha)^N$$

Therefore

$$P(\textit{at least one} \text{ error occurs}) = P(\text{Family-Wise Error}) = 1 - (1 - \alpha)^N$$

When α is small the binomial expansion can be approximated by just the first two terms:

$$P(\text{Family-Wise Error}) \leq N\alpha$$

If we consider P(FWE) to be the acceptable error threshold over all N tests – conventionally set as 0.05 or 0.01 - then we need to adjust α for each individual test. The most stringent but simplest means of correction is the Bonferroni method by which, rearranging the above equation, we set α for each individual test to be P(FWE) / N. Since N for fMRI is in the order of 100,000, this would entail a very high level of statistical significance required for the null hypothesis to be rejected at any single voxel, and so this correction method is too conservative.

The second problem with sampling over many voxels is that they do not reflect independent samples by virtue of their spatial nature. In other words we would generally expect measurements that come from samples spaced closely together to be more correlated than samples taken from highly separated locations. (This is likely to be true for any kind of geographical survey). Spatial autocorrelation in fMRI originates from both inherent smoothness (owing to neural, vascular and MRI spatial properties) and applied smoothness (performed for reasons described earlier). Principles for estimating and correcting spatial autocorrelations are similar to those used for temporal autocorrelations discussed above.

Although spatial autocorrelation is a problem in the sense that it means our spatial resolution is not as good as the number of voxels sampled suggests, it actually mitigates the first problem of multiple comparisons. This is because the number of *independent* comparisons that we need to correct for is significantly less than the number of voxels.

For example, if the smoothness of the dataset is governed solely by the applied smoothing kernel of full-width half maximum (FWHM) then the number of independent observations approximates to the total volume divided by the number of cubes of FWHM side length. In this situation, each FWHM-characterised cube is the true spatial resolution element (known as a 'resel') (Worsley et al, 1992). However, since we do not know the extent of spatial correlation before smoothing is applied it is necessary to estimate smoothness, which is derived from the residual error images (Kiebel et al, 1999).

Having estimated the number of resels that our dataset is composed of, we can calculate the probability that a subset of resels will exceed a statistical threshold by chance. This relationship is derived from an equation of random field theory, which states that the average number of suprathreshold clusters - also referred to as the expected Euler characteristic, or E(EC) - is a function of resel number and the statistical threshold Z. For Z values greater than 1, E(EC) decreases exponentially, and for E(EC) values less than 1, E(EC) approximates to the probability of finding a suprathreshold cluster by chance. In practice, this equation is used in reverse, whereby we set the probability of observing a suprathreshold cluster by chance, E(EC), to be equivalent to P(FWE). Thus we derive the statistical threshold Z we should set for each voxel to assure ourselves of a P(FWE) of <0.05 (or 0.01). Note that this estimated Euler characteristic method is more accurate than the classical Bonferroni method of correction by which Z would simply equal P(FWE) / number of resels.

While using resel number, rather than voxel number, allows us to use a lower statistical threshold, and thus increase sensitivity, the method of correction as described is still relatively conservative. In order to improve sensitivity, or decrease the likelihood of type 2 errors (i.e. false negatives), two alternative methods are sometimes used (including within experiments in this thesis). The first of these methods controls for false-discovery rate (FDR) (Genovese et al, 2002). The critical distinction here is that the FDR refers to the rate of false positives *among only those tests which are all ready positive* (i.e. null hypothesis is rejected), whereas with Bonferonni correction the FWE refers to the rate of false positives *over all tests performed regardless of their result*. Consequently, with the FDR method, for a given FDR value (e.g. 0.05), the α level for individual voxels will differ between statistical contrasts, depending on the overall spatial pattern of activity. In particular, it allows us to be more lenient with our thresholding in contrasts when only a small proportion of voxels have relatively low p values. By contrast, when a large number of voxels have low p values, the correction becomes more conservative and approximates a Bonferroni method.

The second method by which we can improve statistical sensitivity is to restrict the search volume to that framed by the experimental hypothesis. Reducing the number of resels decreases the chance of finding a false cluster, E(EC), for a given statistical threshold Z. Given the inverse relationship between E(EC) and Z for Z>1 (see above), this entails that as resel number decreases, Z can be decreased while keeping E(EC) at a pre-specified level (usually, 0.05). In SPM software, a region of interest (ROI) can be defined by creating a 'mask' of voxels, thereby restricting statistical parameter estimation

to a subset of brain voxels. This mask can be defined anatomically, e.g. based upon recognized cerebral sulci or fissure landmarks, or functionally, e.g. a sphere centred upon activation coordinates quoted in a previous study, and of diameter equal to the smoothing FWHM.

Sometimes an ROI is derived from a separate contrast performed in the same study. However, for statistical validity, and avoidance of bias, this ROI should be derived from separate trials, or separate subjects, than those from which the test is based upon, even if the contrasts are orthogonal (Mitsis et al, 2008). Rather, the main advantage of spatially masking contrasts with other contrasts is to enable functional characterization of regions, and interpretation of interactions between factors. This is elaborated in the Methods subsections for each experiment.

In this thesis, results are reported at a statistical threshold of $p < 0.05$ corrected for whole brain, or corrected for small-volume as defined by the above methods. Additionally, voxels are reported at thresholds of $p < 0.001$, uncorrected, in anatomical regions considered to be relevant to the behavioral paradigm based upon previous work.

Random-Effects Analysis

The statistical values estimated from one subject's dataset relate the experimental effect of interest to the variance of this particular subject's dataset. Since the number of degrees of freedom from which the statistic is computed is based upon the number of measurements, i.e. scans, which is in the order of ~100, the statistical significance of

effects is often found to be very high. Usually the variances of different sessions are pooled, which is acceptable providing there are no good reasons for believing that the within-session variance is different from the between-session variance (acceptable, for example, if they are separated by only a short period of time). However, this fixed-effects method of analysis means that our inference regarding the experimental effect is confined to the subject under study.

Usually, we are interested in making inferences about an experimental effect in a population – for example, of healthy adults. However, it is likely that variance between subjects is different from that within subjects, necessitating a random-effects analysis. In this analysis, the size of an experimental effect – averaged over a number of tested subjects – is compared to the variance *between*, rather than *within*, individuals. Accordingly, in SPM, the results of a contrast performed over a single subject's GLM are reported (and saved) both in terms of effect size (difference between βs; referred to as "con-images"), and t- (or F-) statistics, the latter of which incorporates both effect size and *within* subject variance. Of these two types of result, it is the effect size alone that is utilized for a random-effects analysis, through pooling or comparison with the equivalent effect sizes from other subjects.

Depending on what type of question we are asking at the population level, a random-effects analysis proceeds as one of several types of GLM. This is the second time we construct a GLM – explaining why random-effects is also called a second-level analysis - but now we take *subjects'* pooled effect size, rather than *scans*, as each datapoint

(modeled by individual rows of the design matrix). This often means that the degrees of freedom, and therefore sensitivity is reduced.

If the question is what regions is BOLD greater during state A than state B, then the contrast of A – B generates a single effect size for each subject, which can then be analysed at the second level by a **one-sample t-test**. This would identify those regions in which (A-B) > 0, inferred upon the population of which the tested subjects were representative. If we are interested in comparing the responses between two conditions, or two groups of subjects (for example between patients and age-matched controls: see Experiments 4 and 5), then the second-level GLM is constructed so as to perform a **two-sample t-test**. In these situations, the two explanatory variables (i.e. columns of the design matrix) reflect the two types of condition or subject. If more than two explanatory variables are represented within the second-level GLM, then an **ANOVA** can be performed. Alternatively, as in Experiment 2, we can perform a conjunction analysis, in which we estimate the probability of two or more contrasts being jointly significant (Price & Friston, 1997). The utility of a conjunction is that it isolates cognitive components common to two sets of conditions (and hence is an alternative approach to subtraction methodologies). The conjunction analysis proceeds as a split t-test, and its results can be incorporated within random field theory (Worsley & Friston, 2000).

Sometimes we are interested in correlating an independently-derived parameter with individual subject effect sizes, in which case this parameter is modeled as a separate term within the GLM, allowing for an **ANCOVA**. For example, in Experiment 5, effects of

drug on a particular functional contrast of BOLD activations were correlated between subjects with the drug-effect on each subject's subsequent recognition performance. Sometimes the modeled parameter is a 'covariate of no interest'. For example, in Experiment 4, drug-effects on reaction times were included within the GLM so that the effects of drug on the functional contrast of interest (e.g. deep versus superficial encoding) could be determined separately from the effects of drug on RT. Finally, the parameter of interest can be set to be the effect size for another type of contrast in another brain region – enabling testing of hypotheses regarding functional connectivity. For example, in Experiment 5, the effect of task on stimulus-selectivity is modeled both as a constant term, and as a variable term based upon the size of individuals' task-effects within right parietal cortex.

In estimating the parameters of a GLM, where more than one condition is specified as in repeated measures ANOVA, we assume that variance is identical between conditions. Furthermore, we assume that the sizes of effects between orthogonal contrasts are independent. Since these assumptions are often unlikely to hold, we apply a non-sphericity correction that is equivalent to a Greenhouse-Geisser correction. In SPM this occurs by deriving the error variance-covariance structure over all voxels showing a significant 'effects of interest' F-contrast. This enables estimation of voxel-specific hyperparameters controlling the nonspherical variance components, which themselves can be used to correct for the appropriate statistic and degrees of freedom (Glaser & Friston, 2004).

Experimental Design

The experiments within this thesis are based predominantly upon a factorial design. By this we mean that the behavioural paradigm can be divided into two or more factors that act orthogonally with respect to each other. For example, in Experiments 1 and 2, stimuli were arranged in such a manner as to be distinguishable along three dimensions: attention (faces in attended vs unattended locations); emotional valence (fearful vs neutral faces, irrespective of face location), and repetition (first vs second exposure to face). This is considered to be a 2 x 2 x 2 factorial design in which each factor has two levels. Note that these factors are presented to each subject, enabling within-subject comparisons, whereas treatment (drug vs placebo) in Experiments 1 and 2 was a further factor that acted between subjects.

The main advantage of the factorial approach is that it enables manipulation of several factors that may interact. By this is meant that effect of one factor (e.g. A1-A2) is modulated by whether another factor is operating or not (e.g. B1-B2). For example, in Experiment 4 we note that face, relative to building, stimuli activate fusiform cortex regardless of task (shallow vs deep), whereas the same stimulus comparison activates posterior superior temporal sulci significantly greater during a deep than shallow task. The other advantage of factorial designs is that they enable multiple factors to be investigated within the same experiment. However, whenever an extra factor, F say, has been included within the experimental design, this should be modeled so as to allow for the possibility that it is actually F that is the strongest driver of an effect, rather than a factor A represented in the original model. In this situation, we say that the main effect of

A is being driven by its interaction with F. The problem with modeling too many factors though (especially 4 or more) is that one may be left with too few event types per condition to enable reasonable parameter estimation, and interpretation of interactions becomes complicated. In order to deal with the latter problem, the approach used here is to mask interactions (e.g. stimulus x task x drug) with more rudimentary contrasts (e.g. main effect of stimulus). This avoids having to interpret so-called cross-over interactions in which a region shows opposite effects, rather than different sizes of an effect, under different conditions.

Design Efficiency

The design of a functional imaging experiment, in terms of trial numbers, spacings, durations, and order, affects the efficiency with which an underlying neural signal can be detected. At the simplest level, the more trials we measure the better our estimate of the the signal, meaning that we should try to cram in as many trials (i.e. have short stimulus onset asynchronies) as we can per experimental session. However, as well as possibly interfering with the psychological process that we wish to study, having too short SOAs is not efficient in fMRI because of the sluggishness and long duration of the HRF. For example, if we wish to observe the main effect of photic stimulation on visual cortex, then we need to wait for the HRF to rise and then fall over about 20s for each trial. Were we to space trials too close together (e.g. < 10s), the effect would be to elevate the baseline – that is not sampled because of the high-pass filter - while making individual trials indiscernible. Extending this logic entails that the most efficient protocol for main

effect contrasts (i.e. condition A - 0) are blocked designs in which A can be successively repeated, and then alternated with rest (0), in cycles with periods of ~20s.

Usually our interest is in comparing two conditions rather than performing solely a main effect. Furthermore, blocked designs are problematic from a psychological standpoint since factors of interest may be confounded by time. Moreover the neural processes that we wish to probe are often phasic, rather than tonic, in nature, and in some cases we wish to classify trials according to post-scanning information (e.g. subsequent memory as in Experiment 5). Fortunately, if we wish to compare two trial types the signal difference between them can be discerned in the early parts of the HRF and we are not forced to wait 20s as with main effect discrimination. This fact allows us to perform event-related fMRI in which different trial types are mixed up within a block. The optimal SOA in this situation is determined by the ordering of the two trial types. If the two trial types simply alternate, then placing these together closer in time (e.g. < 3 s apart) than the peak of the HRF, will lead to a poorer discrimination than if we waited about 5 – 10s to sample each trial type. However, if we were to pseudorandomise the order in which the trial types occur, thereby allowing some runs in which the same trial occurs repeatedly, we can allow for a shorter SOA. This is because in runs with identical trial types, there is a summation of overlapping HRFs, during which we can observe more readily differences between trial types. Consequently, for a pseudorandomised design if SOAs are long (e.g. 6 – 25 s) then sensitivity decreases because low-frequency components resulting from long runs of one event type are attenuated by the high-pass filter.

More formally, the ways in which variations in trial ordering, or SOA, affect signal estimability can be simulated with running estimates of the HRF (Josephs & Henson, 1999). The estimated measurable power is equivalent to the total energy (sum of squared signal across scans) divided by the number of scans. This shows that the most powerful designs for experiments that contrast more than one trial type are those in which there is a short SOA (~3s), and a pseudorandomised ordering of trial types. At too short SOAs (<2s), the linear time-invariant assumption for BOLD responses breaks down, and efficiency becomes compromised. If both main and differential effects are desired, then insertion of 'null events' that are not distinguishable to the subject, but serve merely as spacers between events, enable sensitivity to both at short SOAs (Dale & Buckner, 1997).

The experiments in this thesis generally adopt a mixed fMRI design in which task conditions are blocked, and stimuli types are assorted as 'events' within each block. Blocking tasks is often performed to reduce the likelihood that subjects make mistakes (especially important in Experiments 4 and 5 involving dementia patients). Within the blocks, event types are pseudorandomised, and SOAs are varied with a mean of ~ 3s. The use of jittering for the intertrial interval both reduces the likelihood of slice-timing confounding, and increases sensitivity for short SOA designs (Burock et al, 1998).

Finally, it should be noted that efficiency of signal comparison between two conditions or more, is related to the relative timings and orderings of events between the condition types. In general, the greater the orthogonality (i.e. independence) between conditions, the more accurate is the parameter estimation for each condition. This is because with

orthogonal designs all the variance of the dataset can be divided up between the conditions, whereas with non-orthogonal designs some of the variance is common to multiple conditions and so cannot be utilised for parameter estimation. More formally, efficiency for a single contrast is proportional to the variance of a column in a design matrix, and for multiple contrasts, efficiency can be characterised by the trace of the covariance of the contrasted design matrix:

$$\text{Efficiency}(X) = \text{Var}(X) = X^T X$$

$$\text{Efficiency}(c,X) = \{c^T (X^T X)^{-1} c\}^{-1}$$

Pharmacological and Clinical fMRI

Confounding Effects of Drugs and Disease in fMRI

A particular methodological concern to the experiments of this thesis is that drugs (Burke & Buhrle, 2006; Stefanovic et al, 2007; Pattinson et al, 2007) and disease - in this thesis, Alzheimer's disease (Rombouts et al, 2005; Rombouts et al, 2007), as well as ageing (D'Esposito et al, 1999), alter the neurovascular coupling relationship. As discussed above, our assumption that fMRI is a reliable indicator of neural activity is predicated upon the assumption that there is a fairly constant relationship between neural activation on the one hand, and changes in regional blood flow and oxygenation, on the other. Thus any fMRI investigation into how a factor X influences neural activations needs to first question whether X also influences the neural-BOLD relationship. In other words, if X

reduces BOLD activations, this might be because X reduces neural activity, or it could mean that neural activity remains the same, but that the BOLD signal generated for the same amount of neural activity is lessened. It should be noted that this concern is distinct from that which questions whether there is a linear time-invariant relationship between evoked neural activity and BOLD response. Rather, the question is whether the function that maps neural activity to BOLD signal – be it linear or non-linear – differs under different physiological conditions, some of which may happen to encompass the manipulation of interest.

Variations in physiology may alter neural-BOLD coupling at four theoretical stages (Iannetti & Wise, 2007):

1. **Neural**. Whilst pharmacological or disease-associated changes in *evoked* neural responses are usually the subject of interest, it needs to be considered whether the experimental factor also alters the level of *baseline* neural activity. Studies measuring combined neural and metabolic parameters in rats have shown that different baseline firing patterns (invoked by differing degrees of anesthesia) can alter both the magnitude and spatial pattern of BOLD responses (Hyder et al, 2002). For example, for a given stimulus, a low baseline state produces a higher evoked response than a high-baseline state, suggesting that function arises from an absolute, rather than relative, level of neural activity. Furthermore, observations that high-baseline relative to low-baseline states manifest evoked BOLD responses that are spatially more diffuse, but weaker, may reflect a higher

relative proportion of fast-firing (γ-range) to slow-firing (α-range) neurons (van Eijsden et al, 2009). In Alzheimer's disease, background alpha-activity is impaired, which is predicted to reduce evoked BOLD responses according to a unified EEG-fMRI model (Sotero & Trujillo-Barreto, 2008). It is also possible that both drugs and disease alter the spatial distribution of default networks characterized by spatially-correlated baseline activity (e.g. Greicius et al, 2004).

2. **Metabolic signaling and blood flow.** Changes in neural activity - whether ultimately originating from synaptic processes or action potential generation – necessitate changes in metabolic, especially oxygen, flux. The main way in which oxygen supply is varied is through modulation of regional cerebral blood flow (CBF), rather than adjusting the rate of cerebral metabolic oxygen consumption ($CMRO_2$). This is because merely increasing $CMRO_2$ following increased neural activity would rapidly become ineffective since a requisite oxygen concentration gradient from vessel to cell would be removed. Consequently, neuronal activity results in arteriolar vasodilatation and a fractional increase in CBF several times greater than any increase in $CMRO_2$, thereby sustaining a high partial pressure of capillary oxygen.

   Coupling between neural activity and vascular tone may occur homeostatically through neural or glial-associated changes in the local concentrations of metabolites, including nitric oxide and oxygen. Alternatively, it may be controlled indirectly through vascular sensitivity to neurotransmitter release e.g. glutamate

and GABA (Drake & Iadecola, 2007). Which ever pathway is dominant, it is likely that efficiency of neurovascular signaling is susceptible to disease and drugs. Furthermore, in a similar vein to the confound of neural baseline previously mentioned, the values of *basal* metabolism, blood flow and volume, will also determine the *incremental* changes of each parameter, that ultimately determines BOLD signal (see below). For example, states in which there is a greater basal blood volume, e.g. due to vasodilator drugs or diseases characterized by angiogenesis, will exhibit a greater BOLD signal simply by virtue of a linear gain factor. Conversely, increasing basal cerebral blood flow, for example by inducing hypercapnia or administering acetazolamide, reduces the BOLD response to a constant stimulus (Cohen et al, 2002; Brown et al, 2003).

3. **Vascular Reactivity.** Whereas with PET functional imaging, the measured signal is a direct reflection of regional metabolic activity, with fMRI, the BOLD signal is a more derived measure. Specifically, as previously discussed, BOLD ultimately depends upon the ratio of oxyHb to deoxyHB, in a given volume, which is only partially a function of metabolic demand. Changes in BOLD are directly related to changes in CBF and changes in cerebral metabolic rate, or consumption of oxygen ($CMRO_2$) according to the scaling parameter M, described by the relationship:

$$\Delta BOLD = M\ (1 - (\Delta\ CBF^{x}.\ \Delta\ CMRO_2{}^{y}))$$

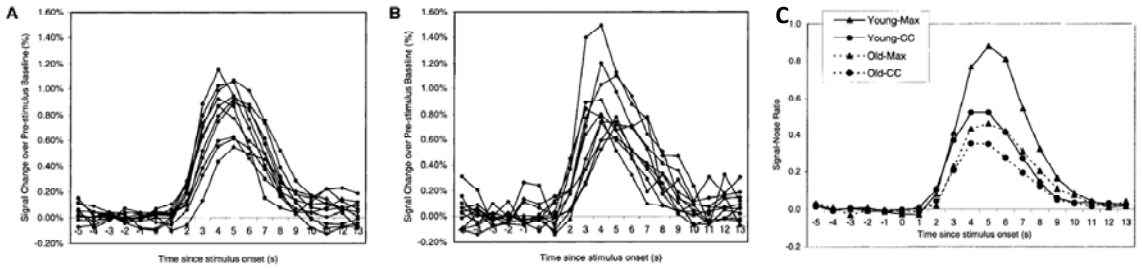
where x relates cerebral blood flow to blood volume, and y is a physiological constant (Davis et al, 1998). M reflects the amount of deoxyHb in the baseline state, and defines the maximum possible BOLD signal change for a particular region. The value of M is inversely proportional to venous blood volume and flow, either of which, as well as parameter y, may alter according to physiological or pathological state. For example, drugs that lower systemic blood pressure are associated with increased venous volumes, due to a cerebral autoregulatory reflex, and thus a reduced BOLD signal (Kalisch et al, 2001). M is also inversely proportional to arterial oxygenation which may be reduced under certain conditions, e.g. smokers, sedating drugs.

4. **BOLD signal.** The relationship between changes in the deoxyHb content of a given voxel and recorded BOLD signal change is governed by factors that influence proton T2* relaxivity. Most of these – e.g. B0 magnet strength, pulse sequence – are clearly unrelated to drugs or disease. However, certain exceptions may exist: such as if bulk head motion were to be increased in dementia patients, or under drug state, then the efficiency of signal measurement would be reduced. Furthermore, disease-associated anatomical differences such as of grey matter density, or microhemorrhages, may alter MR signal-related factors, such as proton density and T2* susceptibility, respectively.

Variations in hemodynamic response to evoked activity have been investigated in elderly people since a major application of fMRI is exploring the cognitive disorders of patients

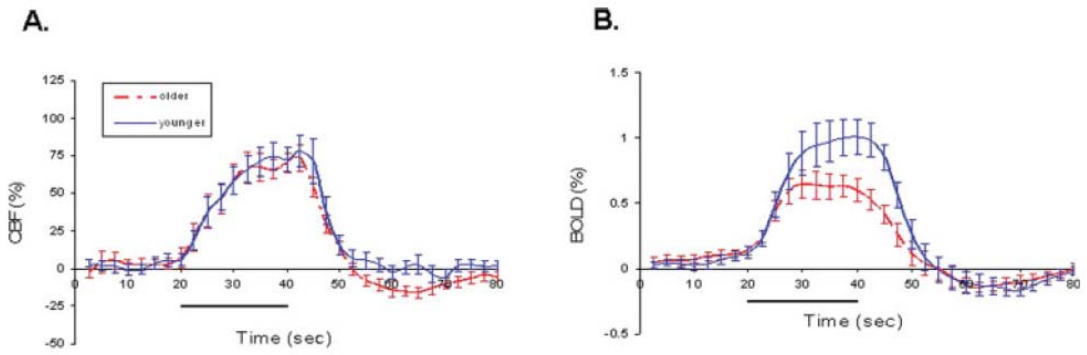
with neurodegenerative diseases as well as of healthy ageing. For the same stimulus intensity, elderly subjects are found to show greater intersubject and intrasubject variability in the evoked haemodynamic response function, leading to smaller signal to noise ratios, and smaller clusters of significant activation (Fig. 4.12; Huettel et al, 2001).

Figure 4.11. BOLD responses in primary visual cortex show less variance in young (A) than old humans (B), with consequent higher signal-to-noise ratio (C) (Huettel et al, 2001). CC = calcarine cortex; Max = maximum signal voxel.



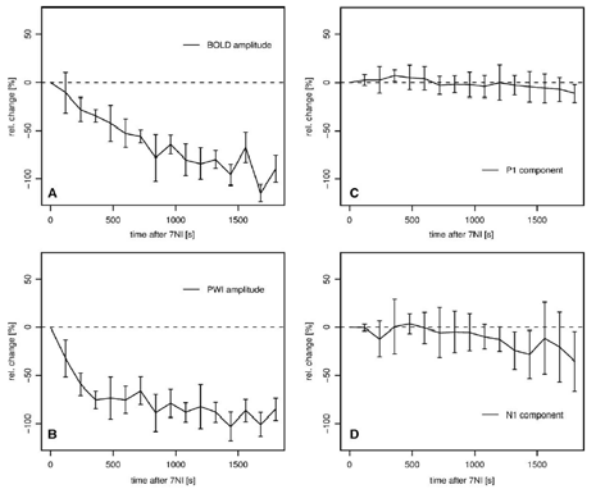
Whether effects of age on the BOLD response are due to alterations in the neural - BOLD relationship, rather than neural differences per se, has been explored by studies measuring both cerebral blood flow (CBF) and BOLD (Ances et al, 2009). Since it was found that ΔBOLD was diminished in elderly under circumstances where both ΔCBF and $\Delta CMRO_2$ remained constant, it appears that one reason for the age-related difference in BOLD response is a difference in the coupling relationship expressed by the parameter M in the 'Vascular Reactivity' equation described above (Figure 4.12; Ances et al, 2009).

Figure 4.12. Old and young subjects show similar visual cortex blood flow changes (A) in response to a stimulus, but decreased BOLD amplitude (B). This suggests a reduction in the neural – BOLD relationship with age (Ances et al, 2009).



It is likely that certain drugs also have the potential to modulate the constants of the neural-BOLD relationship. This can been demonstrated by noting an electrohysiological-BOLD dissociation, such as with the drug 7-nitroindazole (Burke & Buhrle, 2006).

Figure 4.13. Pharmacological decoupling of the neural – vascular – BOLD relationship by 7 nitroindazole demonstrated by separate measurements of somatosensory evoked potentials (C, D) and somatosensory evoked BOLD (A) and blood flow (B) in rats (Burke & Buhrle, 2006).



It is possible that concerns about drug effects on the neural-BOLD relationship are limited to only a few particularly vasoactive drugs. Simultaneous BOLD – neural sampling in monkeys has shown no significant variation in correlations between the two measures comparing anaesthetized and awake monkeys, at least in primary visual cortex (Goense & Logothestis, 2008). It is noteworthy that this result appears to downplay the importance of differences in baseline energy in determining evoked BOLD responses (Hyder et al, 2002). Furthermore, the tight coupling known to occur between local field potential and BOLD is unaffected by direct injection of a serotonergic agonist into visual cortex (Rauch et al, 2008), in spite of this drug having vasoconstrictive properties (e.g. Hamel et al, 1989). Even where a drug significantly affects cerebral blood flow, as shown for cocaine using flow-sensitive inversion recovery MRI sequence, this does not necessarily alter the BOLD response (Gollub et al, 1998).

Measures to Control for Confounding in Clinical and Pharmacological fMRI

As we have reviewed, in the handful of studies that jointly measure BOLD and another dependent physiological parameter – such as neural firing, evoked electrophysiological responses, or metabolic consumption – there are both examples and counterexamples of drugs and clinical states causing interference of the fundamental neural – BOLD relationship. However, in the absence of any such studies for the particular drug regime, patient population, and regions of interest relevant to the current experiments, we remain uncertain whether the disease or drug of interest affect the measured BOLD signal separately from any effect these factors have on underlying neural activations. Nevertheless, certain measures exist that enable us to control for potential confounds, and to verify whether the BOLD effects observed are likely to have been primarily neural, rather than vascular, in origin (Iannetti & Wise, 2007). A subset of these were employed in this thesis.

1. **Systemic Physiological Parameters**. In the current experiments, pulse, capillary oxygen and blood pressure were measured immediately before and after scanning. The first two parameters were also monitored throughout scanning by means of MRI-compatible telemetry.

2. **Subjective Indices**. Psychologically-validated questionnaires (e.g. Bond & Lader, 1974) can be provided to subjects to assess states such as awareness and mood, as well as side-effects. These can provide surrogate markers of the baseline state

(especially arousal) which as discussed can influence magnitude of BOLD signal change.

3. **Global Scaling**. As described above, two types of data scaling were performed so as to eliminate any global (i.e. whole-brain) effects of drug or disease. These operations served to correct both for the session and individual scan global means. It should be noted that the mean corrections are multiplicative, meaning that it corrects for main effects or interactions that are driven by a simple gain factor applying between sessions. Furthermore, the mean values are calculated independently of whether a particular scan samples a baseline period or an 'event'. However, as discussed, we cannot assume that the baseline and evoked responses scale equivalently; in fact, experiments suggest the opposite: viz. that as baseline decreases, the size of the evoked response increases (Hyder et al, 2002). Therefore in order to test for the possibility that changes in global mean may confound changes in event-related signal, including via a relationship other than simple scaling, we can derive the global mean for each session and subject and perform statistical tests on these. For example, we can test for absolute differences in mean BOLD between two pharmacological states with a t-test, or we can correct for a particular comparison in evoked responses between two states by including the global mean as a covariate of no interest in an ANCOVA.

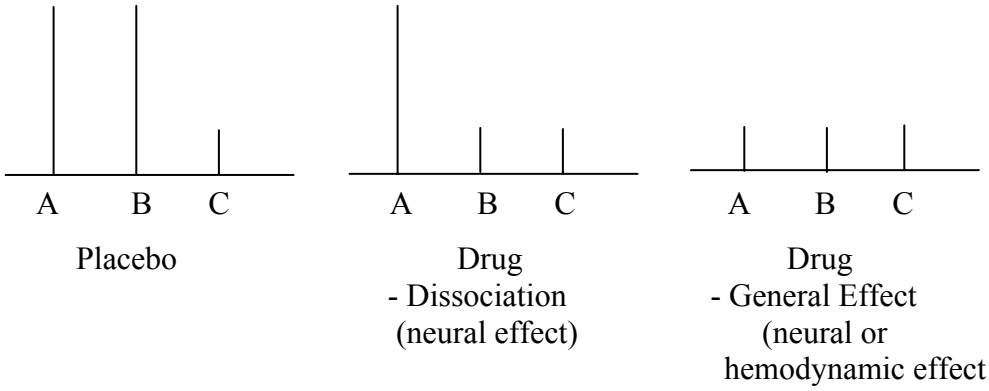
4. **Voxel Session Effects**. The arguments in favour of performing scaling also apply to region-specific effects. In other words, a disease or drug may enhance or

depress the overall level of BOLD signal, independent of event-related activations, in a particular region rather than over the whole brain. Such an effect can be corrected for over a session by the constant term that is included in every design matrix. Note that this serves to subtract the mean, rather than scale by the mean as performed on the global mean value. Furthermore, the possibility that the mean session activity, specific for each voxel, may influence the size of the evoked response in that particular voxel, and hence confound an apparent effect, can be tested for with either t-tests or ANCOVA, as described above.

5. **Task Dissociations**. If a systemic drug or a diffuse brain disease, such as Alzheimer's disease, affects the neurovascular coupling relationship, we would expect consistent alterations in BOLD signal intensity for a given level of neural activation, regardless of task. Conversely, if we were to find that a drug, or disease, results in a modulation of BOLD response for one behavioral condition but not another, where both conditions otherwise result in equivalent BOLD responses, this would be strong evidence for an interaction of the drug, or disease, with neural activity.

Figure 4.14. Hypothetical effects of drug on BOLD response between three different behavioral conditions A, B and C (control). Both drug effects result in a task-by-drug interaction, but only the first type suggests a neural effect specifically. Task C can also be replaced by the baseline providing this has been shown to be unaffected by drug.

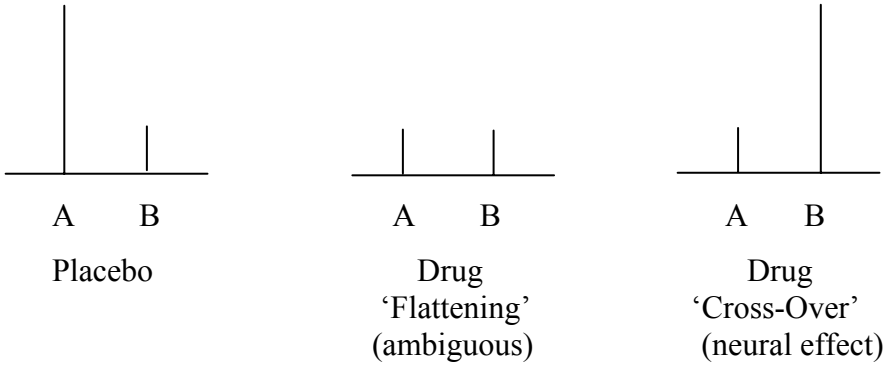
Figure 4.14.



Ideally, the two behavioral conditions for which there is a BOLD dissociation should activate the same, or similar anatomical regions because of the possibility of region-specific neurovascular BOLD relationships (e.g. Schacter et al, 1997).

6. **Cross-Over Interactions**. A further reason why the presence of a task-by-drug (or disease) interaction on BOLD response does not assure us of a neural interpretation is that the interaction may arise from a difference in the level of BOLD response between tasks in the normal state. For example, if the drug (or disease) in question acted to decrease blood flow changes, and hence BOLD excursions, it would limit any apparent effect of the drug to tasks that already generate large changes in BOLD. However, if the drug was found to decrease BOLD responses during certain tasks, but to increase it during others – i.e. show a cross-over interaction – then it is difficult to explain this without resource to the differential neural effects of these tasks.

Figure 4.15. Hypothetical effects of drug on BOLD response between two different behavioral conditions A, B. Both drug effects result in a drug-by-task interaction, but only the latter is immune from haemodynamic confound.



7. **HRF Variation**. Differences in the shape of the hemodynamic response function – in addition to its magnitude, may alter the apparent effect size for a condition modelled with an inflexible HRF. Since evidence exists for age, and disease-related differences in HRF profile (D'Esposito et al, 1999), these may result in spurious interpretations regarding effects of such clinical states on neural activations. Hence inspection of the raw signal, and comparison of this between physiological states of interest is required. One formal method by which this can be done is to decompose different aspects of the HRF into different regressors all of which are included in modeling the data, and orthogonalised hierarchically. In this thesis, the first differential of the HRF was added to the design matrix for every modeled condition, which effectively accounts for HRF delay. A more comprehensive HRF shape characterization can be achieved by inclusion of more regressors. For example, differences in HRF between mild cognitive impairment patients and healthy controls have been observed specifically for fast-BOLD

responses in a model that included seven HRF modeling regressors of different delay (Rombouts et al, 2005).

Finally, methods exist that do not require explicit modeling of the HRF, but rather measure timing properties of the evoked BOLD response. For example, BOLD responses to alternating blocks of photic stimulation / rest in individual visual cortex voxels were classified according to one of two possible phase relationships (positive / negative) relative to a sinusoid fitted to the stimulus. The effects of donepezil on visual cortex activity were then calculated as changes in the proportions of positive versus negative-responding voxels (Silver et al, 2008).

8. **Behavioral Correlations and Confounds**. In some settings, behavioral effects of a drug or disease provide circumstantial support for a neural-based interpretation of imaging data. For example, if an interaction of a drug is observed with a behavioral contrast of BOLD activations, then a correlation of the same interaction with a relevant behavioral effect across subjects would be supportive of the claim that the drug interacted with neural, rather than vascular, processes. Furthermore, group effects at combined BOLD - behavioral levels are mutually supportive if there are prior reasons to expect parallel changes, e.g. sensory cortex activation and detectability, or hippocampal activation and memory performance.

   Saying this, behavioral differences between treatments or clinical groups act as double-edged swords in neurophysiological studies. This is because although such

differences suggest that the pharmacological, or pathological, factor of interest interacts with the neurophysiological process being investigated, there is a danger that performance differences can confound the results. As applied to functional imaging this implies that where we observe changes in brain activation due to a drug or disease, we would like to know whether this neuromodulation is the *cause* of a behavioral difference, or whether it is merely the *effect* of a behavioral difference - itself caused by some other process. Trying to disentangle the two cannot always be done, but some of the strategies that can be used are: using low drug doses or mild disease cases to minimize performance effects (while still observing neural effects); including performance effects (e.g. RT differences secondary to drug) as a covariate of no interest into the imaging ANCOVA; identifying neural – performance dissociations (e.g. under some circumstances a drug causes both neural and performance effects, but under other circumstances, the same neural effects but not performance effects are seen); choosing regions of interest, e.g. visual cortices, that are unlikely to be influenced by limb movements e.g. due to button presses; and separating scanning from sessions measuring performance effects, e.g. on subsequent memory (see Experiment 5).

9. **Multi-modal Imaging**. Interpretation of BOLD effects can be enhanced by simultaneously employing methods that assess cerebral blood flow (e.g. arterial-spin labeling MRI or contrast-based perfusion MRI); cerebral oxygen metabolism (e.g. PET); or vascular reactivity and cerebral blood volume (BOLD-MRI in combination with CO_2 or acetazolamide challenge). Additionally, techniques e.g.

EEG or MEG that measure neural activity directly, without any metabolic or vascular confounds, can check for consistencies with BOLD effects, or alternatively flag up dissociations (Fig. 4.14).

Evidence for Cholinergic Modulation of the Neural - BOLD Relationship

No studies to date have directly measured cholinergic effects on neural and BOLD parameters within the same experiment. However, several lines of evidence exist to suggest that fMRI is vulnerable to confounding by cholinergic drugs.

Endogenous control mechanisms underlying changes in regional cerebral blood flow (rCBF) following neural activity are complex (Drake & Iadecola, 2007), and involve multiple neurotransmitter systems including acetylcholine (Edvinsson et al, 1987). This is suggested by studies showing that cholinergic fibres interact with cortical capillaries and small arterioles (Arneric et al, 1989a), as well as perivascular astroglia or pericytes (Chedotal et al, 1994; Wu et al., 2003). In general, ACh dilates cerebral arterioles, via activation of nitric oxide (NO)-containing endothelial cells, neurons or glia (Parnavelas et al, 1985; Uchida & Hotta, 2009). Thus stimulation of basal forebrain neurons produces vasodilatation, and increases rCBF, without changes in systemic blood pressure (e.g. Sato et al, 2002). Cholinergic-dependent increases in rCBF are predominantly mediated by nicotinic receptors within both cerebral cortex and nucleus basalis (Uchida & Hotta, 2009), although this response decreases with age (Uchida & Hotta, 2009).

Critically, abolition of cortical cholinergic neurotransmission impairs the normal pattern of functional hyperaemia without impairing cerebral glucose metabolism (Kimura et al, 1990; Ogawa et al, 1994), suggesting that this is a vascular, not a neural mechanism. In support of this, nucleus basalis stimulation increases both cortical extracellular ACh release and rCBF, without increasing local lactate concentration (Hallstrom et al, 1990), or changes in electrocorticogram responses (Lacombe et al, 1989). Similarly, cholinergic enhancement with physostigmine increases rCBF without altering cerebral oxygen consumption (Scremin et al, 1982). However, in other circumstances, cholinergic lesions of the basal forebrain impair cortical cerebral glucose metabolism without affecting rCBF, suggesting a specific neural effect (Ouchi et al, 1996; Ogawa et al, 1996). Direct influences of ACh on rCBF occur either through cholinergic cortical interneurons (Fukuyama et al, 1996), or via stimulation of the nucleus basalis (Uchida et al, 1997).

Increases in brain blood flow following nucleus basalis stimulation show regional specificity (Adachi et al, 1990), with neocortical targets showing most response, hippocampus less response (Sato et al, 2004), and subcortical nuclei showing virtually no response. Within neocortices, cholinergic stimulation increases rCBF and oxygen concentration more in prefrontal than parietal regions (Lacombe et al, 1989).

The significance of these findings to the present thesis is that drugs acting on cholinergic neurotransmission may alter the size of rCBF change for a given level of neural activation. Given that ACh increases rCBF (Sato et al, 2004), which itself increases oxyHb:deoxyHb ratio (Davis et al, 1998), we might physostigmine to result in a greater

BOLD response for a fixed level of neural activation. Supporting this assertion, it has been found that cholinesterase inhibitors can reverse scopolamine-induced or age-associated impairments in rCBF response to vibrotactile stimulation in monkey somatosensory cortex (Tsukada et al, 1997; Tsukada et al, 2000). Because these drug effects occurred in the absence of changes in cerebral glucose metabolism, the rCBF modulation appears to be directly due to vascular actions of the drug, rather than indirectly due to neural modulation. Furthermore, physostigmine uncouples the normal relationship between cerebral blood flow and glucose consumption, by increasing global cerebral flow, while decreasing regional glucose metabolism (Blin et al, 1997), or failing to alter oxygen consumption (Scremin et al, 1982). These effects are greater in Alzheimer's disease patients than aged controls (Blin et al, 1997).

The relevance of direct cholinergic influences on rCBF as regards its impact on BOLD is unclear. Although nicotine has been found not to alter the normal photic-driven BOLD response in visual cortex, suggesting relative immunity of BOLD recordings from cholinergic vascular effects (Jacobsen et al, 2002), it cannot be assumed that this holds for other cholinergic drugs such as physostigmine, or in other brain regions. For all of these reasons, wherever significant effects of physostigmine on BOLD responses are observed it is necessary to consider whether these may be because of direct pharmacological influences on vascular properties, rather than because of neural modulation as is intended.

5. EXPERIMENT 1:

Effects of ChEI on Attention and Emotion

Introduction

Numerous lines of evidence indicate that corticopetal cholinergic projections originating in nucleus basalis may modulate attention, through influences both on a frontoparietal network thought to mediate "top-down" control and on sensory cortices subserving "bottom-up" stimulus processing (Sarter et al, 2001; Muir et al, 1996; Robbins, 1998). Cholinergic inputs to frontoparietal cortex have been associated with performance on sustained and selective attention tasks (McGaughy et al, 1996; Himmelheber et al, 2001), with attention-correlated-neural activity (Gill et al, 2000), and covert attentional shifts (Davidson & Marrocco, 2000). On the other hand, cholinergic neurotransmission within primary and secondary sensory cortices can facilitate stimulus processing via positive effects on signal-to-noise ratio (Sato et al, 1987), information flow (Hasselmo, 1995; Xiang et al, 1998), and response selectivity (Murphy & Sillito, 1991). Cholinergic influences on attention are also suggested by the fact that attentional deficits found in dementias associated with cholinergic degeneration (Perry & Hodges, 1999; Ballard et al, 2001) are more amenable to correction with anticholinesterases than other cognitive deficits (Lawrence & Sahakian, 1995; McKeith et al, 2000). A recent fMRI study (Furey et al, 2000a) suggested that effects of acetylcholine on stimulus-processing regions might occur selectively during particular stages of a task e.g., encoding into working memory. It has not yet been shown, however, that cholinergic manipulation can modify activity in sensory cortices specifically attributable to attention, rather than to concomitant changes in stimulus or task (Lawrence et al, 2002).

Other research suggests a role for neocortical cholinergic modulation in emotional processing (e.g., conditioning, fear responses, anxiety). Cholinergic inputs have been shown in rodents to facilitate conditioning via effects on sensory cortices (Weinberger, 1998; Delacour et al, 1990), while cholinergic blockade has recently been shown with a human fMRI study to inhibit conditioning-related responses in auditory cortex (Thiel et al, 2002a). Such data complement anatomical (Kapp et al, 1990; Amaral et al, 1992), neurophysiological (Weinberger et al, 1990; Wilson and Rolls, 1990), and computational (Friston et al, 1994) studies suggesting a role for nucleus basalis cholinergic fibres in relaying evaluative processing within regions such as the amygdala to selection and learning mechanisms in the thalamus and cortex. Increased cholinergic tone in the prefrontal cortex may also be expressed for behaviorally, significant or novel stimuli (Acquas et al, 1996; Pirch et al, 1992; Wilson and Rolls, 1990), which when continually hyperactive may engender clinical anxiety (Berntson et al, 1998; Hart et al, 1999). These findings suggest that cholinergic afferents to specific sensory and prefrontal regions may contribute to "automatic" enhancement of emotional stimulus processing, independently of whether such stimuli are attended.

The present study crossed factors of selective attention and emotion within a fully orthogonal design to examine modulation of condition-specific fMRI responses by cholinergic enhancement with the centrally acting anticholinesterase physostigmine. The paradigm was similar to that of recent functional MRI studies with untreated human subjects, in which the response of the fusiform gyrus to faces was found to be increased by both selective spatial attention and emotional expression (Wojciulik et al, 1998;

Vuilleumier et al, 2001). Since acetylcholine has been found in animals to benefit selective attention and emotional processing, we predicted that the differential response of the fusiform cortex to both factors would be independently enhanced with physostigmine. We also assessed whether regions of the extrastriate cortex preferentially activated for attending houses versus faces—parahippocampal and posterolateral occipital cortices (Vuilleumier et al, 2001)—might also show a greater differential effect under physostigmine. Finally, as cholinergic afferents to parietal (Holland & Gallagher, 1999) and orbitofrontal (Cavada et al, 2000; Aou et al, 1983) cortices have been proposed to mediate attentional recruitment by emotional stimuli, we predicted that these regions would show modulation by physostigmine specifically when emotional faces were task-irrelevant. On the other hand, task-relevant emotional stimuli, being already attended, would not be expected to engage this cholinergic facilitated circuit.

Methods

Subjects

Thirty healthy right-handed volunteers with no history of medical or psychiatric disease gave written informed consent. They were divided into two groups of 15 (placebo, 7 female, 8 male; mean age, 26.8 ± 2.3; physostigmine, 8 female, 7 male; mean age, 23.5 ± 2.0). No subject was taking active medication. While 2 subjects were mild smokers, they were in different groups and refrained from smoking on the test day. A between-subjects design was chosen for the pharmacological manipulation, to avoid habituation effects that may occur in within-subjects designs following repeated exposure to emotional stimuli (Breiter et al, 1996; van Turrennout et al, 2000).

Drug treatment

A double-blind placebo-controlled drug administration technique was used. Each subject received an intravenous cannula into the left cubital fossa and an infusion of either physostigmine or saline. Dosage and rate of physostigmine infused were identical to those used in a recent study (Furey et al, 2000b), which demonstrated significant and stable levels of plasma drug concentration and butyrylcholinesterase inhibition, as well as a significant and stable effect on cognitive performance for 40 min, following a 40-min loading period. The same protocol has also been found to result in changes in both regional cerebral blood flow (rCBF) and blood oxygenation level-dependent (BOLD) activity, during visual working memory tasks (Furey et al, 1997; Furey et al, 2000a).

Subjects in the drug group first received 0.2 mg of intravenous glycopyrrolate—a peripheral muscarinic receptor antagonist—to reduce peripheral side effects. The placebo group were injected with an equivalent volume of saline. Both groups then received an intravenous infusion. For the drug group this consisted of physostigmine at a rate of 1.93 mg/hour for 10 min, followed by a constant rate of 0.816 mg/hour for 40 min, before scanning. The same rate was then continued until the end of study so that each subject received no more than 1.3 mg of physostigmine in total. The placebo group received an equivalent volume of saline over the same time course. Both groups of subjects had their blood pressure checked before and at 40 min into infusion; pulse oximetry was performed continuously throughout the experiment. Subjects were also given questionnaires at these two time points to document side effects and subjective ratings (Bond & Lader, 1974).

Cognitive task

Subjects performed a matching task (Vuilleumier et al, 2001) for two black and white photographs situated in either the north–south or east–west positions of a cross-format display that comprised four concurrent photographs (3° × 5° visual angle each), arranged into a cross around a central fixation point (Fig. 5.1). At the start of each block, subjects were cued (for 2 s) to attend selectively to either the two vertically arranged or two horizontally arranged positions, while the alternative two locations were to be ignored throughout the block. In total, there were four blocks of 40 trials each. Each trial consisted of a central fixation cross (1 s) followed by the four-picture display for 250 ms. Subjects were required to indicate, as accurately and rapidly as possible, whether the two stimuli at task-relevant locations were the same or different, by either of two possible key presses with the right hand. Reaction time (RT) and accuracy were recorded.

Figure 5.1: A stimulus example is shown. Before each block the subject was cued to attend either the two horizontal or the two vertical locations via a pair of highlighted frames. During the block subjects were required to perform a same/different judgment for the pair of stimuli at just the task-relevant locations; the other pair of stimuli were task-irrelevant. Each display contained one pair of faces and one pair of houses, with either type equally likely to be at the relevant or irrelevant locations, in an unpredictable sequence. The pair of faces could both be fearful (emotional trial) or both neutral.

Figure 5.1 For legend see above.

Within each trial, either the two attended or the two unattended locations were occupied by two faces, in an intermingled and unpredictable sequence. The remaining two locations were occupied by two houses. Hence each trial could be classified as faces-attended (A) or faces-unattended (U) in this sense (with the type of attended stimulus thus being determined by spatial location). Furthermore, faces could have either a fearful emotion (E) or a neutral (N) expression, independently of whether they were at task-relevant locations. Thus four conditions existed. AE, AN, UE, and UN (where AE, for example, would represent trials where fearful faces appeared at attended task-relevant locations). The four trial types, and pair identities (i.e., same/different, which was independent between the attended and unattended pair in each trial), were randomly counterbalanced throughout each block. The order of task-relevant locations (i.e., either vertical or horizontal) between blocks was randomly selected from one of four alternatives (HVHV, VHVH, HVVH, VHHV) and counterbalanced across subjects within each group.

Although the task design was identical to that used in our recent study of untreated subjects (Vuilleumier et al, 2001), there were four differences in details: (1) the median intertrial interval (2.5 s; range, 1.5–14.4 s) was half that used previously; (2) the number of trials of each type was reduced from 52 to 40; (3) an alternative set of pictoral stimuli were used (faces taken from The Karolinska Directed Emotional Faces set; (Lundqvist, et al, 1998), with each being repeated only once; (4) 40 "null" trials were included in which a blank screen occurred, following a 1-s central fixation cross (thus enabling measurement of any attentional activity in the absence of stimulation: see (Chawla et al, 1999). The first two changes were implemented because of time constraints imposed by drug administration.

The cross-format spatial array and brief exposure time have previously been shown to be effective at engaging covert attention to the relevant pair of locations without saccades (Vuilleumier et al, 2001; Wojciulik et al, 1998), as well as enabling emotional processing without awareness of unattended fearful faces (Vuilleumier et al, 2001). We nevertheless monitored eye movements throughout the task with an infrared eye tracker (ASL Model 540, Applied Science Group Co., Bedford, MA; refresh rate, 60 Hz). For technical reasons, eye-position data were lost for six subjects (two placebo and four from drug group).

Imaging and image processing

MRI data were acquired from a 2-T VISION system (Siemens, Erlangen, Germany) equipped with a head coil. Functional images were acquired with a gradient echo–planar T2* sequence using BOLD contrast. The acquired image consisted of 32 × 3 mm thickness axial slices that covered the entire brain. Volumes were acquired in a single continuous session with an effective repetition time of 3.26. The first eight volumes were discarded, to allow for T1 equilibration effects. Images were realigned, time corrected, normalized to a standard echo–planar image template, and smoothed with a Gaussian kernel of 8 mm full-width half-maximum.

Statistical analysis of images

Data were analyzed with a general linear model for event-related designs (SPM99; Wellcome Dept. of Cognitive Neurology, London, UK; (Friston et al, 1995) using a random effects analysis. Data were globally scaled and high-pass filtered at 1/120 Hz. Individual events were modeled by a canonical synthetic hemodynamic response function and its temporal derivative, aligned with the onset of the picture array. Time-related changes specific to each event type were included using a linear trend model, after being orthogonalized with respect to time-constant effects (Buchel et al, 1999). The six head movement parameters were included as confounds, and incorrect responses were modeled separately. Since face stimuli were presented twice, and repetition effects may themselves be cholinergically modulated (Thiel et al, 2002b), a second model was generated in which repetition effects were included as a separate factor. None of the drug-by-condition interactions presented here could be accounted for by repetition effects (see Experiment 2).

Linear contrasts of parameter estimates for each subject were used to generate statistical parametric maps (SPMs) of the t statistic. We first examined regions specific to attended stimulus type (i.e., attending faces minus attending houses or vice- versa) in the placebo and physostigmine groups separately. We next performed t-tests that directly compared drug and placebo groups for the same contrasts across the whole brain. Similarly, we identified regions activated by emotional versus neutral faces (independent of attention) in each group separately, before comparing drug and placebo groups for this. For all drug × condition interactions, only regions showing a significant effect of face or house attention, or of emotion ($P < 0.001$, uncorrected), in either group are noted. Finally, to characterize the nature of any three-way interactions of attention, emotion, and drug, we performed post hoc ANOVAs on signal estimates of drug x emotion interactions, separately for trials with faces relevant versus irrelevant. Results are listed according to which of the two levels of attention showed a significant drug × emotion interaction ($P < 0.05$); regions in which a significant interaction occurred under both levels of face attention are noted separately.

Since one major issue concerned any cholinergic modulation of fusiform face-responsive areas in the present paradigm, we derived two regions of interest (ROI) from the bilateral fusiform areas identified from our previous study, which had demonstrated attentional modulation to faces in untreated subjects using a similar paradigm (thresholded at $P < 0.05$, uncorrected; (Vuilleumier et al, 2001). We report areas that achieved significance

after correction either within these prespecified ROIs (Worsley et al, 1996) or for the entire brain volume, plus activations that reached $P < 0.001$, uncorrected.

Results

Physiological data, subjective reports, and eye tracking

Questionnaires detailing possible side effects and subjective feelings as well as measures of blood pressure and pulse were recorded before infusion and just prior to scanner entrance, when a steady state of physostigmine would be expected (Furey et al, 2000b). Although subjects given physostigmine with glycopyrrolate were more likely to experience a dry mouth ($U = 62$, $P < 0.05$) and dizziness ($U = 68$, $P < 0.01$), the mean intensity of these symptoms was small (1.3 ± 0.95 and 0.5 ± 0.40, respectively, on a scale of 0 to 6). Two subjects given physostigmine who vomited were excluded and replaced with alternative subjects. A pooled measure of subjective alertness (Bond & Lader, 1974) suggested that the physostigmine group felt more drowsy at test relative to preinfusion (mean percentages of difference between preinfusion and prescan, $-1.3 \pm 2.2\%$ for placebo and $+8.8 \pm 3.7\%$ for physostigmine; $F(1,28) = 6.4$; $P < 0.05$), although the simple effects of group for absolute subjective alertness at each time point were insignificant. There were no significant cardiovascular main effects or interactions.

The frequency of saccades and median angular deviation of the eye were measured during 250-ms epochs before and after the onset of each stimulus. These measurements were entered into a three-way ANOVA with factors of group, attention, and emotion. The

mean percentages of trials with saccades over both epochs were 3.2 and 3.0 under placebo and physostigmine, respectively. There were no reliable group differences during either of the two peristimulus epochs, either as a main effect or as an interaction with condition, for either saccade number or median ocular position. Finally, the same two measurements were compared within the first block only, given some group differences found in task performance for this block (see below). Once again, no significant main effects or interactions with condition were found.

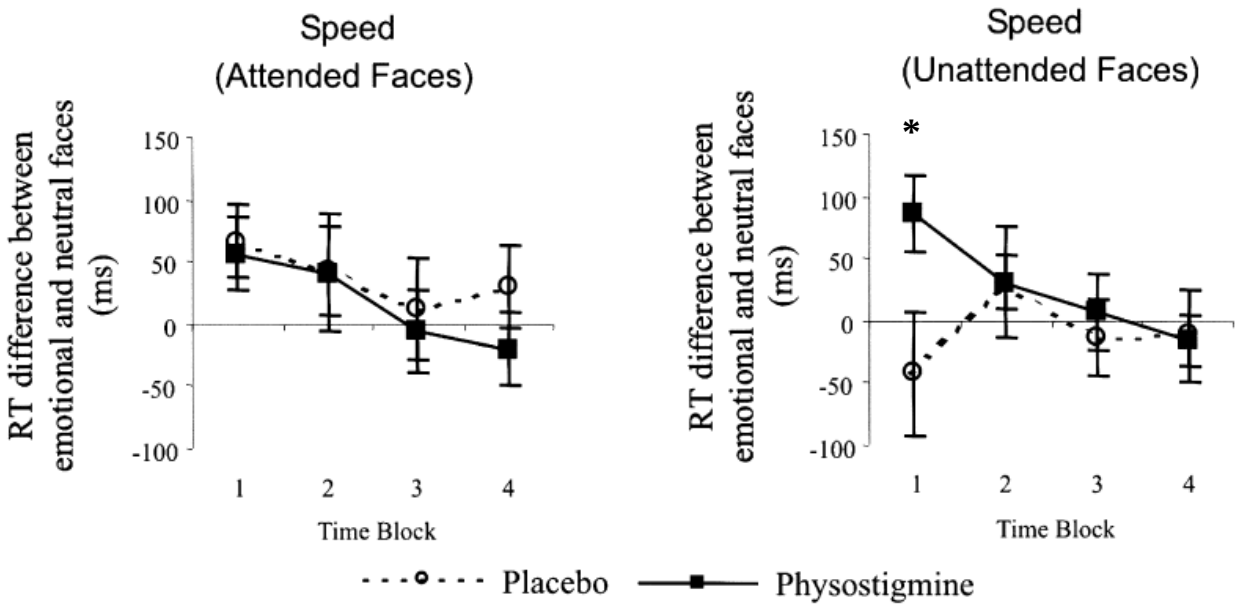
Behavioural

A nonsignificant trend for faster responses was evident with physostigmine (mean RTs, 844 ± 62 ms, 915 ± 62 ms, under drug and placebo, respectively; $t(28) = 1.4$; $P < 0.1$, one-tailed based on (Furey et al, 1997), but there was no difference in accuracy between groups (mean scores, 85 ± 3.2 % and 83 ± 3.2 %, under drug and placebo, respectively; $t(28) = -0.7$; ns; two-tailed hereon). There were no significant effects of group as a function of condition (attention, emotion, or their interaction) for either measure.

It has previously been shown that the effect of emotional and distracting stimuli can habituate with time (Breiter et al, 1996 and Lorch et al, 1984). Consequently, the RT difference between emotional and neutral trials was calculated separately for each of the four successive blocks, with planned group comparisons made in the first block. During face-attended trials, both groups showed a trend to a time-dependent effect of emotion ($F(3,27) = 2.7$; $P < 0.1$), with a significant slowing of RTs to emotional stimuli within the first block only ($t(28) = 2.6$; $P < 0.05$; Fig. 5.2). There were no between-group RT

differences for these face-attended trials. However, during face- unattended trials, the physostigmine group showed significant slowing relative to the placebo group by the presence of an emotional expression in the first block ($t(28) = 2.1$; $P < 0.05$; Fig. 5.2). Because of these behavioral patterns, block sequence was also considered in time-related fMRI analyses.

Figure 5.2: Plots show differences in RT (in milliseconds) between emotional minus neutral trials over block number for placebo and physostigmine groups separately for attended (A) and unattended (B) faces. An asterisk denotes significant between-group difference ($P < 0.05$) for the planned comparison in the first block when emotional effects would be expected to be greatest. Bars denote standard errors.



fMRI data: effects of physostigmine on attentional modulation

We first aimed to replicate the findings from two previous studies using a similar paradigm but with untreated subjects (Wojciulik et al, 1998; Vuilleumier et al, 2001) by examining the placebo group for responses dependent on whether faces or houses fell in the task-relevant locations (i.e., attending faces minus attending houses and vice versa). As in those previous studies, we found bilateral mid-fusiform gyrus activation when faces appeared at task-relevant locations, while bilateral parahippocampal and posterolateral occipital cortices were activated when houses appeared at task-relevant locations (Figs. 5.3A and 5.3B, respectively; all $Z \geq 4.29$; $P < 0.05$, corrected for fusiform ROIs or whole brain). These regions were also all found to be activated by the same contrasts in the physostigmine group (all $Z \geq 3.30$; $P < 0.001$, uncorrected).

We next identified regions whose differential activity for faces relative to houses (or vice versa) was greater under physostigmine relative to placebo (or vice versa; Table 5.1). With faces versus houses in attended locations, physostigmine enhanced differential activation in left anterior fusiform gyrus (Fig. 4.3C) relative to placebo. This region failed to show a significant effect of face attention under placebo. The only regions showing less differential activity under drug, relative to placebo, for faces in attended versus unattended locations were bilateral insula.

With houses versus faces in attended locations there were no enhancements of activity due to physostigmine. However, physostigmine reduced differential activity in the right posterolateral occipital cortex relative to placebo (Fig. 5.3D). Thus, physostigmine

engendered an opposite attentional effect depending on either the stimulus-processing region or the stimulus type falling within attended locations: fusiform gyrus showed increased attentional enhancement (for attending faces minus houses), while posterolateral occipital cortex showed reduced modulation by attention (for attending houses minus faces) under physostigmine relative to placebo.

To unconfound the effect of drug on extrastriate region (fusiform and posterolateral occipital cortices) from stimulus type (faces and houses), we further tested whether the observed region-specific effects of physostigmine might correspond to a modulation of activity even in the absence of stimuli. We tested for this by comparing activations between groups in occipitotemporal regions on "null trials," when subjects were cued but no stimulus appeared (Chawla et al, 1999). Note that these null events were modeled separately from, and hence are orthogonal to, session mean activity (thus any drug-induced changes cannot reflect overall changes in mean activity throughout a session for particular brain areas). Comparing groups, this contrast (Table 5.2) showed that physostigmine, relative to placebo, resulted in greater null trial activity in anteroinferior temporal regions (Figure 4.3E), including left anterior fusiform, which showed the drug-by-face attention interaction described above ($t(28) = 2.3$, $P < 0.05$; Fig. 5.3C). Conversely, physostigmine, relative to placebo, resulted in reduced activity in posterior occipital regions (Fig. 5.3F), although this failed to reach significance in that region showing less differential activity for house attention under physostigmine (Fig. 5.3D). These results indicate that the effects of physostigmine on selective attention may partly reflect region-specific changes in activity, independently of stimulus (but related to

spatial cueing), which may then either increase or decrease the differential response to attended versus unattended stimuli that are selectively processed in these regions. Furthermore, these drug-induced changes occurred only when attention was spatially cued, as shown by the fact there were no between-group differences in session means for either the inferior temporal or posterior occipital regions identified by the group-by-attention and group-by-null trial interactions.

Figure 5.3. (See next page): Regions of inferotemporal cortex showing differing effects of physostigmine on attentional processing in face (A,C,E) and house-selective regions (B,D,F). Red represents regions that in the placebo group showed an increased response to faces (A) or to houses (B) in attended versus unattended locations; the physostigmine group displays similar effects in both fusiform and parahippocampal areas. Yellow represents regions in which physostigmine, relative to placebo, modulated the attentional effect by either increasing the differential response to faces in attended versus unattended locations (C) or decreasing the differential response to houses in attended versus unattended locations (D). Cyan represents regions in which physostigmine, relative to placebo, resulted in increased (E) or decreased (F) responses to null trials, i.e., when subjects were cued, but no stimulus appeared. Contrasts are thresholded at $P < 0.001$, uncorrected. Regions in E and F also showed selectivity for face and house attention, respectively, over both groups ($P < 0.01$, uncorrected). Activation maps are superimposed on a single-subject T1-weighted MRI brain. Graphs show percentages of signal change from baseline with faces in attended locations, houses in attended locations, and null trials for drug and placebo groups, mean-corrected between regions.

Figure 5.3: For legend see previous page.

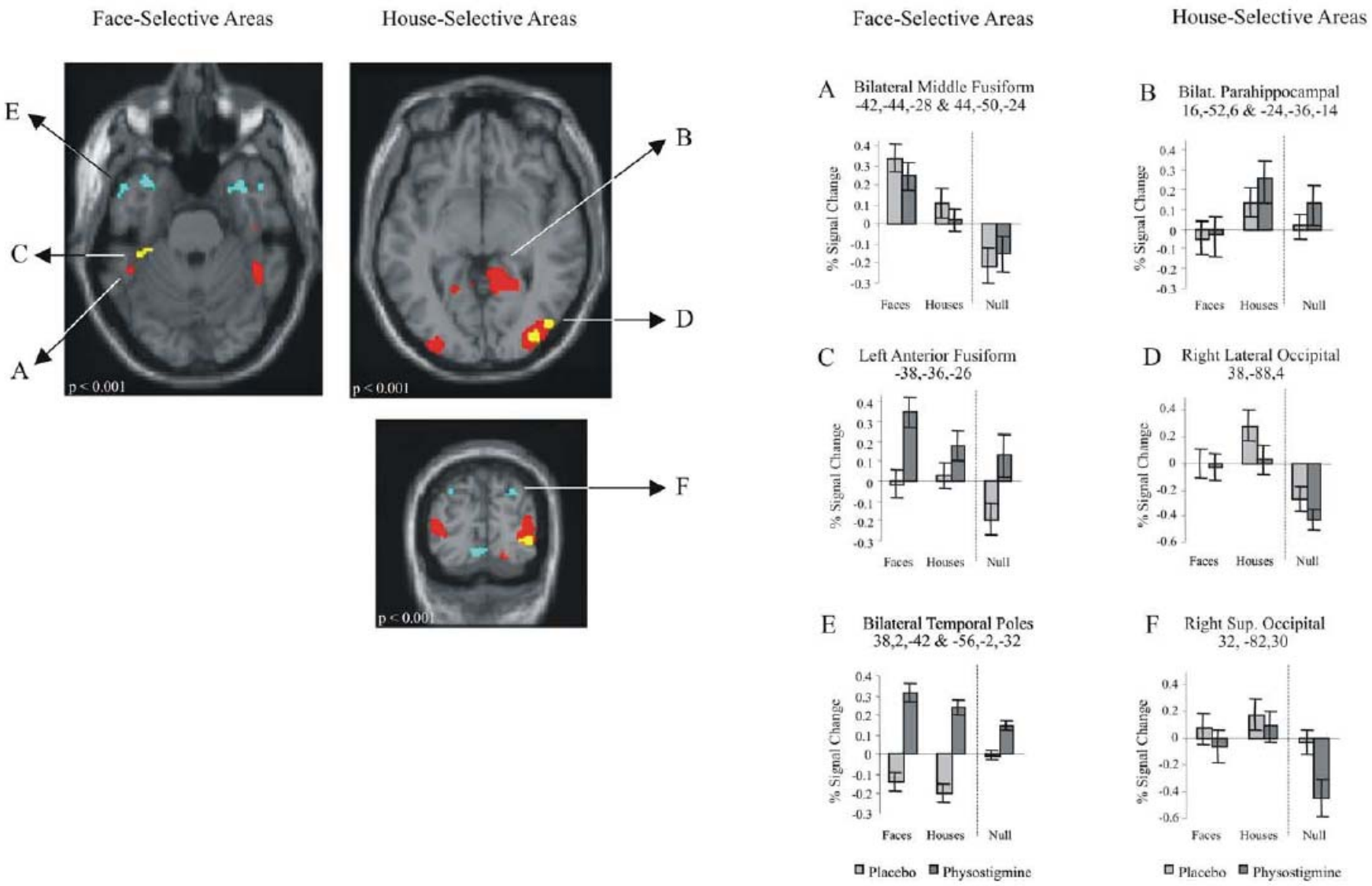


Table 5.1. Effects of drug on attentional modulation of face and house processing

Area	*x*	*y*	*z*	*Z* value
(A) Attentional enhancement of face processing: increased by physostigmine				
Left anterior fusiform gyrus	−38	−36	−26	4.23
(B) Attentional enhancement of face processing: decreased by physostigmine				
Left insula/claustrum	−30	−18	6	4.34
Right insula	44	−20	10	3.57
(C) Attentional enhancement of house processing: increased by physostigmine				
No areas approached significance				
(D) Attentional enhancement of house processing: decreased by physostigmine				
Right lateral occipital gyrus	38	−88	4	4.50

Table 5.2. Effect of drug on the occipitotemporal cortex during null trials

Area	*x*	*y*	*z*	*Z* value
(A) Null trial activity increased by physostigmine				
Left temporal pole	−56	−2	−32	4.67
	−30	6	−36	3.80
Left inferior temporal gyrus	−50	−14	−34	3.37
Right temporal pole	38	2	−44	3.74
Right collateral sulcus	26	−40	2	3.81
(B) Null trial activity decreased by physostigmine				
Lingual gyrus	0	−86	−6	4.41
	16	−72	−12	4.00
Left posterior fusiform gyrus	−30	−70	−22	4.37
Right posterior fusiform gyrus	46	−72	−14	4.18
Right superior occipital gyrus	32	−82	30	3.35
Left middle occipital gyrus	−42	−74	16	3.25

Note. Null trials represent trials in which subjects had previously been spatially cued but no stimuli appeared following an alerting central fixation cross. The mean session activity is modeled separately and was found not to be significantly different between placebo and physostigmine groups in the regions listed.

fMRI data: effects of physostigmine on response to fearful expression

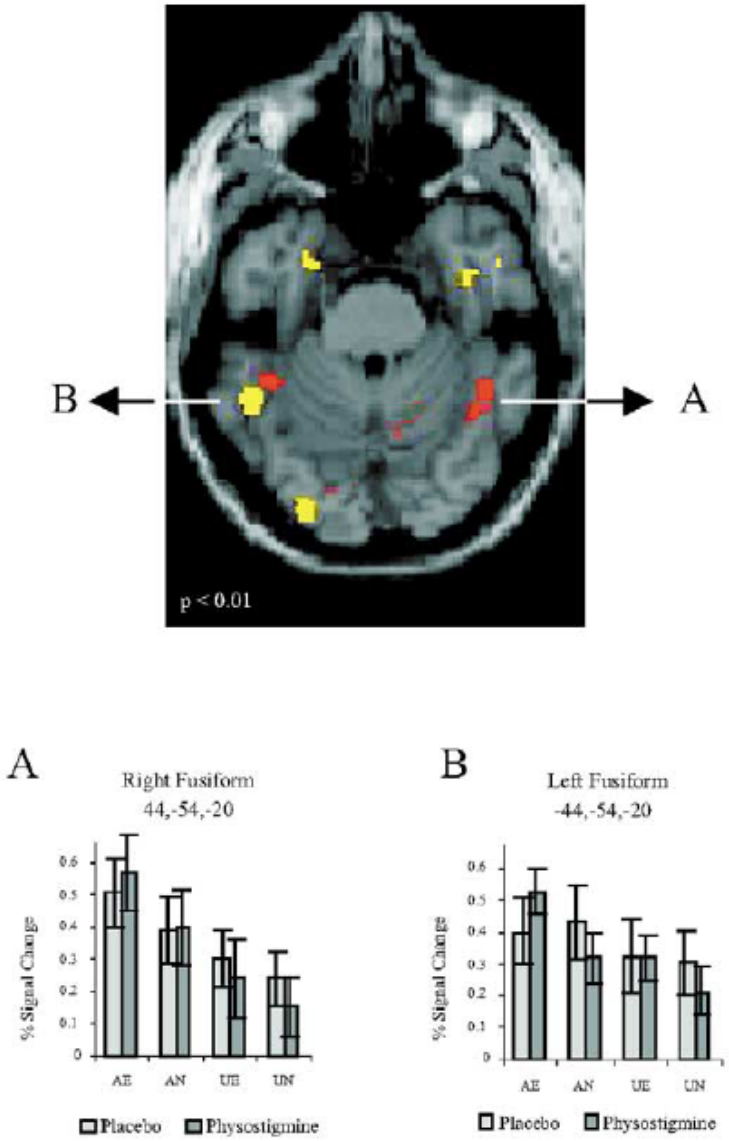
The orthogonal contrast of fearful minus neutral faces was performed, independently of whether faces were attended or unattended, on each trial. Within extrastriate cortices, the placebo group demonstrated heightened activity to fearful relative to neutral faces in left mid-fusiform cortex (−40, −48, −24; $Z = 3.27$; $P < 0.001$, uncorrected). In addition, the same voxel in right mid-fusiform cortex (44, −52, −20) that showed emotion modulation in our previous study (Vuilleumier et al, 2001) demonstrated a similar effect in our data, but at a lower significance ($Z = 2.64$; $P < 0.01$, uncorrected; Fig. 5A). These areas were also activated by emotional stimuli under physostigmine (all $Z > 3.09$, $P < 0.001$,

uncorrected). The main effect of emotion over all subjects also identified a region extending between the hypothalamus and posterior medial amygdala (10, −8, −16; Z = 3.49; P < 0.001, uncorrected; and 12, −10, −16; Z = 3.06; P < 0.05 corrected for right amygdala volume identified in (Vuilleumier et al, 2001), thresholded at P < 0.01, uncorrected). Furthermore, a more lateral amygdala-centred activation was activated just below threshold (30, −6, −18; Z = 2.45; P = 0.007, uncorrected). Activity in both of these areas was independent of attention (in keeping with Vuilleumier et al, 2001) and group.

Comparing drug and placebo groups for the effect of emotion, we found regions in left middle fusiform (−48, −52, −22; Z = 2.85; P < 0.05, corrected for ROI; Figure 4.4B) and inferior occipital cortex (−24, −94, −8; Z = 3.37; P < 0.001, uncorrected) that showed a greater differential response to emotional versus neutral stimuli under physostigmine, relative to placebo. Furthermore, by examining emotional effects in the extrastriate cortex that habituated with time (Buchel et al, 1999, Morris et al, 2001; Rotshtein et al, 2001)—in keeping with the time-dependent patterns observed behaviorally—we found that left mid-fusiform cortex also showed a stronger relative response to emotional stimuli with drug relative to placebo, as a function of time (−34, −52, −6; Z = 3.26; P < 0.001, uncorrected). There were no regions in which an emotional activation under placebo was significantly reduced by physostigmine.

Figure 5.4. (See next page): (A) Regions of the inferotemporal cortex showing the effect of physostigmine on emotional processing. Red represents regions in the placebo group that showed an increased response to emotional versus neutral faces, the physostigmine group can be seen to display similar effects here (A). Yellow represents regions in which physostigmine, relative to placebo, resulted in an enhanced differential response to emotional versus neutral faces (B). Both contrasts are thresholded at $P < 0.01$, uncorrected (so as to illustrate less significant activation of the right fusiform in the placebo group—in the same region previously showing emotional modulation in untreated subjects: (Vuilleumier et al, 2001). Activation maps are superimposed on a single-subject T1-weighted MRI brain, pitched to visualize both contrasts. Graphs represent percentages of signal change from baseline during emotional and neutral trials with faces in attended (AE and AN) or unattended (UE and UN) locations for drug and placebo groups, mean-corrected between regions. The voxels chosen are based upon the two voxels in our previous study (Vuilleumier et al, 2001) showing the most significant modulation of the fusiform cortex by attention. In the right fusiform (A), both groups show a positive main effect of emotion ($P < 0.01$); in the left fusiform, physostigmine shows a main effect of emotion ($P < 0.005$), but not placebo; a group by emotion interaction was also observed here ($P < 0.005$).

Figure 5.4. For legend see previous page.



fMRI data: effects of physostigmine on the interaction of attention with emotion

Finally, we examined physostigmine modulation of emotional responses as a function of whether emotional faces were task-relevant or task-irrelevant. The majority of effects were in regions previously found to exhibit an attention x emotion interaction (Vuilleumier et al, 2001, Armony and Dolan, 2002; Perlstein et al, 2002). Thus physostigmine versus placebo showed enhanced differential activity for emotional faces, specifically when task-irrelevant (i.e. (UE-UN)-(AE-AN)), in left lateral orbitofrontal cortex, temporal pole, and anterior cingulate, while decreasing activity in the right intraparietal sulcus for the equivalent contrast (Table 5.3; Fig. 5.5A).

Areas where physostigmine, relative to placebo, showed enhanced differential activity for emotional faces when task-relevant (i.e. (AE-AN)-(UE-UN)), faces were seen in the left dorsolateral prefrontal and medial prefrontal cortex (Table 5.3). The only areas where physostigmine reduced activity related to the emotion of task relevant faces were in the ventral striatum and medial orbitofrontal cortex.

To complement our RT findings of physostigmine-induced, time-dependent effects for fearful faces in task-irrelevant locations, we also examined fMRI data for an interaction of drug × task-irrelevant emotion that habituated over the course of the experiment (using a linearly decreasing time model (Buchel et al, 1999). Results of this analysis were broadly similar to those of the time-independent fMRI effects. Thus left lateral orbitofrontal cortex (−38, 32, −8), adjacent inferior frontal cortex (−44, 38, 6), right temporal pole (48, 8, −20), plus left intraparietal sulcus (−40, −58, 46; $Z \geq 3.85$ for all

above; Fig. 5.5B) showed stronger time-dependent effects for task-irrelevant emotional versus neutral faces under physostigmine relative to placebo, while left superior parietal (−14, −56, 68) and right occipital cortices (32, −82, 36; $Z \geq 3.36$; $P < 0.001$, uncorrected for all above) showed a reduced effect with physostigmine for the equivalent contrast.

Figure 5.5. (See next page): Regions showing modulation of emotional responses by physostigmine selectively when faces were task-irrelevant on examination of both time-independent (A) and time-dependent (B) effects. (A) The lateral orbitofrontal and right intraparietal regions showing a significant drug by emotion by attention interaction, due to a predominant effect within face-unattended trials. Statistical maps are overlaid on a single-subject T1-weighted MRI Graphs represent percentages of signal change from baseline during face-attended emotional and neutral trials (AE and AN) and face-unattended emotional and neutral trials (UE and UN) for drug and placebo groups. The orbitofrontal cortex demonstrated a significant enhancement to task-irrelevant emotional stimuli under physostigmine only (*$P < 0.005$ for post hoc contrast of UE–UN), whereas the right intraparietal sulcus demonstrated reduced activity to task-irrelevant emotional stimuli under physostigmine only (**$P < 0.001$ for post hoc contrast of UN–UE). (B) A representative profile of activity in the lateral orbitofrontal and left intraparietal regions identified in the interaction of drug x emotion is shown examining condition-specific effects modeled with a linear time-dependent response function, specifically for trials when faces were task-irrelevant. Plots depict the best fitting peak canonical response over trial number of the subject from each of the placebo (dashed) and physostigmine (solid) groups showing the median effect size of task-irrelevant emotion for fearful (red) and

neutral (green) trials. There were no drug by emotion interactions in these two regions for task-relevant faces.

Figure 5.5: For legend see above.

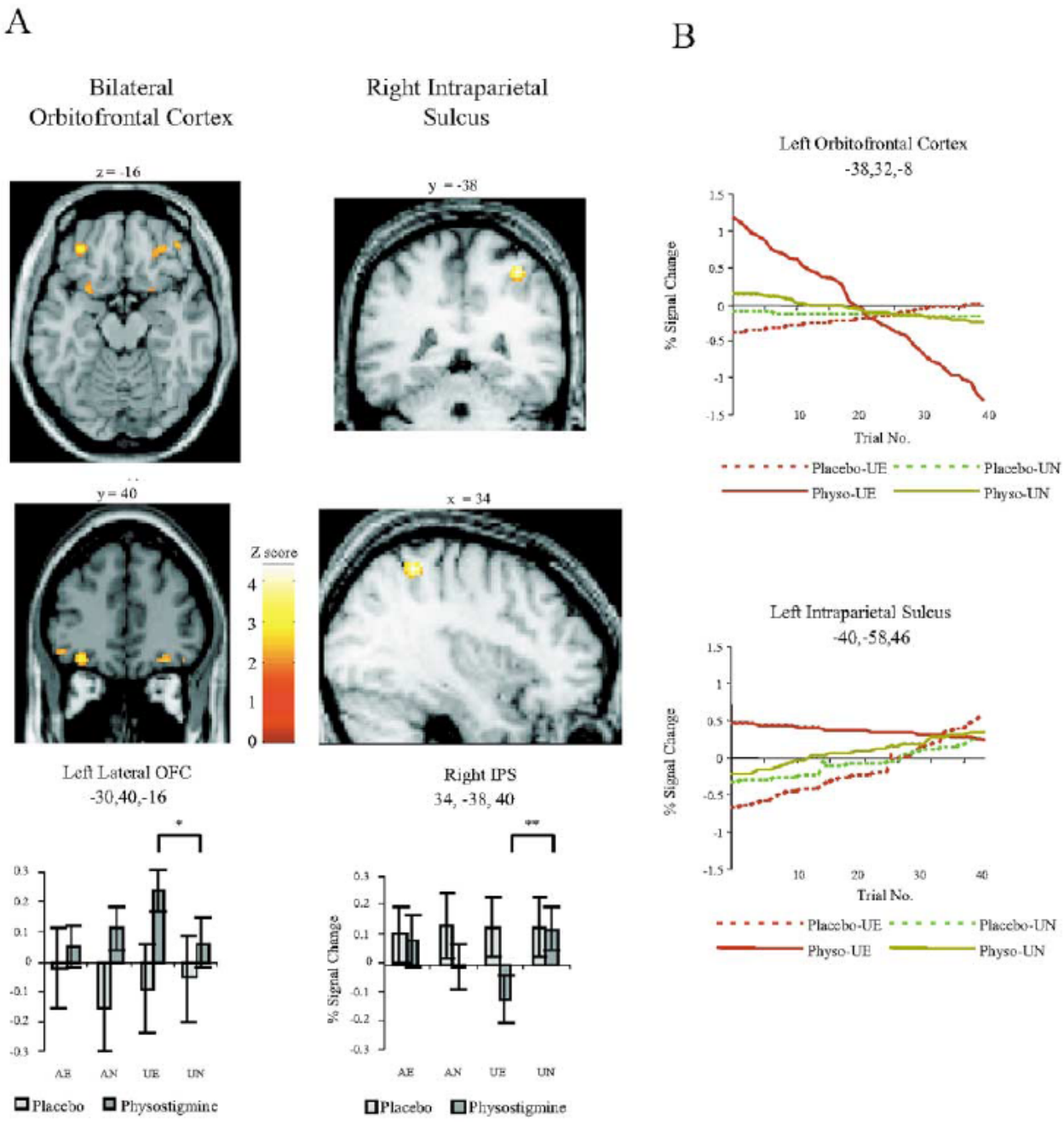


Table 5.3: Areas showing effects of drug on interaction of attention with emotion

Area	*x*	*y*	*z*	*Z* value[b]
(A) Enhanced activity to task-irrelevant emotion by physostigmine				
Left inferior temporal pole	−44	6	−44	4.56
Anterior cingulate	−2	18	22	3.83
Left lateral orbitofrontal cortex	−30	40	−16	3.49
(B) Reduction of activity to task-irrelevant emotion by physostigmine				
Right intraparietal sulcus	34	−38	40	4.17
Left precentral gyrus	−34	−12	52	3.39
Left superior temporal sulcus[a]	−52	−14	−8	4.16
(C) Enhancement of activity to task-relevant emotion by physostigmine				
Left dorsolateral prefrontal cortex[a]	−32	52	26	4.90*
	−40	30	18	4.39
Medial prefrontal cortex	6	46	26	3.64
(D) Reduction of activity to task-relevant emotion by physostigmine				
Ventral striatum[a]	−24	8	−4	3.41
Right medial orbitofrontal cortex	22	48	−8	3.36

Note. The formal notation for the interactions referred to in the table are (A and D) physostigmine [(UE-UN)-(AE-AN] > placebo [(UE-UN)-(AE-AN)]; (B and C) physostigmine [(AE-AN)-(UE-UN]>placebo [(AL-AN)-(UE-UN)].

[a] Areas showing significant drug x emotion interactions with both task-relevant and task-irrelevant faces are listed under the heading appropriate for the stronger effect.

[b] All *Z* scores represent $P < 0.001$, uncorrected, except* (corrected for whole brain volume).

Discussion

Cholinergic modulation of attentional effects within visual cortex

The task employed here has previously been shown in untreated subjects to activate face-related and house-related regions of extrastriate cortex differentially, depending solely on endogenous spatial attention (i.e., when both types of stimuli are presented on every trial, but with only one type falling at the currently attended locations; (Vuilleumier et al, 2001; Wojciulik et al, 1998). We now show that physostigmine enhanced the anterior fusiform gyrus (a region linked to higher level processing of faces: George et al, 1999) for attended versus unattended faces, but suppressed differential responses in posterolateral occipital cortex for attended versus unattended houses. These results suggest that while physostigmine enhances the effect of selective attention within the extrastriate visual cortex, not all stimulus processing regions, or stimulus types, are affected in a similar fashion. The cortical cholinergic system may thus facilitate selective

attention not only via general influences on "top-down" processes within frontoparietal cortices (Sarter et al, 2001; Himmelheber et al, 2001)—which would predict parallel effects for face and house-selective regions—but also through region-specific effects in sensory perceptual areas. The fact that *anterior* fusiform cortex showed this drug interaction rather than *mid*-fusiform is possibly because mid-fusiform was already maximally modulated by selective attention.

To distinguish whether these distinct effects of physostigmine on attentional processing were a function of extrastriate cortical region (fusiform or posterolateral occipital cortex) or stimulus type attended (faces or houses), we examined activity in these same regions on null trials, when subjects were cued but no stimulus appeared. This showed that event-related activity in anterior fusiform cortex was enhanced, while that in occipital cortex was decreased by physostigmine, even in stimulus absence. Thus, drug-induced changes on null trials occurred in the same direction as when a stimulus was present and were associated with similar changes in the differential responses caused by attention. This may suggest that physostigmine modified the general responsiveness of extrastriate cortex according to region, rather than stimulus type. Importantly, these drug-induced regional modulations were observed only to the event related responses to cued trials and were not seen as group differences in baseline (mean session) activity. Furthermore, the profile of response in many regions e.g. left anterior fusiform cortex, cannot be modelled by a simple scaling factor in the presence of drug.

It is noteworthy that similar regional effects of cholinergic modulation have been found in previous functional imaging studies, across a wide variety of tasks. Thus while posterolateral occipital areas have been associated with cholinergic-induced activity decreases (Mentis et al, 2001; Grasby et al, 1995; Thiel et al, 2001), fusiform cortex has been associated with cholinergic-induced increases (Furey et al, 1997; Furey et al, 2000a, Rosier et al, 1999; Thiel et al, 2002b); some of these studies employed cholinergic antagonists to demonstrate the converse effects). Our data extend these findings by showing regional differences in cholinergic modulation for effects of selective attention, when stimuli and task are kept constant.

A further interpretation of the extrastriate region-specific effects of physostigmine observed here was that they were dependent on the stimuli expected. Hence cholinergic enhancement may have biased activations in advance of any stimulation (Chawla et al, 1999) to favor enhancements of face attention (in fusiform) and reductions of house attention effects (in posterolateral occipital cortex). However, any biasing cannot have taken the extreme form of the physostigmine group always attending to the faces, regardless of whether these were task-relevant. This could only have reduced differential activation for task-relevant versus irrelevant faces in the fusiform cortex, whereas in fact physostigmine either enhanced this effect (left anterior fusiform) or did not diminish it (bilateral mid-fusiform). Thus, in terms of brain responses, top-down selection continued to operate under physostigmine, but with task-relevant faces becoming particularly dominant, in keeping with the anteroinferior temporal activations.

Neuropharmacological studies have found that acetylcholine can result in differing relative levels of activation versus inhibition in the visual cortex depending on local factors (Xiang et al, 1998; Muller & Singer, 1989; Sillito & Kemp, 1983). Within the inferior temporal cortex, cholinergic stimulation has been proposed to underlie the diffuse activation seen at the start of new delayed-match-to-sample trials when attention is heightened (Sohal & Hasselmo, 2000; Furey et al, 2000a). Furthermore, the anteroinferior temporal cortex represents a unique sensory region in having projections both to and from the nucleus basalis (Mesulam & Mufson, 1984) and so may influence its own activation through a cholinergic-dependent feedback loop (Sohal & Hasselmo, 2000). Conversely, early visual cortical areas have been found to be inhibited by acetylcholine in all layers except layer IV (Kimura et al, 1999), which may favor feedforward over feedback activity (Hasselmo, 1995) and enhance direction and orientation specificities (Murphy & Sillito, 1991). Consequently, the contrasting activity profile between inferior temporal and occipital cortices observed here in response to systemically administered anticholinesterase may reflect such regional differences of net neural activation versus inhibition. Moreover, our finding that such changes in activity were trial specific may relate to the fact that endogenous cholinergic release elevates with attentional demand (Gill et al, 2000).

Cholinergic modulation of emotional effects within visual cortex

Corticopetal cholinergic fibers appear to be involved in both filtering out distractors (Gill et al, 2000), consistent with the modulation of attentional effects reported here, and enhancing responses to stimuli of emotional significance (Acquas et al, 1996). One

mechanism for this may involve direct cholinergic modulation of the visual cortex, similar to that found in the auditory (Weinberger, 1998) and somatosensory cortices (Delacour et al, 1990) during conditioning in rodents. Moreover, connections between the amygdala, nucleus basalis, and sensory cortical regions may provide one pathway (Amaral et al, 1992) by which emotional discriminations within the amygdala can facilitate relevant stimulus processing (Wilson & Rolls, 1990, Kapp et al, 1990, Weinberger et al, 1990, Friston et al, 1994; Morris et al, 2001).

The results of our placebo group, and those of a previous report employing the present study design (without drug: Vuilleumier et al, 2001) found that the right mid-fusiform gyrus was modulated by both attention and emotion separately (i.e., additively; Figure 4.4A). We now demonstrate that cholinergic enhancement can increase the extent of emotional modulation within the left mid-fusiform gyrus (Figure 4.4B), as well as the posterior occipital cortex. As with the enhancement of face attention discussed earlier, the left laterality of this drug effect may arise because the right fusiform is already highly sensitive to faces and their emotion, while the left fusiform becomes more so with cholinergic enhancement. The fact that left fusiform cortex also showed enhanced responses to emotional stimuli when we modeled time habituating effects (akin to those seen in behavior; see also (Buchel et al, 1999, Breiter et al, 1996, Morris et al, 2001 and Rotshtein et al, 2001) is consistent with cell recordings in the substantia innominata suggesting that cortical cholinergic stimulation occurs selectively with novel, behaviorally relevant stimuli (Wilson & Rolls, 1990).

Finally, we note that in addition to enhancing fusiform gyrus activity, the main effect of fearful versus neutral faces activated a region around the right hypothalamus–dorsomedial amygdala, with only a trend for activation centred on the right amygdala proper. In this respect, our findings in the placebo group did not entirely replicate those in our earlier study (Vuilleumier et al, 2001). However, this difference might be attributable to a change in several parameters, including stimulus set, number of events, interstimulus interval, statistical analysis (see Methods), as well as the stress of venipuncture and infusion. We note that the two amygdala-related regions showing fear-related activations were unaffected by either attention (as in Vuilleumier et al, 2001) or drug (consistent with the amygdala being upstream of nucleus basalis activation).

Cholinergic modulation of attention–emotion interactions in frontoparietal cortex

Cholinergic projections from the nucleus basalis to the frontoparietal cortex and thalamus may provide a means by which emotional processing engages attention (Holland & Gallagher, 1999; Friston et al, 1994); when overactive, this may contribute to clinical anxiety (Berntson et al, 1998; Hart et al, 1999). Previous functional imaging studies have identified distinct frontoparietal regions that respond to emotional stimuli in a manner that depends on the degree to which the stimuli are task-relevant (Vuilleumier et al, 2001, Armony & Dolan, 2002; Perlstein et al, 2002). By examining areas that showed an interaction of attention by emotion by drug, we found that many of these regions also displayed a cholinergic-induced modulation of responses to emotional faces that depended on whether the faces were task relevant.

Physostigmine, relative to placebo, resulted in an enhancement to task-irrelevant fearful faces in the lateral orbitofrontal cortex (OFC), anterior cingulate, and temporal pole, whereas the right intraparietal sulcus showed a decrement in response for the equivalent contrast (Fig. 5.5A). The lateral OFC and left intraparietal sulcus also showed physostigmine-specific enhancements to task-irrelevant fearful faces that decreased with time (Fig. 5.5B), in line with a parallel impairment in RTs under physostigmine that was similarly time-dependent (Fig. 5.2). These data support the view (Armony & Dolan, 2002; Elliott et al, 2000) that this network of areas relays information about the affective value of background stimuli to attentional processes and, furthermore, they show an increase in this effect with cholinergic enhancement. Animal studies have previously shown that the OFC is intimately connected with cholinergic fibers of the nucleus basalis (Cavada et al, 2000) and is activated by acetylcholine during reinforcement (Aou et al, 1983), while cholinergic modulation of the parietal cortex influences covert spatial attention (Davidson & Marrocco, 2000; Cavada et al, 2000), including that toward reward-associated stimuli (Chiba et al, 1995; Bucci et al, 1998). Here we have demonstrated that cholinergic enhancement both modulated activity in the OFC and parietal cortex, and resulted in impairment of performance, specifically under the condition of task-irrelevant fearful faces (in a time-dependent manner for both).

By contrast, physostigmine increased responses to task-relevant fearful faces in dorsolateral and medial prefrontal cortices, which have previously shown emotion-sensitive task-relevant activity (Simpson et al, 2000, Gray et al, 2002; Perlstein et al, 2002). These regions have also been found to depend on cholinergic afferents for both

selective attention (Muir et al, 1996; Gill et al, 2000) and enhancement of cortical responses to emotional stimuli (Mark et al, 1996; Acquas et al, 1996; Thiel et al, 1998; Pirch et al, 1992). The pattern of activity in prefrontal areas under physostigmine seen here is particularly in keeping with a model of anxiety which proposes excessive cholinergic stimulation of the prefrontal cortex as a means by which fearful stimuli are processed excessively (Hart et al, 1999; Berntson et al, 1998). In contrast to the case with task-irrelevant emotional stimuli, RTs were impaired with task-relevant emotional stimuli to a similar extent under physostigmine and placebo, suggesting a ceiling effect in placebo.

Conclusion

Our study has shown that neural correlates of both selective attention and emotional processing can be independently enhanced by physostigmine in the fusiform gyrus. By contrast, physostigmine decreased differential activation due to attention in the posterolateral occipital cortex. As these changes occurred even in the absence of stimuli we suggest that acetylcholine may modulate attention according to extrastriate region, rather than stimulus type. Physostigmine also modulated responses to emotional stimuli depending on whether they were task-irrelevant (in orbitofrontal and intraparietal cortices) or task-relevant (in dorsolateral and medial prefrontal cortices). These results demonstrate that despite their diffuse neocortical innervation, cholinergic projections may modulate attention-related and emotion-related activity in distinct parts of extrastriate and frontoparietal cortices.

6. EXPERIMENT 2:

Effects of ChEI on Repetition Priming

Introduction

Stimulus repetition is associated with decreases in cortical activity (Buckner et al, 1998), which may reflect more efficient stimulus processing and underlie perceptual priming - a type of implicit memory (Schacter & Buckner, 1998). The present study sought to determine whether cholinergic enhancement with the anticholinesterase physostigmine.would modulate behavioural and fMRI repetition effects. Behavioural and haemodynamic measures of priming have both previously been found to be impaired by cholinergic blockade with scopolamine (Thiel et al, 2001, Thiel et al, 2002a), in line with known effects of acetylcholine on cortical plasticity and learning (e.g. Rasmusson, 2000). By contrast, cholinergic enhancement improves an fMRI measure of perceptual processing in extrastriate cortex, selectively for stimuli that must be remembered (Furey et al, 2000a). Cholinesterase inhibition also increases the proportion of studied words used on a subsequent (incidental) word-completion task, in Alzheimer's disease (Riekkinen & Riekkinen, 1999). On this basis, plus the fact that cholinergic modulation is thought to favour processing selectively of attended stimuli (Sarter et al, 2001), we hypothesised that physostigmine should result in greater neural and behavioural repetition effects specifically for attended (task-relevant) stimuli. Furthermore, since acetylcholine has been shown to enhance cortical plasticity specifically for emotional stimuli, as in fear-conditioning (Weinberger et al, 1998; Ji et al, 2001; Thiel et al, 2002b), we predicted that priming effects to fearful, relative to neutral, faces would be greater under physostigmine.

The investigation of cholinergic modulation of repetition effects in the current study is embedded within the design of Experiment 1. As such, the dataset also permits analysis of possible interactions between attention, emotion and repetition, within the

placebo-treated group. This is interesting in itself because the degree to which repetition priming and/or suppression operates automatically (Wiggs & Martin, 1998; Desimone, 1996), versus being influenced by top-down factors (Henson et al, 2002) remains unclear. This is especially so in sensory-perceptual areas, where repetition suppression occurs most robustly (Badgaiyan, 2000). Similar repetition decreases for both target and foil faces have been observed in extrastriate visual cortex during a working memory task (Jiang et al, 2000), and in superior temporal gyrus to written words following both divided and full attention study phases using spoken words (Badgaiyan et al, 2001). On the other hand, some differences in face-repetition effects in visual cortex have been observed depending on task (Henson et al, 2002; Reber et al, 1998), in keeping with electrophysiological data (Puce et al, 1999; Dale et al, 2000) suggesting that sensory repetition effects may be influenced by re-entrant signals from regions involved in higher levels of processing. If repetition effects in sensory cortex are entirely dependent on task-related processing, e.g. in prefrontal cortex, then it might be expected that stimuli which are completely irrelevant to the task (at both study and test) would not engender repetition effects. Our first aim in the current experiment was to determine whether repetition effects in extrastriate visual cortex as measured by fMRI, would be observed for faces both when selectively attended and ignored as distractors.

A further factor that may influence priming, independently of attention, is the emotional value of a stimulus. Emotional stimuli can enhance activity within extrastriate visual areas separately from an effect of attention (Vuilleumier at al, 2001), which appears to be involved in association learning in the context of fear conditioning (Morris et al, 2001). Since priming may represent a similar form of

perceptually-based implicit memory (Schacter & Buckner, 1998), it might also be expected to be enhanced by emotion. Behavioural studies have suggested a priming benefit for emotional stimuli in healthy adults (LaBar and Phelps, 2002), as well as depressed and social phobic patients (Watkins et al, 2000; Lundh and Ost, 1997). On the other hand, unpleasant (versus neutral) faces have been found in one fMRI study to result in less of a repetition decrease in temporo-occipital cortex, which was interpreted in terms of reduced adaptation to negative valence stimuli (Rotshtein et al, 2001). An alternative explanation, however, was that the critical faces in this particular study were not just unpleasant but also bizarre (i.e. mouth and eyes were inverted), which could have influenced repetition effects through indirect attentional factors. In the present experiment we examined the effect of face-emotion on repetition effects, orthogonally from any effect of attention, by using an event-related design which repeated faces only once so as to minimise habituation (Brown and Xiang, 1998).

Methods

Subjects

As for Experiment 1.

Drug treatment

As for Experiment 1.

Cognitive task

Subjects performed the same matching task as described in Experiment 1 (after Vuilleumier et al., 2001). However, now the critical factor lay in analysis of stimulus

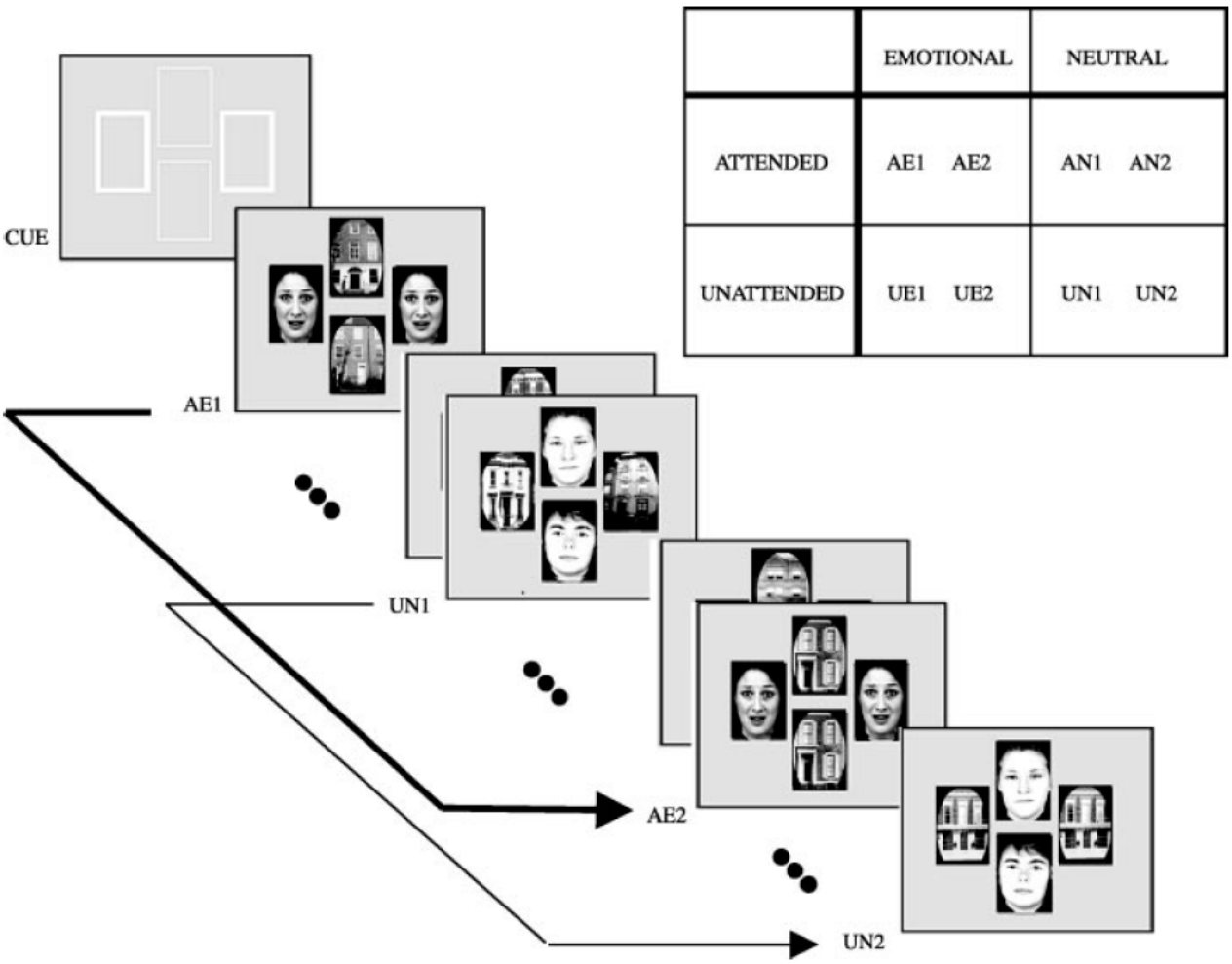
order since each stimulus was shown twice (Fig. 6.1). At the start of each block, subjects were cued (for 2 secs) to attend selectively to either the two vertically-arranged or two horizontally-arranged positions, while the alternative two locations were to be ignored throughout the block. In total, there were four blocks of forty trials each. Each trial consisted of a central fixation cross (1 sec) followed by the four-picture display for 250 ms. Subjects were required to indicate, as accurately and rapidly as possible, whether the two stimuli at task-relevant locations were the same or different, by either of two possible key presses with the right hand. Reaction time (RT) and accuracy were recorded. The median intertrial interval was 2.5 secs (range: 1.5 – 14.4 secs).

Within each trial, either the two attended or the two unattended locations were occupied by two non-famous faces (taken from The Karolinska Directed Emotional Faces set; Lundqvist et al, 1998), in an intermingled and unpredictable sequence. The remaining two locations were occupied by two house pictures. Hence a trial could be classified as faces-attended (A) or faces-unattended (U) in this sense, depending on where the faces were placed with respect to the currently attended locations. Furthermore, both faces could either have a fearful emotion (E) or neutral (N) expression, independent of whether they were at task-relevant locations. This resulted in four conditions (AE,AN, UE and UN faces); these conditions plus the pair identities (i.e. same/different, which was independent between the attended and unattended pair in each trial), were randomly counterbalanced throughout each block. The order of task-relevant locations (i.e. either vertical or horizontal) between blocks was randomly picked from one of four alternatives (HVHV, VHVH, HVVH, VHHV), and counterbalanced across subjects within each group.

Stimulus repetition only occurred for particular face stimuli. Every first presentation of a pair of faces (suffixed 1 henceforth) was followed by only one other repetition of the same pair of faces (suffixed 2) that occurred after an interval of two to five intervening trials (equally distributed across each block). Thus eight conditions existed: AE1, AE2, AN1, AN2, UE1, UE2, UN1 and UN2 (where AE1, for example, would represent first presentation of a particular pair of fearful faces appearing at task-relevant locations, and AE2 would represent their subsequent repetition). There were twenty trials of each condition per subject. Owing to the small number of intervening stimuli between first and second repetitions, the influence of any time confound would be minimal (< 5% trials fell outside of the time window in which stimulus order was fully counterbalanced with respect to time).

The cross-format spatial array and brief exposure time has previously been shown to be effective at engaging covert attention to the relevant pair of locations without saccades (Vuilleumier et al, 2001; Wojciulik et al, 1998), and without awareness of identity, gender or expression of faces when these appear in task-irrelevant locations only (Vuilleumier et al, 2001). Nonetheless, to exclude possible between-group differences in saccade frequency or compliance with the requirement for central fixation, we monitored eye movements throughout the task using an infra-red eye tracker (ASL Model 540, Applied Science Group Co., Bedford, MA; refresh rate = 60 Hz). For technical reasons, eye-position data were lost for six subjects (2 placebo, 4 from drug group).

Figure 6.1. Stimulus format. Each block started with cue stimulus that indicated whether subjects must attend to horizontal or vertical locations in cross-array for performance of subsequent matching task (in this example, this would be horizontal locations). Each trial consisted of a pair of faces that may be either in task-relevant (A) or taskirrelevant (U) locations; together with a pair of houses occupying alternate 2 locations. Face pairs were repeated with lag of 2 to 5 intervening trials; houses were never repeated. Face pairs could be either fearful and thus emotional (E) or neutral (N). Experiment thus had a 2 x 2 x 2 factorial design, with factors of face-repetition, attention (toward or away from faces), and emotion.



	EMOTIONAL	NEUTRAL
ATTENDED	AE1 AE2	AN1 AN2
UNATTENDED	UE1 UE2	UN1 UN2

Imaging and image processing

As for Experiment 1.

Statistical analysis of images

Data were analyzed with a general linear model for event-related designs, using a random-effects analysis, as in Experiment 1. However, in Experiment 2, eight event types were defined for each subject (see above). Data were globally scaled and high-passed filtered at 1/120 Hz. Individual events were modelled by a canonical synthetic hemodynamic response function and its temporal derivative (Friston et al, 1998). The six head movement parameters were included within the model as before.

Linear contrasts of parameter estimates were made for each subject and used to generate statistical parametric maps (SPMs) of the *t*-statistic. In order to test for regions showing face-repetition effects common to different conditions (i.e. to both attended and unattended faces; or to both emotional and neutral faces), contrasts of first versus second presentation under each condition were performed for each subject and entered into a repeated-measures ANOVA corrected for non-sphericity (Glaser et al, 2002). A conjunction analysis was then performed over contrasts from both conditions (Price & Friston, 1997). In order to test for regions showing a different magnitude of repetition decrease between conditions, contrasts representing the repetition x condition interaction for each subject were entered into a one-sample *t*-test; only regions showing a significant repetition decrease ($p < 0.001$, uncorrected) in at least one of the tested conditions are reported. Regions found to show repetition x condition interactions that also showed a significant three-way interaction (repetition x attention x emotion; thresholded at $p < 0.001$, uncorrected) are noted.

Repetition x drug interactions were analysed separately for attended and unattended faces by comparing between-subject repetition effects with *t*-tests. Repetition x condition x drug interactions were similarly assessed by comparing the repetition x condition interactions, for each subject, between groups, but only the volume of voxels showing a repetition main effect or repetition x condition interaction in the placebo group was searched (masks thresholded at $p < 0.001$, uncorrected). Effects of physostigmine on attention and emotion, independent of repetition, are considered in Experiment 1. We emphasise that the drug effects reported here are condition-specific, as mean session-effects are modelled separately. All regions that showed significant repetition x drug, or repetition x condition x drug, interactions were found to show insignificant between-group session effects ($p > 0.05$, uncorrected). Furthermore, the global session mean activity did not differ between groups ($p > 0.05$), suggesting that physostigmine did not engender significant general vascular effects.

We report areas that achieve significance either after correction for whole brain (or effective search volume in the case of repetition x condition x drug interactions; see above), or regions of interest (ROI) where indicated (Worsley et al, 1996). Two ROI's of approximately 20 cm^3 each were defined (Rorden & Brett, 2001) in right and left inferior temporo-occipital cortices so as to encompass coordinates previously found to show repetition decreases in event-related fMRI designs for repetition of unfamiliar faces (Henson et al, 2002; Jiang et al, 2001). Regions surviving a threshold of $p < 0.001$, uncorrected, are also reported descriptively. Any activations smaller than 5 contiguous voxels were discounted.

Results

Physiological data, subjective reports, and eye tracking

Side-effects and subjective reports are described in Experiment 1.

The frequency of saccades and median angular deviation of the eye were measured also as in Experiment 1. However, these parameters were now entered into a four-way ANOVA with factors of group, attention, emotion and repetition,. There were no significant effects or interactions for either saccade number, or median ocular position (all F's < 1.8; $p \geq 0.2$).

Behavioural

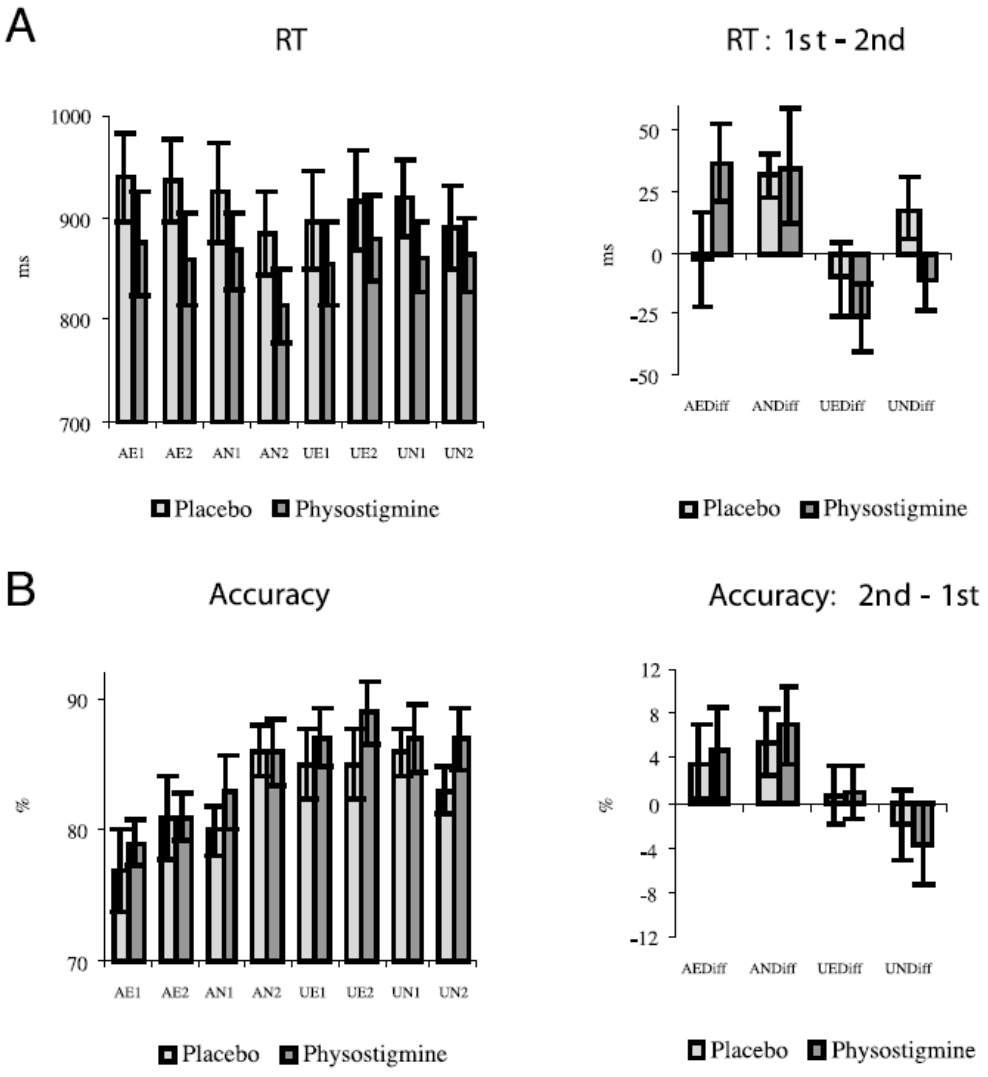
Behavioural effects of priming were determined by calculating median RT and mean accuracy differences between the first and second presentations for each face pair, separately for each of the four conditions in each participant. In the placebo group, a significant speeding of responses when attended neutral (AN) faces were repeated (mean RT difference between first and second presentation = 30.3 ± 17.5ms, $t(14) = 3.5$, $p < 0.005$), plus a trend for more accurate performance with repeated stimuli (mean accuracy difference = 5.3 ± 5.8 %; $t(14) = 1.8$, $p < 0.1$) was evident, consistent with previous studies of repetition priming (e.g. Henson et al, 2002).

To ascertain any effects of condition (attention and emotion) and group (cholinergic enhancement versus placebo), we entered RT and accuracy differences (between first and second presentation) for each subject, for each condition, into a repeated-measures, mixed ANOVA. While there was no main effect of group on the RT

priming effect, there were significant group x attention ($F(1,28) = 4.4$; $p < 0.05$) and group x emotion interactions ($F(1,28) = 4.3$; $p < 0.05$; Fig. 6.2A). These effects can be explained by a significant reduction of the RT priming effect for emotional versus neutral trials under placebo ($F(1,14) = 11.7$; $p < 0.005$), but not under physostigmine ($F(1,14) = 0.7$; ns); while the RT priming effect was significantly greater for attended versus unattended faces under physostigmine ($F(1,14) = 12.8$; $p < 0.005$), but not under placebo ($F(1,14) = 0.3$; ns). A separate ANOVA comparing absolute RTs between groups over condition (attention x emotion x stimulus order) did not reveal any between-group differences, although there was a trend to faster overall RTs in the physostigmine group ($t(28) = 1.4$, $p < 0.1$, one-tailed based on Furey et al, 1997).

There was no group x condition interaction for priming in accuracy measures, but a trend for greater primimg with attended repeated faces versus unattended repeated faces ($F(1,14) = 3.0$; $p < 0.1$; Fig. 6.2B). Overall accuracy (mean score = 85 ± 3.2 %, and 83 ± 3.2 %, under drug and placebo respectively) was comparable to a previous study using the same task in which limited awareness of task-irrelevant compared with task-relevant faces was demonstrated (Vuilleumier, et al, 2001). There were no behavioural effects of specific attended location (i.e. horizontal or vertical pair), or interactions of location x group.

Figure 6.2:. A: median RT for each of 8 conditions (AE, Attended Emotional faces; AN, Attended Neutral; UE, Unattended Emotional; UN, Unattended Neutral; − 1: 1st presentation; − 2: 2nd presentation), separately for placebo and physostigmine groups. Second graph shows itemwise differences in RTs to novel minus primed stimuli for each of 4 main conditions (hence positive values represent repetition advantage). B: mean accuracy for same 8 conditions. Second graph shows itemwise differences of accuracy to primed minus novel stimuli for each of 4 main conditions (hence positive values represent repetition advantage). SE bars are shown. Note that statistical inferences concerning priming are based on subject-specific differences, and that inferences on behavioral effects are based on item-specific repetition (not conveyed by error bars on group means).



fMRI data: Effects of selective attention on face-repetition

The main effect of faces presented in task-relevant, versus task-irrelevant, locations (i.e. independent of repetition) identified bilateral mid-fusiform regions (44, -50, -24 and –42, -44, -28; $Z \geq 4.29$; $p < 0.001$, uncorrected; Fig. 6.3A), while the opposite contrast (houses in task-relevant, versus task-irrelevant, locations) identified bilateral parahippocampal cortex (16, -52, 6 and –24, -36, -14; $Z \geq 5.50$; $p < 0.01$, corrected for whole brain). This replicates previous results using a similar task (Vuilleumier et al, 2001; Wojciulik et al, 1998) and demonstrates that subjects selectively processed the pair of stimuli at cued locations.

We next identified areas that showed fMRI face-repetition effects common to both attended and unattended face stimuli, by performing a conjunction analysis across contrasts comparing first and second presentations of both attended-face and unattended-face trials in the placebo group. Areas showing repetition *decreases* under both levels of attention were bilateral inferior temporo-occipital cortex, left inferior prefrontal gyrus and premotor areas (Table 6.1A, Figure 6.3B). These regions were distinct from those that showed greater repetition decreases for attended versus unattended faces, viz. superior temporal sulcus, middle occipital gyrus, and striatum (Table 6.1C). We note that the inferior temporo-occipital regions showing a repetition effect independent of attention did not show a main effect of face-attention (Fig. 6.3B), and, conversely, the mid-fusiform peaks showing a main effect of attention did not show any repetition effects (Fig. 6.3A). Repetition effects in these inferior occipital regions are likely to reflect processing of stimulus features more general than those encoded by the face-selective mid-fusiform regions, that were located more anteriorly. Areas that showed repetition *increases* across both levels of attention

included bi-parietal cortex and posterior cingulate (Table 6.1B), both of which showed likewise increases to repeated faces in Henson et al, 2002.

Finally, as our behavioural data suggested a performance advantage on house judgements when faces were repeated at irrelevant locations, we examined the contrast of first versus second presentations of task-irrelevant faces (i.e. when house judgements were performed), and restricted the analysis to house-selective regions (by masking with the main effect of house-attention, thresholded at $p < 0.001$, uncorrected). This identified a left parahippocampal region (-22, -32, -10; $Z = 4.09$, $p < 0.05$, corrected) that showed *increased* activity with repetition of unattended face stimuli, presumably as the houses became more dominant when the same faces were ignored. This repetition enhancement was indeed specific to trials when house judgements were required (and faces ignored), as shown by a significant repetition x attention interaction ($F(1,14) = 5.0$; $p < 0.05$).

Figure 6.3. (See next page): Regions of inferior temporo-occipital cortex showing main effect of faces in attended vs. unattended locations, independent of repetition (mauve; A), and conjunction of repetition decreases to faces in both attended and unattended locations (yellow; B); both contrasts $P < 0.001$, uncorrected. Activation map is superimposed on single-subject T1-weighted MRI brain, pitched to visualize inferior cortical surface. Graphs show percentage signal change from baseline in right mid-fusiform (A) and inferior occipital cortex (B) during first and second presentations of faces from each of 4 conditions (AE, AN, UE, UN). On right are plots of mean subject-specific signal differences between first and second presentations for each of 4 conditions (positive values represent repetition decreases;

negative values represent repetition enhancements). Regions showing main effect of face-attention ($P < 0.001$) did not show a significant effect of repetition ($P > 0.05$), whereas regions showing repetitions to both attended and ignored faces ($P < 0.001$) did not show a significant effect of face-attention ($P > 0.05$). Placebo and physostigmine groups are shown separately. Left-sided plots are corrected for mean over all conditions between groups (no significant main effect of drug).

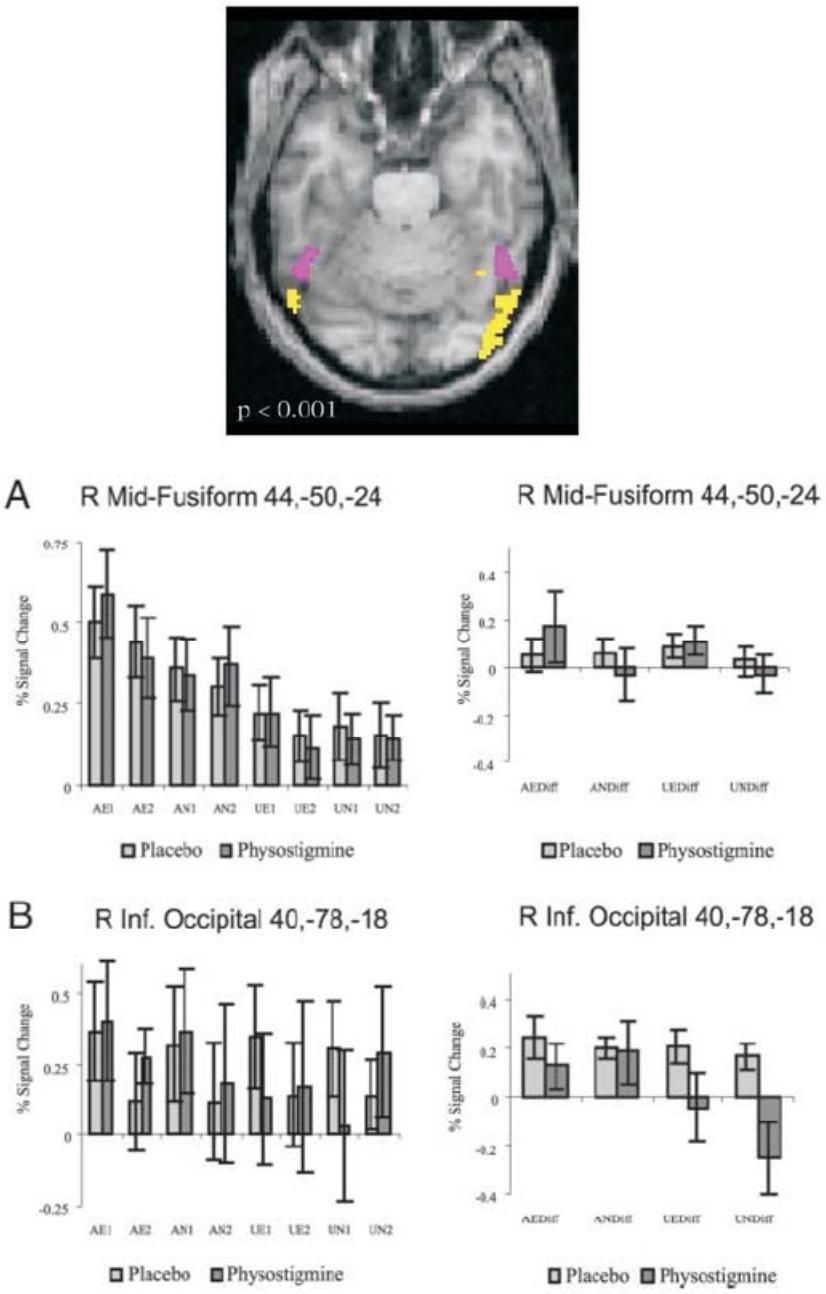


Table 6.1: Common and differential effects of attention on priming in placebo group

Area	x	y	z	Z Value
A. Repetition decreases to faces in both attended and unattended locations				
Right Inferior Occipital	40	−78	−18	6.25**
	36	−86	−16	3.92*
	46	−62	−22	3.98*
Right Inferior Temporal	32	−40	−22	3.85
Left Inferior Occipital	−48	−62	−22	4.49*
Left Temporo-Parietal Junction	−54	−40	34	4.09
Right Lingual Gyrus	12	−56	6	3.94
Cuneus	6	−86	14	4.34
Left Inferior Frontal Gyrus	−52	38	8	4.21
Right Inferior Frontal Gyrus	60	24	10	3.29
Left Posterior Superior Frontal Gyrus	−6	14	50	3.67
Right Posterior Middle Frontal Gyrus	58	16	30	3.95
B. Repetition increases to faces in both attended and unattended locations				
Right Parietal	54	−38	56	4.52
	22	−68	32	4.16
Left Parietal	−32	−56	30	3.42
Posterior Cingulate	8	−34	18	3.67
Left Middle Temporal Gyrus	−54	0	−30	3.85
Right Premotor Cortex	14	−8	68	4.05
Left Central Sulcus	−24	−16	44	3.70
C. Repetition decreases greater for faces in attended than unattended locations				
Left Superior Temporal Sulcus	−62	−36	12	4.26
Left Middle Occipital Gyrus	−34	−80	16	3.91
Right Striatum	22	8	0	3.68
Medial Orbitofrontal Cortex†	−2	42	−22	4.20
D. Repetition decreases greater for faces in unattended than attended locations				
No regions approached significance				

* $P < 0.05$ corrected for ROI (based on Henson et al., 2002; Jiang et al., 2000). ** $P < 0.01$ corrected for whole brain. † Regions also showing a three-way interaction of repetition × attention × emotion, that is, (AN1-AN2)–(AE1-AE2)–[(UN1-UN2)–(UE1-UE2)].

fMRI data: Effects of emotion on face-repetition

Comparison of signal estimates between emotional and neutral face conditions at the peak of the conjunction of face-repetition effects across attended and unattended contrasts suggested a similar repetition decrease in occipital cortex for both emotional and neutral faces (Fig. 6.3B). This was confirmed formally by performing the conjunction of priming effects between fearful and neutral-face trials, independent of attention (Table 6.2, yellow area in Fig. 6.4A). The same posterior visual (and premotor) areas that showed face-repetition decreases for both levels of attention thus also showed repetition decreases to both neutral and emotional faces.

We next examined for repetition effects that were significantly modulated by emotion (i.e. repetition x emotion interaction). This showed that left postero-inferior occipital cortex, as well as a region at posterior amygdala-hippocampal border, exhibited a greater repetition decrease for emotional, relative to neutral, stimuli (Table 6.2; cyan area in Figs. 6.4A, 6.4B). Conversely, the only area showing a greater repetition decrease to neutral, relative to emotional, faces was lateral orbitofrontal cortex (OFC: red area in Fig. 6.4A). The pattern of repetition decreases in this region across conditions (Fig. 6.4B) parallels the RT priming effects observed behaviourally. Furthermore, a correlation analysis comparing the size of the emotional effect on RT priming with the size of the emotional effect on BOLD repetition decreases at the OFC peak showed a positive trend ($r = 0.46$, $p = 0.08$): i.e. subjects showing the greatest attenuation of RT priming with emotional faces also tended to show the greatest diminuition of repetition decreases in OFC towards emotional faces.

We note that none of the extrastriate areas exhibiting repetition effects showed a main effect of emotion, but such a main effect of emotion, independent of attention, was found in left fusiform (-40, -48, -24, Z = 3.27, p < 0.001, uncorrected), and medial amygdala-substantia innominata (10, -8, -16, Z = 3.09, p = 0.001, uncorrected), in keeping with Vuilleumier et al, 2001.

Figure 6.4. (See next page): Regions of inferior temporo-occipital and orbitofrontal cortex showing conjunction of repetition decreases for both emotional and neutral faces (yellow; activity plot similar to that shown in Figure 6.3*B*; a left inferior occipital cluster occurred on lower slice); or greater repetition decreases to neutral, relative to emotional, faces (red; *A*); or greater repetition decreases to emotional, relative to neutral, faces (blue; *B*); all contrasts thresholded at $P < 0.001$, uncorrected. Activation maps are superimposed on single-subject T1-weighted MRI brain (z = -14). Graphs show percentage signal change from baseline in right lateral orbitofrontal cortex (OFC: *A*), and left posterior occipital (*B*) regions during first and second presentations of faces from each of 4 conditions (AE, AN, UE, UN). Scales differ between regions. On right are plots of mean subject-specific signal differences between first and second presentations for each of 4 conditions (positive values represent repetition decreases; negative values represent repetition enhancements). Placebo and physostigmine groups are shown separately for each contrast. Left-sided plots are corrected for mean over all conditions between groups (no significant main effect of drug).

Figure 5.4. For legend see above.

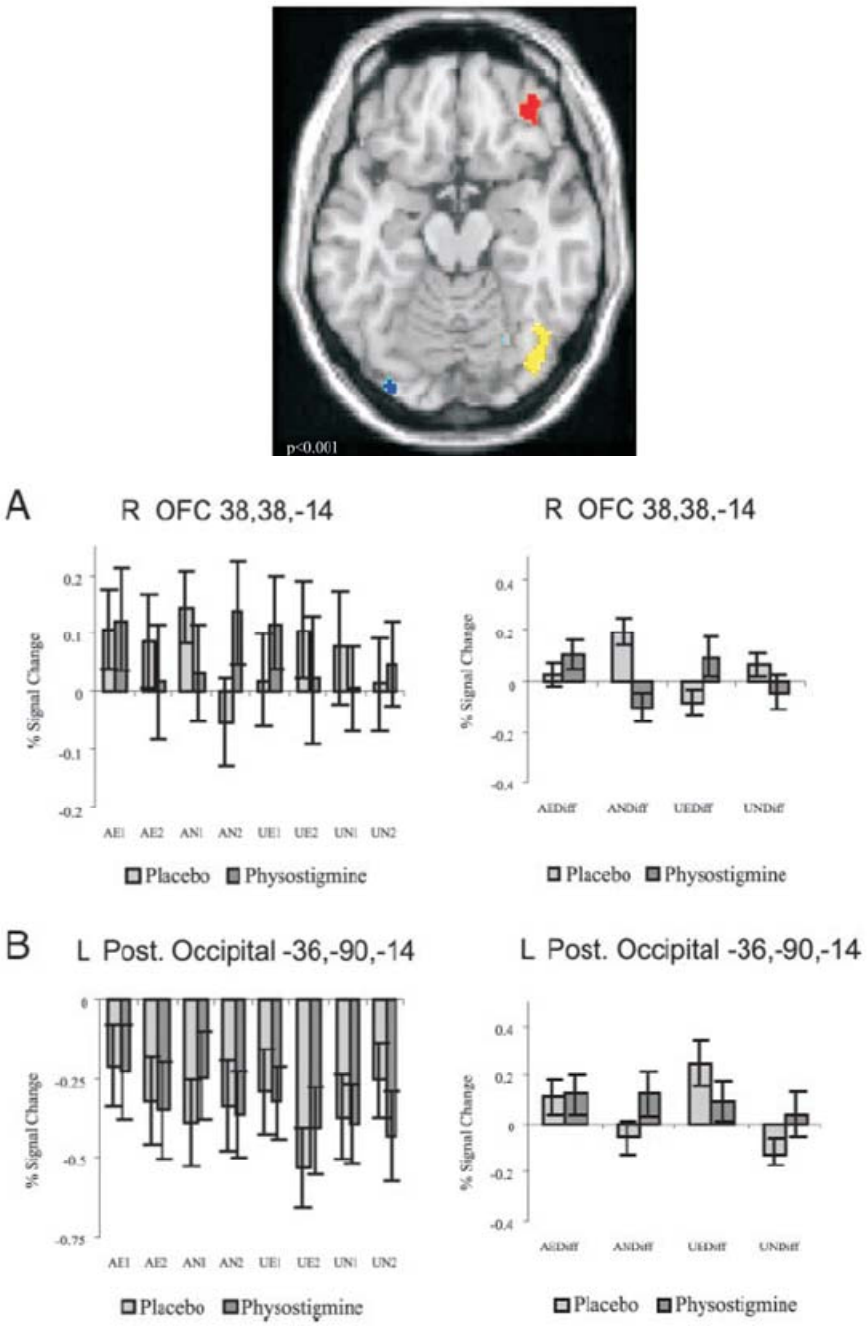


Table 5.2: Common and differential effects of emotion on priming in placebo group

Area	x	y	z	Z Value
A. Repetition decreases to both emotional and neutral faces				
Right Inferior Occipital	40	−78	−16	5.17**
	46	−62	−24	4.17*
Right Inferior Temporal	32	−52	−22	3.42
Left Inferior Occipital	−46	−62	−22	4.57*
Left Middle Occipital Gyrus	−34	−84	16	3.36
Left Temporo-Parietal Junction	−52	−42	34	3.68
Cuneus	−2	−86	6	4.02
Left Amygdala	−26	0	−26	3.34
Right Posterior Middle Frontal Gyrus	58	12	30	3.67
Left Posterior Superior Frontal Gyrus	−8	12	48	3.63
B. Repetition increases to both emotional and neutral faces				
Right Parietal	22	−68	32	3.56
Posterior Cingulate	6	−24	32	3.52
Left Hippocampus	−22	−34	−10	4.13
Left Central Sulcus	−24	−16	42	4.01
Left Superior Frontal Sulcus	−18	38	30	3.70
Right Middle Frontal Gyrus	38	56	−10	3.77
C. Repetition decreases greater for emotional than neutral faces				
Left Posterior Inferior Occipital	−36	−90	−14	3.45
Right Posterior Amygdala–Hippocampus	18	−10	−12	3.47
D. Repetition decreases greater for neutral than emotional faces				
Right Lateral Orbitofrontal Cortex	38	38	−14	4.16
	40	46	−16	4.14

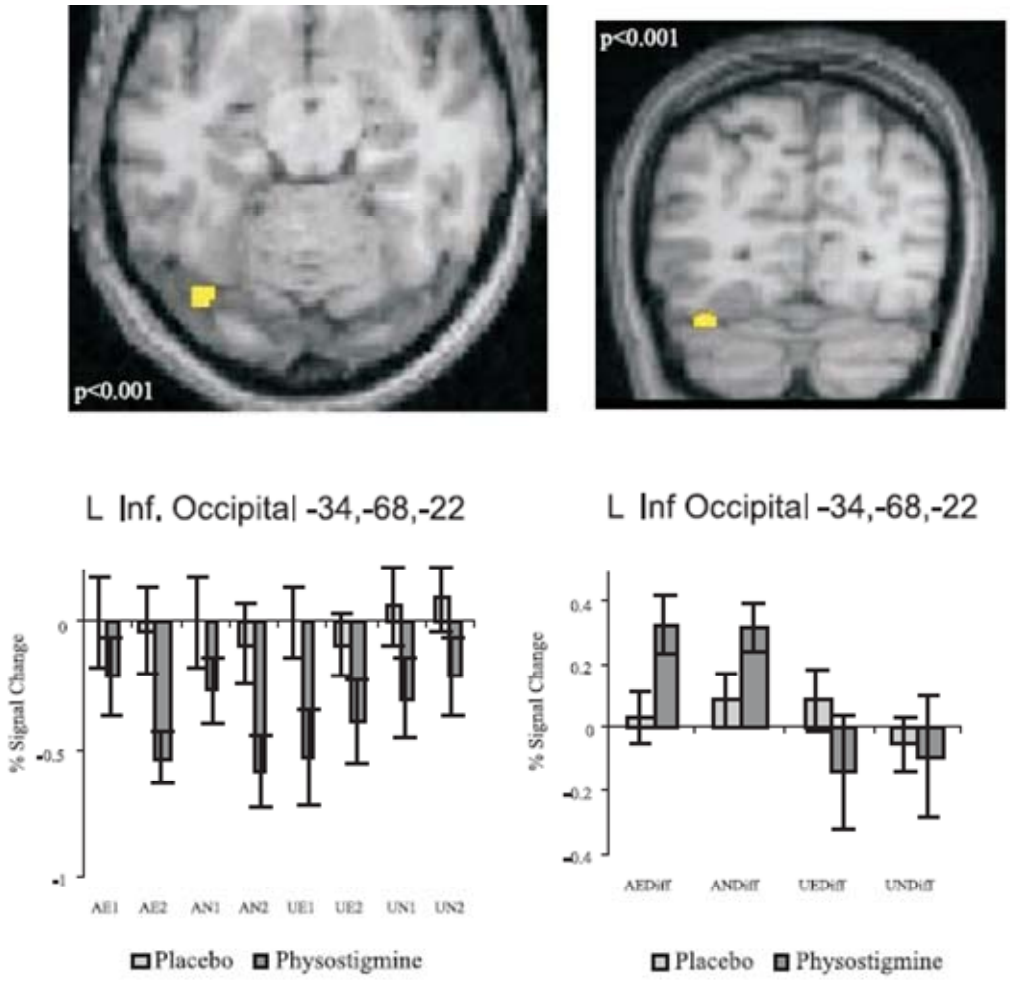
* $P < 0.05$ corrected for ROI (based on Henson et al., 2002; Jiang et al., 2000). ** $P < 0.05$ corrected for whole brain.

fMRI data: Effects of cholinergic enhancement on repetition

Given the role of cholinergic inputs on selective attention, we had hypothesised that physostigmine might enhance repetition suppression effects (see Thiel et al, 2001; Thiel et al, 2002a) specifically for attended faces (see also Furey et al, 2000a). Therefore we examined group x repetition interactions for attended and unattended trials separately. With faces attended, this interaction identified left inferior occipital cortex as showing a greater repetition decrease under physostigmine relative to placebo (-32, -66, -22; $Z = 3.91$; $p < 0.05$, corrected for ROI; Fig. 6.5). This occipital area also showed a repetition x attention x drug interaction ($F(1,28) = 4.0$; $p < 0.05$) that reflected physostigmine, but not placebo, engendering a greater repetition decrease for attended, versus ignored, faces (physostigmine: $F(1,28) = 7.0$; $p < 0.05$; placebo: $F(1,28) = 0.6$; ns). Furthermore, in the same region, we note that physostigmine, versus placebo, resulted in reduced activity to the *repeated* face ($t(28) = 2.4$; $p < 0.05$), but did not change activity to the *first* face ($t(28) = 1.1$; ns). These results complement previous findings of modulation of repetition effects within left inferior occipital cortex by cholinergic blockade, when scopolamine resulted in a reduced repetition decrease, due specifically to an elevation in activity to the repeated stimulus (Thiel et al, 2001).

No regions showed less repetition decreases to attended faces under physostigmine, relative to placebo, and no regions showed a significant drug x repetition interaction for unattended faces.

Figure 6.5: Regions of inferior temporo-occipital cortex showing greater repetition decreases for physostigmine than placebo, specifically for faces in attended locations ($P < 0.001$, uncorrected). Area showing this significant interation was close to region previously showing repetition decreases in face priming study (Henson et al. 2002), and also showing diminution of repetition decrease under cholinergic blockade (Thiel et al. 2001). Activation map is superimposed on single-subject T1-weighted MRI brain. Transverse slice taken at z = -22; coronal slice taken at y =-68. Graph shows percentage signal change from baseline in left inferior occipital cortex during first and second presentations of faces from each of 4 conditions (AE, AN, UE, UN). On right are plots of mean subject-specific signal differences between first and second presentations for each of 4 conditions, at the same point. Placebo and physostigmine groups are shown separately for each contrast, and are not mean-corrected.



Three-way interactions of drug x repetition x condition (for conditions of attention and emotion, separately) were also tested, given that these interactions showed significant effects in the RT data. We restricted our search volumes to those voxels showing a main effect of repetition-decrease (regardless of attention and emotion condition) or a repetition x condition interaction in the placebo group (thresholded at $p < 0.001$, uncorrected). Importantly, no areas showed a repetition x drug interaction over all conditions (i.e. there was no main effect of physostigmine on repetition independent of conditions).

In the repetition x drug x *attention* interaction, we found that the right inferior occipital region which had shown the maximum repetition decrease common to both attended and unattended faces (as well as to both emotional and neutral faces) under placebo, was found under physostigmine, to show a repetition decrease selectively for attended faces only (i.e. repetition x attention x drug interaction: $F(1,28) = 5.5$; $p < 0.05$; a similar interaction was found in the left inferior occipital region showing a repetition x drug interaction for attended faces – see above). As shown in Fig. 6.3B, under physostigmine, the right inferior occipital region manifested a similar degree of repetition decrease to attended faces as under placebo, but showed a trend for repetition increases to unattended faces ($t(14) = 1.7$; $p = 0.1$), in contrast to the placebo group. There were no regions in which this interaction survived correction for search volume.

Finally, the repetition x drug x *emotion* interaction revealed that the lateral orbitofrontal region (38, 38, -12), previously found in the placebo group to display less repetition effects with emotional, relative to neutral, faces, was not associated

with this pattern under physostigmine ($Z = 4.51$; $p < 0.05$, corrected; Fig. 6.4B). In fact, physostigmine produced the opposite effect at this point, viz. greater repetition decreases with emotional than neutral faces ($F(1,28) = 10.3$; $p < 0.01$).

Discussion

Effects of cholinergic enhancement on repetition priming and its neural correlates

Since cholinergic blockade with scopolamine has previously been found to inhibit both behavioural and neuronal correlates of repetition priming to attended stimuli (Thiel et al, 2001; Thiel et al, 2002a), we expected to find an increase of both measures with the cholinergic enhancer physostigmine. Consistent with this prediction, physostigmine produced a greater repetition decrease in left inferior occipital cortex (selectively with attended face-repetition), although this did not benefit RT or accuracy significantly. This occipital area was close to those previously showing repetition decreases to faces (Henson et al, 2002; Jiang et al, 2000), or showing an elimination of repetition effects following cholinergic blockade (Thiel et al, 2001). The fact that no RT improvement was observed with physostigmine suggests that an additional rate-limiting step of the task may lie downstream from perceptual processes in occipital cortex, e.g. response-related.

The nature of the physostigmine interaction with repetition was closely complementary to that previously found with scopolamine (Thiel et al, 2001) in another respect: both cholinergic manipulations only affected response to the repeated occurrence of an item, not to the initial presentation. This contrasts with other drugs, e.g. GABAergic modulators, that disrupt cortical repetition effects and priming

through effects on novel stimulus processing (Thiel et al, 2001; Vidailhet et al, 1999). Our results may also be relevant in the context of Alzheimer's disease (AD), in which perceptual priming is impaired (Shimamura et al, 1987; Schwartz et al, 1996). Since AD patients have shown paradoxical repetition enhancements in inferior occipital cortex, in contrast to healthy age-matched subjects who show repetition decreases (Backman et al, 2000), we speculate that the beneficial effect of anticholinesterases on priming observed in AD (Riekkinen & Riekkinen, 1999) may arise from an enhancement of repetition effects in inferior occipital cortex, as observed here in healthy subjects.

In contrast to the placebo group, who showed similar repetition decreases to both attended and ignored faces in inferior occipital regions, we found in the physostigmine group that repetition decreases occurred preferentially to attended stimuli (Figs. 6.3B, 6.5B). These results complement our RT data in showing that physostigmine, but not placebo, induced a repetition benefit selectively with attended stimuli. Such an effect would be consistent with studies showing the importance of cortical cholinergic modulation on selective attention (Sarter et al, 2001), noise filtering (Sato et al, 1987), and selective perceptual processing of stimuli that need to be remembered (Furey et al, 2000).

Cholinergic modulation has also been shown to enhance processing of emotional stimuli (Holland & Gallagher, 1999), and facilitate experience-dependent cortical plasticity specifically to fear-conditioned stimuli (Weinberger et al, 1998; Ji et al, 2001; Thiel et al, 2002b). Our results extend the role of acetylcholine in emotional learning by showing that physostigmine favoured repetition decreases to emotional,

relative to neutral, faces in orbitofrontal cortex (thus reversing the normal pattern); and eliminated the detrimental effect of emotion on primed RTs seen under placebo. The OFC has especially strong interconnections with cholinergic neurons of the nucleus basalis (Cavada et al, 2000) and is modulated by acetylcholine during reinforcement learning in animals (Aou et al, 1983).

A discussion of the other findings of this Experiment – on attentional and emotional interactions with repetition, independent of cholinergic modulation – is made elsewhere (Bentley et al, 2003).

Conclusion

Experiment 2 demonstrates that cholinergic enhancement can augment the size of repetition effects in inferior occipital cortex, and favour behavioural and neural priming effects for both attended and emotional stimuli (the latter being due to a reversal of the emotion-selective impairment of priming in untreated subjects). The experiment also showed that repetition priming, and its associated haemodynamic marker of extrastriate cortex repetition decrease, can occur by a similar amount for stimuli appearing at task-relevant or irrelevant locations. In contrast, emotional stimuli reduced behavioural priming, associated with an attenuation of repetition decreases in lateral orbitofrontal cortex. These results suggest that cortical mechanisms underlying priming may occur automatically (in extrastriate areas) and yet be influenced by intrinsic stimulus value (in orbitofrontal cortex) and cholinergic modulation (in both regions).

7. EXPERIMENT 3:

Effects of ChEI on Visual Stimulation, Visuospatial Attention and Spatial Working Memory

Introduction

The integrity of cholinergic afferents to cerebral cortex is necessary for normal stimulus discrimination, selection and vigilance (Robbins, 1998). During periods of high attentional demand, acetylcholine is released diffusely throughout neocortex (Phillis & Chong, 1965) and modulates processing within both sensory and prefrontal-parietal cortices (Sarter & Bruno, 1997). Thus, cholinergic input to visual or auditory cortices has been shown to sharpen stimulus representations through a combination of signal amplification and noise suppression (e.g. Sato et al, 1987, Hars et al, 1993). Additionally, cholinergic afferents to prefrontal and parietal areas have been shown to be critical for spatial orientation (Davidson & Marrocco, 2000; Chiba et al, 1999) and sustained attention (McGaughy & Sarter, 1998), especially in the presence of distractors (Gill et al, 2000).

The above effects have been characterised in terms of cholinergic modulation of bottom-up and top-down processes, respectively (Sarter et al, 2001). An issue that remains unaddressed is the manner in which cholinergic modulation of these two types of processes combine. There remains uncertainty as to whether top-down modulation of sensory cortices is enhanced with cholinergic stimulation (as might be expected given the facilitatory effects of acetylcholine on attention generally – Sarter et al, 2001) or whether it is suppressed, so as to favour bottom-up activity (suggested by cell-layer recording studies in sensory cortices – Hasselmo & Cekic, 1996; Kimura et al, 1999, and computer modelling of cholinergic effects – Yu & Dayan, 2002).

One method by which this issue can be investigated is with functional imaging which has reliably demonstrated neural correlates of both bottom-up and top-down activity

within human visual cortex (e.g. Chawla et al, 1999, Hopfinger et al, 2000). While previous studies report modulation of visual cortical activity as a result of cholinergic drug administration, none have compared effects of cholinergic modulation on occipital activation evoked by stimulus, as compared to that due to attention. The anticholinesterase physostigmine has been found to increase extrastriate cortex activity selectively during the encoding-phase of a face working memory task (Furey et al, 2000). However, a more recent fMRI study (Lawrence et al, 2002) failed to find selective cholinergic effects, observing that nicotine (a more selective pro-cholinergic agent) enhanced occipital activity during both easy and difficult versions of a sustained attention (rapid visual information-processing) task. Consequently, the enhancement of occipital cortices may have been a direct effect of nicotine on visual-evoked responses. It is worth noting that both studies employed tasks that involved both attention and working memory components, which themselves may be independently modulated by acetylcholine (Everitt & Robbins, 1997; Ernst et al, 2001a; Heishman et al, 1994). Furthermore, Experiment 1 demonstrated that physostigmine may modulate neural correlates of attention differently between face and non-face, stimuli, which may reflect a bias of acetylcholine towards processing stimuli of high intrinsic valence (e.g. Holland & Gallagher, 1999; Wilson & Rolls, 1990; Acquas et al, 1996).

In the present study, we aimed to distinguish effects of physostigmine on occipital cortex activation attributable to attention from that due to stimulus. We also assessed whether the differential occipital activation engendered by selective spatial attention (e.g. Hopfinger et al, 2000) is itself modulated by physostigmine. This question is motivated by a recent model predicting that excess acetylcholine reduces the degree to

which top-down influences, such as selective attention, modulate activity in sensory cortices (Yu & Dayan, 2002). Finally, to unconfound effects of acetylcholine on attention and working memory (Furey et al, 2000; Lawrence et al, 2002), and mindful that both types of task may engage similar processes or brain areas (LaBar et al, 1999; Awh & Jonides, 2001), we compared physostigmine modulation of cortical activity between spatial attention and spatial working memory.

Methods

Subjects

Eighteen right-handed volunteers (13 female; 5 male; mean age = 23.4 ± 1.0) with no history of medical or psychiatric disease gave written informed consent. No subject was on medication or a smoker. Each subject participated in two sessions separated by 7 – 10 days, performed at similar times of the day. Subjects received physostigmine or placebo (saline infusion) on different sessions, with treatment order counterbalanced across subjects. Three further subjects scanned were excluded due to excessive saccades (> 50% trials).

Drug treatment

A double-blind placebo-controlled drug administration technique was used. Each subject received an intravenous cannula into the left cubital fossa and an infusion of either physostigmine or saline, depending on session. Dosage and rate of physostigmine infused was identical to that used in a recent study (Furey et al, 2000b) providing stable levels of plasma drug concentration and butyrylcholinesterase inhibition, as well as a significant and stable effect on cognitive performance for 40 minutes, following a 40 minute loading period. The same drug protocol has also been

found to result in changes in task-specific occipital activity using fMRI or PET techniques, using working memory (Furey et al, 1997; Furey et al, 2000a) and perceptual-attention (Experiment 1) tasks.

During the drug-session, subjects were first given 0.2 mg intravenous glycopyrrolate (peripheral muscarinic receptor antagonist), before an intravenous infusion of physostigmine was commenced (1.93 mg / hour for 10 minutes, followed by 0.816 mg / hour for 40 minutes). Subjects then performed the task in the scanner while receiving a constant rate of drug for a further 40 minutes (< 1.3mg physostigmine in total delivered). In the placebo-session, an equivalent volume of saline was administered in all steps. On both sessions, blood pressure was checked before, and at 40 minutes into infusion, whilst pulse-oximetry was performed continuously. Subjects were given a questionnaire at 0 and at 40 minutes post-infusion that allowed a ranked measurement (0 – 6 scale) of seven recognised adverse reactions to physostigmine and glycopyrrolate, as well as a list of visual analogue scales for estimating subjective feelings (Bond & Lader, 1974).

Cognitive task

On each session, subjects performed three tasks (spatial attention, spatial working memory, and visual control: Fig. 7.1) in different blocks, and repeated once (e.g. AWCAWC). To minimise order effects, treatment and task order were completely counterbalanced across subjects, with task-order being repeated across sessions. Furthermore, on each session, subjects were given half-hour practice with feedback, outside the scanner, prior to drug delivery. There were fifty-two trials of each condition per session, with an ITI of 0.5 – 3.5 seconds.

In the attention task, subjects were cued to either right or left visual hemi-fields (for 2.1 seconds), before being presented with a 12 Hz alternating, polarised chequerboard (18° height x 22° width; vertical wedges removed) for 3 –14 seconds (mean = 7.8 secs; approximate Poisson distribution). After this delay period, two adjacent 'squares' on either right or left side of the chequerboard (6° eccentricity; 3° wide) reversed in polarity (the target, appearing as a 'hole') for 84 ms, before being replaced by the normal chequerboard for a further 2.5 seconds. Subjects were required to attend to the cued side covertly (i.e. while fixating centrally), and to press either right or left buttons, depending on target-side, immediately on seeing the target. By only including responses within 1.5 seconds of target (accounting for > 95% responses), a measure of accuracy could be obtained (since only < 27 % accuracy could occur by subjects simply pressing after the commonest delay period). Targets either appeared on the same (valid trials: 80%) or opposite (invalid trials: 20%) side to that cued.

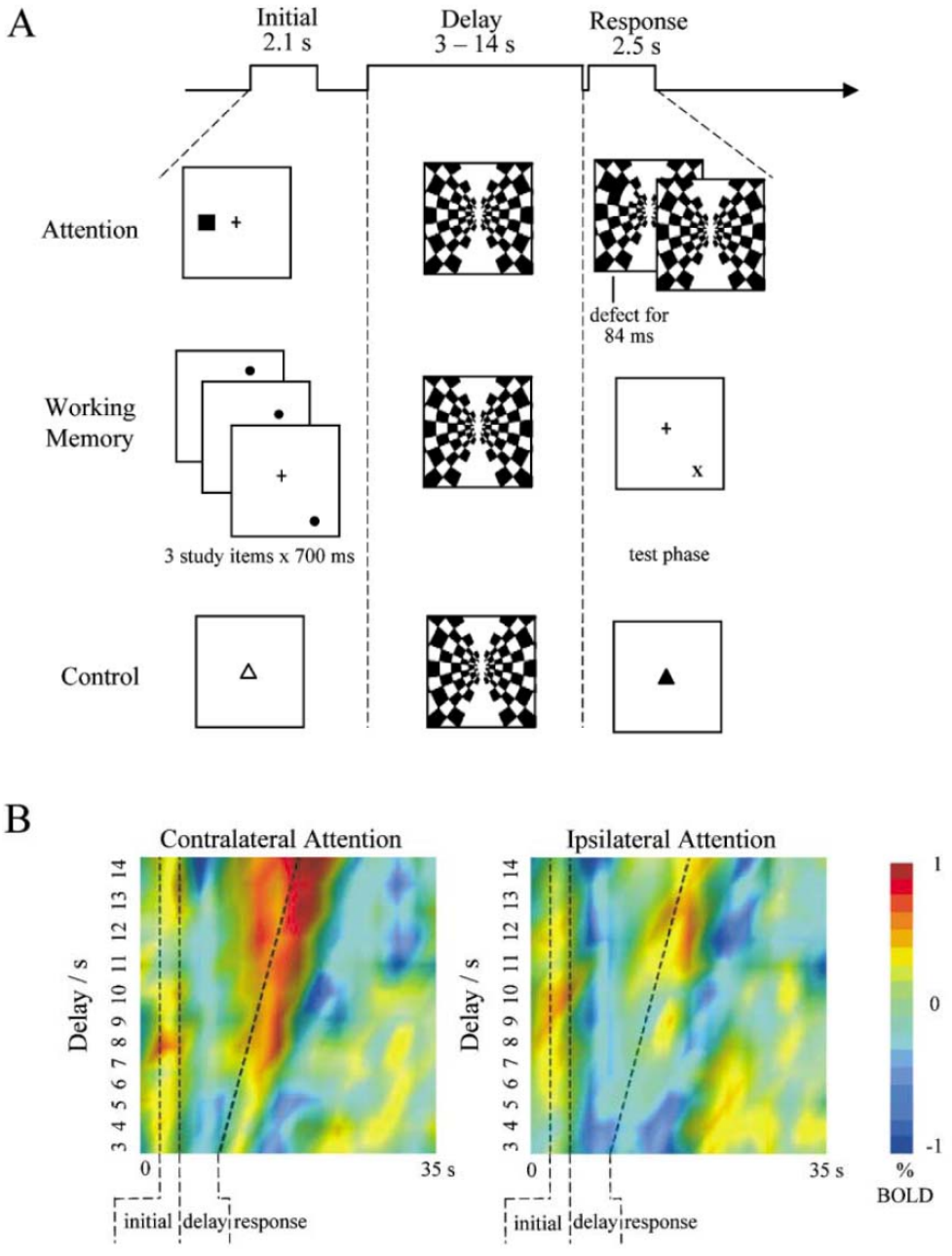
Working memory trials began with three points presented successively (for 700 ms each), each in one of twenty-four, equally-spaced locations in either right or left visual hemi-fields (equivalent to half the chequerboard area). Subjects were than required to rehearse the locations of the three points, while fixating centrally, during presentation of a 3 – 14 seconds, alternating chequerboard (parameters as for Attention task). Following this period, a probe point appeared anywhere in the display (for 2.5 seconds), and subjects had to indicate whether its location was the same as one of the three studied points.

Visual control trials resembled attention and WM trials in temporal composition, with a 3 – 14 second delay period of alternating chequerboard, during which subjects fixated centrally. However, trials began with a central cue for 2.1 seconds, and ended with a large, central triangle for 2.5 seconds, at which subjects had been instructed to press the first key, with no emphasis on speed (hence requiring minimal attention).

The use of variable-duration delay periods enabled us to model delay-period brain activity (in which the stimulus remained identical across conditions, and there was no motor response), separately from transients at either end of the delay-period (that varied between conditions and group – the latter due to effects on response by drug), while minimising the potential correlation between these task components (see Rowe at al, 2001). Furthermore, by monitoring saccades and median eye position during each delay period (using an infra-red eye tracker: ASL Model 540, Applied Science Group Co., Bedford, MA; refresh rate = 60 Hz), we were able to discount those trials in which central fixation did not occur from the behavioural and imaging analysis.

Figure 7.1:. (See next page): Task design and peristimulus- evoked BOLD responses. (A) Schematic time course of three tasks. Each task type consisted of task-specific stimuli at the beginning and end of every trial and a variable intervening delay period (3–14 s of alternating checkerboard) that was identical in stimulus across tasks and in which no motor response occurred. Transients at trial start and end were modeled separately from delay period for each task type, with each task phase being convolved with its own canonical hemodynamic response function. (B) Adjusted data from occipital cortex (averaged over bilateral peaks plotted in Figure 7.5, under placebo) showing changes in BOLD response across attention trials for varying delay periods,

temporally realigned to each trial onset. Trials were divided according to whether the initial cue was in the visual hemifield contralateral or ipsilateral to the occipital side from which the data was acquired. Note the increasing amplitude and duration of BOLD activity with delay duration reflects delay period activity (higher for attention to contralateral than ipsilateral space), unlike responses to cue or target that are delay independent. Effects reported here reflect the degree to which data fits a standardized delay-dependent regressor for each trial type, similar to the actual profile of activity observed here for contralateral attention.



Imaging and image processing

MRI data were acquired from a 2T VISION system (Siemens, Erlangen, Germany) equipped with a head coil. Functional images were acquired with a gradient echo-planar T2* sequence using BOLD (blood-oxygenation level dependent) contrast. The acquired image consisted of 33 x 3 mm thickness axial slices that covered the entire brain. Within each session (drug or placebo), volumes were acquired in two sub-sessions, with an effective repetition time (TR) of 2.51 seconds; echo time (TE), 50ms, and flip angle 90º. The first six volumes were discarded, to allow for T1 equilibration effects. Images were realigned to the first scan of the first session, time-corrected, normalised to a standard echo-planar image template, and smoothed with a Gaussian kernel of 8-mm full-width half-maximum.

Statistical analysis of images

Data were analyzed with a general linear model for blocked, event-related designs (SPM99; Wellcome Dept. of Cognitive Neurology, London, UK; Friston et al, 1995) using a random-effects analysis. Data were globally scaled and high-passed filtered at 1/256 Hz. For each subject, treatment and sub-session, the following events and epoch types were modelled: attention-cue (R and L, separately), attention-delay (R and L), attention-target (R and L, and for both, valid and invalid), WM-study (R and L), WM-delay (R and L), WM-probe, control-cue, control-delay, control-target (all control trials were arbitrarily divided into two to allow for independence in a conjunction analysis of attention and working memory versus control), false alarms, and saccades or eye-deviation (R and L). In those attention trials in which the target was missed, the modelled delay period was extended until the end of chequerboard presentation. All modelled events and epochs were convolved by a canonical hemodynamic

response function; temporal derivatives of these functions were modelled separately (Friston et al, 1998). The six head movement parameters were included within the model as confounding covariates.

In order to minimise the effect of sensorimotor differences between conditions, and performance between treatments, only contrasts of delay period activity were made. Differences of activity between delay period types of interest (and with respect to baseline) were calculated for each subject and treatment (i.e. parameter estimates), before being submitted to one-sample t-tests and generation of statistical parametric maps (SPMs) of the t-statistic. Comparisons of these contrasts were then made between treatments (group-by-condition interactions). Regions showing significant group-by-condition interactions were only reported if activation clusters at least partially overlapped with clusters showing a significant effect of the relevant condition within either treatment group (thresholded at $p < 0.001$, uncorrected). In order to test for regions showing both attention and working memory activity, contrasts of each condition versus its own set of control trials, under placebo, for each subject, were submitted to repeated-measures ANOVA corrected for non-sphericity (Glaser et al, 2002). A conjunction analysis was then performed over contrasts from both conditions (Price & Friston, 1997). We report areas that achieve significance after correction for whole brain, as well as those surviving a threshold of $p < 0.001$, uncorrected (qualified by previous fMRI studies employing similar tasks).

We emphasise that the drug effects reported here are task-specific, as mean session-effects are modelled separately. All regions that showed significant treatment-by-condition interactions were found to show insignificant between-treatment session

effects ($p > 0.10$, uncorrected). Furthermore, the global session-mean activity did not differ between treatments ($p > 0.10$), suggesting that physostigmine did not engender significant general vascular effects.

Results

Physiological data, subjective reports, and eye tracking

Subjects were less alert after physostigmine relative to placebo, comparing subjective rating scores (Bond & Lader, 1974) between 0 and 40 minutes post-infusion (68% vs. 75% alert, respectively; $p < 0.01$). This replicates an effect observed in Experiment 1 and 2. Subjects were also more likely to develop dry mouth ($n = 8$), dizziness ($n = 8$) and nausea ($n = 6$; all $p < 0.05$; mean intensity out of 6 = 1, 0.6, 0.4, respectively) under drug. There were no significant effects of drug on cardiovascular measures. Mean saccade frequency was 8% in attention; 3% in working memory, and <1% in control delay periods; median eye position was <0.5° from fixation in all sessions. There were no treatment effects for either eye position measure.

Behavioural

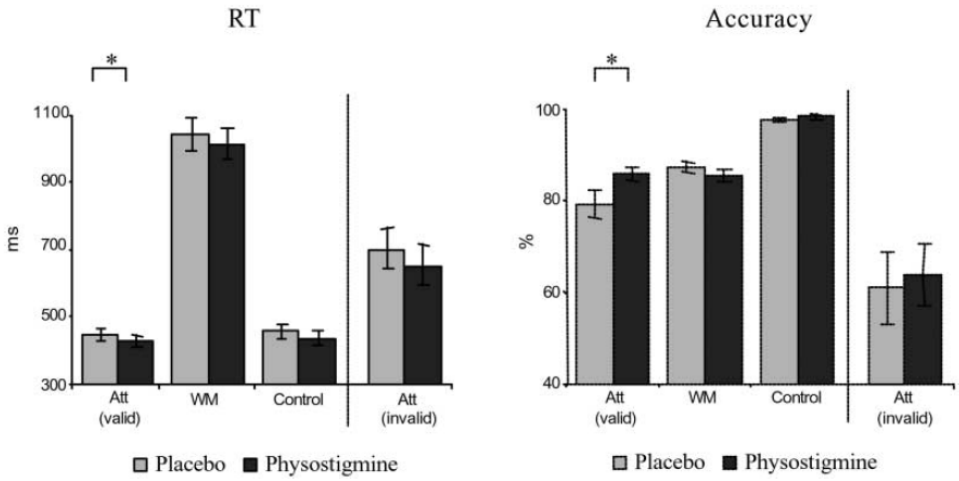
RT and accuracy measures for each subject were submitted to repeated-measures ANOVA with factors of group (drug or placebo) and condition (attention, WM and control; Fig. 7.2). Subjects were faster under physostigmine relative to placebo over all conditions ($F(1,17) = 4.6$; $p < 0.05$; RT's, comparing drug to placebo, for attention, WM and control, were 428 vs. 443 ms; 1014 vs. 1047 ms, and 435 vs. 457, respectively; paired t-tests of this comparison were only significant in the case of attention task). There was no main effect of group for accuracy. Conversely, there was

no group-by-condition interaction for RT, but a group-by-condition interaction was evident for accuracy ($F(1,17) = 5.7$; $p < 0.05$). This interaction reflected physostigmine improving accuracy in attention (86 vs. 79 %; $p < 0.05$), but not in WM (86 vs. 87 %) or control conditions (98 vs. 98 %). Differences in performance across task were also found in the following orders: For RT: WM was slower than attention and control ($p < 0.01$). For accuracy: attention and WM scored less than control ($p < 0.01$); attention also scored less than WM ($p < 0.05$; accountable by an effect in the placebo group alone).

Within the attention condition, a selective spatial processing bias towards cued, versus uncued, hemifields, was indicated by a faster performance (RT = 435 vs. 678 secs.; $p < 0.01$) and greater accuracy (83.3 vs. 62.6; $p < 0.01$) during validly versus invalidly-cued trials. There was no group-by-validity interaction. Finally, we found no difference in false alarm rate between groups (mean across groups = 4.6%; 1.1 %, and 1.6%, for attention, WM and control, respectively).

Figure 7.2. (See next page): Performance compared across conditions (attention, working memory, and control; valid and invalid cue trials are shown separately) and treatments (placebo and physostigmine). For RTs, a main effect of group existed, suggesting faster responses under physostigmine (individual paired t tests for each task revealed a significant effect only for attention). For accuracy, subjects performed better under physostigmine during attention but not working memory or control (at ceiling), as suggested by a treatment x condition interaction. *$p < 0.05$.

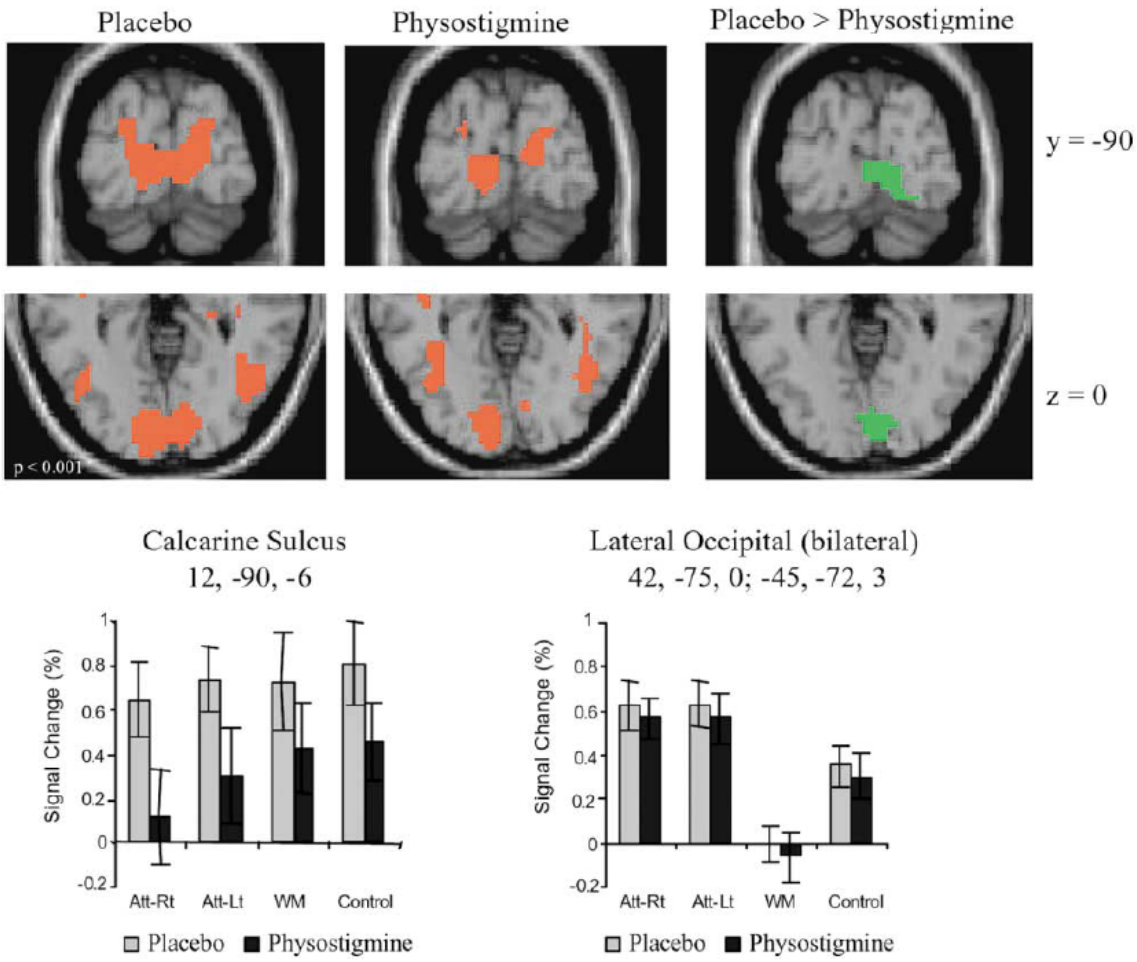
Figure 7.2. For legend see previous page



fMRI data: Effects of physostigmine on visual stimulation

The first fMRI contrast performed was for visual regions showing stimulus-evoked activation to the alternating chequerboard across all three tasks (attention, working memory and control) versus baseline. Under both placebo and physostigmine, robust activations were evident in primary visual (9, -84, -6, $Z = 5.91$, $p < 0.01$, corrected) and lateral occipital (42, -72, 0; -42, -75, 6, $Z \geq 4.24$, $p < 0.001$, uncorrected) cortices. A treatment effect was evident in primary visual cortex, with physostigmine reducing delay-period activations compared to placebo (i.e. main effect of drug, with no interaction: Fig. 7.3, graph 1). Lateral occipital cortices did not show a treatment effect (Fig. 7.3, graph 2; group-by-region interaction for primary visual and lateral occipital regions was $p < 0.005$), suggesting that physostigmine did not simply change the haemodynamic response function across occipital cortex.

Fig. 7.3. Effect of physostigmine on visual stimulation. Regions within occipital cortex showing main effect of visual stimulation (i.e., delay period activity across all tasks) versus baseline, under placebo, physostigmine, and when comparing treatments for this effect (no occipital areas were greater under physostigmine than placebo for the main effect of visual stimulation). Graphs plot % signal change from baseline for the three conditions (separating attend-right and attend-left conditions) in regions showing a main effect of visual stimulation under placebo. Primary visual cortex (calcarine sulcus) showed greater stimulus-evoked activity under placebo than physostigmine, which did not differ significantly across conditions. This effect was unlikely to be due to a general vascular effect of drug, as it was not seen in either lateral occipital cortex that also showed main effects of visual stimulation under placebo (these regions can also be seen to show an effect of condition due to failure of activation during WM but not attention or control).



fMRI data: Effects of physostigmine on spatial attention versus control

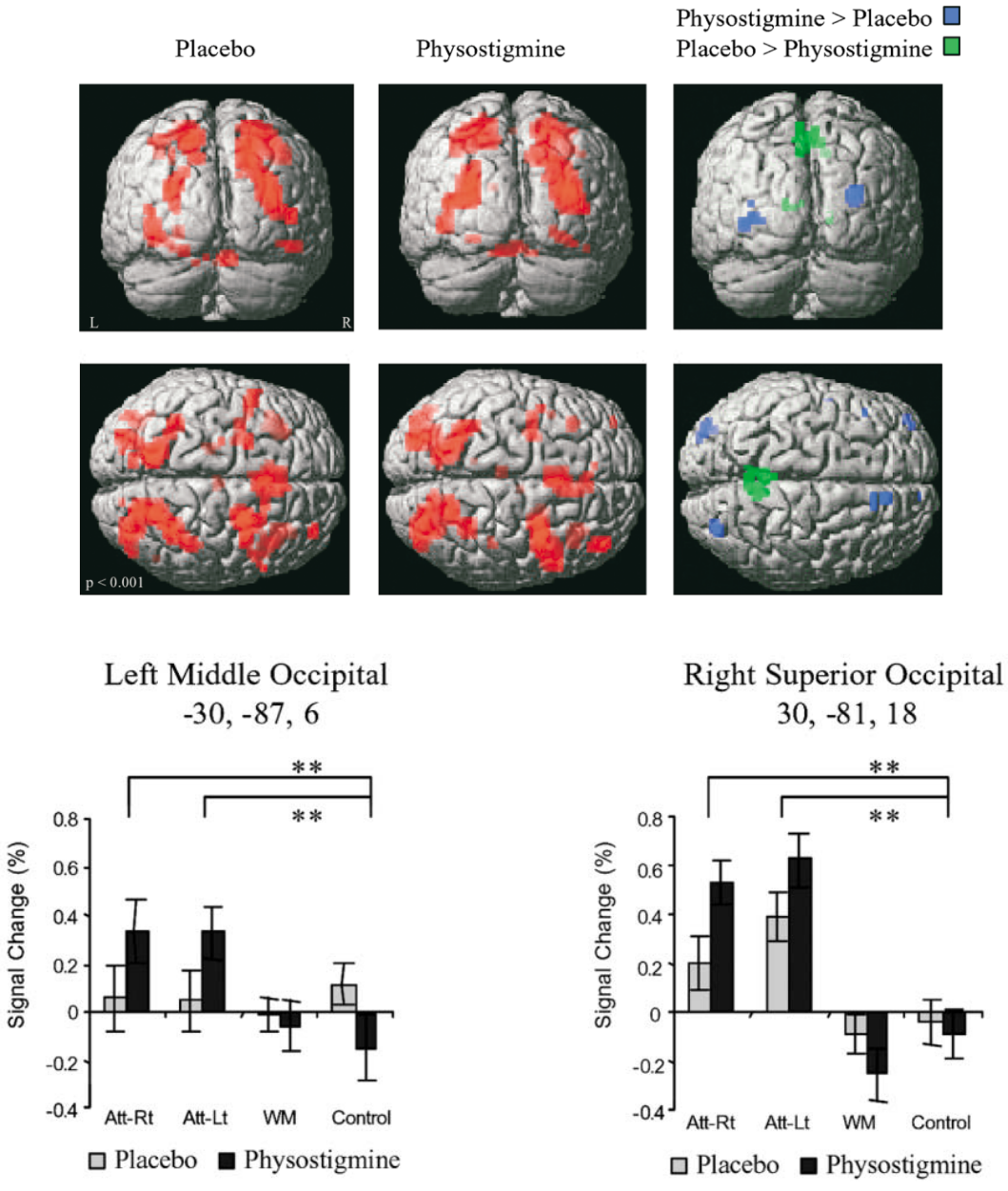
Under placebo, spatial attention versus control activated prefrontal, superior parietal and superior occipital cortices (Table 7.1; Fig. 7.4). These same areas were also activated under physostigmine. The direct comparison (i.e. group-by-task interaction) revealed that these regions were differentially modulated by cholinergic enhancement. Specifically, bilateral occipital and prefrontal cortices showed enhanced differential activity (mauve in Fig. 7.3), while superior-medial parietal cortex (green in Fig. 7.3; yellow in Fig. 7.6) showed reduced differential activity, during spatial attention relative to control, under physostigmine versus placebo. We note that the drug-induced increases in bi-occipital activity with attention occurred to an equivalent degree in both attend-right and attend-left trials (Fig. 7.4B).

Table 7.1: Regions showing effect of attention versus control, under placebo, and interaction of this with physostigmine.

Brain Region	Peak Coordinates			Z Score
Placebo: attention delay > control				
R superior occipital	30	−78	18	5.29
L superior occipital	−24	−75	30	4.10
L fusiform gyrus	−39	−66	−6	4.20
R superior parietal	30	−51	57	4.48
L superior parietal	−15	−66	54	5.60
L inferior prefrontal	−36	21	−3	5.10
	−48	6	33	4.39
R inferior prefrontal	30	27	−12	4.30
Medial prefrontal	0	15	51	4.50
R dorsolateral prefrontal	24	0	57	4.49
L pulvinar	−6	−21	0	4.42
Drug > placebo: attention delay > control				
R superior occipital	30	−81	18	4.84*
L middle occipital	−36	−87	0	3.57*
L anterior prefrontal	−36	54	−6	3.81
R superior prefrontal	18	3	−9	3.69
Placebo > drug: attention delay > control				
R supero-medial parietal	6	−54	57	3.95*

Regions showing main effect of spatial attention under placebo are restricted to those in which clusters are significant at $p < 0.05$ (whole-brain corrected; SPM thresholded at $p < 0.001$, uncorrected). Treatment × condition interactions are thresholded at $p < 0.001$, uncorrected; *$p < 0.05$, corrected for whole-brain, or 12 mm radius spheres centred on MNI coordinates derived from appropriate contrast in Hopfinger et al., 2000. Simple effects of attention > control for strongest treatment level are also significant at $p < 0.001$, except L anterior prefrontal ($p < 0.01$) and R supero-medial parietal ($p < 0.005$).

Figure 7.4. Effects of physostigmine on spatial attention versus control. Surface rendering of regions showing activity during delay periods of attention versus control tasks, under placebo, physostigmine, and when comparing the two treatments for this effect. Graphs plot % signal change from baseline for each task (separating attend-right and attend -left conditions) in right and left occipital regions showing enhancement of attention versus control under physostigmine. Both regions showed an enhancement of attention relative to control activity on both attend right and attend left trials (**p < 0.001, uncorrected). The superior parietal region showing less activation under physostigmine for the same contrast is also shown in Fig. 7.6A.



fMRI data: Effects of physostigmine on right versus left-spatial attention

We next addressed whether physostigmine influenced the differential activation of right-versus-left occipital cortices (and vice versa) as a function of attended location. Under placebo, attending to either hemifield (versus the opposite hemifield) activated contralateral occipital cortex, stronger for right-versus-left attention than vice versa (Table 7.2, Fig. 7.5A). Under physostigmine, activation of occipital cortex contralateral to attended hemifield was also evident, although the volume of activation for this right-versus-left attention contrast was notably less. The direct between-group comparison of right-versus-left attention, and vice versa, showed that contralateral occipital cortex was less differentially activated under physostigmine than placebo for both hemispheres. Inspection of signal estimates indicates that this interaction was driven by greater ipsilateral than contralateral occipital cortex activation under physostigmine (Fig. 7.5B).

Consequently, the effect of physostigmine on superior-middle occipital cortex was two-fold: 1) it increased activity selectively during the Attention task (rather than WM or control tasks); 2) it decreased the differential retinotopic activation observed as a function of visual-hemifeld cueing (i.e. Rvs L and vice versa) during the unmedicated session. The latter effect occurred because physostigmine enhanced occipital activations more within the hemisphere representing the unattended visual hemifield.

Since physostigmine decreased cue-driven spatial biasing of occipital cortex, we determined whether there was an equivalent effect on performance. For the latter measure, we calculated the difference in accuracy between validly and invalidly cued trials ('invalidity effect') for each subject. There was a highly significant correlation

between drug-induced impairment of the invalidity effect and drug-induced attenuation in occipital activity lateralisation reported above ($r = 0.70$; $p = 0.001$; Fig. 7.5C). In other words, where physostigmine decreased the spatial biasing of BOLD activity between right and left occipital cortices, there was a proportionate reduction in the differential processing of task-relevant versus task-irrelevant stimuli.

Furthermore, those occipital regions manifesting a physostigmine-induced enhancement specifically during ipsilateral attention (versus control) showed a correlation of this effect with drug-induced improvement in accuracy of invalid trials ($r = 0.51$, $p < 0.05$; Fig. 7.5C; effect averaged over bilateral occipital peaks showing treatment × task interaction: −33, −87, 0 and 30, −81, 18). These BOLD-behavioral correlations, together with the fact that the peak signal estimates of both superior occipital regions in Fig. 7.5A were less ($p < 0.05$) than those observed elsewhere in superior occipital cortex (e.g., Fig. 7.4), argues against the possibility that a ceiling in the hemodynamic response could explain the treatment × laterality interactions.

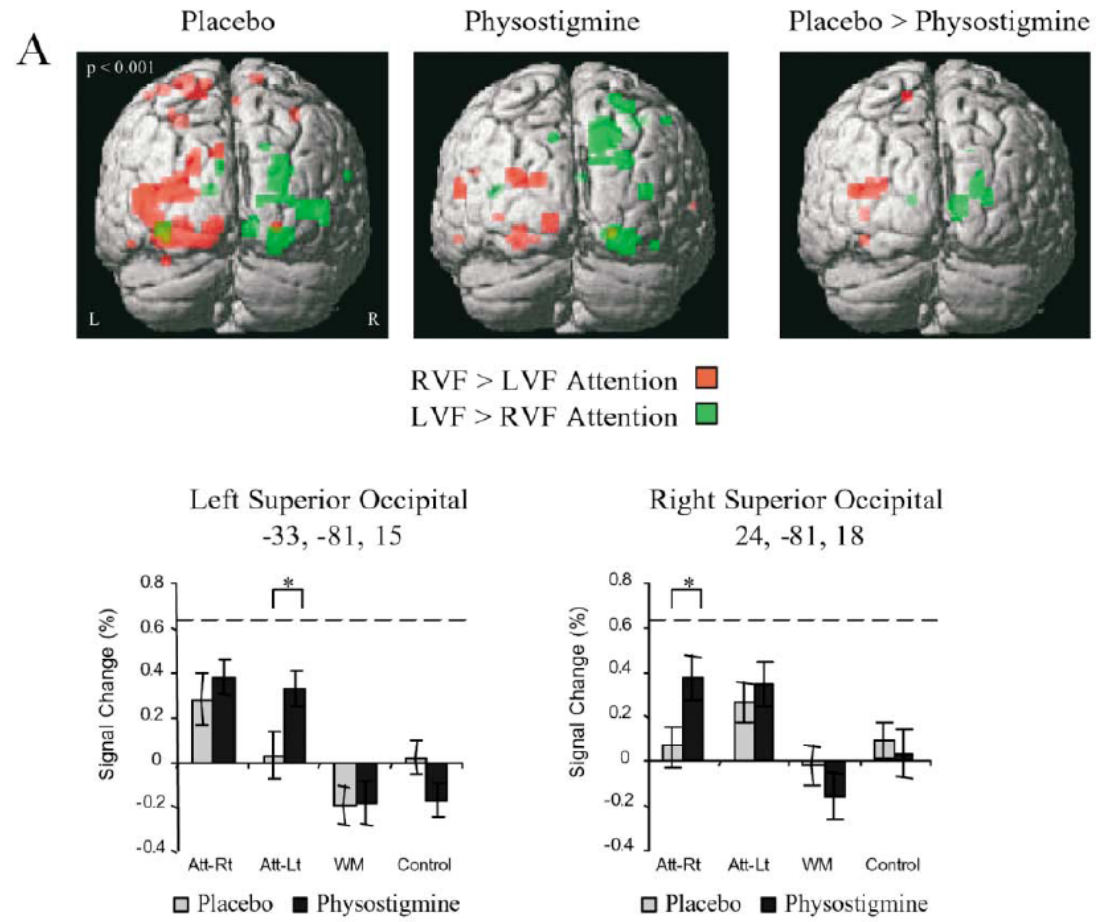
Table 7.2: Regions showing effect of lateralized attention, under placebo, and interaction of this with physostigmine

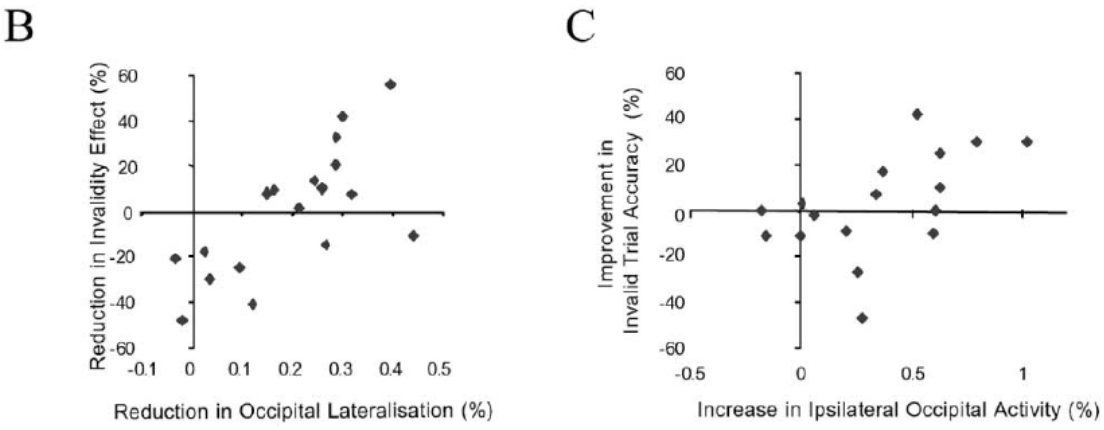
Brain Region	Peak Coordinates			Z Score
Placebo: RVF > LVF attention				
L middle occipital gyrus	−42	−78	12	4.14
Placebo: LVF > RVF attention				
R middle occipital gyrus	45	−75	3	3.72
R fusiform gyrus	24	−69	−12	3.89
R lingual gyrus	9	−81	−9	3.53
L fusiform gyrus	−36	−93	−9	3.91
Placebo > drug: RVF > LVF attention				
L middle occipital gyrus	−33	−81	15	3.83
Placebo > drug: LVF > RVF attention				
R middle occipital gyrus	24	−81	18	3.53

RVF, right visual field; LVF, left visual field. Contrasts are thresholded at $p < 0.001$, uncorrected. All regions are significant at $p < 0.05$, corrected for 12 mm radius spheres centered on MNI coordinates derived from similar contrasts in Martinez et al., 1999 (except for L lingual gyrus). Simple effects of attention laterality for strongest treatment level are also significant at $p < 0.001$ at all coordinates.

Figure 7.5. (See next page): Effects of physostigmine on right versus left spatial attention and vice versa. (A) Surface rendering of visual regions showing activity during delay periods of attend-right versus attend-left and vice versa for placebo, physostigmine, and the difference between treatments for these effects. Graphs plot percent signal change from baseline for the three conditions (separating attend-right and attend-left conditions) in regions from right and left occipital cortices showing reduced differential activity to attend-left versus right (and vice versa) under physostigmine relative to placebo. The physostigmine-induced reductions in differential activity occurred as a result of physostigmine increasing activity during ipsilateral attended trials ($*p < 0.05$) rather than due to drug-induced effects during contralateral attended trials (not significant). Dashed line indicates peak activity observed in right superior occipital region from Fig. 7.4 (which was significantly greater than the peak activity depicted here). RVF, right visual field; LVF, left visual field. (B) Scatter plot illustrates relationship between physostigmine-induced reduction in occipital lateralization and a behavioral measure of physostigmine-induced reduction in stimulus selectivity. Values on x axis calculated as Placebo[contralateral - ipsilateral activity] - Physostigmine[contralateral - ipsilateral activity], averaged over both occipital peaks showing treatment x laterality interaction. Values on y axis calculated as Placebo - Physostigmine Invalidity Effect, where Invalidity Effect = valid trial - invalid trial accuracy. (C) Scatter plot illustrates relationship between physostigmine-induced enhancement of delay period activity in occipital cortex ipsilateral to cue location and accuracy on invalid trials. Values on x axis calculated as Placebo[ipsilateral - control activity] - Physostigmine[ipsilateral - control activity], averaged over both occipital peaks showing equivalent treatment x condition interaction.

Figure 7.5. For legend see previous page.





fMRI data: Effects of physostigmine on working memory

The effect of working memory versus control task, under placebo, engendered activation in superior parietal and prefrontal cortices (Table 7.3). A similar network of fronto-parietal areas was also activated by the attention task, under placebo, as shown by a conjunction analysis over the two tasks (i.e. regions significantly active in both attention and working memory: red in Fig. 7.6). However, in contrast to the case in the attention task where superior-medial parietal cortex showed attenuated responses under physostigmine (yellow in Fig. 7.6), there was no drug-induced modulation of this area in the working memory task. The difference in drug effect on this region between attention and working memory conditions (i.e. group-by-task interaction) just fell short of conventional significance (6, -63, 60; $Z = 2.77$; $p < 0.005$, uncorrected; first graph). The only area showing an effect of drug on working memory activity (versus control) was in left inferior prefrontal cortex that showed less differential activation under physostigmine. There was no group-by-task interaction in this region.

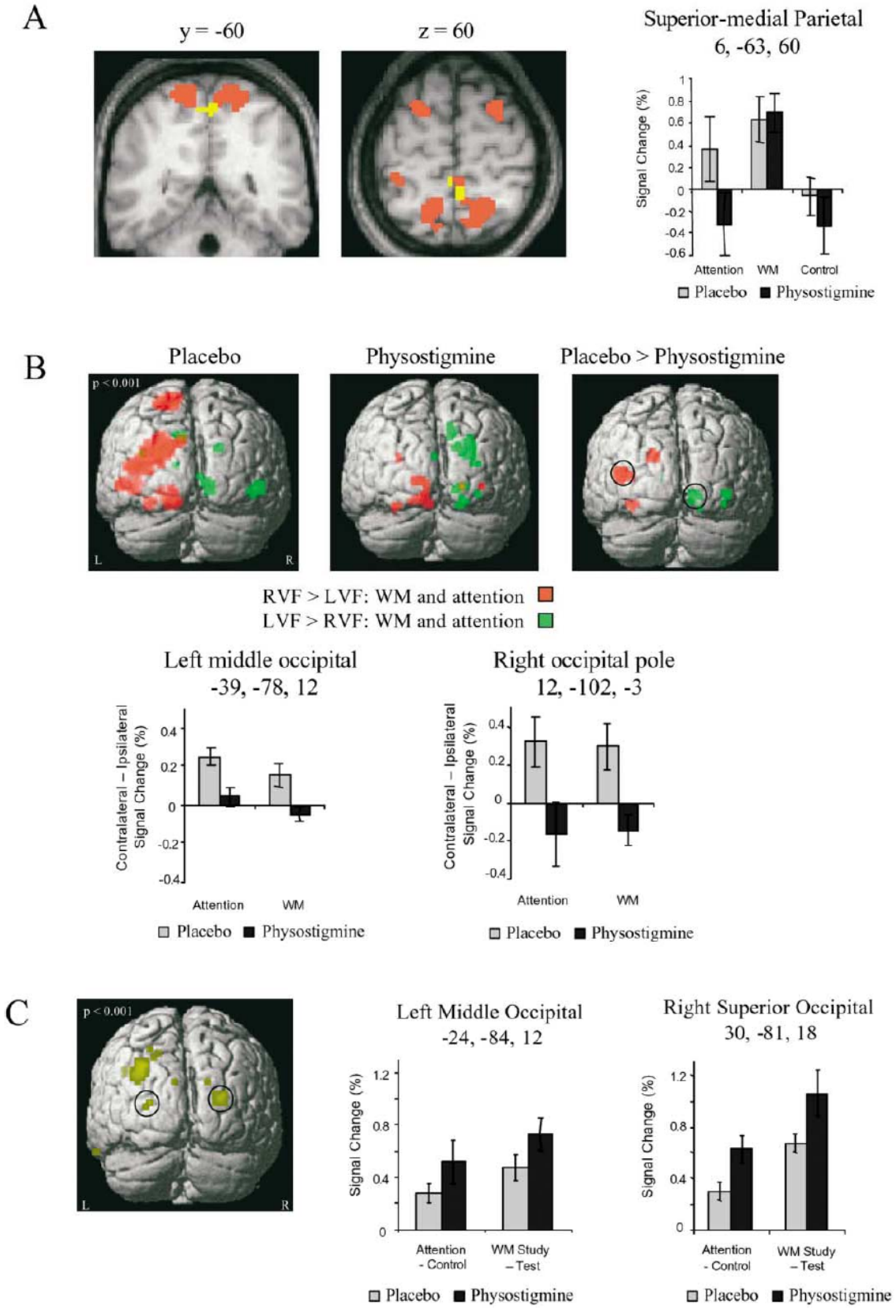
Table 7.3: Regions showing effect of working memory versus control, under placebo, and interaction of this with physostigmine and showing the effects of both lateralized attention and lateralized working memory, under placebo, and interaction of these with physostigmine.

Brain Region	Peak Coordinates			Z Score
A.				
Placebo: spatial WM[1] > control				
R superior parietal	6	−60	57	4.28*
L superior parietal	−24	−57	66	3.43
R dorsolateral prefrontal	27	3	60	4.16*
L inferior prefrontal	−33	18	3	3.38
R inferior prefrontal	48	21	−6	3.35
Drug > placebo: spatial WM > control				
No areas reached significance				
Placebo > drug: spatial WM > control				
L inferior prefrontal	−33	18	0	3.13
B.				
Placebo: RVF > LVF (attention & WM)[2]				
L superior occipital gyrus	−18	−87	27	5.46*
L inferior temporal gyrus	−42	−63	0	5.05*
L fusiform gyrus	−15	−84	−15	4.88*
L occipital pole	−12	−96	−6	3.43
Placebo: LVF > RVF (attention & WM)				
R occipital pole	12	−102	−3	4.37*
R inferior temporal gyrus	45	−66	−3	3.85
Placebo > drug: RVF > LVF (attention & WM)				
L middle occipital gyrus	−39	−78	12	4.97*
L fusiform gyrus	−33	−72	−12	4.33*
L superior occipital gyrus	−18	−87	24	4.02
Placebo > drug: LVF > RVF (attention & WM)				
R occipital pole	12	−102	−3	3.63*
R inferior occipital gyrus	36	−87	−3	3.11

Contrasts are thresholded at $p < 0.001$, uncorrected; *$p < 0.05$, corrected for whole-brain or for 12 mm spheres centred on MNI coordinates derived from similar contrasts in Rowe and Passingham, 2001 (working memory) and Martinez et al., 1999 (attention laterality). Simple effects of laterality for attention and WM under the strongest treatment level were significant at $p < 0.001$, uncorrected, at all coordinates.
[1]Effects presented in this table are restricted to *delay* periods of each task; see text for WM *encode* phase.
[2]This contrast represents the conjunction of (RVF > LVF attention) and (RVF > LVF WM).

Fig. 7.6. Dissociation and commonalities of physostigmine effects on spatial attention and WM. (A) Regions within superior parietal and prefrontal cortices showing increased activity to the conjunction of attention and working memory, relative to control, under placebo (red). Superimposed is that parietal region showing greater differential activity to attention versus control, under placebo relative to physostigmine (yellow). This region was not modulated by physostigmine during WM in spite of showing an even greater effect of WM relative to control under placebo. Graph plots percent signal change from baseline for the three conditions in superiormedial parietal region showing a treatment x condition (attention or WM) interaction ($p < 0.005$). Values have been mean corrected with respect to occipital regions (across groups) to facilitate interregional comparison. (B) Surface rendering of visual regions showing activity during delay periods of both WM-right versus left (i.e., whether study items were presented in right or left visual field) and attend-right versus left and vice versa, as revealed by a conjunction analysis of laterality effects over both tasks, for placebo, physostigmine, and the between-treatment effect. Graphs plot percent signal change difference between trials in which attention or WM were directed contralaterally versus ipsilaterally to each occipital side. Plots are from those coordinates showing the maximal treatment x laterality interaction (ringed) and demonstrate similar physostigmine-induced reductions in selective occipital activation with both attention and WM. (C) Surface rendering of visual regions showing physostigmine-induced enhancement of both attention delay (versus control delay) and WM encode (versus WM test) contrasts. Graphs plot percent signal change difference for both contrasts under placebo and physostigmine, in those occipital coordinates showing the maximal treatment effect on both contrasts (ringed).

Figure 7.6. For legend see previous page.



Discussion

The present study sought to dissociate cortical effects of cholinergic enhancement on attention, from those on visual stimulation and working memory, by employing a design that minimised sensorimotor confounds between tasks and treatments (see Rowe et al, 2000). Physostigmine increased differential activity in bilateral superior occipital cortex during spatial attention, relative to both control and working memory conditions, but decreased differential activity in superior parietal cortex selectively with spatial attention. Physostigmine also modulated prefrontal cortex differently for attention and WM tasks. In contrast to an increase in superior occipital activity with attention, physostigmine decreased activity in primary visual cortex evoked by visual stimulation. Finally, physostigmine-induced enhancement of superior occipital cortex activity was greater on the side ipsilateral, than contralateral, to that spatially attended, resulting in a net reduction in selectivity of visual cortex activation under attention. This finding is supportive of models (Yu & Dayan, 2002; Hasselmo & Cekic, 1996) that predict acetylcholine decreases top-down influences on stimulus processing.

We discount any explanation of our findings in terms of general effects of drug on blood oxygenation level dependent (BOLD) responses. Firstly, all task effects were corrected for session means, which themselves did not differ by treatment across any of the areas highlighted (nor was there a treatment effect in global activity). Secondly, certain task-by-treatment interactions (apparent in Fig. 7.4, 7.5 and 7.6) can only be explained by recourse to an effect of drug on specific cognitive processes. In the case of drug effects across all tasks (Fig. 7.3), we note that the effect reported was specific to only one part of visual cortex, arguing against a general change in BOLD

responsiveness. While a BOLD response ceiling could potentially explain a reduced occipital lateralisation under drug, we observe that the peak response is different between contiguous occipital regions (that would be unlikely in the case of a vascular ceiling). Furthermore, the behavioural-BOLD correlation found strongly suggests that the BOLD effects observed indeed mirror population neuronal activity. Finally, the fact that subjects reported more sedation with physostigmine argues against an 'arousal' explanation for enhanced performance and BOLD responses under drug. Conceivably this effect on subjective alertness might reflects influences of physostigmine on brainstem / reticular formation.

Cholinergic modulation of attention relative to other cognitive processes

Our results of physostigmine-induced enhancement of occipital cortex, selectively with attention, but reduction in primary visual cortex activity to visual stimulation, bear similarity to an fMRI study by Furey et al (2000). Using an identical drug protocol, this group demonstrated physostigmine-induced enhancement of extrastriate cortex selectively during study, versus test, phases of a face working memory task, but a decrease within inferior occipital cortex to non-face stimuli during a control task. The results of our study help to narrow the interpretation of Furey et al, 2000 by suggesting that it was increased attention to study-phase faces, rather than stimulus-related properties (e.g. first presentation of a face; see Experiment 1), or working memory demand, that accounted for the selective facilitation of occipital activity by physostigmine.

One explanation for the specific effects of physostigmine on occipital activity is that more acetylcholine was released during attention, than other, conditions (see

Himmelheber et al, 2000), allowing physostigmine to have a greater local effect. Although the response profile in calcarine sulcus argues against this (by showing non-selective physostigmine modulation: Fig. 7.3), this may reflect the fact that primary, relative to higher, sensory areas possess greater concentrations of cholinergic receptors (Mash et al, 1988; Prusky et al, 1988), and hence their cholinergic responsiveness may occur at lower concentrations of acetylcholine. An alternative explanation for the attention-specificity of physostigmine on extrastriate cortex is due to an effect of drug on higher processing centres (as we found in superior parietal and prefrontal cortices), which may have then indirectly augmented activations in sensory regions (see Sarter et al, 2001). The behavioural consequences of cortical cholinergic deafferentation have suggested that cholinergic inputs to rat prefrontal (Gill et al, 2000), but not visual (Sarter et al, 2001), cortices are necessary for normal sustained attention. A future method by which direct versus indirect effects of cholinergic modulation on sensory cortices may be addressed is through connectivity analyses of human functional imaging data (Friston et al, 2002).

Physostigmine decreased activity over all conditions in primary visual cortex, consistent with previous studies showing cholinergic reductions in posterior occipital cortices (Experiment 1; Mentis et al, 2001;Grasby et al, 1995; Thiel et al, 2001), and indicating that attention-specific cholinergic enhancement of occipital activity is limited to higher parts of the visual stream. We suggest that acetylcholine decreases net neuronal activity in early visual areas due to both noise suppression (e.g. Sato et al, 1987, Murphy & Sillito, 1991) and reduced feedback from higher centres (Hasselmo & Cekic, 1996; Kimura et al, 1999). In so doing, cholinergic modulation of early visual processing may both facilitate a feedforward direction of information-

flow (Hasselmo & Cekic, 1996) and potentiate higher visual processing (as indexed by greater activation under physostigmine in higher visual areas in the present study, or in Furey et al, 2000; Experiment 1).

In contrast to the wide effects of physostigmine on attention-associated activity, physostigmine's modulation of working memory responses was restricted to inferior prefrontal cortex (similar to Furey et al, 2000). Notably, physostigmine decreased attention, but not WM-related, activity in superior parietal cortex, in spite of both conditions activating this region under placebo. This pattern of activity could be explicable, as for occipital cortex, either in terms of differing acetylcholine release between conditions, or as a difference in input to parietal cortex from other cortical regions (occipital and prefrontal cortices were more widely activated under attention than WM). We also note a parallel dissociation of drug effect on accuracy between attention (improved) and WM (no effect), in spite of the tasks being similarly difficult (under placebo, attention was performed slightly less accurately, but faster than WM). Such attention-specific effects of cholinergic manipulation mirror animal studies that de-emphasise the role of cortical acetylcholine in modulating short-term memory (e.g. Chappell et al, 1998; Baxter et al, 1996). We suggest that behavioural and neuronal effects of cholinergic drugs observed in human WM tasks (e.g. Furey et al, 2000; Lawrence et al, 2002; Ernst et al, 2001b; Kumari et al, 2003) act primarily through attentional components within these tasks.

Cholinergic modulation of selective attention

A most significant finding of our study was that, within the attention condition, physostigmine decreased the selectivity of visual cortex activation, through increasing

activation disproportionately on the occipital side ipsilateral (representing the irrelevant visual field), rather than contralateral, to the attended direction. Furthermore, subjects in whom this effect was greatest showed the least performance discrepancy between valid and invalid trials (the fact that this correlation crossed the abscissa suggests an additional factor, e.g. observed drug-induced fatigue, may have worked against the behavioural effect). This data supports psychopharmacological studies showing that cholinergic levels inversely correlate with the detriment engendered by invalid cues (Stewart et al, 2001; Witte et al, 1997; Chiba et al, 1999), and that the hypercholinergic state may be associated with heightened processing of irrelevant information, e.g. as in anxiety (Bernston et al, 1998). Our imaging results suggest a neural substrate for such enhanced processing of task-irrelevant stimuli. Interestingly, we note a similar effect of physostigmine on auditory cortical responses was observed in Thiel et al (2002), when responses to a behaviourally-irrelevant tone were increased disproportionately relative to a relevant (conditioned) tone.

Summarising, we note that while cholinergic enhancement increases selectivity of occipital activation comparing tasks of different attentional demand (see also Furey et al, 2000), and increase selectivity of stimulus-evoked responses (e.g. Sato et al, 1987, Murphy & Sillito, 1991), it appears to decrease the selectivity of attention-driven modulation of occipital cortices. These findings are in keeping with two recent models (Hasselmo & Cekic, 1996; Yu & Dayan, 2002) which suggest that neocortical acetylcholine favours feedforward over feedback, processes. Such models propose that ACh is preferentially released under conditions of high uncertainty, e.g. sustained attention, with the effect of suppressing top down modulation of stimulus processing. Since such top down modulations of sensory cortices may involve a biasing between

mutually-inhibitory areas (e.g. Chelazzi et al, 2001), our results could be explained in terms of Yu & Dayan's model (2002) by assuming that the disproportionate enhancement of occipital cortex, ipsilateral to attention, represented a disinhibition of attentional influences on the spatially-irrelevant side. This account may still be compatible with findings that ACh enhances signal-to-noise ratio (Sato et al, 1987), in that physostigmine increased task-related activations over a wider set of visual areas than placebo, and therefore enhance signal processing generally rather than selectively.

Conclusion

Physostigmine was found to improve subjects' accuracy on a spatial attention, but not spatial working memory, task, while producing modulations of occipital and parietal cortex under the former, but not the latter, condition. Occipital cortex also exhibited different responses to physostigmine comparing attention (drug enhanced superior occipital cortex) with visual stimulation (drug suppressed primary visual cortex over all conditions). Finally, the selectivity of occipital activation normally observed under spatial attention was diminished under physostigmine. These results support a central role for the cortical cholinergic system in specifically attentional processing (Everitt & Robbins, 1997), and suggest that acetylcholine may bias the interaction of top-down with bottom-up processes.

8. EXPERIMENT 4:

Effects of ChEI on Visual and Attentional Processing in Healthy Elderly and Alzheimer's Disease

Introduction

An understanding of how the cholinergic neuromodulatory system interacts with cerebral cortical processing represents an important step in elucidating neuropsychological conditions such as Alzheimer's disease, cortical Lewy body disease, vascular dementia, and head injury (Auld et al, 2002; Tiraboschi et al, 2000; Wilkinson et al, 2005; Conner et al, 2005). Conversely, studies of brain diseases associated with damaged cholinergic structures inform the normal physiology of acetylcholine as a central nervous system neurotransmitter. In Alzheimer's disease, the association of acetylcholine with cognitive impairment is suggested by three principal facts: cortical cholinergic neurons, along with medial temporal structures, are preferential victims of the degenerative process in AD (Geula & Mesulam, 1989); selective lesions of cortical cholinergic neurons in animals reproduce the memory and attentional deficits found in Alzheimer's disease (Everitt & Robbins, 1997); and cholinesterase inhibitors by increasing concentrations of acetylcholine throughout the brain help to improve, or slow the deterioration, in cognitive performance in AD (Rogers et al, 1998).

Experiments 1 and 3 investigated the effects of cholinesterase inhibition on stimulus processing and attention in healthy young adults using fMRI. A starting hypothesis was that attention-related activations of parietal and sensory cortices would be enhanced with raised levels of acetylcholine, since animal-lesion studies have shown that corticopetal cholinergic fibres are necessary for sustained and selective visual attention (Sarter & Bruno, 1997), and administration of cholinesterase inhibitors to healthy humans had been associated with improved performance and heightened

extrastriate cortex responses (Davis et al, 1978; Furey et al, 2000). However, across three different paradigms (Experiment 1 and Experiment 3; also Thiel et al, 2002) the opposite effect was found: parietal activation and the differential activation of sensory cortices as a function of task or emotional memory (i.e. top-down influences) was mostly decreased by physostigmine in spite of subjects tending to be faster. One explanation for this lay in the findings that cholinergic enhancement increased sensory cortex activity non-specifically which may have a greater relative effect during conditions that do not normally activate sensory cortices compared to those conditions that, in the healthy brain, are already activated near to maximum (Experiment 3; Thiel et al, 2002). This is consistent with neurobiological models predicting that acetylcholine favors bottom-up over top-down sensory processing (Hasselmo & Giocomo, 2006; Yu & Dayan, 2005), and with data suggesting that excessive cholinergic stimulation underlies heightened processing of irrelevant stimuli (Thiel et al, 2005), including in anxiety (Bernston et al, 1998).

In Alzheimer's disease degeneration of cholinergic neurons that connect substantia innominata with cerebral cortex is an early pathological finding, whereas the intrinsic structure of sensory cortices is relatively spared (Geula & Mesulam, 1989). Animal studies have demonstrated that cholinergic stimulation of sensory cortices has facilitatory effects on stimulus-processing parameters such as selectivity and signal-to-noise ratio (e.g. Sato et al, 1987, Murphy & Sillito, 1991), while cholinergic inputs to frontoparietal cortices provide a necessary contribution to tasks requiring sustained or selective attention (Sarter & Bruno, 1997). Behavioral testing in mild-to-moderate Alzheimer's disease have recognised defects in both early sensory processing e.g. visual contrast sensitivity (Tippett et al, 2003) as well as with attentional manipulation

of sensory information (Perry et al, 2000; Baddeley et al, 2001). Consequently, it is reasonable to hypothesise that at least part of both the visual and attentional deficits in AD are due to a reduction of cholinergic input to sensory and frontoparietal cortices (see also Perry & Hodges, 1999). Using fMRI this hypothesis was tested by firstly examining differences in both visual and attentional processing between AD and healthy controls, and secondly by enquiring as to whether a restoration of normal activity can be achieved following administration of a cholinesterase inhibitor. Following on from the results of Experiments 1 and 3 in healthy young adults, the following predictions were made for an fMRI study comparing AD patients with healthy age-matched controls:

1) Stimulus-selectivity in extrastriate cortex (BOLD-responses to face versus building visual stimuli, and vice versa) would be decreased in AD relative to controls, but this would be corrected with cholinesterase inhibition.

2) Attention-dependent activations of frontoparietal cortex with attention would be diminished in Alzheimer's disease relative to controls, but this would, at least partially, be reversed by cholinesterase inhibition. The direction of this pharmacological effect would therefore be opposite to what we expected in controls (see also Experiment 3; Furey et al, 2000; Thiel et al, 2005).

3) Attention-driven modulation of extrastriate cortex (stimulus-selectivity compared between high and low attention-demanding tasks) would be decreased in AD relative to controls due to impaired recruitment of sensory cortex in the more attention-demanding condition. Furthermore, this failure would be remedied by cholinesterase

inhibition. In other words, cholinesterase inhibition would produce the opposite attentional effect in AD as that seen in controls: an increase in stimulus-selectivity under the harder condition (in AD), rather than an increase in stimulus-selectivity under the easier condition (in controls).

Methods

Subjects

Sixteen right-handed patients with newly-diagnosed Alzheimer's disease and MMSE of 21 – 26 were recruited from the Dementia Research Group, National Hospital for Neurology and Neurosurgery (London, United Kingdom) over a fifteen month period. Seventeen right-handed healthy subjects, matched for age and sex, were recruited over the same period. No subjects were active smokers. Characteristics of the two groups are listed in Table 8.1. All subjects gave written informed consent. The inclusion criteria for patients were: (i) probable Alzheimer's disease according to international criteria (National Institute of Neurological and Communication Disorders/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) and the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSMIV). Exclusion criteria for patients were: (ii) alternative or additional diagnosis contributing to cognitive symptoms considered possible; this was assessed following a full neuropsychological, neurological and general clinical examination, as well as dementia-screening blood tests, chest x-ray, brain MRI, electroencephalography, and cerebrospinal fluid examinations (where felt to be appropriate for diagnosis); (iii) mild cognitive impairment; (iv) major visuospatial or visuo-perceptual impairment or severe apraxia; (v) coexistent significant central

nervous system disease, e.g. no epilepsy, movement disorder, head injury, drug nor alcohol abuse; (vi) receiving psychoactive drugs, including cholinesterase inhibitors, N-methyl-D-aspartate antagonist, or antidepressants. Patients or healthy subjects found to have significant lesions on brain MRI (other than Alzheimer's disease-associated changes in the case of the Alzheimer's disease group) such as ischemic changes, were excluded.

All patients were started on therapeutic oral cholinesterase inhibitor following the second experimental session, and were followed up for a minimum of one year to ensure that no other features developed that would suggest an alternative cause for dementia.

Table 8.1: Characteristics of control and Alzheimer disease subjects (±95% confidence intervals).

	Controls	AD
Number	17	16
Males	8	9
Age	64.9 (±4.0)	66.4 (±4.4)
Education (in years)	12.7 (±0.8)	12.5 (±0.9)
Baseline blood-pressure	129/75.8 (±9.0/4.9)	135/82.4 (±6.5/3.5)
MMSE	29.6 (±0.2)	23.9 (±1.2)*
Verbal IQ (WAIS)	n/a	94.2 (±5.7)*
Performance IQ (WAIS)	n/a	92.7 (±7.9)*
Verbal IQ (NART)	115 (±1.1)	n/a
Performance IQ (NART)	115 (±1.1)	n/a

IQ scores in controls are estimated from National Adult Reading Test (NFER-NELSON Publishing Co. Ltd., Berkshire, England, 2nd Edition, 1991).
*$P < 0.01$ between-group difference.

Drug-treatment

A double-blind placebo-controlled drug administration technique was used. Each subject received an intravenous cannula into the left cubital fossa and an infusion of either physostigmine or saline, depending on session. In the drug-session, subjects first received 0.2 mg intravenous glycopyrrolate (peripheral muscarinic receptor antagonist) before being administered an infusion of physostigmine at a rate of 1mg / hr. Testing took place at 25 minutes from the start of the infusion. In the placebo-session, an equivalent volume of saline was administered in all steps. We employed a lower dosage of physostigmine relative to Experiments 1, 2 and 3 that had used subjects aged between 20 and 30, since a pilot study showed an unacceptably high level of adverse effects (predominantly nausea and vomiting in 4/6 subjects) in the age-range of the present study. The dosage and timing schedule of physostigmine that we used was based upon previous studies in which performance improvements were observed over a range of tasks in Alzheimer's disease (Christie et al, 1981; Asthana et al, 1995; Davis &Mohs, 1982; Muramoto et al, 1984).

Cognitive task

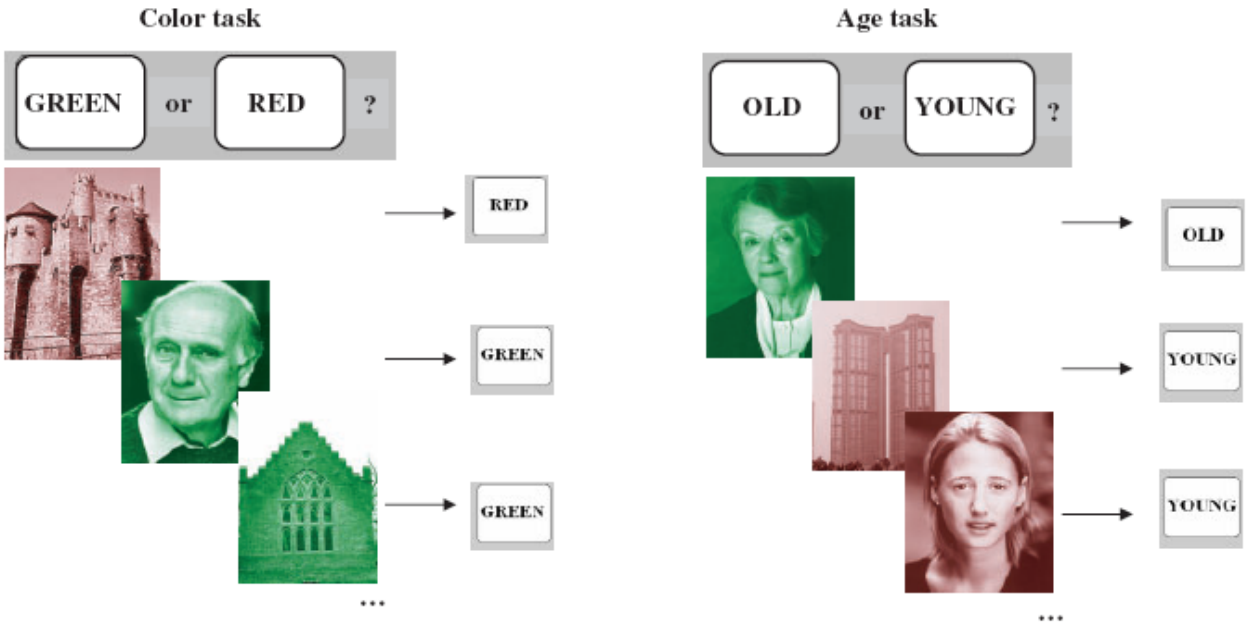
On each of two sessions (placebo / physostigmine), subjects performed two tasks (Colour and Age: Figure 8.1) separated into blocks of 48 trials each, and repeated once in one of the following orders: CACA, ACAC, CAAC, or ACCA. Treatment and task order were counterbalanced across subjects, with task-order being repeated across sessions. The two sessions were separated in time by 1 – 2 weeks. Both tasks comprised serial presentation of either faces or buildings with no image being repeated across both sessions. The images for both tasks were presented in isoluminant red or green monochrome. The Colour task required an indication as to

whether an image was red or green; the Age task required a judgment as to whether the featured face or building was old or young (the latter choice denoting 'modern' in the case of buildings).

The stimulus set comprised an equal number of 'young' (individuals aged 21-35) and 'old' faces (individuals aged over 65), as well as the same and an equal number of modern (e.g. office-blocks) and old buildings (e.g. castles). We excluded faces and buildings that were famous or depicted from a non-canonical view, and faces with overtly emotional expressions. The particular stimuli comprising any session were counterbalanced for task, treatment and group between subjects. Responses were recorded by one of two possible button-presses made with the right-hand. The SOA was 4.05 seconds with images being presented for 1 sec. A reminder of the button meanings for that block preceded each image. Subjects were taught and practiced the tasks with repeating stimuli sixty minutes prior to scanner entry (at each session) for as long it took them to achieve a stable performance. A short practice run was also performed before each block in the scanner. Subjects were informed that a recognition test of faces was to be carried out after scanning but were told to perform their best on the judgements tasks and not to concentrate on memorising items. Images were presented at central fixation and subtended 5º vertically and 3º horizontally.

Subjects were fitted with appropriate MRI-compatible refractive lenses where required. Eye movements were monitored with an infra-red eye tracker (ASL Model 540, Applied Science Group Co., Bedford, MA; refresh rate = 60 Hz) in 16 control and 11 AD subjects.

Figure 8.1: Task design. In the scanner, subjects performed one of two tasks in block-fashion: Colour task: subjects were prompted as to whether the image was red or green; Age task: subjects were prompted as to whether the depicted object was old or young /modern. Face and building-stimuli occurred with equal frequency in each task. Subjects were reminded of the key-press meanings prior to each stimulus.



Imaging and image processing

Data were collected on a 1.5 T MRI scanner (Siemens, Erlangen, Germany) using gradient echo T2*-weighted echo-planar images, with blood oxygenation level dependent (BOLD) contrast. Volumes consisted of 39 horizontal slices through the whole brain, each 2mm thick with a 1mm gap between slices (field-of-view, 192 x 192 mm^2; matrix size 64 x 64). In-plane resolution was 3mm×3mm; effective repetition time (TR), 3.51 s; echo time (TE), 50ms, and flip angle 90°. For each block 63 volumes were acquired, with the task only beginning after the sixth volume to allow for T1 equilibration

effects. Imaging data were pre-processed and analysed using SPM2 (Wellcome Department of Imaging Neuroscience, London; http://www.fil.ion.ucl.ac.uk/spm). Preprocessing consisted of determining and applying rigid affine transformations to the image series to realign the scans (Friston et al, 1995a), normalization (Friston et al, 1995a) to a standard EPI template in MNI space and smoothing with a three-dimensional 8mm Gaussian kernel to account for residual inter-subject anatomical differences.

Statistical analysis of images

Data were analyzed with a general linear model for blocked, event-related designs (SPM2; Wellcome Dept. of Cognitive Neurology, London, UK; Friston et al, 1995) using a random-effects analysis. Data were globally scaled and high-passed filtered at 1/256 Hz. Events were modelled by delta functions convolved with a synthetic hemodynamic response function (Friston et al, 1998); temporal derivatives of these functions were modelled separately (Friston et al, 1998). Within-subject conditions of interest were stimulus-type, task, and treatment. Stimuli in different scanning-blocks were modelled separately to enable estimation of session effects. Six-dimensional head movement parameters derived from image-realignment were included within the model as confounding covariates.

Differences of activity between conditions of interest (stimulus-type, task and their interaction) were estimated for each subject and treatment (i.e. parameter estimates), before being submitted to one-sample t-tests and generation of statistical parametric maps (SPMs) of the t-statistic. The analyses report effects for stimulus-selectivity, task and task x stimulus interactions in control subjects in the drug-free state where

voxels are significant at $p<0.05$, corrected (false-discovery rate) for a visual cortex mask for stimulus-dependent effects, or for whole-brain volume for task effect. The visual cortex mask was constructed manually using MRIcro software (www.mricro.com) and the combined-group mean EPI image so as to include the entire occipital, temporal and parietal lobes but excluding somatosensory and auditory cortices - thereby encompassing activations from Experiment 1 that employed similar stimulus classes. The interaction of task x stimulus was qualified by masking with simple effects of stimulus-selectivity at each task level (thresholded at $p<0.01$, uncorrected). In the task analysis, the threshold was dropped to $p<0.001$, uncorrected, in order to explore effects in prefrontal cortex that was an a priori region of interest and that did not show significance at the whole-brain level. Having identified regions showing the primary effects (stimulus, task and stimulus x task) in drug-free controls, we then interrogated within these areas (thresholded at $p<0.01$, uncorrected) for interactions of these primary effects with group; with treatment in each group separately, and with a treatment-by-group interaction (reported at $p<0.001$, uncorrected). We also report regions that showed enhanced stimulus and/or task-effects in AD relative to controls and inspected for interactions with treatment (x group) within these regions ($p<0.001$, uncorrected). Group-effects were overlaid on mean-normalised functional images of the appropriate group(s) to enable anatomical localisation.

Results

Physiological data, subjective reports, and eye tracking

On both sessions, blood pressure was checked before and after scanning, whilst pulse-oximetry was performed continuously. Subjects were given a questionnaire before and after scanning that allowed a ranked measurement (0 – 6 scale) of seven recognised adverse reactions to physostigmine and glycopyrrolate, as well as visual analogue scales for alertness and physical wellbeing. For blood pressure, there were no effects of drug, time-point, or group, nor interactions between these factors ($p>0.05$). The only physical side-effects reported after the physostigmine (with glycopyrrolate) session, and documented in more than one subject, were nausea (controls: 4; AD: 4 subjects; median severity 1.5/7 within these subjects) and dry mouth (controls: 8; AD: 7; median severity 3/7). Subjective scores of alertness and physical wellbeing both showed an interaction of time-point with treatment ($p<0.01$) reflecting mean reductions over time by 0.14 and 0.15, respectively (on a scale of 0-1) under physostigmine, compared to 0.05 and 0.03, respectively under placebo. However, there was no effect of group or interaction of group with treatment and time ($p>0.1$) for either measure. The frequency and type of side-effects associated with physostigmine are similar to those reported in Experiments 1 and 3.

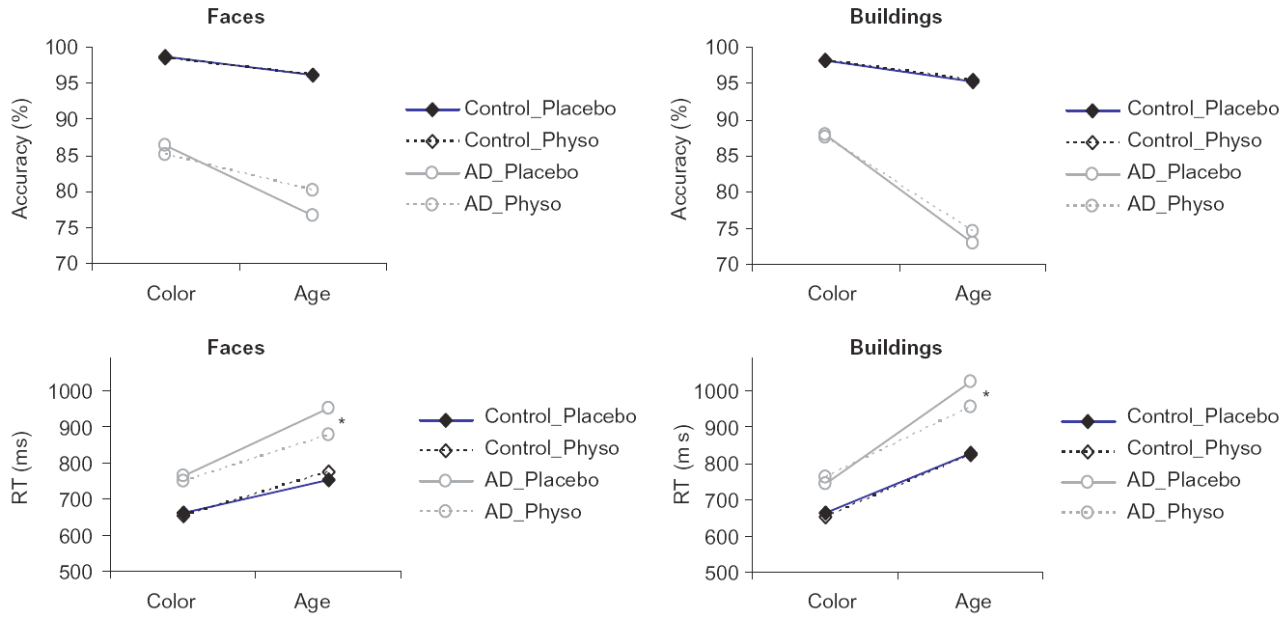
Saccade frequency was 0.8% in controls and 1% in patients. There were no interactions of eye-movement with stimulus-type, task, treatment or group.

Behavioural

RT and accuracy were submitted to between-subject (controls versus AD) repeated-measures ANOVAs with factors of stimulus (building, face), task (Colour, Age), and treatment (placebo, physostigmine) (Figure 8.2). For both RT and accuracy, there were main effects of task ($F(1,31)>24$, $p<0.01$),and group ($F(1,31)>4$, $p< 0.05$), as

well as a task x group interaction for accuracy ($F(1,31)=9$, $p<0.01$) reflecting a greater impairment of performance by AD relative to controls with Age task relative to Colour task (task effect in AD: $F(1,15)=16$, $p<0.01$; in controls: $F(1,16)=8$, $p<0.05$). The equivalent interaction for RT showed a non-significant trend ($F(1,31)=2$, $p=1.3$). The effect of treatment manifested itself as a strong interaction of treatment x group x task ($F(1,31)=9$, $p<0.01$) for RT. Hence whilst there was no treatment effect on performance in controls, physostigmine in AD shortened RTs during Age ($F(1,15)=14$, $p<0.01$) but not Colour ($F(1,15)=0$, ns) tasks ($F(1,31)=10$, $p<0.01$, for the treatment x task interaction). This effect was also present when face and house stimuli were analysed separately ($p<0.05$ for each stimulus-class; there was no treatment x group x task x stimulus interaction) even though Age judgements were more difficult for buildings than faces across all subjects (task x stimulus interaction ($F(1,31)>4$, $p<0.05$ for both measures).

Figure 8.2: RT and accuracy responses separated by stimulus-type and task for each combination of group and treatment. * denotes significant task x treatment interactions for the AD group ($p < 0.05$).



fMRI data: Session effects

Estimates of the mean BOLD signal across each session were obtained both for the whole-brain (global) and in regions described above showing stimulus and task effects in controls. These were subjected to between-group repeat-measures ANOVAs with group, task and treatment as factors. Both global and regional session BOLD estimates were influenced neither by a main-effect nor by an interaction between any of these factors ($p>0.05$).

fMRI data: Effects of physostigmine on stimulus-selectivity

We first identified regions of extrastriate cortex that were selective in their response to faces versus buildings, and to buildings versus faces. The main effects of stimulus type in controls under placebo are listed in Table 8.2 (1st column; also Figs. 8.3A, 8.3G, 8.5A). The regions listed are similar to those found in numerous previous studies for corresponding contrasts of faces versus houses (instead of buildings, as here) and vice versa (see Experiment 1). AD activated a similar set of areas (8.3B, 8.3H, 8.5B), but a direct comparison of stimulus-selectivity between groups in the drug-free state revealed a subset of these regions for which selectivity of both classes of stimulus was reduced in AD relative to controls (Table 8.2, 2nd column; Figs. 8.3E, I). There were no regions in which AD showed greater stimulus selectivity.

Physostigmine was found to reduce both face and building selectivity in many of the regions identified in controls under placebo (3rd column; Figs. 8.3C, J). In AD (4th column), physostigmine modulated stimulus-selectivity in one of two ways that corresponded to whether there had been a difference in stimulus-selectivity between AD and controls in the drug-free state: in right fusiform cortex, the region showing

the strongest face-selectivity, and where there was no difference between groups in stimulus-selectivity ($p>0.1$; peak coordinate in AD being 40, -54, -24; $Z = 4.26$), physostigmine resulted in a similar *decrease* of stimulus-selectivity as had been observed in controls (Fig. 8.3D, M – 1st graph). By contrast, in another face-selective region, precuneus, and in one building-selective region, right posterior parahippocampal cortex, where AD had shown reduced selectivity relative to controls, physostigmine resulted in *increased* selectivity in AD (Figs. 8.3F, K, M – 2nd and 3rd graphs). Consequently, both regions responded to physostigmine in an opposite manner comparing controls and AD as demonstrated by the group x treatment x stimulus-selectivity interactions (Table 8.2, final column; Fig. 8.3L).

Figure 8.3: (See next page): A, B – Main-effect of face > building in controls (A) and AD (B) on placebo at the level of mid-fusiform cortex and precuneus (y = -50). C, D – Interaction of face-selectivity x treatment in controls (C) and AD (D) demonstrating *reduced* selectivity in right fusiform cortex with physostigmine in both groups (y = -50 and -54, respectively). There was no between-group difference in face-selectivity or in the interaction of selectivity x treatment in the right fusiform cortex ($p>0.1$). E, F – E: interaction of face-selectivity x group (on placebo) demonstrating reduction of selectivity in AD relative to controls in precuneus. F: interaction of face-selectivity x treatment in AD demonstrating *increased* selectivity in precuneus with physostigmine relative to placebo. G, H, I – Main-effect of building > face in controls (G), AD (H) and the difference between them (I), on placebo, at the level of parahippocampal cortices (z = -16), demonstrating reduction of selectivity in AD relative to controls in posterior parahippocampal cortex. J, K, L – Interaction of building-selectivity x treatment in controls (J) and AD (K) demonstrating that physostigmine induces a *reduction* of selectivity in controls (J) but an *increase* in selectivity in AD (K) in right posterior parahippocampal cortex. L depicts the interaction of building-selectivity x treatment x group. M – Plots of %-signal change for face > building contrast in right fusiform cortex, and precuneus, and for building > face contrast in right posterior parahippocampal cortex, under each combination of treatment and group. Coordinates plotted are those at the maxima of selectivity x treatment interaction in controls (first graph); and selectivity x treatment in AD (second and third graphs). Activations are thresholded at $p<0.001$, uncorrected, and are superimposed on the mean normalised EPI of controls or patients as appropriate (group interactions are overlaid on patients' mean).

Figure 8.3. For legend see previous page.

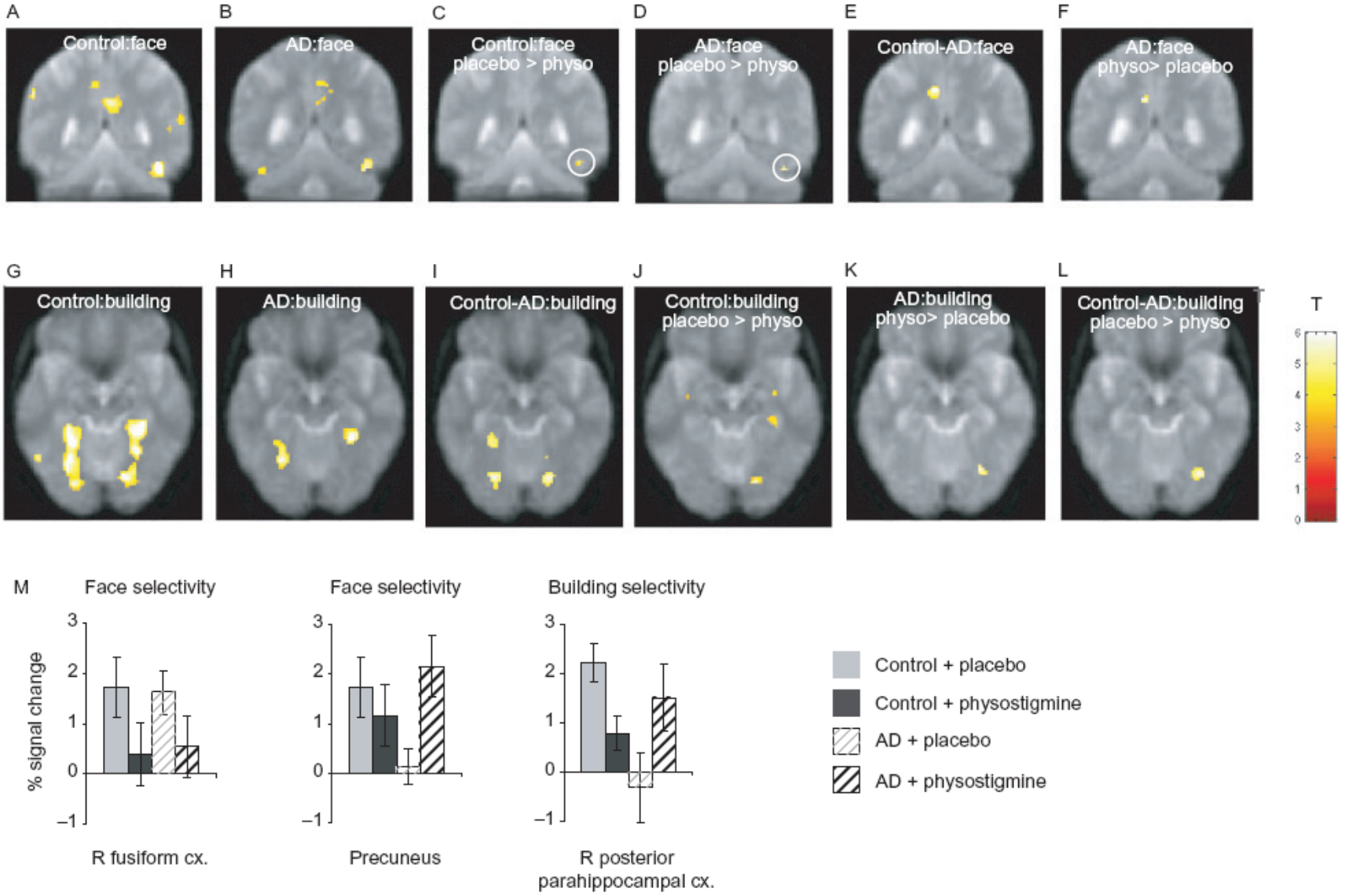


Table 8.2: Effect of stimulus-type on extrastriate cortex.

	Control				Control > AD				Control: Physo > Placebo				AD: Physo > Placebo				(AD > Control) × (Physo > Placebo)			
	x	*y*	*z*	Z-score	*x*	*y*	*z*	Z-score	*x*	*y*	*z*	Z-score	*x*	*y*	*z*	Z-score	*x*	*y*	*z*	Z-score
Faces > Buildings																				
R fusiform cortex	42	−50	−22	4.93					46	−48	−18	−3.95	40	−54	−24	−3.74				
R STS/occipital junction	58	−12	−14	4.04	60	−12	−16	3.74												
	46	−18	−20	4.09																
	56	−38	0	3.64																
	62	−54	10	4.00																
	48	−70	0	4.03																
L STS/occipital junction	−54	−66	14	3.92					−48	−66	14	−3.54								
Precuneus/post. cingulate	−6	−56	38	4.45	−8	−50	40	4.19	−14	−56	40	−3.31	−10	−48	30	4.05	14	−46	18	3.32
	6	−48	28	4.34													−12	−48	32	3.12
Buildings > Faces																				
L sup.lat. occipital cortex	−30	−92	12	5.71	−22	−90	12	4.00	−18	−100	22	−3.79					−28	−90	8	3.87
R sup.lat. occipital cortex	32	−82	18	5.56	28	−98	8	3.10												
	34	−96	10	5.12	30	−90	12	3.68												
R parahippocampal cortex	20	−74	−18	6.01	24	−32	−26	4.05	24	−32	−26	−3.74					26	−32	−26	3.35
					20	−74	−16	3.94	18	−76	−16	−4.11	28	−68	−16	3.63	30	−72	−18	4.23
L parahippocampal cortex	−26	−72	−16	5.33	−24	−74	−16	3.99												
					−24	−42	−20	3.62												
R retrosplenial cortex	14	−54	6	5.24																
L retrosplenial cortex	−12	−56	8	4.80	−12	−52	4	3.90												

First column lists effects in controls under placebo; second column lists differences in stimulus-selectivity between controls and AD under placebo. Remaining columns lists regions showing modulation of stimulus-selectivity by physostigmine relative to placebo in controls, AD, and the difference between groups in their response to physostigmine. Interactions with group and treatment are confined to regions showing effects in controls in drug-free state.

Regions listed under control are significant at $P < 0.05$, corrected; interactions within these regions are thresholded at $P < 0.001$, uncorrected. Negative *Z*-values denote drug effect in opposite direction to that stated (i.e. placebo > physostigmine). STS = superior temporal sulcus.

fMRI data: Effects of physostigmine on task-related activity independent of stimulus type

The contrast of Age – Colour in controls in the drug-free state yielded strong activations within right posterior parietal cortex (Table 8.3 – 1st column; Fig. 8.4A). At a less conservative statistical threshold ($p < 0.001$, uncorrected for whole-brain) there were also activations of right dorsolateral and left inferior prefrontal cortices; there was no effect of stimulus-type in any of these areas ($p>0.5$). In AD, Age – Colour activation was found most strongly in bilateral posterior parietal cortices (46, -56, 52; -40 -70 42; $Z>4.07$; Fig. 8.4B). However, the right parietal and left prefrontal cortex activations were less in AD than in controls (group x task interaction under placebo; 2nd column: Figure 8.4C). There were no regions for which the task effect was greater under AD than controls. Physostigmine administered to controls resulted in a reduction of the task effect in both right parietal and left prefrontal cortex (treatment x task interaction; 3rd column: Fig. 8.4D); simple-effect analysis revealed that this was contributed to by both a drug-induced increase of activity in Colour task and decrease in Age task relative to placebo ($p<0.05$ for both). When administered to AD, physostigmine had the opposite effect: task-dependent activations increased in right parietal cortex (treatment x task interaction; 4th column; Fig. 8.4E; there was a trend for the same effect in left inferior prefrontal cortex at $p = 0.006$, uncorrected) although this was due exclusively to an effect during the Age task ($p<0.5$ in both regions). Therefore the regions that had shown decreases in task effect comparing AD with controls in the drug-free state were also those that showed enhancements of task-related activity following physostigmine. The differences in response to physostigmine between groups were confirmed by significant group x treatment x task interactions at both points (5^{th} column; Figure 8.4F). Furthermore, the drug-induced

increase in relative parietal activation in AD was not explained by the relative change in RT between tasks: the effect was still significant (Z = 3.49) when individual RT difference (between treatment and task) was modelled as a nuisance variable in an analysis of covariance.

Figure 8.4. (See next page): A, B, C – Main-effect of task (Age > Colour) in controls (A), AD (B), and the difference between them (C), on placebo. There were no interactions with stimulus-type in regions shown ($p>0.1$). D, E, F – Interaction of task x treatment in controls (D), AD (E) and the difference between them (F): regions shown are those in which the task-effect is *decreased* by physostigmine relative to placebo in controls (D) but *increased* by physostigmine relative to placebo in AD (E). G – Plots of %-signal change for Colour and Age tasks, for each treatment and group at the maxima for the 3-way interaction (from F). Activations are thresholded at $p<0.001$, uncorrected, and are superimposed on the mean normalised EPI of controls or patients as appropriate (group interactions are overlaid on patients' mean).

Figure 8.4. For legend see previous page.

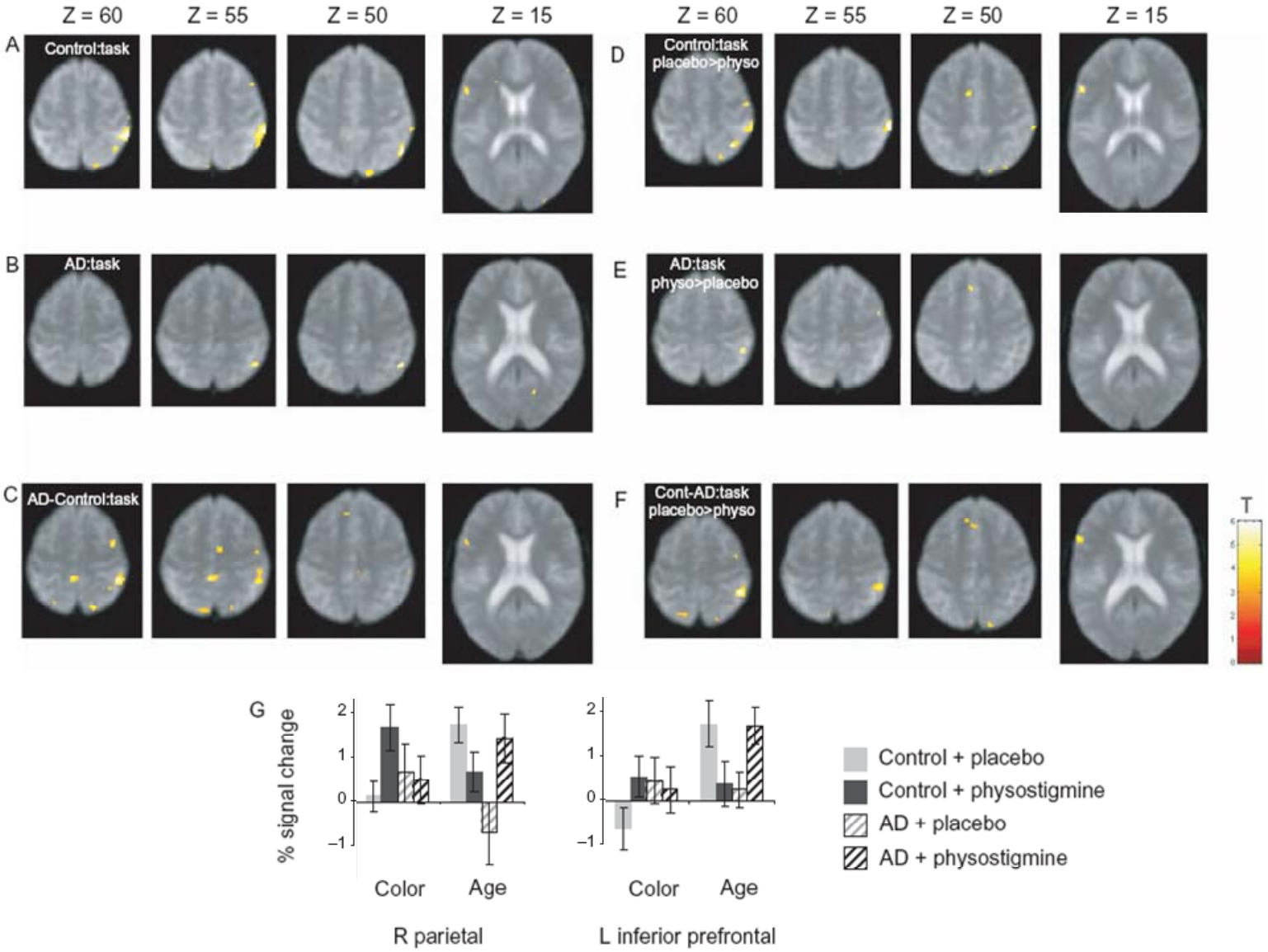


Table 8.3. Effects of task independent of stimulus-type (first row section) and task on stimulus-selectivity effects (second row section).

	Control				Control > AD				Control: Physo > Placebo				AD: Physo > Placebo				(AD > Control) >
	x	*y*	*z*	Z-score	*x*	*y*	*z*	Z-score	*x*	*y*	*z*	Z-score	*x*	*y*	*z*	Z-score	*x*	*y*
Task (independent of stimulus)																		
R posterior parietal cortex	52	−58	48	5.48	36	−50	60	3.14										
	46	−42	58	4.82	44	−42	60	4.81	56	−34	56	−4.78	42	−42	60	3.76	44	−42
	38	−54	62	3.87	58	−32	50	3.27	32	−60	62	−4.33					32	−58
R dorsolateral PFC	44	12	54	3.58														
L inferior PFC	−54	20	16	3.47	−54	16	14	3.40	−54	20	16	−4.00	−58	18	12	2.50*	−58	20
Task × (Faces > Buildings)																		
R posterior STS	60	−64	12	4.80	56	−68	12	5.23	56	−66	10	−3.46	56	−70	14	3.24	58	−66
	54	−58	8	3.62														
	48	−54	12	3.43														
Task × (Buildings > Faces)																		
L lateral occipital cortex	−24	−100	2	5.01	−24	−98	2	4.43	−28	−102	4	−3.46	−26	−100	0	3.88	−26	−100
	−36	−94	6	3.80														

Task effects were observed for Age > Colour, but not vice versa for both stimulus-dependent and stimulus-independent effects. First column lists effects in con second column lists task-effect differences between controls and AD under placebo (i.e. group × task × stimulus and group × task interactions). Third and fourt effects showing modulation by physostigmine relative to placebo in each group (i.e. treatment × task × stimulus and treatment × task interactions); fifth column group comparison of these treatment effects. Interactions with group and treatment are confined to regions showing effects in controls in drug-free state. Regions listed under control are significant at $P < 0.05$, corrected, except for PFC regions that were significant at $P < 0.001$ uncorrected (*a priori* region of interes these regions are thresholded at $P < 0.001$, uncorrected, except for for which $^*P = 0.006$, uncorrected. Negative *Z*-values denote drug effect in opposite directio (i.e. placebo > physostigmine). PFC = prefrontal cortex.

fMRI data: Effects of physostigmine on task x stimulus-selectivity interaction

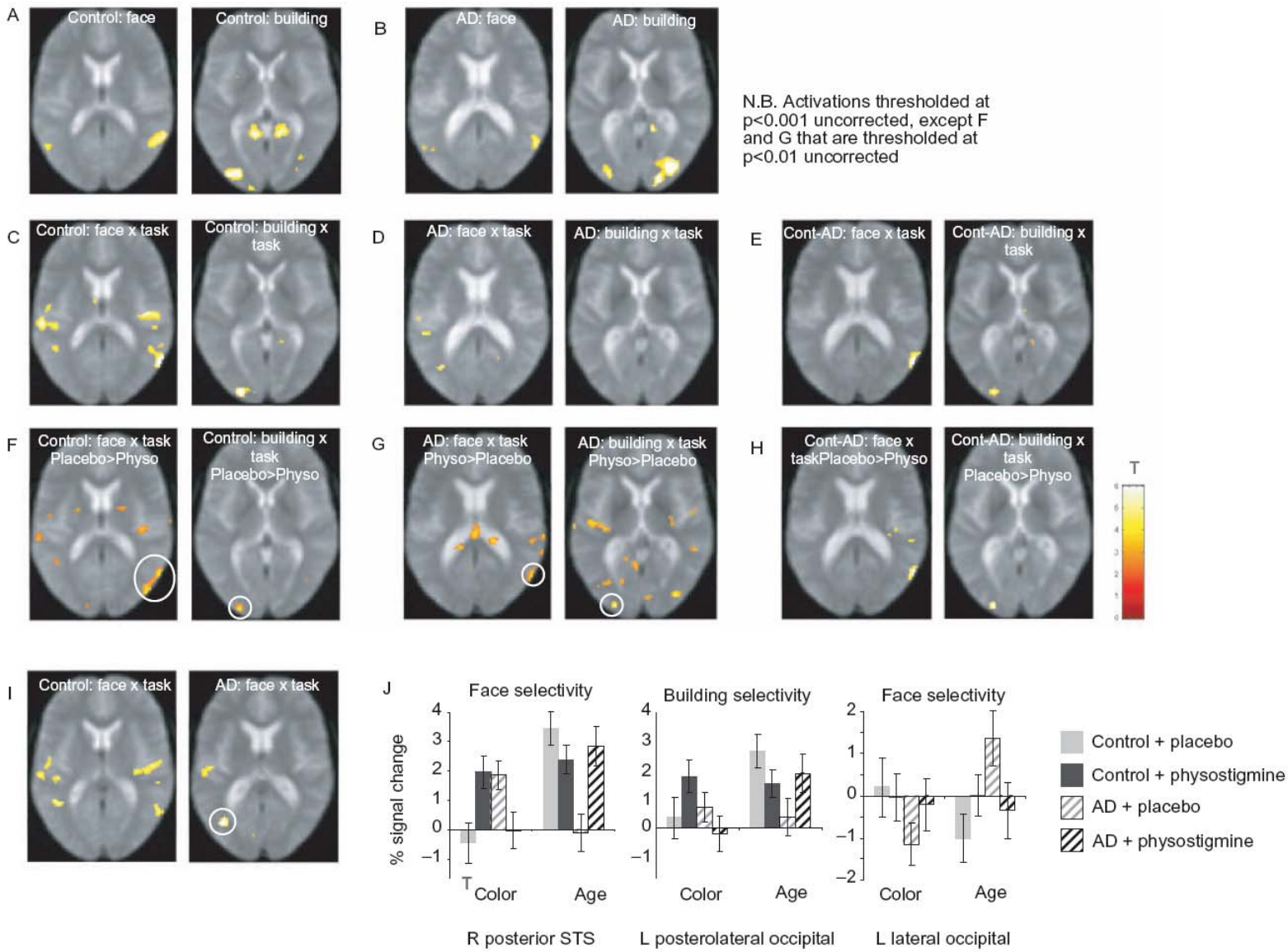
The next analysis examined the interaction of stimulus-selectivity with task. In the control group, under placebo, face-selectivity was enhanced with Age versus Colour task in right posterior superior temporal sulcus (pSTS), while building-selectivity was increased in left posterior occipital cortex for the same task-comparison (Table 8.3 – 1st column; Fig. 8.5C). There were no regions in which stimulus-selectivity was greater with Colour than Age. In AD, the effect of task on selectivity in these two regions was less than that in controls (2nd column; Fig. 8.5D, E). Physostigmine given to controls also lessened the effect of task on stimulus-selectivity in the same two areas (3rd column; Fig. 8.5F). However, the manner by which task-driven modulation of selectivity was lessened differed between effect of group and effect of treatment as revealed by separate analyses of each task. Hence for the factor of group, significant AD-associated *diminutions* of stimulus-selectivity were seen with Age task in both areas, although right pSTS also showed an additional AD-associated increase in selectivity with Colour task ($p < 0.05$ for all). On the other hand, the treatment x task x stimulus interactions in the control group were driven by *increases* in selectivity with the Colour task ($p < 0.05$) rather than by decreases in selectivity with the Age task ($p > 0.1$). In the AD group, physostigmine increased stimulus-selectivity in both areas comparing Age to Colour tasks (4th column; Fig. 8.5G), effectively restoring a similar relationship between task and selectivity as had been observed in controls in the drug-free state. This drug effect in AD was achieved through an increase in selectivity in Age tasks in both regions as well as a decrease in selectivity in Colour task in right pSTS ($p<0.05$ for all). The effect of physostigmine on the task x stimulus-selectivity interaction was therefore opposite between controls and AD, showing itself as strong group x treatment x task x stimulus interactions in both

regions ($p<0.0001$, uncorrected; Fig. 8.5H). A subject-based correlation analysis of the task x stimulus x treatment interaction in the above two extrastriate regions with the task x treatment interaction identified in right parietal cortex, in AD, identified a significant correlation with the right pSTS ($r = 0.50$, $p<0.05$); but not left posterior occipital region ($r = -0.05$).

The AD group showed alternative patterns of stimulus-selectivity x task interactions compared to controls. Left lateral occipital cortex showed enhanced face-selectivity under Age versus Colour (-38, -74, 8; $Z = 4.93$; $p<0.05$, corrected; Fig. 8.5I), whilst right superior occipital cortex showed enhanced house-selectivity under Age versus Colour (36, -86, 18; $Z = 3.90$; $p<0.0001$, uncorrected). The former region differed significantly from controls who did not demonstrate task-modulation of selectivity in this area (group x task x stimulus interaction: $Z = 5.79$; $p<0.05$ corrected). When physostigmine was administered to AD this region just lost its task-dependency (treatment x task x stimulus interaction: $Z = 3.01$; $p = 0.001$ uncorrected) and so reverted to the control pattern. Controls were uninfluenced by physostigmine in this area (treatment x task x stimulus interaction: $Z = 3.87$; $p < 0.001$ uncorrected; Fig. 8.5J; third graph).

Figure 8.5. (See next page): A, B – Main-effect of face > building (first slice) and building > face (second slice) stimuli in control (A) and AD (B) subjects on placebo. The slices chosen include regions which additionally show interactions with task, treatment and group as illustrated below. Regions shown for face-selectivity are bilateral posterior STS (z = +12); and for building-selectivity are lateral occipital and retrosplenial cortices (z = +2). C, D, E – Interaction of stimulus-selectivity x task in controls (C), AD (D) and the difference between them (E) on placebo: regions shown are those in which Age relative to Colour task results in greater face-versus-building (first slice) and building-versus-face (second slice) responses. F, G, H – Interaction of stimulus-selectivity x task x treatment in controls (F), AD (G) and the difference between them (H): circled regions are those in which task-enhancements of face- and building- selectivity are *decreased* by physostigmine relative to placebo in controls (F) but *increased* by physostigmine relative to placebo in AD (G). I – Interaction of stimulus-selectivity (face > building) x task in controls (first slice) and AD (second slice); region circled shows greater task task-modulation of stimulus-selectivity in AD relative to controls, that itself is cholinergic dependent (z = +8; see text). J – Plots of %-signal change for face > building (first and third graphs) and building > face (second graph) contrasts, under each task, treatment and group at the maxima for the 4-way interaction (from H; first two graphs) and at the maximum task x stimulus interaction in AD (from I; third graph). Activations are thresholded at $p<0.001$, uncorrected, except for F and G that are thresholded at $p<0.01$, uncorrected, and are superimposed on the mean normalised EPI of controls or patients as appropriate (group interactions are overlaid on patients' mean).

Figure 8.5. For legend see previous page.



Discussion

The present experiment set out, firstly, to compare neurophysiological responses to stimulus and task-effects between Alzheimer's disease and healthy age-matched controls and, secondly, to examine how cholinergic enhancement modulates these effects. Our results can be summarised as follows:

1. Across two visual discrimination tasks Alzheimer's disease patients performed poorer and showed weaker levels of stimulus-selectivity in several regions of extrastriate visual cortex than healthy age-matched controls; physostigmine improved selectivity in two of these regions specifically in AD. Right fusiform cortex, by contrast, showed an equivalent level of face-selectivity compared with controls, and was negatively modulated by physostigmine in a manner that matched controls.

2. The performance of AD subjects relative to controls was more impaired in the Age than Colour discrimination task, which corresponded with AD relative to controls showing less task-dependent activity in right parietal and left prefrontal cortices. The administration of physostigmine to Alzheimer's disease subjects resulted in both a task-specific improvement in performance and a relative increase in appropriate task-related activity in right parietal cortex (and a trend for this effect in prefrontal cortex).

3. Appropriate task-dependent modulations of stimulus-selectivity seen in controls in two extrastriate regions (i.e. greater for Age than Colour tasks) were reduced in AD. Physostigmine reversed this AD-associated impairment by enhancing stimulus selectivity during the Age task in both regions as well as reducing selectivity during

the Colour task in one region. Conversely, an additional extrastriate cortex region that showed maladaptive task-dependent modulation of stimulus-selectivity in AD reverted to the control pattern of being task-independent with physostigmine.

4. In contrast to AD, when physostigmine was administered to healthy controls there were decreases in cortical stimulus-selectivity as well as reductions in task-dependent effects in frontoparietal cortex and in the interaction of task on stimulus-selectivity in extrastriate cortex. The effect of drug on task modulation of stimulus-selectivity in controls was primarily driven by enhancements in selectivity during the Colour task, although drug effects in frontoparietal cortex were contributed to by opposing effects in each task.

Cholinergic modulation of stimulus-selectivity

Psychophysical and functional imaging studies in mild-to-moderate Alzheimer's disease have demonstrated defects in both early and late stages of visual processing (Tippett et al, 2003; Prvulovic et al, 2002), some of which correlate with the degree of cognitive impairment (Rizzo et al, 2000; Pietrini et al, 2000). However, given that sensory cortices are relatively spared from degeneration until the disease becomes advanced, one possible explanation for this impaired performance is that a deficiency of cholinergic input to sensory regions secondary to the recognised degeneration of basal forebrain in AD is responsible. Since stimulus-selective responses of occipital neurons have been shown to be influenced either positively or negatively by cholinergic enhancers or antagonists, respectively (e.g. Sato et al, 1987, Murphy & Sillito, 1991), we might expect AD to show an impaired level of stimulus-selectivity that is to some extent correctible with cholinergic enhancement. We tested this by

employing a comparison of ‘faces versus places’, which although differ from each other across numerous physical properties, nevertheless, offer a robust fMRI measure of high-order visual processing that is more likely to detect disparities between AD and controls than using simple visual stimuli (Mentis et al, 1998; Dannhauser et al, 2005). Our results show that stimulus-selectivity of extrastriate cortical regions is diminished in AD relative to controls in a significant proportion of areas that are normally activated in controls. In two of these areas – precuneus (face-selective) and posterior parahippocampal cortex (building-selective) - cholinergic enhancement increased selectivity in AD, thereby supporting the proposal that a cholinergic deficiency is, in part, responsible for the visual processing deficit in AD.

A further notable aspect of our data is that whereas superior occipital, precuneus and parahippocampal cortices showed impaired stimulus-selectivity in AD relative to controls, right fusiform cortex – the region showing the strongest face-selective responses - was unaffected. The finding is consistent both with previous functional imaging studies in AD that demonstrate a relatively greater attenuation of activations in dorsal parieto-occipital (Prvulovic et al, 2002) and medial parietal (Bradley et al, 2002) than temporo-occipital areas, and with the association of AD with atrophy in medial relative to lateral temporal structures (Chan et al, 2003). Additionally, the fact that functionally-impaired parahippocampal and precuneus regions showed stimulus-selectivity increases with physotigmine, while functionally-intact fusiform cortex showed the control pattern of a decrease, is evidence for a selective, rather than uniform, loss of cortical cholinergic inputs in AD. Of relevance to our findings is that precuneus was also the region most strongly enhanced by the cholinesterase inhibitor galantamine in a visual working memory task in patients with mild cognitive

impairment (MCI) (Goekoop et al, 2004). The fact that physostigmine reduced stimulus selectivity in many visual extrastriate regions, in healthy elderly, may reflect electrophysiological findings from non-human studies showing that acetylcholine potentiates responses more to inputs that prior to drug are non-dominant, relative to those that were dominant (Kuo et al, 2010); or reflect acetylcholine inducing increases in sensory responsiveness at the cost of reducing stimulus selectivity (e.g. Sato et al, 1987a; Zinke et al, 2006; Herrero et al, 2008; but see also Murphy & Sillito, 1991).

Cholinergic modulation of attention: frontoparietal effects

Whilst amnesia is the hallmark of Alzheimer's disease, attentional impairments are well described even in early stages of the disease (Perry et al, 2000; Baddeley et al, 2001). Furthermore, whereas the memory impairment of AD seems to derive largely from selective atrophy of medial temporal structures (Chan et al, 2003), the attentional defects of AD most likely reflect a deficiency of input – both cortico-cortical and cholinergic – to areas that are relatively structurally intact (Perry & Hodges, 1999). This is consistent with the observations that cholinesterase inhibitors improve attention more than memory scores in AD (Lawrence & Sahakian, 1995; Blin et al, 1998) and that lesions to basal forebrain cholinergic neurons induce deficits in visual-attention more than memory tasks (Everitt & Robbins, 1997). One of the principal aims of our study was to test whether AD-associated impairments in attention, at combined behavioral and neurophysiological levels, are cholinergic dependent. We found that AD patients compared to healthy age-matched controls showed relatively greater impairment of both performance and frontoparietal responses with a more attention-demanding task, and that both relative differences decreased following administration of a cholinergic enhancer.

The strongest task-related activation in our design was right parietal cortex – a region that has previously been found to show impaired activation in AD during attentional paradigms (Hao et al, 2005; Prvulovic et al, 2002; Parasuraman et al, 1992; Buck et al, 1997). We expected this region to manifest cholinergic sensitivity given a wealth of animal studies, largely using visuospatial paradigms, that show a critical dependency of attention on cholinergic inputs to parietal cortex (see Sarter & Bruno, 1997 for review). As well as replicating the previous finding of impaired task-related attention in right parietal cortex in AD, we have shown that cholinergic enhancement can partially restore the normal pattern of task-dependent parietal activation. This result still stood even after controlling for drug effects on reaction time, suggesting that the drug-induced differences in BOLD activity were not merely *caused by* differences in motor activity (Honey et al, 2000). Equally, however, it suggests that the drug-induced differences in BOLD activity cannot be the sole *cause of* the behavioural improvement.

Control subjects were also found to activate two prefrontal regions, one of which – left inferior prefrontal cortex - was underactivated in AD. Recent fMRI studies in mild AD / MCI have also found hypoactivation of left prefrontal regions during divided attention (Dannhauser et al, 2005) and visual search (Hao et al, 2005) tasks. Physostigmine showed a non-significant trend to enhance left prefrontal cortex in our study, that may reflect the weak activation of this region in controls. By contrast, an fMRI-study employing a working memory task that engaged prefrontal cortex strongly in controls but not MCI, demonstrated enhancement within this region following a six-week course of donepezil (Saykin et al, 2004).

Cholinergic modulation of attention: extrastriate effects

A central role of the cortical cholinergic system is in balancing executive-attentional control of sensory processing with stimulus-driven sensory activity (Sarter & Bruno, 1997). Cholinergic inputs to frontoparietal cortex are necessary for selective visual attention, by which is meant a preferential facilitation of task-relevant stimulus-encoding neurons. Since frontoparietal activity in AD is impaired during attentional tasks (see above), we predicted a 'knock-on' detrimental effect in the attentional-modulation of extrastriate cortex; furthermore, we predicted that this would be sensitive to cholinergic manipulation. In order to test for this we chose two tasks that differed from each other in the required level of visual processing while using the same two types of visual stimuli between tasks. The difference between stimulus types – face versus building - was incidental in the Colour task, but contained feature information critical to performance in the Age task. We avoided adopting a selective spatial attention task as had been employed in our previous studies as we anticipated AD patients would have difficulty maintaining central fixation.

The results in healthy elderly subjects showed that face-selectivity was modulated by task in right pSTS, while right fusiform cortex was unaffected – in keeping with the distinct roles ascribed to face-sensitive regions of extrastriate cortex (Haxby et al, 2000). Building-selectivity was modulated by task in early visual regions (approximately V2/3) that encode for features such as orientations and angles, and are strongly activated by houses versus faces (e.g. Experiment 1). While the overall level of stimulus-selectivity did not differ significantly in these two regions between controls and AD, the influence of task on selectivity in these areas was impaired in

AD. Importantly, physostigmine given to AD enhanced the degree to which stimulus-selectivity was favored with Age relative to Colour tasks in the same regions that AD had exhibited an impairment of this effect. Hence the action of cholinesterase inhibition within these extrastriate regions was neither on baseline activity, nor on the main-effect of stimulus selectivity, but specifically on the executive-attentional control of stimulus selectivity. Given the diffuse innervation pattern of cortical cholinergic neurones (Sarter & Bruno, 1997), this may have been due to either (or both) a direct facilitation of top-down inputs within these sensory regions, or due to an indirect action within frontoparietal cortex. The facts that the drug- and group-dependent profile of task-related activity in frontoparietal regions was so similar to that seen in extrastriate regions, and that the response of one extrastriate region (pSTS) correlated in its response profile with right parietal cortex (across AD subjects), support the latter explanation. Furthermore, as well as enhancing the normal pattern of task-influence on sensory cortex, physostigmine reduced an abnormal pattern of the same effect (in left lateral occipital cortex) in AD, suggesting a coordinated response to cholinergic modulation between regions.

In a recent fMRI study comparing face-encoding to a simple baseline task, Kircher et al (2005) reported hypoactivation of fusiform cortex in AD that reversed following a 10-week course of donepezil. Rombouts et al (2002) similarly found rivastigmine-induced enhancement of fusiform cortex activity in seven AD patients on comparing a similar pair of tasks. Both results may have been due to cholinergic modulation of either stimulus-processing or attentional recruitment of sensory cortices. Our study distinguishes these possibilities by finding that face-selectivity of fusiform cortex, independent of attention, was not impaired in AD and showed negative modulation by

cholinesterase inhibition. However, where face-selectivity was dependent on attention (in our task, pSTS) there was an AD-associated impairment, and a positive modulation with cholinesterase inhibition.

Cholinergic modulation in healthy elderly relative to Alzheimer's disease

A striking aspect of our results was the consistent finding that the influence of physostigmine on stimulus-selectivity and/or task-related responses was opposite between AD and controls. The pattern we observed in controls of reduced task-dependent activity in parietal and sensory cortices following cholinergic enhancement is in keeping with several previous studies (Experiments 1 and 3; Thiel et al, 2005). Physostigmine-induced reduction of sensory cortex modulation by task or conditioning have been attributable to disproportionate enhancement of sensory cortex processing during conditions in which sensory cortex is normally at a low activity level (Experiment 3; Thiel et al, 2002). Similarly, reduction of parietal activation with pro-cholinergic treatment has been interpreted in terms of 'broadening of attention' to include stimuli that normally are processed only weakly (Thiel et al, 2005). In the current study, we found that the reduction of task-modulation of sensory cortex by physostigmine was predominantly due to an enhancement of stimulus-selectivity during the low-attention task, although drug-induced reduction of task-dependent parietal activity was due equally to levelling effects in both tasks. By contrast, AD patients compared to controls showed impaired attentional responses primarily due to defective differential activation during the high-attention task in frontoparietal and extrastriate areas, and physostigmine had its impact in these areas primarily during this condition. Combining both results, it would seem that a normal level of acetylcholine is required both for frontoparietal activation and for stimulus-selectivity

enhancement specifically during attention-demanding conditions; whereas excessive acetylcholine enhances parietal and selective extrastriate responses during low-attention conditions that do not normally engage such areas. The facts that physostigmine in controls also tended to reduce parietal and sensory effects during the high-attention task, and that elsewhere, stimulus-selectivity was reduced, suggests that there are also costs to visual processing with excessive cholinergic stimulation. Findings or reduced stimulus selectivity following acetylcholine application to sensory cortices has been found in non-human studies (e.g. Zinke et al, 2006; Herrero et al, 2008). Furthermore, the finding that similar deficits of cortical function may occur with both deficient and excessive levels of neuromodulator (depicted by an 'inverted-U' function) is well recognised in another instance – namely, dopaminergic modulation of prefrontal cortex in working memory performance (Williams & Castner, 2006).

Limitations

We draw our conclusions from the effect of disease and drug on event-related BOLD responses, rather than neural activity directly. Consequently, the results are potentially susceptible to confounds that derive from differences in metabolism, blood-flow and neurovascular coupling between the two groups and two treatments, independent of cognitive factors (Blin et al,1997; Tsukada et al, 2000). In our study we found that baseline BOLD levels did not differ between treatments or between groups at the level of whole brain or within the regions that exhibited task x group and/or treatment interactions. Furthermore, the profile of drug effects on event-related BOLD activity that we found – mostly increases and one decrease in AD, and decreases in control – cannot be explained merely on the basis of a unidirectional influence of

physostigmine on baseline metabolism or cerebral blood-flow as recorded by Blin et al In fact, the pattern of 'cross-over' effects – i.e. where drug enhanced activity during one condition but decreased it in during another in the same voxel – that we observed in several regions, necessarily involves an explanation in terms of the cognitive factor of interest. We also discount explanations in terms of drug-induced reductions in alertness or side-effects such as nausea because both groups were affected equally along these dimensions (in contrast to the effects of interest that were opposite between groups), and because these factors would be expected to act in an opposite direction to the attention-enhancing results found in AD.

Conclusion

Experiment 4 aimed to study the neural correlates of two fundamental cognitive categories known to be impaired in Alzheimer's disease – visual processing and attention – and their sensitivity to cholinergic manipulation, by employing a task that enabled orthogonal manipulation of each variable. The study showed that AD patients manifest deficits in cerebral activations associated with both types of process, as well as their interaction, compared to age-matched controls. Many of these deficient activations were reversed by cholinesterase inhibition thus providing novel insights into how cortical-cholinergic deficiency contributes to the neurophysiological and performance impairments of mild-to-moderate AD. Finally, we have demonstrated that excessive cholinergic stimulation in controls also disrupts the normal pattern of visual-attentional processing, although the mechanisms by which these occur differ from those of AD.

9. EXPERIMENT 5:

Relationship between Effects of ChEI on Visuo - Attentional Processing and Subsequent Memory

Introduction

Among its numerous cognitive impacts, the basal forebrain - neocortical cholinergic system exerts important influences on sensory processing (Everitt & Robbins, 1997; Sarter et al, 2005a). For example, acetylcholine release in sensory cortices enhances stimulus-evoked responses (Sato et al, 1987); modifies stimulus-selectivity (Sillito & Kemp, 1983), and alters the configurations of sensory representation maps (Weinberger, 2007). Indeed, the ability of acetylcholine to influence plasticity mechanisms within sensory cortices during stimulus-encoding – in addition to its separate actions on the hippocampus – has been proposed to contribute to the well-established effects of acetylcholine on memory (Kirkwood et al, 1999; Boroojerdi et al, 2001; Gu, 2003; Hasselmo & McGaughy, 2004; Schon et al, 2005). The present study was designed to test this hypothesis by examining whether effects of cholinesterase inhibitors on processing in higher sensory cortex processing, for healthy subjects and in mild Alzheimer's disease (Furey et al, 2000; Rombouts et al, 2002), may be directly related to its effects on subsequent memory (Davis et al, 1978; Davis & Mohs, 1982).

Previous functional imaging studies using visual paradigms have shown that pro-cholinergic drugs increase stimulus-driven extrastriate visual cortex responses in a task-dependent fashion (Furey et al, 2000; Lawrence et al, 2002; Experiments 1 and 3). In a similar way, we note from psychopharmacological studies that the pro-mnemonic effects of cholinergic-enhancing drugs are also related to encoding-task, with a greater memory improvement noted for stimuli that have undergone 'deep' relative to 'shallow' processing (Rusted & Warburton, 1992; Warburton et al, 2001;

Fitzgerald et al, 2008). In other words, cholinergic manipulation interacts with the well-recognised depth-of-processing effect on memory (Craik & Tulving, 1975; Baddeley, 1990). Here we sought to bridge these two effects, by testing whether cholinergic enhancement of task-dependent activity in visual extrastriate cortex relates to the impact on subsequent memory. We predicted that the cholinergic enhancer physostigmine would increase memory selectively for deeply relative to shallowly encoded faces, and, critically, that this would correlate with the degree to which physostigmine enhances face-selective fusiform cortex activity during the deep relative to shallow encoding task.

A further question we addressed was whether effects of cholinesterase inhibition on the relationship between face-encoding and subsequent recognition differ between healthy older subjects and Alzheimer's disease. Previous studies in Alzheimer's disease have shown impaired extrastriate visual cortex activation during memory tasks, associated with poor subsequent recall (Golby et al, 2005; Gron & Riepe, 2004; Machulda et al, 2003; Rombouts et al, 2005); while cholinesterase inhibition may reverse impairments in sensory cortex activity (Rombouts et al, 2002; Kircher et al, 2005; Gron et al, 2006). No studies however, have shown or assessed any direct relationship between enhanced extrastriate cortex activity following cholinesterase inhibitor treatment in Alzheimer's disease and improved subsequent recognition. Furthermore, it remains unknown whether impairments in depth of processing (Beauregard et al, 2001; Bird & Luszcz, 1991) or task-modulation of sensory cortex activity (Mandzia et al, 2004; Gazzaley A, D'Esposito M, 2007) seen in Alzheimer's disease and ageing, are reversible with pro-cholinergic treatments. Since both pathological (Mesulam et al, 2004) and pharmacological (Lawrence & Sahakian,

1995) studies have suggested that cholinergic deficits or manipulation produce more impact upon attentional than memory processes, and given that stimulus depth-of-processing effects may partly depend upon attentional processes (Baddeley, 1990), we tested whether effects of cholinesterase inhibition on memory in Alzheimer's disease are dependent upon encoding task.

Finally, given the likely importance of sensory- frontoparietal – hippocampal cortex interactions in memory and depth-of-processing (Celone et al, 2006; Rissman et al, 2008), we tested in both healthy and Alzheimer's disease groups the relationship between activity in fusiform cortex with that in wider brain regions, and the effects of cholinergic manipulation on such co-variations between areas.

It should be noted that this experiment involves a secondary analysis of data acquired from Experiment 4, but now incorporating the additional factor of subsequent (i.e. post-scanning) memory performance that was ignored previously.

Methods

Subjects

Eighteen right-handed healthy older subjects (mean age 64.8 ±4.2; hereon referred to as 'healthy subjects') participated, plus thirteen right-handed patients with newly-diagnosed Alzheimer's disease (MMSE of 20 – 26; mean age 64.8 ±4.4) who were recruited from the Dementia Research Group, National Hospital for Neurology and Neurosurgery (London, UK) over a sixteen month period. Data sets were from fifteen of the healthy controls and twelve of the patients included in Experiment 4 (additional

subjects were recruited to make up for subjects in Experiment 4 who did not complete the post-scan memory task). No subjects were active smokers. Summary characteristics of the two groups are listed in Table 9.1. We used the NART-R test to assess IQ in healthy subjects as previous studies have shown that its score correlates robustly with verbal and performance IQ scores from the WAIS-R (e.g. Schretlen et al, 2005) that Alzheimer's disease subjects underwent as part of their clinical management. All subjects gave written informed consent. Inclusion and exclusion criteria for patients were the same as for Experiment 4.

Table 9.1: Summary characteristics of healthy elderly and Alzheimer disease subjects (±95% confidence intervals).

	Healthy	Alzheimer's disease
Number	18	13
Age	10	9
Males	64.8 (±4.2)	64.8 (±4.4)
Education	12.4 (±0.9)	12.9 (±1.0)
Hypertension	4	5
Baseline blood-pressure	128/75 (±10/6.2)	138/84 (±7.3/4.5)
MMSE	29.4 (±0.4)	23.6 (±1.3)*
Verbal IQ	114 (±2.4)	94.2 (±7.0)*
Performance IQ	114 (±2.3)	94.2 (±9.8)*

IQ scores in controls are estimated from National Adult Reading Test (NFER-NELSON Publishing Co. Ltd., Berkshire, England, 2nd Edition, 1991).

* $P < 0.01$ between-group difference.

Drug-treatment

A double-blind, placebo-controlled drug administration technique was used. Details of the physostigmine challenge are described in Experiment 4. The encoding task took place at 25 minutes from the start of the infusion. The infusion was continued until the end of the encoding phase, (i.e. ~45 minutes from the start of the infusion), but then terminated in order to minimise drug side-effects and permit subject mobility. The recognition task took place 10 minutes after termination. Since previous data (Asthana et al, 1995; Christie et al, 1981; Muramoto et al, 1984) indicate a pharmacodynamic half-life for intravenous physostigmine of ~60 minutes, there will have been significant cholinesterase inhibition during both encoding and recognition phases here.

Blood pressure was checked before and after scanning, and pulse-oximetry was performed continuously. Subjects were given a questionnaire before and after scanning that allowed a ranked measurement (0 – 6 scale) of seven recognised adverse reactions to physostigmine and glycopyrrolate, as well as visual analogue scales for alertness and physical wellbeing.

Cognitive task

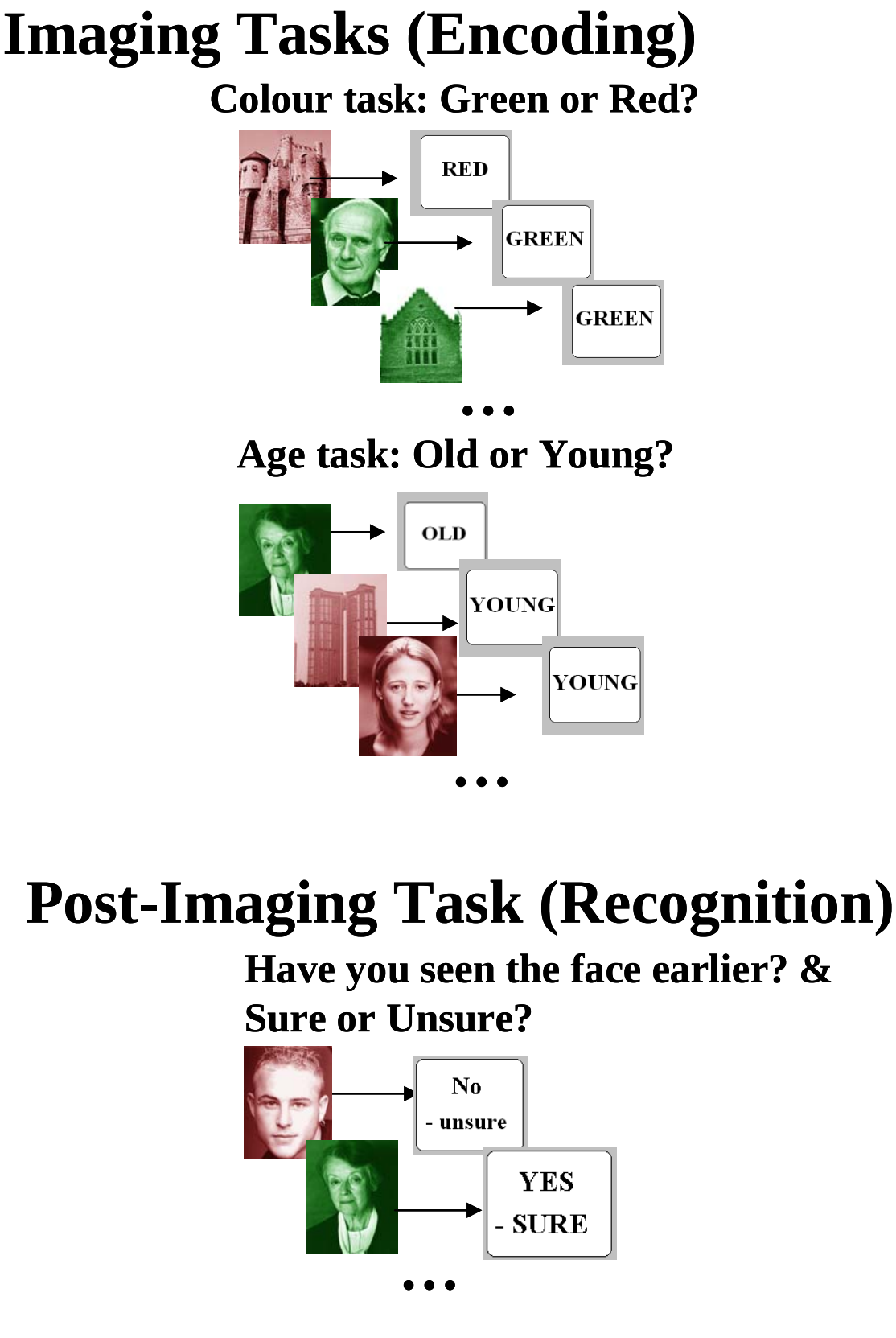
The task performed within the scanner is identical to that described in Experiment 4. To recap, on each of two sessions (placebo or physostigmine), subjects performed two tasks of varied processing depth. For the shallow task they judged the Colour (C) of colour-washed red or green faces or building stimuli. For the deep task they judged instead the Age (A; young/old) of comparable face or building stimuli. Subjects were informed that a recognition test of faces would be carried out after scanning but were

instructed simply to perform their best on the within-scanner colour or age tasks, rather than trying specifically to memorise items. A short practice run (without scanning) was also performed before each block in the scanner. Subjects wore appropriate MRI-compatible refractive lenses if required to correct their visual acuity (i.e. for individuals who would normally wear spectacles).

Eye position was monitored during scanning and task performance, with a remote infra-red eye tracker (ASL Model 540, Applied Science Group Co., Bedford, MA; refresh rate = 60 Hz) for 16 control and 11 Alzheimer's disease subjects.

Recognition memory for exposed faces (versus foils) was tested 10 minutes following the end of the encoding. Subjects were removed from the scanner for testing and sat in front of a laptop computer. Test stimuli were presented singly, and together consisted of the 96 faces that had appeared during the encoding task (presented in the same colour used for either the colour- or age-task during exposure), randomly intermixed with 96 foils (equally divided into red and green) that were also presented singly. Thus each trial comprised either a previously shown face or a foil face. These recognition probe stimuli subtended ~7 º x ~4 º visual angle. Subjects were prompted on the screen to say whether they had seen each face or not during the encoding phase, and whether they were confident or not of this judgement. Subjects' verbal responses were recorded by an examiner blind to the test stimuli. Recognition accuracy was scored using a discrimination index (DI) calculated as p(hit) minus p(false alarm); see Snodgrass & Corwin (1988).

Figure 9.1: Schematics of tasks performed during fMRI scanning (encoding) and afterwards (recognition task).



Imaging and imaging processing

fMRI data were collected during the encoding tasks on a 1.5 T MRI scanner (Siemens, Erlangen, Germany) using gradient echo T2*-weighted echo-planar images, with blood oxygenation level dependent (BOLD) contrast. Volumes consisted of 39 horizontal slices through the whole brain, each 2mm thick with a 1mm gap between slices (field-of-view, 192 x 192 mm^2; matrix size 64 x 64). In-plane resolution was 3mm×3mm; effective repetition time (TR), 3.51 s; echo time (TE), 50ms, and flip angle 90°. For each block 63 volumes were acquired, with the task only beginning after the sixth volume to allow for T1 equilibration effects.

Imaging data were pre-processed and analysed using SPM2 (Wellcome Centre for Neuroimaging at UCL; http://www.fil.ion.ucl.ac.uk/spm). This consisted of determining and applying rigid affine transformations to the image series to realign the scans with respect to the first scan (Friston et al., 1995). Scans were then normalized to a standard EPI template (Montreal Neurological Institute) with a resampled voxel size of 3 x 3 x 3mm (Friston et al., 1995), and smoothed using a Gaussian kernel with a full width at half maximum (FWHM) of 8 mm. The same template was used for healthy subjects and Alzheimer's disease in order to allow for unbiased between-group comparison.

Statistical analysis of behaviour

Behavioural data were analysed with SPSS software (v16.0). DI scores were entered into mixed ANOVAs, with task (shallow or deep), treatment (physostigmine or placebo) and recognition confidence (confident or not) as repeated-measure factors, and group (healthy or Alzheimer's disease) as a non-repeat factor. For completeness,

performance during initial encoding (RT and accuracy for colour or age tasks) underwent comparable ANOVAs with the same factors. Treatment order (physostigmine given in first or second session) produced neither main effects nor interactions with other factors, so was not considered further.

Statistical analysis of images

Imaging data were analyzed with a general linear model for combined blocked (here, colour- or age-task at encoding) and event-related (here, face or building stimuli in a randomly intermingled sequence within each block) factors, using SPM2 with a random-effects approach. Data were globally scaled so as to remove the possibility that between-treatment or between-group effects were caused by any differences in baseline BOLD values, and high-passed filtered at 1/256 Hz. Events were modeled by delta functions convolved with a synthetic hemodynamic response function (Friston et al., 1998); temporal derivatives of these functions were modelled separately for completeness (Friston et al, 1998). Within-subject conditions of interest were stimulus-type, task, and treatment. Stimuli in different scanning-blocks were modelled separately to enable estimation of any session effects. Six-dimensional head movement parameters derived from image-realignment were included within the model as confounding covariates of no interest.

For each of 31 subjects, BOLD differences were estimated for the following contrasts of interest: i) face-selectivity under placebo, i.e. face > building; ii) physostigmine-induced enhancement of face-selectivity, i.e. two-way interaction of treatment x stimulus [physostigmine (face >building)] > [placebo (face > building)]; iii) task-modulation of face-selectivity under either treatment, i.e. two-way interaction of

stimulus x task under placebo, or physostigmine [age (face >building)] > [colour (face > building)], and iv) physostigmine-induced enhancement of task-modulation of face-selectivity, i.e. three-way interaction of treatment x task x stimulus, {physostigmine[age (face > building)] > [colour (face > building)]} > {placebo[age (face > building)] > [colour (face > building)]}.

We next calculated depth-of-processing effects on later behavioural recognition scores for each subject (i.e. DI for deep- minus shallow-encoded faces) under placebo, and the change in this score when comparing physostigmine with placebo. These values for each subject were then correlated respectively with each subject's own BOLD-derived measure of the task x stimulus (under placebo) (contrast iii, above) and treatment x task x stimulus (contrast iv, above) interactions, separately for the two groups. Since the Alzheimer's disease group showed a treatment effect on memory that was independent of task, we also correlated subjects' treatment effects on recognition score (i.e. DI for all faces) with subjects' treatment x stimulus BOLD effect (contrast ii, above), separately for healthy and Alzheimer's disease subjects. Group comparisons of correlation coefficients were performed at the peak estimates for each group using Fisher's Z-test (i.e. for balance we compared between groups the strongest correlations found within each group, rather than the strongest within one against an unselected score for the other). We were guided by behavioural effects of drug on recognition at the group level in deciding whether to use all recognition responses, or instead just confident recognition responses, as the covariate with BOLD activity during the encoding-phase. In order to facilitate interpretation of interactions we limited the search volume to those regions also showing a main effect

of face-selectivity in the appropriate subject group under placebo (thresholded at $p < 0.001$, uncorrected).

In a separate model, for each subject incorporating the same factors as before (stimulus, task, treatment), we re-classified face stimuli according to whether they were later recalled confidently, recalled non-confidently, or forgotten. In this way we could identify any areas that showed heightened BOLD responses at initial exposure for faces that were later recognised or forgotten - i.e. a 'subsequent-memory' analysis (Rugg et al, 2002). This was performed for all recognised faces in healthy subjects, but with a focus on confidently-recalled faces in Alzheimer's disease patients, given the specific physostigmine effect that we found on later recognition confidence for this patient group (see below). Interactions of a subsequent-memory effect with task, treatment and group were also performed within those regions also showing a main-effect of subsequent memory (thresholded at $p < 0.001$, uncorrected).

Face-selective regions were initially identified by performing a one-sample t-test in healthy or Alzheimer's disease subjects separately to generate corresponding statistical parametric maps (SPMs), thresholded at $p < 0.05$, corrected for whole-brain volume (false-discovery rate). Behavioural – BOLD correlations and subsequent memory effects were first explored within 8mm (i.e. the smoothing kernel) of the fusiform peaks of face-selectivity (as identified initially without considering behaviour) for each group. We then explored face-selective regions of interest more widely – namely fusiform gyri and superior temporal sulci (Haxby et al, 2000) – that were defined functionally from the face > building SPM contrast in the corresponding subject group under placebo, itself thresholded at $p < 0.001$, uncorrected (Worsley et

al, 1996). The medial temporal lobes were also interrogated as regions of interest given their central role in episodic memory (Rugg et al, 2002), and were defined anatomically here (see Rorden & Brett, 2000). We used a conventional statistical threshold of $p < 0.001$ (uncorrected) within these regions of interest. The rest of the brain was also examined for these correlations and contrasts, but for those areas we applied a threshold of $p < 0.05$ (false-discovery rate; corrected for whole-brain). Group-effects were overlaid on mean normalised T1 structural images of the appropriate group(s) to enable anatomical localisation.

In order to ascertain whether those regions implicated in differences for behavioural – BOLD correlations between healthy subjects and Alzheimer's disease groups also differed in grey matter volume, we analysed T1 structural images with voxel-based morphometry using SPM5 software (see Mechelli et al, 2005). Essentially, this process involves segmenting volumes to extract grey matter; normalising to an asymmetric T1-weightedtemplate in Montreal Neurological Institute (MNI) stereotactic space; modulating for total volume changes; smoothing (by 8mm kernel), for each subject's scan, before applying a 2-sample t-test to compare healthy subjects with Alzheimer's disease.

Finally, we tested for relationships between right-fusiform effects of task x stimulus, and stimulus, and inter-regional covariation with wider brain regions showing task-effects and subsequent-memory effects respectively. For the first of these connectivity analyses, we first identified regions showing a task-effect (Age > Colour) under placebo over all healthy subjects (and separately for Alzheimer's disease), thresholded at $p < 0.001$ uncorrected, and smoothed with a 8mm kernel. Within this predetermined

area we then tested for subregions in which this task effect for individual subjects covaried with task modulation of face-selective fusiform activity (contrast iii, above) sampled from the peak of the pharmacological behavioural – BOLD correlation, separately for subject group and treatment. We then compared differences in correlation coefficients between treatments for each group. Similarly we tested for regions that showed covariation of a subsequent-memory effect (see above) with face-selective fusiform activity (contrast i, above) also sampled from the peak of the pharmacological behavioural – BOLD correlations. These results are reported at $p < 0.001$ uncorrected, within regions showing a main effect of task, or in medial temporal lobe regions of interest (no other brain areas exhibited these correlations when thresholding at $p < 0.05$, whole-brain corrected).

The influence of physostigmine on group effects of stimulus-selectivity and task-modulation independent of subsequent recognition are reported in Experiment 4.

Results

Physiological data, subjective reports and eye-tracking

The only side-effects reported in the treatment group in more than one subject were nausea and dry mouth. Blood pressure was unaffected. Subjective scores of alertness and physical wellbeing reduced between beginning and end of session somewhat more for physostigmine than placebo (time x treatment interaction $p < 0.05$). There was no effect or interaction concerning group (healthy or Alzheimer's disease) for any of these measures (all $p > 0.1$). Saccades arose on only 0.8% of trials in controls and

only 1% in patients. Moreover, there were no interactions of saccade-rate with stimulus-type, task, treatment or group, so eye position was not considered further.

Behavioural

The expected difference in attentional demand for deep versus shallow encoding tasks was found in controls and Alzheimer's disease groups, expressed as slower RTs and decreased accuracy for the Age versus Colour task ($p < 0.01$ for each measure and group). Alzheimer's disease subjects performed worse than controls for both tasks in RT and accuracy (both $F(1,28) > 4$, both $p < 0.05$). A task x group interaction arose for accuracy, due to Alzheimer's disease patients showing a greater difference between the two tasks than controls ($F(1,28) = 5.5$, $p < 0.05$). Physostigmine led to faster RTs selectively in Alzheimer's disease but not healthy subjects, during the Age but not the Colour task ($F(1,28) = 9.0$, $p < 0.01$).

Recognition memory performance is shown in Fig. 9.2, separately for all responses and for just confident responses. Healthy subjects demonstrated superior memory to Alzheimer's disease patients (main effect of group ($F(1,29) = 5.4$, $p < 0.05$); dividing up recognition score by encoding task identified a selective group difference for Age-encoded ($t(29)= 3.0$; $p < 0.01$), but not Colour-encoded faces ($p = 0.13$). Furthermore, healthy subjects showed a strong benefit in memory when comparing Age with Colour encoding tasks ($F(1,17) = 14.2$; $p<0.01$; also significant at $p < 0.05$ under each treatment), whereas there was no such effect in Alzheimer's disease patients ($F(1,12) = 0.3$, n.s; no task effect under either treatment) leading to a significant task x group interaction for recognition memory scores ($F(1,29) = 4.4$, $p < 0.05$). Among Alzheimer's disease subjects, there was a trend for a correlation between MMSE

scores and recognition memory of deep- versus shallow-encoded faces ($r(12) = 5.3$; $p = 0.06$). There was also a confidence x task x group interaction ($F(29,1) = 4.9$; $p < 0.05$), that reflected healthy subjects showing a task effect for confident ($p < 0.01$), but not un-confident judgements, while Alzheimer's disease subjects showed no task-effect for either.

Physostigmine had distinct influences on the impact of encoding-task upon memory for healthy subjects versus Alzheimer's disease patients, leading to a three-way group x task x treatment interaction ($F(1,29) = 4.5$, $p < 0.05$). In healthy subjects, physostigmine increased the difference in memory between the two types of encoding-task, relative to placebo, specifically enhancing the depth-of-processing effect ($F(17,1) = 4.7$, $p < 0.05$). This effect occurred regardless of recognition confidence. By contrast in Alzheimer's disease patients, there was no effect ($p > 0.1$) of physostigmine on task-dependent memory, relative to placebo, i.e. no tendency for it to restore the depth-of-processing effect found in healthy subjects. However, when analysing only those recognition judgements that Alzheimer's disease patients rated with confidence (see Fig. 9.2, rightmost graph), we found that physostigmine exerted a beneficial effect on their memory ($F(12,1) = 5.2$; $p < 0.05$), although this was equivalent for faces encoded during the Age and Colour task (i.e. there was no task x treatment interaction for the Alzheimer's disease group, $p > 0.1$).

Analyses of false-alarm rates showed an effect of group (higher for Alzheimer's disease: $t(29)=2.44$, $p < 0.05$), but not treatment, either as a main-effect, or as an interaction with group or task (Table 9.2).

Figure 9.2: Behavioural recognition results for each group. Discrimination indices [P(hit) – P(false alarm)] plotted separately for faces that had earlier been encoded during 'shallow' colour task, or encoded during 'deep' age task, under placebo or physostigmine, in control or AD subjects. The left graph scores all recognition responses as hits, while the right graph scores only confident recognition judgements as hits.

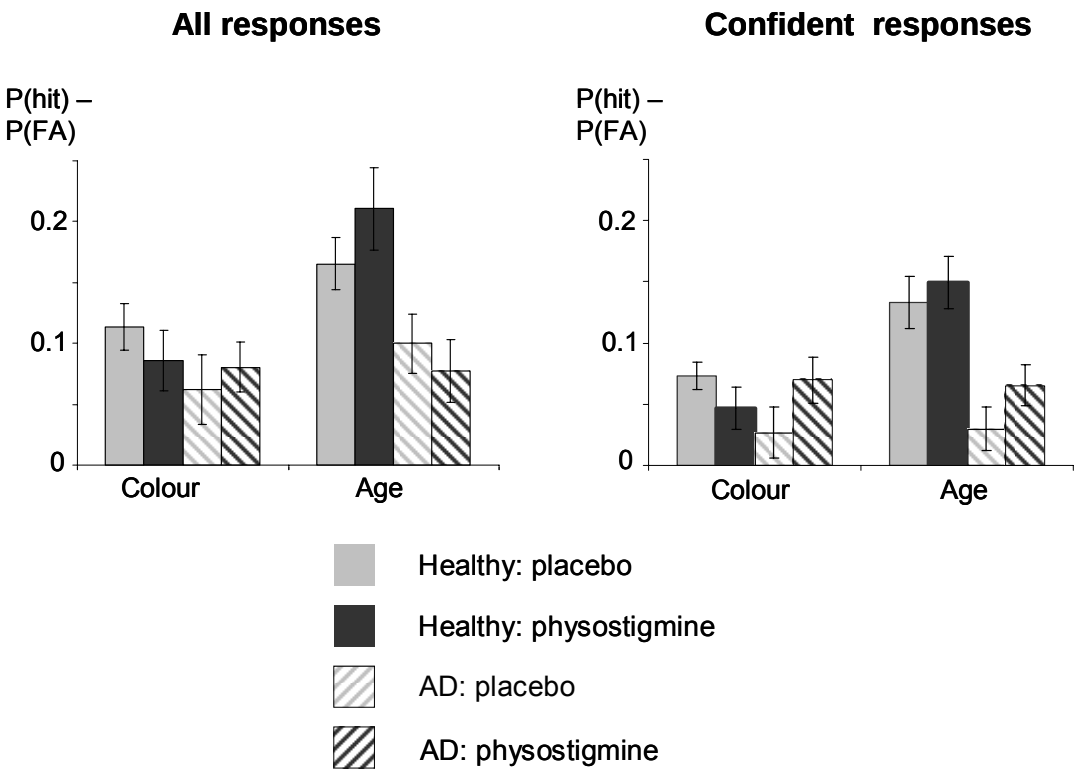


Table 9.2: Probabilities of correct hits and false alarms (with standard errors)

All Responses

Healthy						Alzheimer's Disease					
Placebo			Physostigmine			Placebo			Physostigmine		
Colour P(Hit)	Age P(Hit)	P(FA)	Colour P(Hit)	Age P(Hit)	P(FA)	Colour P(Hit)	Age P(Hit)	P(FA)	Colour P(Hit)	Age P(Hit)	P(FA)
0.43 (0.03)	0.48 (0.02)	0.29 (0.03)	0.39 (0.04)	0.52 (0.04)	0.31 (0.03)	0.47 (0.06)	0.50 (0.05)	0.40 (0.05)	0.49 (0.05)	0.49 (0.05)	0.41 (0.05)

Confident Responses

Healthy						Alzheimer's Disease					
Placebo			Physostigmine			Placebo			Physostigmine		
Colour P(Hit)	Age P(Hit)	P(FA)	Colour P(Hit)	Age P(Hit)	P(FA)	Colour P(Hit)	Age P(Hit)	P(FA)	Colour P(Hit)	Age P(Hit)	P(FA)
0.18 (0.02)	0.24 (0.02)	0.11 (0.02)	0.16 (0.02)	0.26 (0.02)	0.11 (0.02)	0.18 (0.04)	0.18 (0.04)	0.15 (0.04)	0.24 (0.05)	0.23 (0.05)	0.16 (0.05)

fMRI data: Session effects

We obtained estimates of the mean BOLD signal per session for the whole-brain (global) and in functionally-defined (face>house) face-selective extrastriate cortical regions. Importantly, neither global (whole-brain) nor regional (face-selective areas) session BOLD estimates were influenced by group or treatment overall, and there was no significant interaction between these factors. This means that the specific results reported later below cannot be a trivial outcome of any non-specific drug or group influences on whole brain or face-selective BOLD signals.

fMRI data: Face-selectivity, subsequent memory and depth of processing

Extrastriate cortical regions showing higher BOLD-signals for face than building stimuli in healthy subjects were most apparent in right fusiform cortex (Fig. 9.3A; Table 9.3). In Alzheimer's disease patients, the same contrast showed activation of bilateral fusiform cortices (Fig. 9.4A; Table 9.3), with no significant group-differences in face-selectivity (i.e. no interaction of face>building with group, all $p > 0.1$) for fusiform cortex in either hemisphere. Effects of task, treatment and group on face-selective responses that do not take into account individuals' subsequent recognition performance are described in Experiment 4.

We next investigated the relationship between face-selective fusiform cortex activations during encoding with memory performance post-scanning. Specifically, we tested: i) whether the strength of fusiform responses to faces was associated with subsequent successful recognition, and ii) whether task-modulation of face-selective responses in this region was associated with task-dependent recognition scores – i.e. the depth of processing memory effect. For the first question, we compared responses

to faces that were later correctly recognised to those which were incorrectly rejected later as foils. This 'subsequent-memory' contrast in healthy subjects under placebo showed higher BOLD for faces later recognised than forgotten in anterior right fusiform cortex (Fig. 9.3B; Table 9.3). The right hippocampus, as an a priori anatomical region of interest (Rugg et al, 2002), also showed this subsequent memory effect at a lower statistical threshold (28, -4, -24; $Z = 2.10$; $p < 0.05$, uncorrected). In Alzheimer's disease subjects under placebo, there was no such subsequent-memory effect in fusiform cortex for either hemisphere, leading to a between-group difference for this in right fusiform cortex (44, -38, -18; $Z = 3.95$; $p < 0.001$, uncorrected). However, on comparing faces later recognised confidently by Alzheimer's disease patients to those forgotten by them (for which a drug-effect had been observed behaviourally – see above), a subsequent-memory effect did emerge for this patient group under placebo in left fusiform cortex, within 8mm of the local peak of face-selectivity for the Alzheimer's disease group (Fig. 9.4B). The Alzheimer's disease group also showed a subsequent-memory effect in left hippocampus (-18, -16, -8; $Z = 3.35$; $p < 0.001$, uncorrected) but only under physostigmine. Apart from the right fusiform cortex region mentioned showing a greater subsequent-memory effect for healthy subjects than Alzheimer's disease, there were no other interactions of subsequent-memory with task, treatment or group (thresholded at $p < 0.001$, uncorrected in regions of interest; $p < 0.05$ corrected in other brain regions).

For the second question, we examined whether the behavioural improvement in recognition for faces encoded deeply (Age task) relative to faces encoded shallowly (Colour task) found in healthy subjects would correlate in a subject-by-subject manner with task-modulation of face-selective responses extrastriate cortex at encoding. Right

fusiform cortex, at the peak of face-selectivity identified above in a behaviour-independent manner for healthy subjects under placebo, showed a correlation between task-modulation of BOLD signal at exposure, and the behavioural depth-of-processing effect on later recognition ($r(30) = 0.49$; $Z = 2.82$, $p < 0.01$), with no difference between patients and controls ($p > 0.1$). Left fusiform cortex did not show a significant correlation in either group. We note that the extrastriate regions showing the strongest effects for this correlation in healthy subjects were in bilateral superior temporal sulci (60, -38, -2; $r(17) = 0.86$, $Z = 4.52$; and -44, -48, -8; $r(17) = 0.80$, $Z = 3.93$; both $p < 0.0001$, uncorrected). For these superior temporal regions, Alzheimer's disease subjects failed to show positive correlations ($r(12) = -0.25$ and -0.12, n.s.) leading to between-group differences in this respect ($Z = 2.56$ or 1.65, $p < 0.05$ or $p < 0.1$, respectively).

Summarising this section, we found that fusiform cortices in both healthy and Alzheimer's disease groups showed activations that were: i) greater for faces than buildings; ii) greater for faces subsequently remembered than forgotten, and iii) greater for faces shown during the deep, relative to the shallow, encoding task in subjects showing a greater depth-of-processing subsequent memory effect.

Figure 9.3. (See next page): BOLD responses obtained from fMRI scanning during encoding in healthy control participants. A) Face-selective responses (faces>houses) regardless of task during encoding, under placebo, and physostigmine, show strongest activation in right mid-fusiform gyrus. B) Regions where higher BOLD signals during face encoding (independent of task or drug) predict subsequent recognition (i.e. faces reclassified as later recognised or forgotten), under placebo and physotigmine. C) Regions where physostigmine-induced enhancements of task-modulation at encoding (i.e. face-selective BOLD responses for deep minus shallow task) correlate with physostigmine-induced enhancements of depth-of-processing effect on later recognition (i.e. discrimination indices for deeply minus shallowly encoded faces), across healthy participants. Graphs show individual subject scatterplots for this relation in right fusiform cortex, which was significant in healthy (scatter plot shown at left, with diamond symbols for each healthy participant) but not for the Alzheimer patients (scatter plot shown centrallym with open-circle symbols for each Alzheimer patient). The BOLD-behavioral relation found for healthy controls in right fusiform cortex can also be seen (right bar graph) by dividing subjects into tertile sub-groups according to the degree that physostigmine increased memory for Age-encoded relative to Color-encoded faces. The extent to which physostigmine increased face-selective responses during encoding, specifically for the Age relative to Color tasks, mirrored the degree to which physostigmine induced enhancements in the depth-of-processing effect for subsequent memory. SPM contrasts shown are thresholded for display purposes at $p<0.001$ uncorrected, in A and C, or $p < 0.01$, uncorrected, in B, and overlaid on mean T1-weighted MRI of the healthy subjects. Percent BOLD signal changes for the conditions making up each contrast are plotted for the peaks in each circled cluster.

Figure 9.3: For legend see previous page.

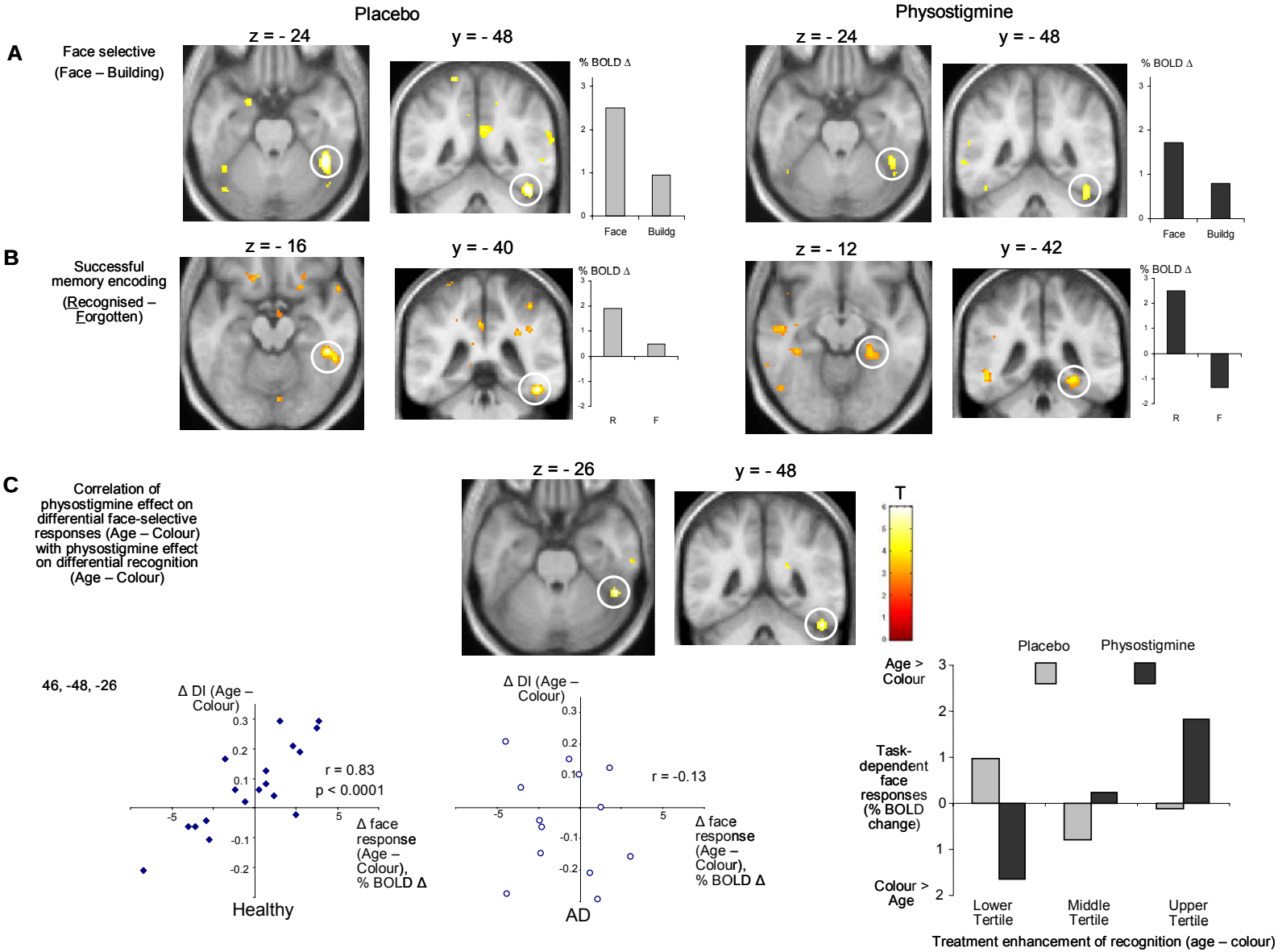


Table 9.3: Coordinates in fusiform cortex showing maxima of face-selective and subsequent- memory effects.

	Placebo	Z	P	Physostigmine	Z	P
Face-selective effects (face–building)						
Healthy	42, −48, −24	4.92	0.001	42, −48, −22	3.82	<0.05
Alzheimer's disease	44, −52, −30	5.06	<0.01	44, −52, −28	3.75	<0.0001*
	−38, −56, −22	4.68	<0.05	−40, −48, −22	4.14	<0.0001*
Subsequent-memory effects (recognized–forgotten)						
Healthy	44, −40, −18	4.28	<0.0001*	−36, −34, −16	3.25	<0.001*
	50, −46, −14	3.68	<0.001*	24, − 42, −10	3.17	<0.001*
Alzheimer's disease[a]	−44, −56, −24	3.91	<0.0001*	34, −44, −16	3.45	<0.001*
	−42, −36, −14	3.53	<0.001*	−28, −50, −20	2.89	<0.01*

Significance values are corrected for whole-brain volume (false-discovery rate) except * that are reported uncorrected for completeness.

[a] The AD group only showed subsequent memory effects using the contrast of confidently recognised – forgotten faces (for which the healthy group did not show effects).

fMRI data: Effects of physostigmine on task-dependent encoding in health

The principle hypothesis of this experiment was that physostigmine-induced enhancement of extrastriate visual cortex activations during encoding would relate systematically to effects of physostigmine on subsequent recognition performance. Since in healthy subjects, the behavioural effect of physostigmine on recognition was dependent upon encoding task (i.e. greater improvement for deeply- than shallowly-encoded faces) we assessed whether this effect related to physostigmine-induced enhancements of face-responses, during the deep relative to the shallow-encoding tasks. As predicted, we found in healthy controls a correlation of exactly this type - i.e. higher subject-by-subject recognition for deeply-studied, relative to superficially-studied, faces under physostigmine, associated with higher face-selective BOLD responses during deep versus superficial encoding tasks, under physostigmine in right mid-fusiform cortex (peak at 46, -48, -26, this being within 8mm of the peak for face-selectivity reported above in healthy subjects; $r(17) = 0.79$; $Z = 4.22$; $p < 0.0001$, uncorrected; Fig. 9.3C). The impact of this relationship can also be seen by ordering healthy subjects into tertile sub-groups, according to the degree to which physostigmine increased memory of deeply-encoded faces relative to shallow-encoded faces, i.e. Physostigmine[DI (Age) > DI (Color)] > Placebo[DI (Age) > DI (Color)]; see Fig. 9.3C. While all 3 subgroups showed positive face-selective responses at this fusiform peak under both placebo and drug, the relative strength by which face-selective fusiform responses were increased by physostigmine during Age versus Colour tasks paralleled the drug's enhancement of memory for faces presented during the Age relative to Colour tasks. There were no other face-selective regions showing this BOLD-behavioural correlation ($p > 0.05$).

The equivalent correlation analysis for Alzheimer's disease subjects showed no such relationship at the right fusiform peak identified above ($r(12) = -0.13$, n.s.), leading to a reliable between-group difference there in this respect ($Z(12) = 3.71$, $p < 0.01$). The Alzheimer's disease group did not show such a correlation in any other face-selective area, whether using all recognition responses or only those judged as being confident. A voxel-based morphometric analysis showed that there was no significant structural difference ($p > 0.05$ uncorrected) in grey matter density at this right fusiform peak between groups.

fMRI data: Effects of physostigmine on task-independent encoding in Alzheimer's disease

Since the behavioural influence of physostigmine in the Alzheimer's disease group had arisen specifically for confident recognition of faces, regardless of encoding task (see above), we next examined in this patient group whether physostigmine-induced enhancements of face-selective BOLD signals (at exposure) correlated with physostigmine-induced increases in later confident recognition, regardless of task. This analysis revealed such a positive BOLD-behaviour correlation for the patient group in left fusiform cortex (peak at -40, -54, -20; $r(12) = 0.89$; $Z = 4.44$; $p < 0.0001$, uncorrected) within 8mm of the left fusiform peak effect of face-selectivity already described above for the Alzheimer's disease-group (see Fig. 9.4B, circled); as well as in right fusiform cortex, (34, -40, -24; $r(12) = 0.89$; $Z = 4.06$; $p < 0.001$, uncorrected), and posterior hippocampus (24, -24, -20; $r(12) = 0.81$; $Z = 3.50$; $p < 0.001$, uncorrected). The impact of this relationship in left fusiform can also be appreciated by dividing up patients into three ordered tertile sub-groups, according to the degree to which physostigmine increased confident face recognition; see Fig. 9.4C.

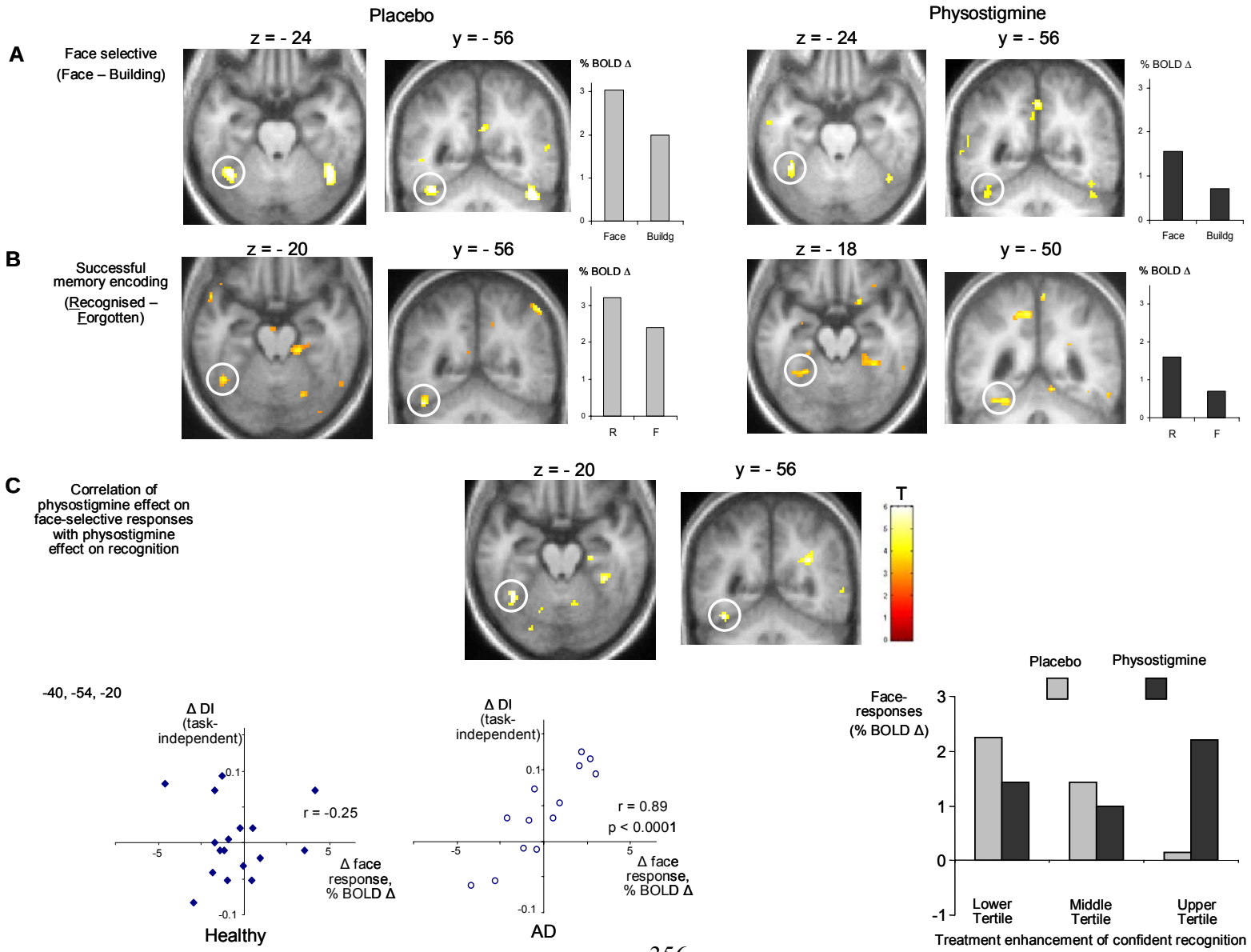
Physostigmine increased face responses selectively in the sub-group showing the greatest drug-induced enhancement of subsequent memory.

The equivalent analysis for healthy subjects found no reliable correlation of physostigmine-modulation of face-selective BOLD responses with physostigmine-modulation of later confident recognition, regardless of task, in any region (all $r \leq 0.104$, n.s.). This led to reliable between-group differences between all the brain-regions showing a significant brain-behaviour correlation of this type for the Alzheimer's disease patients (as listed above) but not for the healthy participants (all $Z \geq 2.56$, all $p \leq 0.01$). Voxel-based morphometric comparison of grey-matter density between groups showed no significant structural differences at any of these these voxels ($p > 0.05$, uncorrected).

There were no correlations between drug modulation of task-independent face-selectivity and subsequent recognition for healthy subjects if using all recognition judgements, rather than just confident responses.

Figure 9.4. (See next page): BOLD responses obtained from fMRI scanning during encoding in Alzheimer patients. A) Face-selective responses (faces>houses) regardless of task during encoding, under placebo and physotigmine, show strongest activations in bilateral mid-fusiform gyri. B) Regions where heightened BOLD signal during face encoding (independent of task or drug) predict subsequent confident recognition in AD patients under placebo and drug. C) Regions where physostigmine-induced enhancements of face-selective BOLD responses (independent of task) correlate with physostigmine-induced enhancements of confident recognition performance, across AD patients. Regions showing a significant BOLD-behaviour relation of this specific type included middle left fusiform, anterior right fusiform and right hippocampal cortex. Graphs show subject-by-subject scatterplots for this relation in left fusiform gyrus, separately for controls (scatterplot shown at left with diamond symbols for each healthy participant, no significant relation) and for Alzheimer patients (scatterplot shown centrally, with open-circle symbols for each patient, illustrating the significant relationship found only for this pathological group). The rightmost bar graph further illustrates the relation in left fusiform cortex by dividing patients into three tertile sub-groups, ordered by the effect of physostigmine on confident recognition. The upper-tertile subgroup shows the strongest impact of physostigmine on left fusiform at encoding. SPM contrasts shown are thresholded for display purposes at $p<0.001$ uncorrected, in A and C, or $p < 0.01$, uncorrected, in B, and overlaid on mean T1-weighted MRI of the AD patients. Percent signal changes of the conditions making up each contrast are plotted for the peaks in each circled cluster.

Figure 9.4: For legend see previous page.



fMRI data: effects of physostigmine on fusiform-parietal and fusiform-hippocampal functional coupling

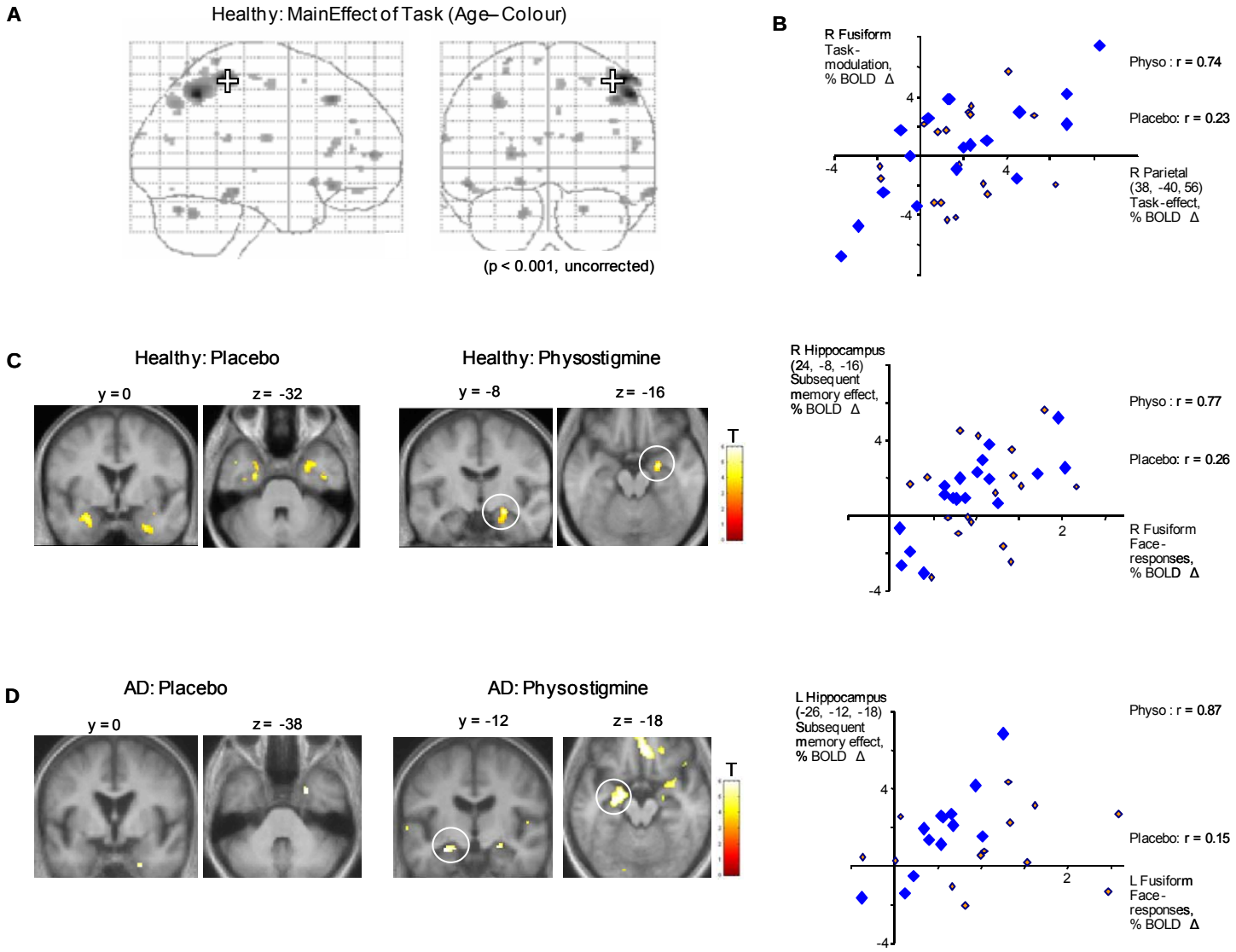
Finally, remote brain regions whose task-related, or memory-related, activity co-varied (in a subject-by-subject manner) with the relevant fusiform activations described above as showing BOLD-behavioural correlations (i.e. at 46, -48, -26 for healthy subjects; plus at -40, -54, -20 and 34, -40, -24 for Alzheimer's disease) were explored. Whether physostigmine impacts on any such inter-regional relationships was also assessed. In healthy subjects, the main-effect of task (Age versus Colour) under placebo activated right superior parietal cortex (peak: 48, -42, 58; $Z = 5.46$; $p < 0.001$, corrected; Fig. 9.5A); no other regions were significant after whole-brain correction. We found that the task effect within this right parietal region also correlated with the task-modulation of face-selective responses in right fusiform cortex under both placebo (66, -36, 40; $Z = 3.37$; $p<0.001$, uncorrected) and physostigmine (38, -40, 56; $Z = 3.49$; $p<0.001$, uncorrected). Comparing each of these two parietal peaks with the equivalent two voxels under the alternative treatment showed a significant between-treatment difference only for the latter peak, i.e. at 38, -40, 56 there was a greater correlation coefficient under physostigmine than under placebo ($Z(17) = 1.96$, $p < 0.05$; Fig. 9.5B). In Alzheimer's disease, task-related regions beyond fusiform cortex did not show correlations with task-modulation of face-selective fusiform cortex under either treatment.

We next investigated any association of fusiform face-selective responses with regions showing a subsequent-memory effect (i.e. higher responses for faces during encoding that were subsequently recognised relative to those forgotten). This showed that healthy subjects showing greater face-selective responses at the right fusiform

peak (46, -48, -26) also showed a greater subsequent memory effect in bilateral amygdala (36, 6, -42, $Z = 3.42$; -30, -4, -24; $Z = 3.31$; $p < 0.001$, uncorrected) under placebo, and in right hippocampus (24, -8, -16; $Z = 3.85$; $p < 0.0001$, uncorrected) under physostigmine. The latter region also showed a greater correlation coefficient under physostigmine than under placebo ($Z(17) = 2.07$; $p < 0.05$; Fig. 9.5C). In Alzheimer's disease, correlations were found between left fusiform face-selective responses and a subsequent-memory effect in right amygdala (24, 2, -36; $Z = 3.62$; $p < 0.0001$, uncorrected) under placebo, and in extensive regions of bilateral hippocampus - amygdala under physostigmine (-26, -12, -18; $Z = 4.03$; 26, -8, -14; $Z = 3.83$; $p < 0.0001$, uncorrected; Fig. 9.5D; note that confident responses only were included, in line with the preceding results for the Alzheimer's disease group). Each of these Alzheimer's disease fusiform – medial temporal correlations as specified were greater than under the alternative treatment (all $Z(12) > 2.08$; $p < 0.05$). Face-selective activations in right fusiform cortex did not show correlations with subsequent memory responses in any brain region in Alzheimer's disease under either treatment.

Figure 9.5. (See next page): Regions showing main effect of Age>Color task (A) or correlations of task effects (B), or subsequent-memory effects (C, D), with task-modulation and face-selective responses of fusiform cortex, respectively. A) SPM depicting regions in the whole brain showing a main-effect of task (shown at $p < 0.001$ uncorrected, depicted as a maximum-intensity projection), within which were found sub-regions where that effect correlated on a subject-by-subject basis with task-modulation of face-selective responses in right fusiform cortex (at peak of treatment effect: 46, -48, -26) under both placebo and drug conditions. The cross indicates the voxel showing the greatest fusiform – parietal covariation under physostigmine, at which there was a significantly greater correlation coefficient than under placebo as shown (B) in the scatterplot; C) Medial temporal regions in which a subsequent memory effect (i.e. recognised versus forgotten faces) correlated with face-selective responses in right fusiform cortex (peak: 46, -48, -26) under placebo and physostigmine in healthy subjects; scatterplot at right depicts fusiform – hippocampal covariance for the hippocampal site showing the greatest difference in correlation coefficients between treatments ($p < 0.05$); D) As for C, except now in Alzheimer disease subjects, with correlations of medial temporal regions' subsequent memory effect (for confident judgements) with face-selective responses in left fusiform cortex (at peak of treatment effect: -40, -54, -20) under placebo and physostigmine. Scatterplot at right depicts fusiform – hippocampal covariance at a hippocampal region showing greater correlation coefficient under physostigmine than placebo ($p < 0.05$).

Figure 9.5: For legend see previous page.



Discussion

Cholinesterase inhibitors are one of the most widely used symptomatic treatments for dementia (Gruber-Baldini et al, 2007), but the physiological basis for their performance benefits are unclear. We show here for the first time a direct relationship between the behavioural and neural effects of a single challenge with a cholinesterase inhibitor in both health and dementia. The principal findings are: i) the cholinesterase inhibitor physostigmine produced small overall improvements in face-recognition memory, that in healthy subjects but not Alzheimer's disease were dependent upon encoding-task; ii) in healthy subjects, the degree to which physostigmine improved the memory of faces studied deeply (relative to those studied shallowly) correlated with the degree to which physostigmine enhanced face-selective fusiform cortex activity during the deep- (relative to the shallow-) encoding task; iii) in Alzheimer's disease, improvements in confidently-judged face recognition caused by physostigmine correlated with drug-induced enhancements of fusiform face-selective responses during encoding, that unlike the case for healthy subjects, were independent of encoding task; iv) the fusiform cortex regions showing these neural-behavioural correlations also showed increases in their functional coupling with parietal and hippocampal regions following physostigmine. We discuss the results of the healthy and Alzheimer's disease groups in turn.

Cholinergic modulation of encoding in healthy subjects

A recent integrative model of memory suggests that the physiological actions of acetylcholine on both sensory and entorhinal cortices enable the cortical dynamics necessary for new memory formation (Hasselmo, 2006). For example, acetylcholine

increases both sensitivity and specificity of stimulus-evoked visual cortical responses (Sato et al, 1987; Murphy & Sillito, 1991), while suppressing feedback connections to the same areas (Kimura et al, 1999), thereby potentiating the formation of novel input associations (Hasselmo & McGaughy, 2004). Additionally, plastic changes in the response pattern of sensory cortices to specific stimuli (e.g. as seen with fear-conditioning) are dependent on cholinergic inputs from basal forebrain to sensory cortices (Weinberger, 2007; Gu, 2003). In the current study we sought to bridge the neurophysiological actions of acetylcholine on sensory cortices with the well-recognised influences of cholinergic-enhancing drugs on memory performance (Gron et al, 2005) through the use of functional imaging.

The design of our study married together two previous sets of observations. First, previous experiments (e.g. Experiment 3; Furey et al, 2000; Lawrence et al, 2002) have shown that pro-cholinergic drugs can increase visual-evoked responses in visual extrastriate cortex, with this effect appearing to be greater for stimuli that are attended than for those that are incidental to the task. For example, in Experiment 1, in a healthy, young adult population, physostigmine increased fusiform cortex responses for faces that were task-relevant, rather than those that were task-irrelevant, thereby enhancing the usual pattern by which task demands, independent of stimulus changes, can modify sensory cortex activity (Vuilleumier et al, 2001). Secondly, psychophysical studies in humans suggest that nicotine or cholinesterase inhibitors enhance memory through effects during the encoding phase when stimuli are first presented (Ghoneim & Mewaldt, 1977; Wetherell, 1992; Rusted & Warburton, 1992), rather than during consolidation or recall, when they may exert a negative effect instead (Edginton & Rusted, 2003; Gais & Born, 2004). Moreover, the pro-mnemonic

actions of these drugs are experienced more for stimuli that are presented during deep, than shallow, encoding tasks (Warburton et al, 2001; Fitzgerald et al, 2008) – thereby mirroring the pattern of extrastriate cortex modulation found in functional imaging studies. Consequently, we predicted that physostigmine would increase memory more for faces studied during a deep task (of judging age) than during a shallow task in which the particular facial characteristics were incidental to the task (of ascertaining picture colour). Critically, we hypothesised that this behavioural effect (measured at later recognition) would correlate with enhancements in face-selective activity of fusiform cortex (measured during initial encoding), that should also be more pronounced during the Age than the Color tasks. As Fig. 9.3C illustrates such a BOLD-behavioural correlation was found to occur very close to the peak of face-selective responses in right fusiform cortex. In other words, in those subjects for whom physostigmine improved memory more for faces studied deeply than shallowly, physostigmine was also found to increase fusiform face-responsiveness during the encoding phase when the faces were first presented, more during the deep than shallow task.

Several features of our results suggest that the observed pharmacological modulation of fusiform cortex was instrumental to the drug's effects on subsequent memory performance. First, although our conclusion rests in part on a brain-behaviour correlation, it should be noted that this relationship was directional in time - i.e. physostigmine enhancement of face-responses during encoding predicted later effects on memory. Since the behavioural performance of healthy subjects during the encoding tasks was unaltered by physostigmine our results at that time are unconfounded by performance considerations. Furthermore, although physostigmine

would have been present during both encoding and recognition phases, the pharmacological effect observed here in healthy subjects occurred as an interaction with task that differed only during encoding. Second, both the data from our subjects in the placebo condition, and those from several previous studies (Grady et al, 1998; Bernstein et al, 2002; Otten et al, 2002; Mandzia et al, 2004) show that task-modulation of face-selective responses of fusiform cortex during encoding correlates with a subsequent depth-of-processing (i.e. encoding-task-dependent) effect on memory. Third, a separate 'subsequent-memory' analysis of the same subjects showed that faces later recognised, ascompared to faces subsequently forgotten, elicited higher activity in right fusiform cortex during the encoding task (now independent of task or treatment) - again indicating the crucial role of fusiform activity at encoding for subsequent face memory. Previous (but non-drug) studies have analogously observed a subsequent memory effect to visual stimuli in fusiform cortex (Wagner et al, 1998; Kirchhoff et al, 2000; Golby et al, 2001; Sperling et al, 2003; Dickerson et al, 2007; Kircher et al, 2007). Fourth, we found a correlation of face-selective activity in right fusiform cortex with a subsequent-memory effect in hippocampal/amygdala regions that was enhanced under physostigmine specifically in right hippocampus (Fig. 9.5C). Thus the observed effects of drug on memory here may arise from a combination of enhanced fusiform responses, specific to the encoding task, and increases in functional connectivity between sensory cortex and the medial temporal cortices that are thought to be critical for memory formation (Rissman et al, 2008).

It is important to distinguish physostigmine-induced response increases in fusiform cortex shown here that are task-dependent (and which mirror subjects' greater depth

of processing memory effects), from physostigmine-induced decreases in fusiform activity that are task-independent (as reported in Experiment 9.4, and which did not take into account subsequent memory effects). This combination of findings seems consistent with previous fMRI studies showing that, on the one hand, physostigmine increases visual cortex BOLD activity selectively during encoding (Furey et al, 2000) or high-attention tasks (Experiments 1 and 3); but, on the other hand, that the same treatment causes decreases, or no change, in activity in the same regions during low-attention (Experiment 3: visual stimulation main-effect) or passive viewing tasks (Furey et al, 2000; Silver et al, 2008). This profile of functional imaging results parallels observations made using more basic neurophysiological techniques: viz. direct acetylcholine application to visual cortex decreases the net stimulus-driven field potential of cortical columns (Kimura et al, 1999) due to suppressed intracortical signalling (Levy et al, 2006), while increasing differential activity in visual cortical units as a function of spatial attention (Herrero et al, 2008).

A likely source for task-driven as opposed to stimulus-driven activation changes in sensory cortex would seem to be frontoparietal regions within the so-called dorsal attention network (Kastner et al, 1999). Hence one possible explanation for the depth-of-processing memory effect is an enhancement of resource allocation through attentional mechanisms (Baddeley, 1990; Chun & Turk-Browne, 2007). Given that attention is critically dependent on cholinergic innervation to frontoparietal cortices (Sarter et al, 2005a), we explored the possibility that the modulation of task-effects by physostigmine in fusiform cortex (seen here as correlating with drug effects on subsequent memory) may reflect an impact of the drug on functional coupling between fusiform cortex and regions traditionally associated with attention. The main

effect of task in our study (i.e. Age > Colour task) activated right parietal cortex most strongly (Fig. 9.5A). We found that this task effect in parietal cortex correlated across subjects with task-modulation of face-selective right fusiform cortex under both placebo and physostigmine, supporting the idea of a functional connection between these regions. The strength of this relationship was greater under physostigmine than placebo, suggesting that cholinergic modulation of task-responses in fusiform cortex, along with associated depth of processing subsequent memory effects, may involve cholinergic modulation of influences from regions such as parietal cortex that can exert top-down influences on sensory cortices. We note that drug-induced changes in the correlation coefficients for subject-by-subject effect sizes in fusiform and parietal cortex are distinct from drug-effects on mean task-related parietal activity (which is depressed by the drug overall: see Experiment 4). Similar physostigmine-induced reductions in task-related activity in frontoparietal cortices, associated with performance improvements, have been reported before (Experiment 3; Furey et al, 2000) and may reflect either a reduced demand for resource allocation in the face of enhanced sensory processing (Furey et al, 2000) or improved parietal –sensory coupling as suggested here.

Cholinergic modulation of encoding in Alzheimer's disease

Cholinesterase inhibitors enable modest improvements in memory performance in Alzheimer's disease (Almkvist et al, 2004), although whether these occur primarily through direct effects on memory processes (Gron et al, 2005; Gron et al, 2006), or via indirect actions on executive - attentional processes (Alhainen et al, 1993; Lawrence & Sahakian, 1995) is unclear. In our study, we were able to address this issue at both behavioural and neural levels by testing for interactions between drug-

induced memory enhancement and the encoding task. Contrary to what might be expected from a purely attentional account, we found in the Alzheimer's disease group that physostigmine-induced memory improvement was both independent of encoding task, and did not correlate with task-modulations of face-selective extrastriate cortex. Instead we found that physostigmine-induced improvement in recognition performance correlated with enhancement of face-selectivity in left fusiform cortex, that was also independent of encoding-task. Importantly therefore we show that both behavioural and physiological consequences of cholinesterase inhibition may differ between healthy subjects and dementia patients.

In contrast to healthy subjects, Alzheimer's disease subjects did not benefit from a depth-of-encoding manipulation in their subsequent recognition performance (as also shown behaviourally in Beauregard et al, 2001; Bird & Luszcz, 1991). In our situation, this was not due merely to Alzheimer's disease patients failing to follow task instructions, because Alzheimer's disease patients actually showed a greater performance difference between tasks during encoding than healthy subjects. A possible neurophysiological basis for this lack of depth-of-processing in Alzheimer's disease may lie in impaired top-down modulation of sensory cortices by frontoparietal regions (Gazzelley & D'Esposito, 2007; Walla et al, 2005). We found some support for this from our data in two respects: first we found that healthy subjects showed correlations between depth-of-processing memory effects and task modulation of face-selective cortices (in superior temporal sulci) that were reduced in the Alzheimer's disease group. Second we also found some correlations between task-modulation of face-selective fusiform cortex and task effects in right parietal cortex in healthy subjects that were absent in dementia patients. A similar pattern of

correlations arising between encoding-related activity and subsequent recognition in healthy subjects, but not in mild cognitive impairment patients, has recently been reported (Mandzia et al, 2007). Although in the latter study the main between-group differences arose in parahippocampal and hippocampal regions, the contrasts in Mandzia et al (2007) were based upon stimulus-related activations, as opposed to task-related modulations as we report here which more closely reflect the depth-of-processing effect.

In Experiment 4, that employed the same task, but analysed responses without taking into account subsequent memory performance, physostigmine partially reversed Alzheimer's disease-associated deficits in task-related frontoparietal activity, that was associated with a lesser performance impairment during the encoding task (of visual discrimination). However, by directly correlating task-related responses with effects on subsequent memory, the current results show that even under circumstances where physostigmine enhances frontoparietal task-related activity - as in Experiment 4 – this may be insufficient to restore a depth-of-processing effect on subsequent memory. Two previous fMRI studies in mild cognitive impairment patients have similarly shown enhancements of task-related frontoparietal activity following cholinesterase inhibitor therapy that were associated with improvements in working memory / attention, but <u>not</u> in episodic memory (Goekoop et al, 2004; Saykin et al, 2004). Taken together, these observations argue for the existence of dissociable effects for cholinesterase inhibitors on episodic memory versusattention (Sahakian et al, 1993; Lindner et al, 2006), that parallel dissociable pathological correlates of episodic memory and attention impairments in Alzheimer's disease (Perry & Hodges, 1999; Perry et al, 2000; Buckner, 2004). One possible reason for the pharmacological /

functional dissociation observed is that memory, and especially depth of processing memory effects, rely on frontoparietal-extrastriate-hippocampal functional connections (Grady et al, 2001; Celone et al, 2006; Bokde et al, 2006), whose impairments in dementia may be less reversible by physostigmine than strength of activation for each of these regions considered in isolation. Our finding that physostigmine did not impact on fusiform – parietal functional coupling in Alzheimer's disease subjects, unlike in healthy subjects, is consistent with this.

Although physostigmine did not influence depth- of-processing recognition-memory effects in our Alzheimer's disease patients, the drug did exert a significant benefit in (confident) recognition that was independent of encoding task (Fig. 9.4C). Moreover, this behavioural memory effect of the drug in Alzheimer's disease correlated with physostigmine-induced enhancements of bilateral face-selective fusiform cortices at initial encoding, but did so regardless of encoding task. Left fusiform cortex also showed a subsequent memory effect for faces in Alzheimer's disease, suggesting that enhancement of activity in this region by physostigmine was related to subsequent recognition in these patients. This aspect of our results suggest that Alzheimer's disease-associated impairments in fusiform cortex activity (see also Golby et al, 2005; Gron & Riepe, 2004; Machulda et al, 2003; Rombouts et al, 2005) may not only be reversible with cholinergic enhancement (see also Rombouts et al, 2002; Kircher et al, 2005), but that a functional consequence of this can be a proportionate improvement in subsequent recognition memory. The fact that, unlike healthy subjects, the effects of physostigmine on encoding-related activity in Alzheimer's disease patients was independent of task also seems consistent with reports that cholinesterase inhibition may modulate sensory cortices in Alzheimer's disease under both low and high-

attention conditions (Rombouts et al, 2002; Teipel et al, 2006). Our findings also complement studies showing that cholinergic antagonism in healthy subjects impairs both encoding-related activity in fusiform cortices, and recognition performance (Schon et al, 2005; Sperling et al, 2002; Rosier et al, 1999; Thiel et al, 2002).

We found that Alzheimer's disease patients only showed a treatment effect on memory when selectively analysing confident judgements. To the extent that confident judgements can be thought of as indexing hippocampus-based recollection memory, as opposed to familiarity (see Hudon et al, 2009; Wais, 2008), this behavioural result complements studies showing that Alzheimer's disease memory impairment is relatively specific for the former type of memory process (Dalla Barba et al, 1997; Rauchs et al, 2007). Indeed, we found that the drug-induced (confident) memory improvement in Alzheimer's disease correlated with activation enhancement, not only in fusiform cortex but also in hippocampus (Fig. 9.4C), as well as increasing functional coupling between these two regions (Fig. 9.5D). These findings complement a recent study showing that scopolamine reduces perirhinal activations specifically during contextual recollection, rather than for familiarity judgements (Bozzali et al, 2006), as well as supporting behavioural evidence suggesting a specificity of cholinergic actions for explicit relative to implicit memory (Knopman, 1991; Kopelman & Corn, 1988).

Conclusion

The current study unifies three previous sets of results: first, for behavioural studies showing that the memory-enhancing effects of pro-cholinergic drugs interact with encoding task (Warburton et al, 2001; Fitzgerald et al, 2008); second, functional

imaging studies showing that cholinergic-enhancing drugs increase visual extrastriate cortex activity in a task-dependent pattern (Furey et al, 2000; Lawrence et al, 2002; Experiments 1 and 3); and third, a range of studies showing that cholinergic antagonism of higher sensory cortices (as well as perirhinal-entorhinal cortices) correlates with impaired encoding (Kirkwood et al, 1999; Boroojerdi et al, 2001; Dotigny et al, 2008; Sperling et al, 2002; Schon et al, 2005). Here we show that the improvement in face-recognition memory induced by a cholinesterase inhibitor challenge directly correlates with drug-induced increases in visual extrastriate cortex activity during encoding, that in healthy subjects, but not Alzheimer's disease, are task-dependent. As well as lending further support to theoretical models that integrate cholinergic actions on sensory, attentional and memory processes (Sarter et al, 2003; Hasselmo & McGaughy, 2004), the BOLD-behavioural relations that we present here support aspirations to apply functional imaging technology to predict treatment responses in patients in future (Matthews et al, 2006).

10. General Discussion

Summary of Experimental Findings

The experiments of this thesis explore how a hypercholinergic state (as induced by a single-challenge with the cholinesterase inhibitor physostigmine) interacts with brain activation patterns observed under specific sensory, attentional and memory paradigms, and how this relates to behavioural effects. Given a theoretical neurobiological framework of cholinergic function, as discussed in Chapter 2, specific hypotheses were generated regarding how elevation of ACh levels would interact with patterns of cerebral processing measurable with human functional neuroimaging. The experiments also complement an expanding literature of human cholinergic functional imaging studies reviewed in Chapter 3. The main findings are as follows (listed thematically to assist interpretation):

1. Stimulus-evoked activity within higher (extrastriate) and lower (striate) visual cortices is *diminished* with physostigmine where there is no prior interaction of attention with sensory response and /or during low-attention. This is seen most clearly in Experiment 3 where physostigmine decreases striate cortex activity response to a flashing visual chequerboard regardless of task, and decreases superior and lateral occipital activity during low-attention conditions. Experiment 4 also showed that face-evoked fusiform cortex activity is generally decreased by physostigmine - during a depth-of-processing manipulation that did not itself modulate fusiform cortex activity.

2. By contrast, stimulus-evoked activity within higher visual cortex is *enhanced* with physostigmine selectively during tasks demanding high attention and where there is

already an interaction of attention with sensory response. This is seen in Experiment 3 where superior-lateral occipital visual-evoked responses are increased by physostigmine selectively during a demanding visual orienting task; and in Experiment 4 where occipital regions already demonstrating task-by-sensory interactions showed an overall enhancement of stimulus-evoked activity by physostigmine. Experiment 1 too showed an enhancement of fusiform cortex activity with physostigmine - this region also showing an interaction with attention (unlike the paradigm of Experiment 4 in which fusiform cortex activity did not apparently interact with task, and in which physostigmine decreased activations in this region). Experiment 1 also revealed a drug-induced enhancement of fusiform activations specifically to emotional, relative to neutral valence, stimuli, suggesting that physostigmine can also boost responses to bottom-up forms of attention, e.g. as elicited by stimulus salience.

3. Although physostigmine enhances sensory cortex activations during attention-demanding tasks (see above), this enhancement is often greater for task-irrelevant than task-relevant stimuli. Consequently, top-down *differential* activation of sensory cortices may actually be reduced. This is seen in Experiment 3 where physostigmine preferentially increased occipital activations on the hemisphere side opposite to that expected from the cue direction. A functional correlate of this was also expressed in that treated subjects also tended to show faster responses to targets in the visual hemifield opposite to that cued. Experiment 1 showed similarly a reduction in selective attention-driven modulation of lateral occipital cortex with physostigmine. Furthermore, Experiment 4 demonstrated a reduction in task-dependent differential modulation of sensory cortices with physostigmine, due primarily to a drug-induced enhancement of

sensory activations during a 'superficial encoding' task, in which higher sensory processing was irrelevant to task.

4. One of the functional consequences of physostigmine increasing higher sensory cortex activations, selectively under attention-demanding conditions, is that it may enhance stimulus encoding, as measured by a subsequent recognition memory test. Hence in Experiment 5, a strong positive correlation was found between physostigmine-induced enhancement of fusiform cortex activity and the extent to which physostigmine enhanced subsequent recognition memory scores.

5. Repetition suppression - a well-recognised neural correlate of repetition priming – is increased by physostigmine in early sensory cortices, although only for attended, rather than unattended, stimuli (Experiment 2). In so doing, physostigmine shifted the normal pattern of repetition decreases – in which they occur equally for all stimuli regardless of task relevance, to one in which repetition decreases occurred selectively for task-relevant stimuli. A concordant behavioural effect was also observed, in that priming (i.e. faster responses to repeated stimuli) became under physostigmine confined to trial pairs in which repeated stimuli were task-relevant, rather than occurring to repeated stimuli regardless of task relevance, as seen under placebo.

6. Cerebral responses to physostigmine differed importantly and consistently between healthy elderly people and age-matched Alzheimer's disease patients. Specifically, where physostigmine had decreased differential activations in healthy subjects, e.g. in sensory or frontoparietal cortices as a function of depth of processing, the same manipulation in

Alzheimer's disease had caused increases in these same contrasts (Experiment 4). Furthermore, whereas correlations between drug-induced fusiform cortex enhancements and recognition memory improvements had in healthy subjects been found to interact with depth of processing (in that physostigmine preferentially increased both fusiform responses and recognition to 'deeply encoded' stimuli), in Alzheimer's disease, where BOLD – memory correlations did occur, these were not related to depth of encoding (Experiment 5).

Interpretation

1. Directionality of sensory cortex modulations depends upon task or stimulus type

The general findings of Experiments 1, 3 and 4 that probed interactions of physostigmine with sensory cortex function are in line with previous functional imaging studies, and accounts of cholinergic modulation of sensory and attentional functions described earlier. Thus, cholinergic stimulation enhanced sensory cortex activation, but only under high-attention conditions where there is already evidence for top-down modulation of sensory activations. In other words, physostigmine does not enhance sensory cortex activity generally, or even enhance stimulus-evoked responses, but instead enhances top-down recruitment of sensory regions. This is entirely in keeping with a key postulated role for the nucleus basalis - neocortical system - namely, to enhance sensory and attentional processes in posterior regions during periods of performance challenges - for example with distracter insertion, or with sustained attention paradigms when performance typically declines with time (Sarter et al, 2006).

Experiments 3 and 4 also concur with another general point noted in the earlier review of human cholinergic functional imaging studies (Chapter 3) - that during periods of low-attention or rest, cholinergic stimulation decreases sensory cortex activity (or conversely, cholinergic antagonists increase sensory cortex activity). This was interpreted with reference to cortical-slice studies, showing that ACh decreases overall activation of cortical columnar activity, by virtue of decreasing transmission in all cortical layers, except in input layer IV (e.g. Kimura et al, 1999). Since the functional effect of this laminar-selective suppression by ACh is to enhance feedforward, relative to feedback, information flow (Hasselemo & McGaughy, 2004), the general neuroimaging finding that cholinergic-stimulating drugs decrease sensory cortex activity, might be regarded as indicating enhanced bottom-up processing. In other words, rises in ACh levels in sensory cortex, when top-down signalling is at a low level, result in a processing mode favouring input potentiation, although the observed effect is a net suppression of feedback synapses.

That effects of physostigmine in higher sensory regions interact with attention is highlighted by comparing the results of Experiments 1 and 4. Thus in Experiment 1, physostigmine increased attentional modulation of fusiform cortex, whereas in Experiment 4, the same drug caused a general decrease in stimulus-evoked activations of fusiform cortex, there being no effects of task (attention) in this region, for this experimental design. This difference in fusiform cortex drug response parallels the region-specific responses found in Experiment 3, whereby striate cortex –which showed no task-modulation – was suppressed by physostigmine, whereas lateral occipital cortex – which did show task-modulation – showed task-selective enhancement under physostigmine. Thus physostigmine appears to enhance attention-driven modulations of

(higher) sensory cortex, while decreasing sensory-driven modulations of sensory cortex; although, as discussed, this latter neuroimaging finding may still be compatible with a model in which bottom-up processing is enhanced with acetylcholine.

As well as enhancing attention-dependent activations of sensory cortex, Experiment 1 demonstrated that physostigmine can enhance fusiform cortex activations driven by the emotional valence of a stimulus type. This is perhaps not too surprising given that stimulus salience can act as a source of 'bottom up' attention, and may employ common downstream machinery to that recruited by top-down forms of attention. Two types of experiments in rodents have demonstrated analogous effects. In the first, cholinergic lesions in rodents disrupt attentional increments to conditioned stimuli secondary to stimulus contingency violations (Chiba et al, 1995). In this particular paradigm, attention shifting is found to be critically dependent upon a circuit comprising amygdala central nucleus (Holland & Gallagher, 1993), substantia innominata – nucleus basalis, and posterior parietal cortex (Bucci et al, 1998). In the second set of experiments, fear-conditioning-induced remapping of tone-specific representations within auditory cortex is similarly found to depend upon both amygdala, nucleus-basalis and neocortical cholinergic integrity (Weinberger et al, 2007). The results of Experiment 1 accord with these two sets of findings since the valence of the stimuli employed activated both amygdala, and higher sensory cortex, with physostigmine increasing emotion-driven responses in the latter. Hence Experiment 1 supports a model by which either top-down or motivation-driven recruitment of sensory processing is mediated, at least partially, by the nucleus basalis – neocortical cholinergic system (Sarter et al, 2006). Moreover it

shows that cholinesterase inhibition can increase both attention-driven and salience-driven activations of higher sensory cortex.

2. Sensory cortex modulations may depend upon anatomical region

A further factor that may determine responsiveness of sensory regions to cholinergic stimulation is anatomical location. In Experiment 3, physostigmine decreased stimulus-induced visual striate cortex activations regardless of task, whereas higher visual regions e.g. lateral occipital cortex, showed the task-dependent pattern of drug-induced modulation discussed above. Other functional neuroimaging studies have also suggested that cholinergic stimulation increases activation in higher more than lower visual processing regions. For example, cholinesterase inhibition suppressed stimulus-evoked activations in striate cortex, (Silver et al, 2007), while increasing them in a task-dependent fashion in higher extrastriate visual regions (Furey et al, 2000a). Nicotine also is found to decrease posterior visual cortical activations while increasing those in more anterior visual regions (Thiel et al, 2005; Hahn et al, 2009). Furthermore, scopolamine decreases activations in extrastriate visual cortex specifically during face-name learning, whereas no modulation is observed in striate cortex (Sperling et al, 2002). Such anatomical variations of cholinergic response would be in keeping with a model (Fig. 3.1) in which cholinergic stimulation modifies both bottom-up effects (recorded as net deactivation of *early* sensory cortices) and top-down effects (recorded as net enhancements of *higher* sensory cortices); as in most of these studies top-down effects were seen more readily in higher visual regions. One of the ways by which such anatomical-specificity of ACh effects occurs is through receptor segregation. For

example, a preferential expression of muscarinic receptors in V2 relative to V1 cortex parallels a spatial gradient in attentional modulation (Disney et al, 2006).

A further consistent anatomical division by which visual regions differ according to cholinergic response is that between ventromedial and posterolateral visual regions, with the former showing increased, and the latter decreases, in activity secondary to cholinergic stimulation. This dichotomous pattern of responses is seen most readily in Experiment 1, where neural correlates of spatial attention were increased by physostigmine in fusiform cortex, but decreased in posterolateral occipital cortex. Experiment 2 and 4 also found that physostigmine decreased task-related differential responses in posterolateral occipital cortex, while Experiment 5 found evidence for physostigmine-induced increases in task-related activations within fusiform cortex (apparent only on correlating with subsequent recognition performance). It is striking that a very similar anatomical dissociation in responses to physostigmine has also been observed by a separate group using a face working memory paradigm – with physostigmine increasing stimulus-induced, ventromedial extrastriate activations (including fusiform gyrus), while decreasing activations in posterolateral occipital regions (Furey et al, 2000b; Furey et al, 2008a; Ricciardi et al, 2009; Mentis et al, 2001). A similar profile of modulations is also seen with nicotine in higher visual areas (Thiel et al, 2005; Hahn et al, 2007). Conversely, muscarinic blockade results in activation decreases in fusiform cortex (Thiel et al, 2002c; Sperling et al, 2002; Schon et al, 2005; Rosier et al, 1999) but activation increases in lateral occipital cortices (Grasby et al, 1995; Bahro et al, 1999; Mentis et al, 2001; Thienel et al, 2009b).

Cholinergic-induced enhancements of inferior-medial temporal cortex might relate to this region's critical role in encoding. Since activations in inferior temporal cortex may index subsequent memory (Grady et al, 1998), cholinergic-induced enhancements here may reflect facilitation of encoding (Experiment 5), possibly due to processes such as sustained-spiking (Schon et al, 2005). This might explain why cholinergic modulation of medial, but not lateral, occipital regions is delay-dependent (Furey et al, 2008a). Conversely, lateral occipital cortex, that is heavily influenced by top-down or lateral connections (Vinberg & Grill-Spector, 2008), might be expected to show depressed activity following cholinergic stimulation, given that ACh generally inhibits intracortical transmission (Roberts et al, 2005). It is also noteworthy that cholinergic innervation to human occipital cortex segregates into medial and lateral pathways (Selden et al, 1998).

3. Effects on top-down modulation of sensory activations

Given that ACh plays a key role in selective attention (Sarter et al, 2006), with evidence from single-unit studies of ACh potentiating attentional modulation of visual (Herrero et al, 2008) and parietal (Broussard et al, 2009) responses, it was initially hypothesized that pro-cholinergic drugs would enhance selectivity of sensory processing. Thus an unexpected finding across several experimental paradigms is that cholinesterase inhibition reduces top-down selectivity of sensory cortices. This is seen for both spatial attention (Experiment 1; Experiment 3) and depth-of-processing (Experiment 4) visual tasks, in which physostigmine reduces task-driven (as opposed to stimulus-driven) *differential* modulation of extrastriate visual cortices. Similarly, in a fear-conditioning paradigm, physostigmine reduced the differential activation of auditory cortex to a conditioned stimulus (i.e. previously paired with a shock) relative to a non-conditioned

stimulus (i.e. no shock association) (Thiel et al, 2002b). Physostigmine has also been shown to increase the spatial extent of sensory activations during stimulus processing implying a reduction in stimulus-selectivity (Furey et al, 2000a).

Importantly, in order to reconcile this set of findings with the previous observation that pro-cholinergic drugs elevate functional activations during attention-demanding tasks, these experiments also showed that a main reason for such decrease in attentional selectivity is because of a disproportionate increase in sensory activity for task-irrelevant, or non-conditioned, rather than task-relevant, or conditioned, stimuli. Moreover, accompanying behavioural data suggest that enhancement of irrelevant stimulus processing associated with a hypercholinergic state has functional correlates. For example, physostigmine-induced stimulation of visual cortex coding for the visual hemifield opposite to that cued correlates with speeding to invalidly-cued targets (Experiment 3). Furthermore, high-ACh states enhance behavioral (Holley et al, 1995) and autonomic (Quigley et al, 1994) responses to irrelevant or low salience (Furey et al, 2008b) stimuli. Thus by heightening activity in sensory regions away from those favoured by top-down commands, a hypercholinergic state increases detectability of unexpected signals. Once again this fits with a model in which cortical ACh levels increase under conditions of high uncertainty by reducing internally-derived weighting of inputs (Yu & Dayan, 2005).

Whether nicotine works in a similar regard to cholinesterase inhibition is unclear. Behaviourally, nicotine reduces the penalty incurred by invalid cueing, suggesting that - like physostigmine - it counteracts selective attention by balancing out competing inputs

(Witte et al, 1997; Thiel et al, 2005). Furthermore, nicotine reduces a correlation between occipital deactivations and increasingly precise spatial cueing, suggesting that it enhances activity in task-irrelevant retinotopic areas (Hahn et al, 2007). However, nicotine does not consistently modulate cue-driven sensory cortex selectivity (Thiel et al, 2008), suggesting that more regionally abundant muscarinic receptors may account for the profile seen with physostigmine (Paterson & Nordberg, 2000; Zilles et al, 2002).

Does evidence from other sources indicate that a hypercholinergic state can decrease sensory cortex selectivity? As mentioned earlier, local ACh application in visual cortex increases the difference in firing rates between cells coding for task-relevant versus task-irrelevant locations (Herrero et al, 2008). However, the same study also found that ACh increased the overall firing rate, and, moreover, in some neurons increased it disproportionately more for stimulus-attribute values (e.g. bar length) that were non-optimal. Other studies have also noted ACh-induced reductions in selectivity to stimulus features (Zinke et al, 2006) or spatial coding (Kuo et al, 2009), at the same time as enhancing overall activity. This concords with functional imaging findings of enhanced sensory cortex activations following cholinergic stimulation, concomitantly with reduced selectivity (Figure 3.1).

Conceivably, under hypercholinergic conditions – i.e. those achievable pharmacologically, but not encountered under usual physiological states – weak top down signals are boosted more than strong ones, because the latter have all ready reached a ceiling. This may explain why some cholinergic-functional imaging results seem maladaptive in the sense that they favour task-irrelevant over task-relevant stimulus

processing. Support for this interpretation comes from an animal model of anxiety and psychosis, in which excessive ACh neurotransmission produces a hypervigilant state - including heightened sensitivity to distractor and irrelevant stimuli (Berntson et al, 1998).

4. Frontoparietal modulations

Since physostigmine was found to reduce top-down modulation of sensory cortices (see above), we might also expect the same drug to modulate those frontoparietal regions – especially right parietal cortex - that are believed to exert top-down control (Yantis et al, 2002). Consistent with this, Experiment 3 demonstrated that physostigmine reduced right superior parietal cortex activity (as well as left inferior prefrontal cortex) during maintenance of orienting, coincident with a reduction in occipital cortex selectivity. In Experiment 4 too, healthy subjects responded to physostigmine with a decrease in task-related right parietal activity, while also showing decreases in task-dependent differential modulation of extrastriate visual cortex. The interpretation of these findings is that physostigmine enhances sensory processing generally during tasks requiring high attention, whilst reducing selectivity of sensory processing during these very conditions – thus enhancing activity relatively more in occipital regions coding for task-irrelevant visual locations (Experiment 3), or in regions processing facial characteristics where this is irrelevant to task (Experiment 4). To the extent that right parietal activation might reflect the source of selective attention it is conceivable that the reductions in sensory cortex selectivity secondary to physostigmine were themselves due primarily to cholinergic effects in parietal cortex.

A related set of findings are from those studies investigating effects of nicotine on a spatial (Posner) cueing task, in which nicotine decreased parietal activation, at the same time as speeding responses to invalidly cued targets (Phillips et al, 2000; Thiel et al, 2008; see Chapter 3). In this context, nicotinic stimulation was proposed to increase sensory processing in general ('vigilance'), whilst diminishing the selectivity of sensory processing induced by spatial cueing. Both this interpretation and that advanced for Experiments 3 and 4, are in line with a model by which acetylcholine increases the influence of bottom-up relative to top-down processing (Yu & Dayan, 2005).

5. ***Memory-associated modulations***

Experiments 2 and 5 tested effects of physostigmine on behavioural and neural measures of one form of implicit memory – repetition priming, and one form of explicit memory – recognition memory. Hypotheses motivating these experiments spawned from studies demonstrating multiple memory mechanisms susceptible to cholinergic manipulation (see Chapter 2).

Repetition priming – the phenomenon by which repeated stimuli are processed more efficiently than unprecedented ones – is believed to be strongly related to the neural phenomenon of repetition suppression – by which neural activity decreases specific to repeated stimulus details (see Henson et al, 2002). The cellular processes that underlie repetition suppression are likely to be sensitive to a range of neuromodulatory influences (Rasmusson, 2000), including acetylcholine that is known to interact with sensory cortices, where most sensory-related repetition decreases are observed. Consistent with this, two previous human fMRI studies employing scopolamine showed a diminution of

both repetition priming and neural repetition decreases in prefrontal and visual extrastriate cortices to either word or face stimuli (Thiel et al, 2001; Thiel et al, 2000a). Although repetition suppression in monkey inferior temporal cortex has not, by contrast, been found to be cholinergic-dependent (Miller & Desimone, 1993), it is possible that this discrepancy may have arisen because of restricted neural sampling or shorter lag times in the latter case.

Given that scopolamine suppresses functional imaging signatures of repetition priming, and given that cholinesterase inhibition improves repetition priming in Alzheimer's disease (Riekkinen and Riekkinen, 1999), it was hypothesised that physostigmine would enhance neural correlates of repetition priming. This prediction was borne out in Experiment 2 in which physostigmine reduced neural activity to the repeated item more than was already the case without drug, thereby *enhancing* the repetition decrease effect. This result stands in contrast to effects of physostigmine on conditioning-associated sensory cortex remapping (Thiel et al, 2002b), in which physostigmine increased neural activity to unconditioned stimuli, effectively *decreasing* the neural conditioning effect. Thus the neural and behavioural influences of physostigmine are not always the opposite of those induced by cholinergic-blocking drugs, even within the restricted set of neural mechanisms underlying implicit memory within sensory cortices.

Experiment 5, in testing effects of physostigmine on recognition memory, also corresponded with previous functional imaging studies investigating effects of scopolamine on recognition memory, and its neural correlates (Sperling et al, 2002; Bullmore et al, 2003; Schon et al, 2005). These studies had shown that cholinergic

antagonism reduces visual extrastriate, as well as perirhinal, cortex activations during encoding, which itself correlated with reduced subsequent memory. In so doing, such studies had corroborated studies in monkeys demonstrating a dependency of recognition memory on cholinergic inputs to equivalent higher sensory regions during encoding (e.g. Tang et al, 1997). Conversely, pro-mnemonic effects of cholinesterase inhibition and nicotine, having also been expressed selectively during encoding (Warburton et al, 2001; Fitzgerald et al, 2008), led to the prediction that task-specific enhancements of sensory cortical processing by physostigmine – as witnessed in Experiments 1 and 3, as well as in the context of a working memory task (Furey et al, 2000b) – would be associated with enhanced subsequent memory. Experiment 5 was able to confirm this prediction by demonstrating positive correlations between effects of physostigmine on face-selective responses in fusiform cortex at encoding, and effects of the same drug on subsequent recognition, at the subject level, in both healthy elderly and mild Alzheimer disease.

An interesting commonality in the results of Experiments 2 and 5 (healthy subjects group), is that the positive effects of physostigmine on memory-associated sensory cortex activity were expressed only through an interaction with attention (or task requirements). In other words, physostigmine enhanced visual cortex repetition decreases, but only for attended, not unattended, repeated stimuli; and correlations between physostigmine-associated increases in fusiform cortex activity, and subsequent recognition, were only apparent for the contrast of deeply-encoded versus superficially-encoded stimuli. Such data concord well with the notion that pro-attentional affects of ACh in sensory cortices are conducive to effective encoding (Warburton et al, 2001; Sarter et al, 2005a). For example, selective effects of ACh on sensory cortical laminae encourage a feedforward

relative to feedback direction of inputs, and as such are likely to enhance both stimulus processing during attention-demanding conditions, and the formation of novel associative connections required for effective encoding (Hasselmo & McGaughy, 2004).

Combining results of Experiments 4 and 5, it is apparent that physostigmine can enhance both non-specific visual processing (in sensory regions showing task-specific activity initially) and task-specific visual processing (in sensory regions not showing task effects initially), and as such support a model by which ACh potentiates both bottom-up and top-down processes (Sarter et al, 2001). This observation may be related to the general anatomical point made earlier (based upon Experiments 1, 3 and 4) that physostigmine enhances task-related effects in ventromedial occipital-temporal regions (including fusiform cortex) while decreasing them in posterolateral occipital regions. The findings from Experiments 2 and 5 suggest a functional consequence of this anatomical dissociation in responses to physostigmine: namely, that the drug-induced increases in task- (or attentional-)dependent differential responses in ventral extrastriate cortex are hallmarks of cholinergic influences on sensory cortex-based memory mechanisms.

6. Differences between health and Alzheimer's disease in response to physostigmine

The use of cholinesterase inhibitors in Alzheimer's disease (AD) followed logically from two sets of facts: firstly, from the pathological insight that the AD brain is distinguished by loss of cortical cholinergic innervation; and secondly, from appreciating that similar profiles of cognitive dysfunction could be induced by selective cholinergic lesions in animals (Bartus et al, 1982). Consequently, drugs that enhance, and therefore which may partially restore, cholinergic neurotransmission might be expected to reverse AD-

associated patterns of neural activation. This prediction was directly tested in Experiment 4, in which each AD patient was scanned both on and off drug (counterbalanced for order), and compared with healthy subjects. As expected from the AD 'cholinergic hypothesis' (Mesulam et al, 2004), physostigmine partially restored patterns of stimulus or task-selective activations in AD patients that had differed significantly between groups. However, the same drug challenge in healthy subjects, rather than further increasing the differential responses to stimulus or task type (as might be expected for a monotonic relationship between ACh levels and cortical activations), actually decreased many of these differential responses. Consequently, both hypocholinergic states (i.e. as recognised in AD), and hypercholinergic states (as here induced by physostigmine), impaired the normal pattern of differential activations, in both sensory and frontoparietal regions – implying an 'inverted-U shaped' pattern of neuromodulation.

A similar, more general finding summarises a range of other cholinergic functional imaging studies that have scanned subjects under different physiological or pathological states. Thus pro-cholinergic drugs normalise task-evoked activation levels in states - such as sleep-deprivation (Chuah & Chee, 2008), aging (Ricciardi et al, 2009) or disease (Blin et al, 1997; Jacobsen et al, 2004; Goekoop et al, 2006), or with certain genetic polymorphisms (Jacobsen et al, 2006) - where such activations are abnormally low or high to begin with. By contrast, many of these studies also show either no modulation, or a *reverse* pattern of modulation, in the same regions, under the same paradigm, in healthy controls tested with the same drugs (Fig. 10.1B). Furthermore, disparate neuromodulatory signatures between patients and controls may be matched by equivalent behavioural dissociations, with performance enhancements selectively in those with abnormal

physiological states to begin with, and deteriorations in controls (Jacobsen et al, 2004). This matches data demonstrating that performance benefits of pro-cholinergic drugs are inversely correlated with baseline performance (Ernst et al, 2001; Kukolja et al, 2009; Newhouse et al, 2004; Thiel et al, 2005; Beglinger et al, 2005).

There are two further patterns of inverted-U response to cholinergic drugs that might be related (Fig. 10.1). Firstly, cortical response to a cholinergic drug often depends upon the level of regional activation *prior* to drug challenge (Figure 4A). Thus pro-cholinergic drugs enhance frontoparietal activity most readily under task conditions where such activity is relatively low under placebo, but decrease activity within the same regions, when activations are high to begin with (seen in Experiment 3 in parietal regions; also see Kumari et al, 2003; Hahn et al, 2007; Hahn et al, 2009). It is notable that the relative activation levels under placebo are not consistently related to the relative attentional demands, suggesting that the inverted U-shaped profile of cholinergic modulations may occur independently of the cognitive process tested. In fact, many examples of task-by-drug interactions, where say cholinergic stimulation decreases activations in frontoparietal regions selectively during demanding conditions (Furey et al, 2008a; Ricciardi et al, 2009; Thiel et al, 2005), may relate to the height of activations at baseline, rather than because of a specific cognitive interaction per se. Similar baseline-dependent inverted-U shaped cholinergic responses have been observed in sensory (Hahn et al, 2007) and hippocampal (Schon et al ,2005; Kukolja et al, 2009) regions.

A related phenomenon consists of activation decreases under both cholinergic blockade and stimulation, for a given brain region and paradigm. As examples, working memory-

associated prefrontal activity is suppressed by both physostigmine (Furey et al, 1997; Furey et al, 2000b), and scopolamine (Grasby et al 1995; Dumas et al, 2008), whilst stimulus-evoked activations of primary visual cortex are suppressed both by donepezil (Silver et al, 2008) and scopolamine (Mentis et al, 2001).

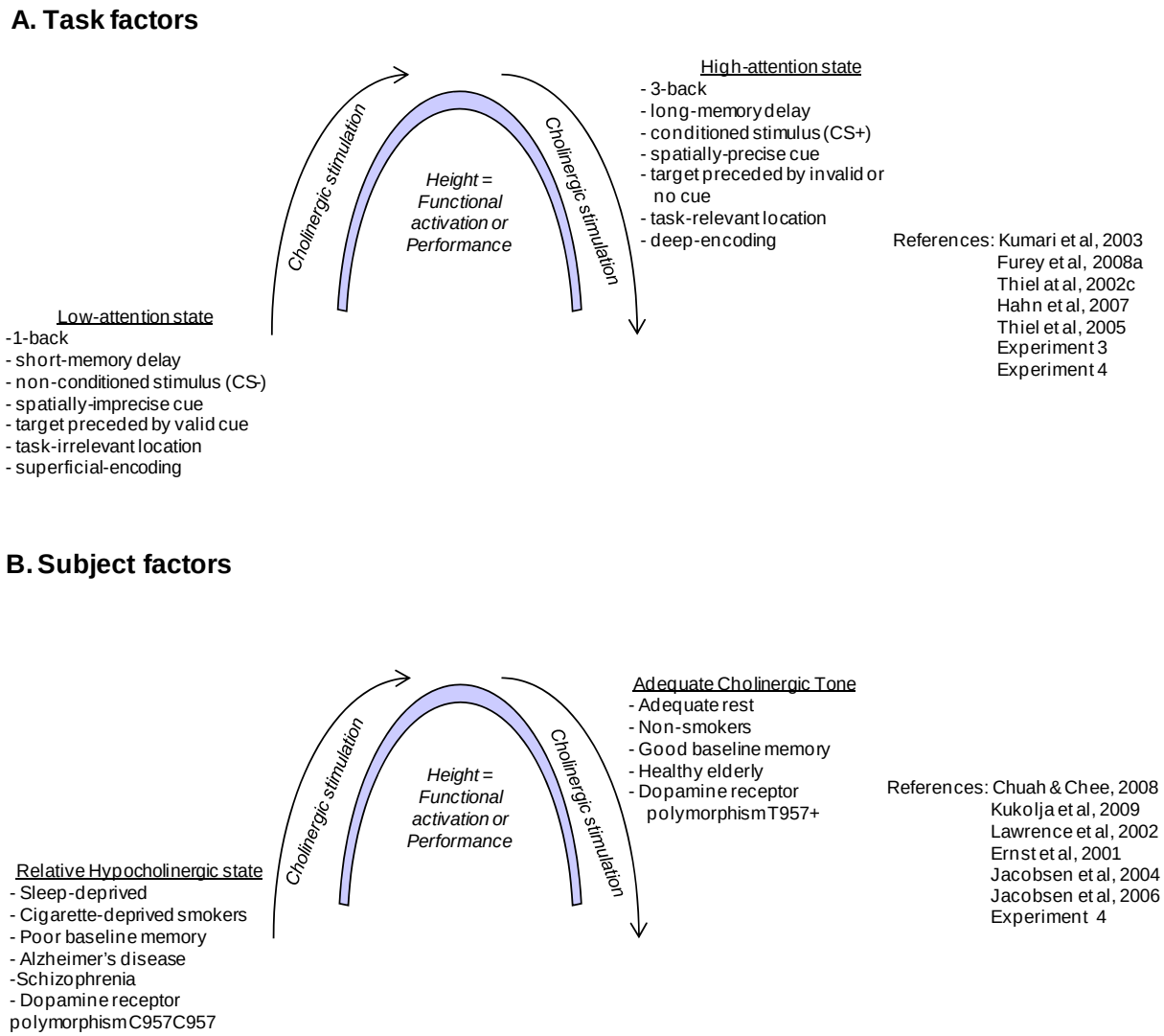
One explanation for these phenomenona might relate to methodology. For example, if a drug reduces all activations by 10%, then this may be discerned only in conditions with high activations to begin with. Conversely, if the hemodynamic response to a particular condition is at ceiling, then drug-induced increases in neural activity may only be manifest in conditions where the hemodynamic response starts off low. Furthermore, we should be wary of 'regression to the mean' artefacts - arising from the fact that floor activations can only get higher, and ceiling activations can only get lower.

However, there are several plausible neurobiological reasons why we might expect this profile. According to the 'attentional effort' hypothesis (Sarter et al, 2006), cholinergic stimulation activates both anterior and posterior cortical regions in response to performance challenges, which may explain why exogenous pro-cholinergic drugs elevate activations that begin low, typically in undemanding conditions. Conversely, the suppression of both frontoparietal task-dependent activity and sensory cortex task-dependent selectivity with pro-cholinergic therapies may correspond to decreases in top-down, or feedback, concordant with information-processing models of cholinergic function (Hasselmo & McGaughy, 2004; Yu & Dayan, 2005). This would explain why the pattern of impaired differential sensory cortex responses seen with physotigmine in healthy subjects is due to *excess activation during task-irrelevant* conditions

(Experiments 3, 4; also Thiel et al, 2000b); whereas the pattern of impaired differential sensory cortex responses observed during disease or with cholinergic antagonists, is accountable more through *under-activation during task-relevant* conditions. More generally, where cholinesterase inhibition or nicotine increases task-related cortical activity, or performance, this appears to reflect subject factors, e.g. due to genetic variation, disease, sleep-deprivation or task conditions, or task factors, e.g. with low attentional demands, where there is a relative reduction in ACh neurotransmission to begin with, and vice versa for subject groups or tasks in which there is a relatively high baseline level of cholinergic activation.

It is noteworthy that inverted-U shaped functions of neuromodulation are also seen with dopamine (Williams & Castner, 2006), and norepinephrine (Introini-Collison & McGaugh, 1986). For instance, amphetamine increases performance and prefrontal activation in subjects with low baseline measures of each, but decreases both in subjects who begin with high values for each (Mattay et al, 2000). Thus a common property of neuromodulators is that their process-optimising capabilities exist within a narrow concentration range. Two practical implications of this are that ‘performance-enhancing’ drugs are unlikely to benefit high-performers, and that the effects of such drugs may be predictable from individuals' baseline behaviour or brain activity (Giessing et al, 2007). Furthermore, differences in performance accountable by genetic polymorphisms in dopaminergic neurotransmission produce an inverted-U pattern of response to nicotine (Jacobsen et al, 2006), providing further evidence for cholinergic - dopaminergic interactions (Dewey et al, 1993).

Figure 10.1 Responses to cholinergic drugs, both behaviourally and as recorded by functional imaging, may correspond to an inverted-U shaped pattern. Hence whether a pro-cholinergic drug increases or decreases performance / relative activity depends upon the value of either measure before drug is given, that itself depends upon task demands (A) and subject-specific factors (B).



Conclusion

The physiological consequences of ingesting cholinergic-active substances have been observed since the Ancient Greek era, with scopolamine (bella donna), nicotine and physostigmine being recognised first as poisons, and only much more recently, as medicines. Applying these compounds to specific clinical scenarios, such as glaucoma, myasthenia gravis and Alzheimer's disease, followed only after a basic pathophysiological appreciation of each condition, and from models of how such drugs act. Functional imaging promises to advance such understandings of disease and drug by relating traditional animal-based or post-mortem lines of enquiry with signatures of brain activity during real-time human performance.

Focusing on cholinergic neurotransmission, the experiments of this thesis, and the subsequent review of like-minded studies, have shown how functional imaging can be used to inform, support or refute, existing accounts of neuromodulatory physiology. For example, although selective attention has previously been shown to be critically dependent upon an intact cholinergic system (Sarter et al, 2005a), functional imaging has shown that pharmacological elevation of cholinergic neurotransmission in healthy humans, cannot in general further improve this process (Experiment 1; Experiment 3; Experiment 4; Thiel et al, 2002c; Thiel et al, 2008). Rather, a range of studies has shown that such drugs modulate sensory and parietal cortex activity in a way that may enhance sensory processing of irrelevant items (Experiment 3; Thiel et al, 2002c; Thiel et al, 2005; Vossel et al, 2008). From a pharmaceutical standpoint, such findings are useful in

showing why pro-cholinergic drugs may be limited in their use amongst relatively-well people, and contrast with 'restorative' patterns of neuromodulation in Alzheimer's disease (Experiment 4). Furthermore, fMRI findings that cholinergic stimulation increases irrelevant sensory processing support independently-derived animal models which suggest that a hypercholinergic state contributes to 'hypervigilant' symptoms of anxiety and psychosis (Berntson et al, 1998; Sarter et al, 2005b). Consequently, a natural prediction from such results is that anti-cholinergic therapies may have a role in disorders characterised by distraction or hypervigilance.

A further example by which functional imaging results may inform therapeutic pathways is suggested by the wealth of studies showing modulatory effects of pro-cholinergic drugs on parietal activations and attentional performance combined (see Table 2). These complement animal studies showing cholinergic dependence of parietal cortex for normal attentional orienting (Bucci et al, 1998; Davidson & Marrocco, 2000). Assuming therefore that parietal cortex is essential for the pro-orienting effects of cholinergic stimulation, a reasonable prediction is that patients with attentional neglect may benefit from pro-cholinergic drugs only if there is some sparing of (right) parietal cortex. A recent study seems to bear this out, with nicotine enabling reorienting away from invalid.cues, selectively in neglect patients with an intact parietal lobe (Vossel et al, 2009).

A different type of conclusion is suggested by those pharmacological functional imaging studies demonstrating correlations between drug-modulations and drug-associated

behavioural effects. In keeping with the observation that most pro-cholinergic drugs show only small or no behavioural effects, these studies demonstrate that subjects can differ widely in their neuromodulatory response to such drugs. Inspecting only group-effects of drugs on brain activations may ignore important neuromodulatory influences in a subset of subjects. For example, whereas group-level analysis suggested that physostigmine only decreases task-related activations in sensory cortex in healthy subjects (Experiment 4), a correlation analysis based upon subsequent memory scores revealed that in certain healthy subjects, physostigmine increases task-related responses (Experiment 5). Other cholinergic imaging studies have similarly shown a range of drug-associated neuromodulations associated with a spread of drug influences on performance (e.g. Ernst et al, 2001; Hahn et al, 2007).

It is critical that drug-induced neural effects are not confounded by the subsequent behavioural influences, that in certain cases can be controlled methodologically, e.g. by restricting analyses to correct responses only (Kukolja et al, 2009) , or temporally separating neural sampling from performance effects (Experiment 5). However, even when such controls are made, it is difficult to know whether performance-correlated neural effects are causative, downstream or even epiphenomenal. For a more complete understanding therefore of *variation* in drug-responses, pharmacological functional imaging studies will need to be complemented by human studies employing factors such as genes, lesions or TMS, as well as more traditional animal experiments.

Yet, while functional imaging may point to neural processes of interest in accounting for performance effects, without actually proving causation, the same technology may also serve therapeutic innovation in other ways. For example, in separating drug-induced modulation of brain activity from performance effects, Experiment 5 demonstrates how functional imaging, in combination with a single drug-challenge, could be used as a predictive tool. A similar concept also underlies the finding that partial-least squares analysis of individual functional imaging data, prior to drug-use, can be used to predict subsequent behavioral effects of a drug, in this case, nicotine (Giessing et al, 2008). Furthermore, response to a cholinesterase inhibitor over a prolonged period has been correlated with both structural atrophy of substantia innominata, and baseline regional perfusion of frontal regions, as measured by baseline SPECT imaging (Kanetaka et al, 2008). With increasing accuracy by which human cholinergic systems can be anatomically localized (Zaborsky et al, 2008; Selden et al, 1998), it is also possible that relationships between focal brain lesion site and drug response may emerge.

One criticism often leveled against functional imaging is that its principle datum exists on a spatiotemporal scale, orders of magnitude greater than that at which neural processing occurs. This thesis has hopefully shown how in spite of this limitation, its results may still be both neuroscientifically meaningful, and offer the potential for clinical application.

Bibliography

Acquas E, Wilson C, Fibiger HC. Conditioned and unconditioned stimuli increasefrontal cortical and hippocampal acetylcholine release: effects of novelty,habituation, and fear. J Neurosci. 1996;16(9):3089-96.

Adams CE, Cepeda C, Boylan MK, Fisher RS, Hull CD, Buchwald NA, Wainer BH, Levine MS. Basal forebrain neurons have axon collaterals that project to widely divergent cortical areas in the cat. Brain Res. 1986;397(2):365-71.

Aigner TG, Mitchell SJ, Aggleton JP, DeLong MR, Struble RG, Price DL, WenkGL, Mishkin M. Effects of scopolamine and physostigmine on recognition memory in monkeys with ibotenic-acid lesions of the nucleus basalis of Meynert. Psychopharmacology (Berl). 1987;92(3):292-300.

Alhainen K, Helkala EL, Riekkinen P. Psychometric discrimination of tetrahydroaminoacridine responders in Alzheimer patients. Dementia. 1993;4(1):54-8.

Allen MT, Padilla Y, Gluck MA. Ibotenic acid lesions of the medial septum retard delay eyeblink conditioning in rabbits (Oryctolagus cuniculus). Behav Neurosci. 2002;116:733-8.

Almkvist O, Darreh-Shori T, Stefanova E, Spiegel R, Nordberg A. Preserved cognitive function after 12 months of treatment with rivastigmine in mild Alzheimer's disease in comparison with untreated AD and MCI patients. Eur J Neurol. 2004;11(4):253-61.

Amaral DG, Price JL, Pitkanen A, Carmichael ST. Anatomical organization of the primate amygdaloid complex, in: Aggleton, J.P. (Ed.), The Amygdala: Neurobiological Aspects of Emotion, Memory, and Mental Dysfunction. 1992. Wiley-Liss, New York, pp. 1–66.

Anagnostaras SG, Murphy GG, Hamilton SE, Mitchell SL, Rahnama NP, Nathanson NM, Silva AJ. Selective cognitive dysfunction in acetylcholine M1 muscarinic receptor mutant mice. Nat Neurosci. 2003;6(1):51-8.

Ances BM, Liang CL, Leontiev O, Perthen JE, Fleisher AS, Lansing AE, Buxton RB. Effects of aging on cerebral blood flow, oxygen metabolism, and blood oxygenation level dependent responses to visual stimulation. Hum Brain Mapp. 2009;30(4):1120-32.

Anderson HK. The paralysis of involuntary muscle: Part III. On the action of pilocarpine, physostigmine, and atropine upon the paralysed iris. J Physiol. 1905;33(4-5):414-38.

Aou S, Oomura Y, Nishino H. Influence of acetylcholine on neuronal activity in monkey orbitofrontal cortex during bar press feeding task. Brain Res. 1983;275, 178–182.

Apparsundaram S, Martinez V, Parikh V, Kozak R, Sarter M. Increased capacity and density of choline transporters situated in synaptic membranes of the right medial prefrontal cortex of attentional task-performing rats. J Neurosci. 2005;25(15):3851-6.

Armony JL, Dolan RJ. Modulation of spatial attention by fear conditioned stimuli: an event-related fMRI study. Neuropsychologia. 2002;40, 817–826.

Arneric SA. Cortical cerebral blood flow is modulated by cholinergic basal forebrain neurons: effects of ibotenic acid lesions and electrical stimulation. In J. Seylaz & E. T. MacKenzie (Eds.), Neurotransmission of cerebrovascular function. 1989a;381–384. Amsterdam: Elsevier.

Arnold HM, Burk JA, Hodgson EM, Sarter M, Bruno JP. Differential cortical acetylcholine release in rats performing a sustained attention task versus behavioral control tasks that do not explicitly tax attention. Neuroscience. 2002;114(2):451-60.

Asthana S, Raffaele KC, Berardi A, Greig NH, Haxby JV, Schapiro MB, Soncrant TT.Treatment of Alzheimer disease by continuous intravenous infusion of physostigmine. Alzheimer Dis Assoc Disord. 1995;9(4):223-32.

Atri A, Sherman S, Norman KA, Kirchhoff BA, Nicolas MM, Greicius MD, Cramer SC, Breiter HC, Hasselmo ME, Stern CE. Blockade of central cholinergic receptors impairs new learning and increases proactive interference in a word paired-associate memory task. Behav Neurosci. 2004;118:223–236.

Auld DS, Kornecook TJ, Bastianetto S, Quirion R. Alzheimer's disease and the basal forebrain cholinergic system: relations to beta-amyloid peptides, cognition, and treatment strategies. Prog Neurobiol. 2002;68(3):209-45.

Awh E, Jonides J. Overlapping mechanisms of attention and spatial working memory. Trends Cogn Sci. 2001;5:119 –126.

Backman L, Almkvist O, Nyberg L, Andersson J. Functional changes in brain activity during priming in Alzheimer’s disease. J Cogn Neurosci 2000;12:134–141.

Baddeley A. Human memory: theory and practice. Erlbaum, Hillside, New Jersey. 1990.

Baddeley AD, Baddeley HA, Bucks RS, Wilcock GK. Attentional control in Alzheimer's disease. Brain. 2001;124(Pt 8):1492-508.

Badgaiyan RD. Neuroanatomical organization of perceptual memory: an fMRI study of picture priming. Hum Brain Mapp 2000;10:197–203.

Badgaiyan RD, Schacter DL, Alpert NM. Priming within and across modalities: exploring the nature of rCBF increases and decreases. Neuroimage 2001;13:272–282.

Bahro M, Molchan SE, Sunderland T, Herscovitch P, Schreurs BG. The effects of scopolamine on changes in regional cerebral blood flow during classical conditioning of the human eyeblink response. Neuropsychobiology. 1999;39(4):187-95.

Ballard C, Sauter M, Scheltens P, He Y, Barkhof F, van Straaten EC, van der Flier WM, Hsu C, Wu S, Lane R. Efficacy, safety and tolerability of rivastigmine capsules in patients with probable vascular dementia: the VantagE study. Curr Med Res Opin. 2008;24(9):2561-74.

Ballard C, O'Brien J, Gray A, Cormack F, Ayre G, Rowan E, Thompson P, Bucks R, McKeith I, Walker M, Tovee M. Attention and fluctuating attention in patients with dementia with Lewy bodies and Alzheimer disease. Arch Neurol. 2001;58(6):977-82. .

Baratti CM, Opezzo JW, Kopf SR. Facilitation of memory storage by the acetylcholine M2 muscarinic receptor antagonist AF-DX 116. Behav Neural Biol. 1993;60(1):69-74.

Bartus RT, Dean RL 3rd, Beer B, Lippa AS. The cholinergic hypothesis of geriatric memory dysfunction. Science. 1982;217(4558):408-14.

Bartus RT, Dean RL, Beer B. An evaluation of drugs for improving memory in aged monkeys: implications for clinical trials in humans. Psychopharmacol Bull. 1983;19(2):168-84.

Bartus RT. On neurodegenerative diseases, models, and treatment strategies: lessons learned and lessons forgotten a generation following the cholinergic hypothesis. Exp Neurol. 2000;163(2):495-529.

Baskerville KA, Schweitzer JB, Herron P. Effects of cholinergic depletion on experience-dependent plasticity in the cortex of the rat. Neuroscience. 1997;80(4):1159-69.

Baxter MG, Holland PC, Gallagher M. Disruption of decrements in conditioned stimulus processing by selective removal of hippocampal cholinergic input. J Neurosci. 1997;17(13):5230-6.

Baxter MG, Bucci DJ, Sobel TJ, Williams MJ, Gorman LK, Gallagher M. Intact spatial learning following lesions of basal forebrain cholinergic neurons. Neuroreport. 1996;7(8):1417-20.

Beatty WW, Butters N, Janowsky DS. Patterns of memory failure after scopolamine treatment: implications for cholinergic hypotheses of dementia. Behav Neural Biol. 1986;45(2):196-211.

Beauregard M, Chertkow H, Gold D, Bergman S. The impact of semantic impairment on word stem completion in Alzheimer's disease. Neuropsychologia. 2001;39:302-14.

Beglinger LJ, Tangphao-Daniels O, Kareken DA, Zhang L, Mohs R, Siemers ER. Neuropsychological test performance in healthy elderly volunteers before and after donepezil administration: a randomized, controlled study. J Clin Psychopharmacol. 2005;25(2):159-65.

Behl P, Bocti C, Swartz RH, Gao F, Sahlas DJ, Lanctot KL, Streiner DL, Black SE. Strategic subcortical hyperintensities in cholinergic pathways and executive function decline in treated Alzheimer patients. Arch Neurol. 2007;64(2):266-72.

Behrends JC, ten Bruggencate G. Cholinergic modulation of synaptic inhibition in the guinea pig hippocampus in vitro: excitation of GABAergic interneurons and inhibition of GABA-release. J Neurophysiol. 1993;69(2):626-9.

Bentley P, Vuilleumier P, Thiel CM, Driver J, Dolan RJ. Effects of attention and emotion on repetition priming and their modulation by cholinergic enhancement. J Neurophysiol. 2003;90(2):1171-81.

Bernstein LJ, Beig S, Siegenthaler AL, Grady CL. The effect of encoding strategy on the neural correlates of memory for faces. Neuropsychologia. 2002;40(1):86-98.

Berntson GG, Sarter M, Cacioppo JT. Anxiety and cardiovascular reactivity: the basal forebrain cholinergic link. Behav.Brain Res. 1998; 94, 225-248.

Berntson GG, Shafi R, Knox D, Sarter M. Blockade of epinephrine priming of the cerebral auditory evoked response by cortical cholinergic deafferentation. Neuroscience. 2003;116(1):179-86.

Bird M, Luszcz M. Encoding specificity, depth of processing, and cued recall in Alzheimer's disease. J Clin Exp Neuropsychol. 1991;13(4):508-20.

Birn RM, Saad ZS, Bandettini PA. Spatial heterogeneity of the nonlinear dynamics in the FMRI BOLD response. Neuroimage. 2001;14(4):817-26.

Bizzarro A, Marra C, Acciarri A, Valenza A, Tiziano FD, Brahe C, Masullo C. Apolipoprotein E epsilon4 allele differentiates the clinical response to donepezil in Alzheimer's disease. Dement Geriatr Cogn Disord. 2005;20(4):254-61

Blin J, Ivanoiu A, Coppens A, De Volder A, Labar D, Michel C, Laterre EC. Cholinergic neurotransmission has different effects on cerebral glucose consumption and blood flow in young normals, aged normals, and Alzheimer's disease patients. Neuroimage. 1997;6:335-43.

Blin J, Ivanoiu A, De Volder A, Michel C, Bol A, Verellen C, Seron X, Duprez T, Laterre EC. Physostigmine results in an increased decrement in brain glucose consumption in Alzheimer's disease. Psychopharmacology (Berl). 1998;136:256-63.

Blin J, Ray CA, Piercey MF, Bartko JJ, Mouradian MM, Chase TN. Comparison of cholinergic drug effects on regional brain glucose consumption in rats and humans by means of autoradiography and position emission tomography. Brain Res. 1994;635(1-2):196-202.

Bokde AL, Lopez-Bayo P, Meindl T, Pechler S, Born C, Faltraco F, Teipel SJ, Möller HJ, Hampel H. Functional connectivity of the fusiform gyrus during a face-matching task in subjects with mild cognitive impairment. Brain. 2006;129(Pt 5):1113-24.

Bond A, Lader M. The use of analogue scales in rating subjective feelings. B J Med Psychol. 1974;47:211–218.

Boroojerdi B, Battaglia F, Muellbacher W, Cohen LG. Mechanisms underlying rapid experience-dependent plasticity in the human visual cortex. Proc Natl Acad Sci U S A. 2001;98(25):14698-701

Botly LC, De Rosa E. Cholinergic deafferentation of the neocortex using 192 IgG-saporin impairs feature binding in rats. J Neurosci. 2009;29(13):4120-30.

Bowen DM, Benton JS, Spillane JA, Smith CC, Allen SJ. Choline acetyltransferase activity and histopathology of frontal neocortex from biopsies of demented patients. J Neurol Sci. 1982;57(2-3):191-202.

Bowen DM, Smith CB, White P, Davison AN. Neurotransmitter-related enzymes and indices of hypoxia in senile dementia and other abiotrophies. Brain. 1976;99:459-96.

Boynton GM, Engel SA, Glover GH, Heeger DJ. Linear systems analysis of functional magnetic resonance imaging in human V1. J Neurosci. 1996;16:4207-21.

Bozzali M, MacPherson SE, Dolan RJ, Shallice T. Left prefrontal cortex control of novel occurrences during recollection: a psychopharmacological study using scopolamine and event-related fMRI. Neuroimage. 2006;33(1):286-95.

Bradley KM, O'Sullivan VT, Soper ND, Nagy Z, King EM, Smith AD, Shepstone BJ. Brain. Cerebral perfusion SPET correlated with Braak pathological stage in Alzheimer's disease. 2002;125(Pt 8):1772-81.

Breiter HC, Etcoff NL, Whalen PJ, Kennedy WA, Rauch SL, Buckner RL, Strauss MM, Hyman SE, Rosen BR. Response and habituation of the human amygdala during visual processing of facial expression. Neuron. 1996;17(5):875-87.

Bröcher S, Artola A, Singer W. Agonists of cholinergic and noradrenergic receptors facilitate synergistically the induction of long-term potentiation in slices of rat visual cortex. Brain Res. 1992:573:27-36.

Broussard JI, Karelina K, Sarter M, Givens B. Cholinergic optimization of cue-evoked parietal activity during challenged attentional performance. Eur J Neurosci. 2009;29(8):1711-22.

Brown GG, Eyler Zorrilla LT, Georgy B, Kindermann SS, Wong EC, Buxton RB. BOLD and perfusion response to finger-thumb apposition after acetazolamide administration: differential relationship to global perfusion. J Cereb Blood Flow Metab. 2003;23(7):829-37.

Brown MW, Xiang JZ. Recognition memory: neuronal substrates of the judgement of prior occurrence. Prog Neurobiol 1998;55:149–189.

Bucci DJ, Holland PC, Gallagher M. Removal of cholinergic input to rat posterior parietal cortex disrupts incremental processing of conditioned stimuli. J Neurosci. 1998;18(19):8038-46.

Buchel C, Dolan RJ, Armony JL, Friston KJ. Amygdalahippocampal involvement in human aversive trace conditioning revealed through event-related functional magnetic resonance imaging. J Neurosci. 1999;19:10869–10876.

Büchel C, Holmes AP, Rees G, Friston KJ. Characterizing stimulus-response functions using nonlinear regressors in parametric fMRI experiments. Neuroimage. 1998;8(2):140-8.

Buckner RL. Memory and executive function in aging and AD: multiple factors that cause decline and reserve factors that compensate. Neuron. 2004;44(1):195-208.

Buckner RL, Goodman J, Burock M, Rotte M, Koutstaal W, Schacter D, Rosen B, Dale AM. Functional-anatomic correlates of object priming in humans revealed by rapid presentation event-related fMRI. Neuron 1998;20:285–296.

Bullmore E, Brammer M, Williams SC, Rabe-Hesketh S, Janot N, David A, Mellers J, Howard R, Sham P. Statistical methods of estimation and inference for functional MR image analysis. Magn Reson Med. 1996;35(2):261-77.

Bullmore E, Suckling J, Zelaya F, Long C, Honey G, Reed L, Routledge C, Ng V, Fletcher P, Brown J, Williams SC. Practice and difficulty evoke anatomically and pharmacologically dissociable brain activation dynamics. Cereb Cortex. 2003;13(2):144-54.

Bullock R, Dengiz A. Cognitive performance in patients with Alzheimer's disease receiving cholinesterase inhibitors for up to 5 years. Int J Clin Pract. 2005;59(7):817-22.

Buresová O, Bolhuis JJ, Bures J. Differential effects of cholinergic blockade on performance of rats in the water tank navigation task and in a radial water maze. Behav Neurosci. 1986;100(4):476-82.

Burke M, Bührle Ch. BOLD response during uncoupling of neuronal activity and CBF. Neuroimage. 2006;32(1):1-8.

Burock MA, Buckner RL, Woldorff MG, Rosen BR, Dale AM. Randomized event-related experimental designs allow for extremely rapid presentation rates using functional MRI. Neuroreport. 1998;9(16):3735-9.

Bushnell PJ, Chiba AA, Oshiro WM. Effects of unilateral removal of basal forebrain cholinergic neurons on cued target detection in rats. Behav Brain Res. 1998;90:57-71.

Buzsaki G, Bickford RG, Ponomare G, Thal LJ, Mandel RJ, Gage FH. Nucleus basalis and thalamic control of neocortical activity in the freely moving rat. J Neurosci. 1988;8:4007-26.

Calabria M, Geroldi C, Lussignoli G, Sabbatini F, Zanetti O. Efficacy of acetyl-cholinesterase-inhibitor (ACHEI) treatment in Alzheimer's disease: a 21-month follow-up "real world" study. Arch Gerontol Geriatr. 2009;49(1):e6-11.

Carey RG, Rieck RW. Topographic projections to the visual cortex from the basal forebrain in the rat. Brain Res. 1987;424(2):205-15.

Casamenti F, Deffenu G, Abbamondi L, Pepeu G. Changes in cortical acetylcholine output induced by modulation of the nucleus basalis, Brain Res. Bull. 1986.16:689–695.

Casu MA, Wong TP, De Koninck Y, Ribeiro-da-Silva A, Cuello AC. Aging causes a preferential loss of cholinergic innervation of characterized neocortical pyramidal neurons. Cereb Cortex. 2002;12(3):329-37.

Catsman-Berrevoets CE, Van Harskamp F, Appelhof A. Beneficial effect of physostigmine on clinical amnesic behaviour and neuropsychological test results in a patient with a post-encephalitic amnesic syndrome. J Neurol Neurosurg Psychiatry. 1986;49(9):1088-90.

Cavada C, Compañy T, Tejedor J, Cruz-Rizzolo RJ, Reinoso-Suárez F. The anatomical connections of the macaque monkey orbitofrontal cortex. A review. Cereb Cortex. 2000;10(3):220-42.

Celone KA, Calhoun VD, Dickerson BC, Atri A, Chua EF, Miller SL, DePeau K, Rentz DM, Selkoe DJ, Blacker D, Albert MS, Sperling RA. Alterations in memory networks in mild cognitive impairment and Alzheimer's disease: an independent component analysis. J Neurosci. 2006;26(40):10222-31.

Chan D, Janssen JC, Whitwell JL, Watt HC, Jenkins R, Frost C, Rossor MN, Fox NC. Change in rates of cerebral atrophy over time in early-onset Alzheimer's disease: longitudinal MRI study. Lancet. 2003;362(9390):1121-2.

Chappell J, McMahan R, Chiba A, Gallagher M. A re-examination of the role of basal forebrain cholinergic neurons in spatial working memory. Neuropharmacology. 1998;37(4-5):481-7.

Chawla D, Rees G, Friston KJ. The physiological basis of attentional modulation in extrastriate visual areas. Nat Neurosci. 1999;2(7):671-6.

Chédotal A, Umbriaco D, Descarries L, Hartman BK, Hamel E. Light and electron microscopic immunocytochemical analysis of the neurovascular relationships of choline acetyltransferase and vasoactive intestical polypeptide nerve terminals in the rat cerebral cortex. J. Comp. Neurol. 1994;343:57–71.

Chelazzi L, Miller EK, Duncan J, Desimone R. Responses of neurons in macaque area V4 during memory-guided visual search. Cereb Cortex. 2001;11(8):761-72.

Chiba AA, Bucci DJ, Holland PC, Gallagher M. Basal forebrain cholinergic lesions disrupt increments but not decrements in conditioned stimulus processing. J Neurosci. 1995;15(11):7315-22.

Chiba AA, Bushnell PJ, Oshiro WM, Gallagher M. Selective removal of cholinergic neurons in the basal forebrain alters cued target detection. Neuroreport. 1999;10(14):3119-23.

Christie JE, Shering A, Ferguson J, Glen AI. Physostigmine and arecoline: effects of intravenous infusions in Alzheimer presenile dementia.Br J Psychiatry. 1981;138:46-50.

Chuah LY, Chee MW. Cholinergic augmentation modulates visual task performance

in sleep-deprived young adults. J Neurosci. 2008;28(44):11369-77.

Chun MM, Turk-Browne NB. Interactions between attention and memory. Curr Opin Neurobiol. 2007;17(2):177-84.

Cohen ER, Ugurbil K, Kim SG. Effect of basal conditions on the magnitude and dynamics of the blood oxygenation level-dependent fMRI response. J Cereb Blood Flow Metab. 2002;22(9):1042-53.

Cohen RM, Gross M, Semple WE, Nordahl TE, Sunderland T. The metabolic brain pattern of young subjects given scopolamine. Exp Brain Res. 1994;100(1):133-43.

Collier B, Mitchell, J.F. The central release of acetylcholine during stimulation of the visual pathway, J. Physiol. 1966:184:239–254.

Conner JM, Chiba AA, Tuszynski MH. The basal forebrain cholinergic system is essential for cortical plasticity and functional recovery following brain injury. Neuron. 2005;46(2):173-9.

Conner JM, Culberson A, Packowski C, Chiba AA, Tuszynski MH. Lesions of the basal forebrain cholinergic system impair task acquisition and abolish cortical plasticity associated with motor skill learning. Neuron. 2003;38(5):819-29.

Courtney SM, Ungerleider LG, Keil K, Haxby JV. Transient and sustained activity in a distributed neural system for human working memory. Nature. 1997;386:608-11.

Craig MC, Fletcher PC, Daly EM, Rymer J, Brammer M, Giampietro V, Stahl D, Maki PM, Murphy DG. The interactive effect of the cholinergic system and acute ovarian suppression on the brain: an fMRI study. Horm Behav. 2009;55(1):41-9.

Craik FIM, Tulving E. Depth of processing and the retention of words in episodic memory. J Exp Psych 1975;104:268–94.

Crow TJ, Grove-White IG. An analysis of the learning deficit following hyoscine administration to man. Br J Pharmacol. 1973;49(2):322-7.

Dale A, Buckner R. Selective averaging of rapidly presented individual trials using fMRI. Hum. Brain Mapping. 1997;5:329–340.

Dale AM, Liu AK, Fischl BR, Buckner RL, Belliveau JW, Lewine JD, Halgren E. Dynamic statistical parametric mapping: combining fMRI and MEG for high-resolution imaging of cortical activity. Neuron 2000;26: 55–67.

Dalla Barba, 1997 G. Dalla Barba, Recognition memory and recollective experience in Alzheimer's disease. Memory 1997;5 (6): 657–672.

Dalley JW, McGaughy J, O'Connell MT, Cardinal RN, Levita L, Robbins TW. Distinct changes in cortical acetylcholine and noradrenaline efflux during contingent and noncontingent performance of a visual attentional task. J Neurosci. 2001;21(13):4908-14.

Dannhauser TM, Walker Z, Stevens T, Lee L, Seal M, Shergill SS. The functional anatomy of divided attention in amnestic mild cognitive impairment. Brain. 2005;128(Pt 6):1418-27.

Davidson MC, Marrocco RT. Local infusion of scopolamine into intraparietal cortex slows covert orienting in rhesus monkeys. J Neurophysiol. 2000;83(3):1536-49.

Davies P, Maloney AJ. Selective loss of central cholinergic neurons in Alzheimer's disease. Lancet. 1976;2(8000):1403.

Davis KL, Hollister LE, Overall J, Johnson A, Train K. Physostigmine: effects on cognition and affect in normal subjects. Psychopharmacology (Berl). 1976;51(1):23-7.

Davis KL, Mohs RC, Marin D, Purohit DP, Perl DP, Lantz M, Austin G, Haroutunian V. Cholinergic markers in elderly patients with early signs of Alzheimer's disease. J. Am. Med. Assoc. 1999:281: 1401–1406.

Davis KL, Mohs RC, Tinklenberg JR, Pfefferbaum A, Hollister LE, Kopell BS. Science. Physostigmine: improvement of long-term memory processes in normal humans. 1978;201(4352):272-4.

Davis KL, Mohs RC, Tinklenberg JR. Enhancement of memory by physostigmine. N Engl J Med. 1979;301(17):946.

Davis KL, Mohs RC. Enhancement of memory processes in Alzheimer's disease with multiple-dose intravenous physostigmine. Am J Psychiatry. 1982;139(11):1421-4.

Davis TL, Kwong KK, Weisskoff RM, Rosen BR. Calibrated functional MRI: Mapping the dynamics of oxidative metabolism. Proc Natl Acad Sci USA. 1998;95:1834–1839.

DeKosky, ST, Ikonomovic MD, Styren SD, Beckett L, Wisniewski S, Bennett DA, Cochran EJ, Kordower JH, and Mufson EJ. Upregulation of choline acetyltransferase activity in hippocampus and frontal cortex of elderly subjects with mild cognitive impairment. Ann. Neurol. 2002;51:145–155.

Delacour J, Houcine O, Costa JC. Evidence for a cholinergic mechanism of "learned" changes in the responses of barrel field neurons of the awake and undrugged rat. Neuroscience. 1990;34(1):1-8.

Descarries L, Gisiger V, Steriade M. Diffuse transmission by acetylcholine in the CNS. Prog Neurobiol 1997;53:603–625.

Desimone R. Neural mechanisms for visual memory and their role in attention. Proc Natl Acad Sci USA 1996;93:13494–13499.

D'Esposito M, Zarahn E, Aguirre GK, Rypma B. The effect of normal aging on the coupling of neural activity to the bold hemodynamic response. Neuroimage. 1999;10(1):6-14.

Détári L, Rasmusson DD, Semba K. The role of basal forebrain neurons in tonic and phasic activation of the cerebral cortex. Prog Neurobiol. 1999;58(3):249-77.

Dewey SL, Smith GS, Logan J, Brodie JD, Simkowitz P, MacGregor RR, Fowler JS, Volkow ND, Wolf AP. Effects of central cholinergic blockade on striatal dopamine release measured with positron emission tomography in normal human subjects. Proc Natl Acad Sci U S A. 1993;90(24):11816-20.

DeYoe EA, Bandettini P, Neitz J, Miller D, Winans P. Functional magnetic resonance imaging (FMRI) of the human brain. J Neurosci Methods. 1994;54(2):171-87.

Dichgans M, Markus HS, Salloway S, Verkkoniemi A, Moline M, Wang Q, Posner H, Chabriat HS. Donepezil in patients with subcortical vascular cognitive impairment: a randomised double-blind trial in CADASIL. Lancet Neurol. 2008 ;7(4):310-8.

Dickerson BC. Functional magnetic resonance imaging of cholinergic modulation in mild cognitive impairment. Curr Opin Psychiatry. 2006;19(3):299-306.

Dickerson BC, Miller SL, Greve DN, Dale AM, Albert MS, Schacter DL, Sperling RA. Prefrontal-hippocampal-fusiform activity during encoding predicts intraindividual differences in free recall ability: an event-related functional-anatomic MRI study. Hippocampus. 2007;17(11):1060-70.

Diniz BS, Pinto JA Jr, Gonzaga ML, Guimarães FM, Gattaz WF, Forlenza OV. To treat or not to treat? A meta-analysis of the use of cholinesterase inhibitors in mild cognitive impairment for delaying progression to Alzheimer's disease. Eur Arch Psychiatry Clin Neurosci. 2009;259(4):248-56.

Disney AA, Domakonda KV, Aoki C. Differential expression of muscarinic acetylcholine receptors across excitatory and inhibitory cells in visual cortical areas V1 and V2 of the macaque monkey. J Comp Neurol. 2006;499(1):49-63.

Dotigny F, Ben Amor AY, Burke M, Vaucher E. Neuromodulatory role of acetylcholine in visually-induced cortical activation: behavioral and neuroanatomical correlates. Neuroscience. 2008;154(4):1607-18.

Drachman DA, Leavitt J. Human memory and the cholinergic system. A relationship to aging? Arch Neurol. 1974;30(2):113-21.

Drachman DA, Sahakian BJ. Memory and cognitive function in the elderly. A preliminary trial of physostigmine. Arch Neurol. 1980;37(10):674-5.

Drake CT, Iadecola C. The role of neuronal signaling in controlling cerebral blood flow. Brain Lang. 2007;102(2):141-52.

Dumas JA, Saykin AJ, McDonald BC, McAllister TW, Hynes ML, Newhouse PA. Nicotinic versus muscarinic blockade alters verbal working memory-related brain activity in older women. Am J Geriatr Psychiatry. 2008;16(4):272-82.

Easwaramoorthy B, Pichika R, Collins D, Potkin SG, Leslie FM, Mukherjee J. Effect of acetylcholinesterase inhibitors on the binding of nicotinic alpha4beta2 receptor PET radiotracer, (18)F-nifene: A measure of acetylcholine competition. Synapse. 2007;61(1):29-36.

Ebmeier KP, Hunter R, Curran SM, Dougal NJ, Murray CL, Wyper DJ, Patterson J, Hanson MT, Siegfried K, Goodwin GM. Effects of a single dose of the acetylcholinesterase inhibitor velnacrine on recognition memory and regional cerebral blood flow in Alzheimer's disease. Psychopharmacology (Berl). 1992;108(1-2):103-9.

Edeline JM. Learning-induced physiological plasticity in the thalamo-cortical sensory systems: a critical evaluation of receptive field plasticity, map changes and their potential mechanisms. Prog Neurobiol. 1999;57(2):165-224.

Edginton T, Rusted JM. Separate and combined effects of scopolamine and nicotine on retrieval-induced forgetting. Psychopharmacology (Berl). 2003;170(4):351-7.

Edvinsson L, MacKenzie ET, McCulloch J, Uddman R. Perivascular innervation and receptor mechanisms in cerebrovascular bed. In: Wood, J.H., Editor. Cerebral Blood Flow. Physiologic and Clinical Aspects, McGraw-Hill, New York. 1987:145–172.

Egorov AV, Hamam BN, Fransén E, Hasselmo ME, Alonso AA. Graded persistent activity in entorhinal cortex neurons. Nature. 2002;420(6912):173-8.

Ekstrom A. How and when the fMRI BOLD signal relates to underlying neural activity: the danger in dissociation. Brain Res Rev. 2010;62(2):233-44.

Ellenbogen JM, Payne JD, Stickgold R. The role of sleep in declarative memory consolidation: passive, permissive, active or none? Curr Opin Neurobiol. 2006;16(6):716-22.

Elliott R, Dolan RJ, Frith CD. Dissociable functions in the medial and lateral orbitofrontal cortex: evidence from human neuroimaging studies. Cereb Cortex. 2000;10(3):308-17.

Ernst M, Matochik JA, Heishman SJ, Van Horn JD, Jons PH, Henningfield JE, London ED. Effect of nicotine on brain activation during performance of a working memory task. Proc Natl Acad Sci U S A. 2001a;98(8):4728-33.

Ernst M, Heishman SJ, Spurgeon L, London ED. Smoking history and nicotine effects on cognitive performance. Neuropsychopharmacology. 2001b;25(3):313-9.

Erskine FF, Ellis JR, Ellis KA, Stuber E, Hogan K, Miller V, Moore E, Bartholomeusz C, Harrison BJ, Lee B, Phan KL, Liley D, Nathan PJ. Evidence for synergistic modulation of early information processing by nicotinic and muscarinic receptors in humans. Hum Psychopharmacol. 2004;19(7):503-9.

Esterman M, Verstynen T, Robertson LC. Attenuating illusory binding with TMS of the right parietal cortex. Neuroimage. 2007;35(3):1247-55.

Ettinger U, Williams SC, Patel D, Michel TM, Nwaigwe A, Caceres A, Mehta MA, Anilkumar AP, Kumari V. Effects of acute nicotine on brain function in healthy smokers and non-smokers: estimation of inter-individual response heterogeneity. Neuroimage. 2009;45(2):549-61.

Everitt BJ, Robbins TW. Central cholinergic systems and cognition. Annu Rev Psychol. 1997;48:649-84.

Fine A, Hoyle C, Maclean CJ, Levatte TL, Baker HF, Ridley RM. Learning impairments following injection of a selective cholinergic immunotoxin, ME20.4 IgG-saporin, into the basal nucleus of Meynert in monkeys. Neuroscience. 1997;81(2):331-43.

FitzGerald DB, Crucian GP, Mielke JB, Shenal BV, Burks D, Womack KB, Ghacibeh G, Drago V, Foster PS, Valenstein E, Heilman KM. Effects of donepezil on verbal memory after semantic processing in healthy older adults. Cogn Behav Neurol. 2008;21(2):57-64.

Forster C, Ringler R, Handwerker HO. Regional brain oxygenation during phasic and tonic painful stimulation. Prog Brain Res. 2000;129:303-11.

Fransen E, Alonso AA, Hasselmo ME. Simulations of the role of the muscarinic-activated calcium-sensitive nonspecific cation current INCM in entorhinal neuronal activity during delayed matching tasks. J Neurosci 2002;22:1081–1097.

Freo U, Ricciardi E, Pietrini P, Schapiro MB, Rapoport SI, Furey ML. Pharmacological modulation of prefrontal cortical activity during a working memory task in young and older humans: a PET study with physostigmine. Am J Psychiatry. 2005;162(11):2061-70.

Fries P, Reynolds JH, Rorie AE, Desimone R. Modulation of oscillatory neuronal synchronization by selective visual attention. Science. 2001;291(5508):1560-3.

Friston KJ, Ashburner J, Frith CD, Poline JB, Heather JD, Frackowiak RSJ. Spatial registration and normalization of images. Human Brain Mapping, 1995a;2:165-189.

Friston K, Holmes A, Poline J-B, Grasby P, Williams S, Frackowiak R, Turner R. Analysis of fMRI time series revisited. Neuroimage. 1995b;2: 45–53.

Friston KJ, Holmes AP, Worsley KJ, Poline JB, Frith CD, Frackowiak RSJ. Statistical parametric maps in functional imaging: A general linear approach. Hum. Brain Mapp. 1995c; 2: 189–210.

Friston KJ, Buechel C, Fink GR, Morris J, Rolls E, Dolan RJ. Psychophysiological and modulatory interactions in neuroimaging. Neuroimage. 1997;6:218-229.

Friston KJ, Fletcher P, Josephs O, Holmes A, Rugg MD, Turner R. Event-related fMRI: Characterizing differential responses, Neuroimage 1998; 1: 30–40.

Friston KJ, Tononi G, Reeke GN Jr, Sporns O, Edelman GM. Value-dependent selection in the brain: simulation in a synthetic neural model. Neuroscience. 1994;59(2):229-43.

Froemke RC, Merzenich MM, Schreiner CE. A synaptic memory trace for cortical receptive field plasticity. Nature. 2007;450(7168):425-9.

Fukuyama H, Ouchi Y, Matsuzaki S, Ogawa M, Yamauchi H, Nagahama Y, Kimura J, Yonekura Y, Shibasaki H, Tsukada H. Focal cortical blood flow activation is regulated by intrinsic cortical cholinergic neurons. Neuroimage. 1996;3(3 Pt 1):195-201.

Furey ML, Pietrini P, Haxby JV, Alexander GE, Lee HC, VanMeter J, Grady CL, Shetty U, Rapoport SI, Schapiro MB, Freo U. Cholinergic stimulation alters performance and task-specific regional cerebral blood flow during working memory. Proc Natl Acad Sci U S A. 1997;94(12):6512-6.

Furey ML, Pietrini P, Haxby JV. Cholinergic enhancement and increased selectivity of perceptual processing during working memory. Science. 2000a;290(5500):2315-9.

Furey ML, Pietrini P, Alexander GE, Mentis MJ, Szczepanik J, Shetty U, Greig NH, Holloway HW, Schapiro MB, Freo U. Time course of pharmacodynamic and pharmacokinetic effects of physostigmine assessed by functional brain imaging in humans. Pharmacol Biochem Behav. 2000b;66(3):475-81.

Furey ML, Pietrini P, Alexander GE, Schapiro MB, Horwitz B. Cholinergic enhancement improves performance on working memory by modulating the functional activity in distinct brain regions: a positron emission tomography regional cerebral blood flow study in healthy humans. Brain Res Bull. 2000c;51(3):213-8.

Furey ML, Ricciardi E, Schapiro MB, Rapoport SI, Pietrini P. Cholinergic enhancement eliminates modulation of neural activity by task difficulty in the prefrontal cortex during working memory. J Cogn Neurosci. 2008a;20(7):1342-53.

Furey ML, Pietrini P, Haxby JV, Drevets WC. Selective effects of cholinergic modulation on task performance during selective attention. Neuropsychopharmacology. 2008b;33(4):913-23.

Gais S, Born J. Low acetylcholine during slow-wave sleep is critical for declarative memory consolidation. Proc Natl Acad Sci U S A. 2004;101(7):2140-4.

Gazzaley A, D'Esposito M. Top-down modulation and normal aging. Ann N Y Acad Sci. 2007;1097:67-83.

Geaney DP, Soper N, Shepstone BJ, Cowen PJ. Effect of central cholinergic stimulation on regional cerebral blood flow in Alzheimer disease. Lancet. 1990;335(8704):1484-7.

Genovese CR, Lazar NA, Nichols T. Thresholding of statistical maps in functional neuroimaging using the false discovery rate. Neuroimage. 2002;15(4):870-8.

George N, Dolan RJ, Fink GR, Baylis GC, Russell C, Driver J. Contrast polarity and face recognition in the human fusiform gyrus. Nat Neurosci. 1999;2(6):574-80.

Geula C, Mesulam MM Cholinergic systems in Alzheimer's disease. In Alzheimer disease 2nd Ed. (eds. R.D. Terry et al.), 1999:69–292. Lippincott, Williams & Wilkins, Philadelphia, PA.

Geula C, Mesulam MM. Cortical cholinergic fibers in aging and Alzheimer's disease: a morphometric study. Neuroscience. 1989;33(3):469-81.

Geula C, Mesulam MM. Systematic regional variations in the loss of cortical cholinergic fibers in Alzheimer's disease. Cerebral Cortex 1996. 6: 165–177.

Ghatan PH, Ingvar M, Eriksson L, Stone-Elander S, Serrander M, Ekberg K, Wahren J. Cerebral effects of nicotine during cognition in smokers and non-smokers. Psychopharmacology (Berl). 1998;136(2):179-89.

Ghoneim MM, Mewaldt SP. Studies on human memory: the interactions of diazepam, scopolamine, and physostigmine. Psychopharmacology (Berl). 1977;52(1):1-6.

Giacobini E. Cholinesterase inhibitors: from the Calabar bean to Alzheimer therapy. In Cholinesterases and Cholinesterase Inhibitors. Ed. Giacobini E. 2000. Martin Dunitz.

Giarman Nj, Pepeu G. Drug-induced changes in brain acetylcholine. Br J Pharmacol Chemother. 1962;19:226-34.

Giessing C, Thiel CM, Rösler F, Fink GR. The modulatory effects of nicotine on parietal cortex activity in a cued target detection task depend on cue reliability. Neuroscience. 2006;137(3):853-64.

Giessing C, Fink GR, Rösler F, Thiel CM. fMRI data predict individual differences of behavioral effects of nicotine: a partial least square analysis. J Cogn Neurosci. 2007;19:658-70.

Gil Z, Connors BW, Amitai Y. Differential regulation of neocortical synapses by neuromodulators and activity. Neuron. 1997;19(3):679-86.

Gill TM, Sarter M, Givens B. Sustained visual attention performance-associated prefrontal neuronal activity: evidence for cholinergic modulation. J Neurosci. 2000;20(12):4745-57.

Giovannini MG, Rakovska A, Benton RS, Pazzagli M, Bianchi L, Pepeu G. Effects of novelty and habituation on acetylcholine, GABA, and glutamate release from the frontal cortex and hippocampus of freely moving rats. Neuroscience.2001;106:43-53.

Gitelman DR, Prohovnik I. Muscarinic and nicotinic contributions to cognitive function and cortical blood flow. Neurobiol Aging. 1992;13(2):313-8.

Giuliano C, Parikh V, Ward JR, Chiamulera C, Sarter M. Increases in cholinergic neurotransmission measured by using choline-sensitive microelectrodes: enhanced detection by hydrolysis of acetylcholine on recording sites?. Neurochem Int. 2008;52(7):1343-50.

Glaser D, Friston K. Variance Components. In Human Brain Function. 2004. (2nd Edition). Eds: Frackowiak RSJ, Friston K, Frith C, Dolan R, Price C, Zeki S, Ashburner J, Penney W.

Glaser D, Penny W, Henson R, Friston K. Coping with the variance structure of neuroimaging data (Abstract). Proc Cogn Neurosci Soc 141:E92, 2002.

Goekoop R, Rombouts SARB, Jonker C, Hibbel A, Knol DL, Truyen L, Barkhof F, Scheltens P. Challenging the cholinergic system in mild cognitive impairment: a pharmacological fMRI study. Neuroimage. 2004;23:1450-1459.

Goense JB, Logothetis NK. Neurophysiology of the BOLD fMRI signal in awake monkeys. Curr Biol. 2008;18(9):631-40.

Golby A, Silverberg G, Race E, Gabrieli S, O'Shea J, Knierim K, Stebbins G, Gabrieli J. Memory encoding in Alzheimer's disease: an fMRI study of explicit and implicit memory. Brain. 2005;128(Pt 4):773-87.

Golby AJ, Gabrieli JD, Chiao JY, Eberhardt JL. Differential responses in the fusiform region to same-race and other-race faces. Nat Neurosci. 2001;4(8):845-50.

Gollub RL, Breiter HC, Kantor H, Kennedy D, Gastfriend D, Mathew RT, Makris N, Guimaraes A, Riorden J, Campbell T, Foley M, Hyman SE, Rosen B, Weisskoff R. Cocaine decreases cortical cerebral blood flow but does not obscure regional activation in functional magnetic resonance imaging in human subjects. J Cereb Blood Flow Metab. 1998;18:724-34.

Golmayo L, Nunez A, Zaborszky L. Electrophysiological evidence for the existence of a posterior cortical-prefrontal-basal forebrain circuitry in modulating sensory responses in visual and somatosensory rat cortical areas. 2003. Neuroscience 119:597–609.

Grady CL, Furey ML, Pietrini P, Horwitz B, Rapoport SI. Altered brain functional connectivity and impaired short-term memory in Alzheimer's disease. Brain. 2001;124(Pt 4):739-56.

Grady CL, Horwitz B, Pietrini P, Mentis MJ, Ungerleider LG, Rapoport SI, Haxby JV: Effect of task difficulty on cerebral blood flow during perceptual matching of faces. Hum Brain Mapp 1996; 4:227–239.

Grady CL, McIntosh AR, Rajah MN, Craik FI. Neural correlates of the episodic encoding of pictures and words. Proc Natl Acad Sci U S A. 1998;95(5):2703-8.

Grasby PM, Frith CD, Paulesu E, Friston KJ, Frackowiak RS, Dolan RJ. The effect of the muscarinic antagonist scopolamine on regional cerebral blood flow during the performance of a memory task. Exp Brain Res. 1995;104(2):337-48.

Gray JR, Braver TS, Raichle ME. Integration of emotion and cognition in the lateral prefrontal cortex. Proc Natl Acad Sci U S A. 2002;99(6):4115-20.

Green A, Ellis KA, Ellis J, Bartholomeusz CF, Ilic S, Croft RJ, Phan KL, Nathan PJ. Muscarinic and nicotinic receptor modulation of object and spatial n-back working memory in humans. Pharmacol Biochem Behav. 2005;81(3):575-84.

Greicius MD, Srivastava G, Reiss AL, Menon V. Default-mode network activity distinguishes Alzheimer's disease from healthy aging: evidence from functional MRI. Proc Natl Acad Sci U S A. 2004;101(13):4637-42.

Griffin AL, Asaka Y, Darling RD, Berry SD. Theta-contingent trial presentation accelerates learning rate and enhances hippocampal plasticity during trace eyeblink conditioning. Behav Neurosci. 2004;118(2):403-11.

Grön G, Brandenburg I, Wunderlich AP, Riepe MW. Inhibition of hippocampal function in mild cognitive impairment: targeting the cholinergic hypothesis. Neurobiol Aging. 2006;27(1):78-87.

Grön G, Kirstein M, Thielscher A, Riepe MW, Spitzer M. Cholinergic enhancement of episodic memory in healthy young adults. Psychopharmacology (Berl). 2005;182(1):170-9.

Grön G, Riepe MW. Neural basis for the cognitive continuum in episodic memory from health to Alzheimer disease. Am J Geriatr Psychiatry. 2004;12(6):648-52.

Gruber-Baldini AL, Stuart B, Zuckerman IH, Simoni-Wastila L, Miller R. Treatment of dementia in community-dwelling and institutionalized medicare beneficiaries. J Am Geriatr Soc. 2007;55(10):1508-16.

Gu Q. Contribution of acetylcholine to visual cortex plasticity. Neurobiol Learn Mem. 2003;80(3):291-301.

Gu, Q. Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity. Neuroscience. 2002;111: 815–835.

Gulledge AT, Park SB, Kawaguchi Y, Stuart GJ. Heterogeneity of phasic cholinergic signaling in neocortical neurons. J Neurophysiol. 2007;97(3):2215-29.

Gustafson L, Edvinsson L, Dahlgren N, Hagberg B, Risberg J, Rosén I, Fernö H. Intravenous physostigmine treatment of Alzheimer's disease evaluated by psychometric testing, regional cerebral blood flow (rCBF) measurement, and EEG. Psychopharmacology (Berl). 1987;93(1):31-5.

Haj-Dahmane S, Andrade R. Muscarinic activation of a voltage-dependent cation nonselective current in rat association cortex. J Neurosci. 1996 ;16(12):3848-61.

Hallstrom A, Sato A, Sato Y, Ungerstedt U. Effect of stimulation of the nucleus basalis of Meynert on blood flow and extracellular lactate in the cerebral cortex with special reference to the effect of noxious stimulation of skin and hypoxia. Neurosci. Lett. 1990;116:227–232.

Hahn B, Ross TJ, Wolkenberg FA, Shakleya DM, Huestis MA, Stein EA. Performance effects of nicotine during selective attention, divided attention, and simple stimulus detection: an fMRI study. Cereb Cortex. 2009;19(9):1990-2000.

Hahn B, Ross TJ, Yang Y, Kim I, Huestis MA, Stein EA. Nicotine enhances visuospatial attention by deactivating areas of the resting brain default network. J Neurosci. 2007;27(13):3477-89.

Hamel E, Robert JP, Young AR, MacKenzie ET. Pharmacological properties of the receptor(s) involved in the 5-hydroxytryptamine-induced contraction of the feline middle cerebral artery. J Pharmacol Exp Ther. 1989;249(3):879-89.

Hao J, Li K, Li K, Zhang D, Wang W, Yang Y, Yan B, Shan B, Zhou X. Visual attention deficits in Alzheimer's disease: an fMRI study. Neurosci Lett. 2005;385(1):18-23.

Harding TH, Wiley RW, Kirby AW. A cholinergic-sensitive channel in the cat visual system tuned to low spatial frequencies. Science. 1983;221(4615):1076-8.

Harold D, Macgregor S, Patterson CE, Hollingworth P, Moore P, Owen MJ, Williams J, O'Donovan M, Passmore P, McIlroy S, Jones L. A single nucleotide polymorphism in CHAT influences response to acetylcholinesterase inhibitors in Alzheimer's disease. Pharmacogenet Genomics. 2006;16(2):75-7.

Hars B, Maho C, Edeline JM, Hennevin E. Basal forebrain stimulation facilitates tone-evoked responses in the auditory cortex of awake rat. Neuroscience. 1993;56, 61-74.

Hart S, Sarter M, Berntson GG. Cholinergic inputs to the rat medial prefrontal cortex mediate potentiation of the cardiovascular defensive response by the anxiogenic benzodiazephine receptor partial inverse agonist FG 7142. Neuroscience. 1999;94(4):1029-38.

Hasenfratz M, Bättig K. Action profiles of smoking and caffeine: Stroop effect, EEG, and peripheral physiology. Pharmacol Biochem Behav. 1992;42(1):155-61.

Hasselmo ME, Bower JM. Cholinergic suppression specific to intrinsic not afferent fiber synapses in rat piriform (olfactory) cortex. J. Neurophysiol. 1992.67, 1222–1229.

Hasselmo ME, Cekic M. Suppression of synaptic transmission may allow combination of associative feedback and self-organizing feedforward connections in the neocortex. Behav Brain Res. 1996;79(1-2):153-61.

Hasselmo ME, Fehlau BP. Differences in time course of ACh and GABA modulation of excitatory synaptic potentials in slices of rat hippocampus. J Neurophysiol. 2001;86(4):1792-802.

Hasselmo ME, Giocomo LM. Cholinergic modulation of cortical function. J Mol Neurosci. 2006;30(1-2):133-6.

Hasselmo ME, McGaughy J. High acetylcholine levels set circuit dynamics for attention and encoding and low acetylcholine levels set dynamics for consolidation. Prog Brain Res. 2004;145:207-31.

Hasselmo ME. Neuromodulation and cortical function: modeling the physiological basis of behavior. Behav Brain Res. 1995;67(1):1-27.

Hasselmo ME. The role of acetylcholine in learning and memory. Curr Opin Neurobiol. 2006;16(6):710-5.

Haubrich DR, Chippendale TJ. Regulation of acetylcholine synthesis in nervous tissue. Life Sci. 1977;20(9):1465-78.

Haxby JV, Hoffman EA, Gobbini MI. The distributed human neural system for face perception. Trends Cogn Sci. 2000;4(6):223-233.

Heishman SJ, Taylor RC, Henningfield JE. Nicotine References and smoking: a review of effects on human performance. Exp.Clin. Psychopharmacol. 1994;2:345–395.

Henson RN, Rugg MD. Neural response suppression, haemodynamic repetition effects, and behavioural priming. Neuropsychologia. 2003;41(3):263-70.

Henson R, Shallice T, Gorno-Tempini ML, Dolan R. Face repetition effects in implicit and explicit memory tests as measured by fMRI. Cereb Cortex 2002;12:178–186.

Hernandez CM, Terry AV Jr. Repeated nicotine exposure in rats: effects on memory function, cholinergic markers and nerve growth factor. Neuroscience. 2005;130(4):997-1012.

Herrero JL, Roberts MJ, Delicato LS, Gieselmann MA, Dayan P, Thiele A. Acetylcholine contributes through muscarinic receptors to attentional modulation in V1. Nature. 2008;454(7208):1110-4.

Himmelheber AM, Sarter M, Bruno JP. Increases in cortical acetylcholine release during sustained attention performance in rats. Brain Res Cogn Brain Res. 2000;9(3):313-25.

Himmelheber AM, Sarter M, Bruno JP. The effects of manipulations of attentional demand on cortical acetylcholine release. Brain Res Cogn Brain Res. 2001;12(3):353-70.

Holland PC, Gallagher M. Amygdala central nucleus lesions disrupt increments, but not decrements, in conditioned stimulus processing. Behav Neurosci. 1993;107(2):246-53.

Holland PC, Gallagher M. Amygdala circuitry in attentional and representational processes. Trends Cogn Sci. 1999;3(2):65-73.

Holley LA, Turchi J, Apple C, Sarter M. Dissociation between the attentional effects of infusions of a benzodiazepine receptor agonist and an inverse agonist into the basal forebrain. Psychopharmacology (Berl). 1995;120(1):99-108.

Homma A, Imai Y, Tago H, Asada T, Shigeta M, Iwamoto T, Takita M, Arimoto I, Koma H, Takase T, Ohbayashi T. Long-term safety and efficacy of donepezil in patients with severe Alzheimer's disease: results from a 52-week, open-label, multicenter, extension study in Japan. Dement Geriatr Cogn Disord. 2009;27(3):232-9.

Honey GD, Bullmore ET, Sharma T. Prolonged reaction time to a verbal working memory task predicts increased power of posterior parietal cortical activation. Neuroimage. 2000;12(5):495-503.

Honey GD, Suckling J, Zelaya F, Long C, Routledge C, Jackson S, Ng V, Fletcher PC, Williams SC, Brown J, Bullmore ET. Dopaminergic drug effects on physiological connectivity in a human cortico-striato-thalamic system. Brain. 2003;126(Pt 8):1767-81.

Hong LE, Schroeder M, Ross TJ, Buchholz B, Salmeron BJ, Wonodi I, Thaker GK, Stein EA. Nicotine Enhances but Does Not Normalize Visual Sustained Attention and the Associated Brain Network in Schizophrenia. Schizophr Bull. 2009 Aug 27.

Hopfinger JB, Buonocore MH, Mangun GR. The neural mechanisms of top-down attentional control. Nat Neurosci. 2000;3(3):284-91.

Howseman AM, Bowtell RW. Functional magnetic resonance imaging: imaging techniques and contrast mechanisms. Philos Trans R Soc Lond B Biol Sci. 1999;354(1387):1179-94.

Hsieh, C.Y., Cruikshank, S.J. & Metherate, R. Differential modulation of auditory thalamocortical and intracortical synaptic transmission by cholinergic agonist. Brain Res.2000;880, 51–64.

Hudon C, Belleville S, Gauthier S. The assessment of recognition memory using the Remember/Know procedure in amnestic mild cognitive impairment and probable Alzheimer's disease. Brain Cogn. 2009;70(1):171-9.

Huerta PT, Lisman JE. Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. Nature. 1993;364(6439):723-5.

Huettel SA, Singerman JD, McCarthy G. The effects of aging upon the hemodynamic response measured by functional MRI. Neuroimage. 2001;13(1):161-75.

Iannetti GD, Wise RG. BOLD functional MRI in disease and pharmacological studies: room for improvement? Magn Reson Imaging. 2007;25(6):978-88.

Introini-Collison IB, McGaugh JL. Epinephrine modulates long term retention of an aversively motivated discrimination. Behav Neural Biol. 1986;45:358 –365.

Jacobsen LK, Gore JC, Skudlarski P, Lacadie CM, Jatlow P, Krystal JH. Impact of intravenous nicotine on BOLD signal response to photic stimulation. Magn Reson Imaging. 2002;20(2):141-5.

Jacobsen LK, D'Souza DC, Mencl WE, Pugh KR, Skudlarski P, Krystal JH. Nicotine effects on brain function and functional connectivity in schizophrenia. Biol Psychiatry. 2004;55(8):850-8.

Jacobsen LK, Pugh KR, Mencl WE, Gelernter J. C957T polymorphism of the dopamine D2 receptor gene modulates the effect of nicotine on working memory performance and cortical processing efficiency. Psychopharmacology (Berl). 2006;188(4):530-40.

Jasper HH, Tessier J. Acetylcholine liberation from cerebral cortex during paradoxical (REM) sleep. Science. 1971;172(983):601-2.

Jenden DJ, Jope RS, Weiler MH. Regulation of acetylcholine synthesis: does cytoplasmic acetylcholine control high affinity choline uptake? Science. 1976;194(4265):635-7.

Ji W, Gao E, Suga N. Effects of acetylcholine and atropine on plasticity of central auditory neurons caused by conditioning in bats. J Neurophysiol 2001;86:211–225.

Jiang Y, Chun MM. Selective attention modulates implicit learning. Q J Exp Psychol A. 2001;54(4):1105-24.

Jiang Y, Haxby JV, Martin A, Ungerleider LG, Parasuraman R. Complementary neural mechanisms for tracking items in human working memory. Science 2000;287:643–646.

Jones GM, Sahakian BJ, Levy R, Warburton DM, Gray JA. Effects of acute subcutaneous nicotine on attention, information processing and short-term memory in Alzheimer's disease. Psychopharmacology (Berl). 1992;108(4):485-94.

Josephs O, Henson RN. Event-related functional magnetic resonance imaging: modelling, inference and optimization. Philos Trans R Soc Lond B Biol Sci. 1999;354(1387):1215-28.

Kalisch R, Elbel GK, Gössl C, Czisch M, Auer DP. Blood pressure changes induced by arterial blood withdrawal influence bold signal in anesthesized rats at 7 Tesla: implications for pharmacologic mri. Neuroimage. 2001;14(4):891-8.

Kametani H, Kawamura H. Alterations in acetylcholine release in the rat hippocampus during sleep-wakefulness detected by intracerebral dialysis. Life Sci. 1990;47(5):421-6.

Kanetaka H, Hanyu H, Hirao K, Shimizu S, Sato T, Akai T, Iwamoto T, Koizumi K. Prediction of response to donepezil in Alzheimer's disease: combined MRI analysis of the substantia innominata and SPECT measurement of cerebral perfusion. Nucl Med Commun. 2008;29(6):568-73.

Kapp BS, Wilson A, Pascoe JP, Supple W, Whalen PJ. A neuroanatomical systems analysis of conditioned bradycardia in the rabbit, in: Gabriel, M., Moore, J. (Eds.), Learning and Computational Neuroscience: Foundations of Adaptive Networks. 1990. MIT Press, Cambridge, MA, pp. 53–90.

Kastner S, Pinsk MA, De Weerd P, Desimone R, Ungerleider LG. Increased activity in human visual cortex during directed attention in the absence of visual stimulation. Neuron. 1999;22(4):751-61

Kiebel SJ, Glaser DE, Friston KJ. A heuristic for the degrees of freedom of statistics based on multiple variance parameters. Neuroimage. 2003;20(1):591-600.

Kiebel SJ, Poline JB, Friston KJ, Holmes AP, Worsley KJ. Robust smoothness estimation in statistical parametric maps using standardized residuals from the general linear model. Neuroimage. 1999;10(6):756-66.

Kikuchi T, Okamura T, Fukushi K, Takahashi K, Toyohara J, Okada M, Zhang MR, Irie T. Cerebral acetylcholinesterase imaging: development of the radioprobes. Curr Top Med Chem. 2007;7(18):1790-9.

Kilgard MP, Merzenich MM. Cortical map reorganization enabled by nucleus basalis activity. Science. 1998;279(5357):1714-8.

Kimura A, Sato A, Takano Y. Stimulation of the nucleus basalis of Meynert does not influence glucose utilization of the cerebral cortex in anesthetized rats. Neurosci Lett. 1990;119(1):101-4.

Kimura F, Baughman RW. Distinct muscarinic receptor subtypes suppress excitatory and inhibitory synaptic responses in cortical neurons. J Neurophysiol. 1997;77(2):709-16.

Kimura F, Fukuda M, Tsumoto T. Acetylcholine suppresses the spread of excitation in the visual cortex revealed by optical recording: possible differential effect depending on the source of input. Eur J Neurosci. 1999;11(10):3597-609.

Kimura F. Cholinergic modulation of cortical function: a hypothetical role in shifting the dynamics in cortical network. Neurosci. Res. 2000.38, 19–26.

Kimura F, Fukuda M, Tsumoto T. Acetylcholine suppresses the spread of excitation in the visual cortex revealed by optical recording: possible differential effect depending on the source of input. Eur. J. Neurosci. 1999.11, 3597–3609.

Kircher TT, Weis S, Freymann K, Erb M, Jessen F, Grodd W, Heun R, Leube DT. Hippocampal activation in patients with mild cognitive impairment is necessary for successful memory encoding. J Neurol Neurosurg Psychiatry. 2007;78(8):812-8.

Kircher TTJ, Erb M, Grodd W, Leube DT. Cortical Activation During Cholinesterase-Inhibitor Treatment in Alzheimer Disease - Preliminary Findings From a Pharmaco-fMRI. Am J Geriatr Psychiatry 2005; 13:1006–101

Kirchhoff BA, Wagner AD, Maril A, Stern CE. Prefrontal-temporal circuitry for episodic encoding and subsequent memory. J Neurosci. 2000;20(16):6173-80.

Kirkwood A, Rozas C, Kirkwood J, Perez F, Bear MF. Modulation of long-term synaptic depression in visual cortex by acetylcholine and norepinephrine. J Neurosci. 1999;19(5):1599-609.

Klink R, Alonso A. Muscarinic modulation of the oscillatory and repetitive firing properties of entorhinal cortex layer II neurons. J Neurophysiol. 1997;77(4):1813-28.

Knopman D. Unaware learning versus preserved learning in pharmacologic amnesia: similarities and differences. J Exp Psychol Learn Mem Cogn. 1991;17(5):1017-29.

Koene RA, Gorchetchnikov A, Cannon RC, Hasselmo ME. Modeling goal-directed spatial navigation in the rat based on physiological data from the hippocampal formation. Neural Netw. 2003;16:577–584.

Kopelman MD, Corn TH. Cholinergic 'blockade' as a model for cholinergic depletion. A comparison of the memory deficits with those of Alzheimer-type dementia and the alcoholic Korsakoff syndrome. Brain. 1988;111 (Pt 5):1079-110.

Kopelman MD. The cholinergic neurotransmitter system in human memory and dementia: A review. Q. J. Exp. Psychol. 1986.38A:535–573.

Kosasa T, Kuriya Y, Matsui K, Yamanishi Y. Effect of donepezil hydrochloride (E2020) on basal concentration of extracellular acetylcholine in the hippocampus of rats. Eur J Pharmacol. 1999b;380(2-3):101-7.

Kosasa T, Kuriya Y, Yamanishi Y. Effect of donepezil hydrochloride (E2020) on extracellular acetylcholine concentration in the cerebral cortex of rats. Jpn J Pharmacol. 1999a;81(2):216-22.

Kozak R, Bruno JP, Sarter M. Augmented prefrontal acetylcholine release during challenged attentional performance. Cereb Cortex. 2006;16(1):9-17.

Krnjević K, Phillis JW. Acetylcholine-sensitive cells in the cerebral cortex. J Physiol. 1963;166:296-327.

Kukolja J, Thiel CM, Fink GR. Cholinergic stimulation enhances neural activity associated with encoding but reduces neural activity associated with retrieval in humans. J Neurosci. 2009;29(25):8119-28.

Kumari V, Aasen I, ffytche D, Williams SC, Sharma T. Neural correlates of adjunctive rivastigmine treatment to antipsychotics in schizophrenia: a randomized, placebo-controlled, double-blind fMRI study. Neuroimage. 2006;29(2):545-56.

Kumari V, Gray JA, ffytche DH, Mitterschiffthaler MT, Das M, Zachariah E, Vythelingum GN, Williams SC, Simmons A, Sharma T. Cognitive effects of nicotine in humans: an fMRI study. Neuroimage. 2003;19(3):1002-13.

Kuo MC, Rasmusson DD, Dringenberg HC. Input-Selective Potentiation and Re-Balancing of Primary Sensory Cortex Afferents by Endogenous Acetylcholine. Neuroscience. 2009 Jun 13. [Epub ahead of print].

LaBar KS, Gitelman DR, Parrish TB, Mesulam M. Neuroanatomic overlap of working memory and spatial attention networks: a functional MRI comparison within subjects. Neuroimage. 1999;10(6):695-704.

LaBar KS, Phelps EA. Perceptual priming of emotionally-arousing scenes is spared following bilateral amygdala damage (Abstract). In: The Amygdala in Brain Function: Basic and Clinical Approaches, Galveston, TX, 2002.

Lacombe P, Sercombe R, Verrecchia C, Philipson V, MacKenzie ET, Seylaz J. Cortical blood flow increases induced by stimulation of the substantia innominata in the unanesthetized rat. Brain Res. 1989;491:1–14.

Lambe EK, Picciotto MR, Aghajanian GK. Nicotine induces glutamate release from thalamocortical terminals in prefrontal cortex. Neuropsychopharmacology. 2003;28:216 –225.

Lawrence AD, Sahakian BJ. Alzheimer disease, attention, and the cholinergic system. Alzheimer Dis Assoc Disord. 1995;9 Suppl 2:43-9.

Lawrence NS, Ross TJ, Stein EA. Cognitive mechanisms of nicotine on visual attention. Neuron. 2002;36(3):539-48.

Lee MG, Chrobak JJ, Sik A, Wiley RG, Buzsáki G. Hippocampal theta activity following selective lesion of the septal cholinergic system. Neuroscience. 1994;62(4):1033-47.

Levin ED, Kaplan S, Boardman A. Acute nicotine interactions with nicotinic and muscarinic antagonists: working and reference memory effects in the 16-arm radial maze. Behav Pharmacol. 1997;8(2-3):236-42.

Levin ED, Simon BB. Nicotinic acetylcholine involvement in cognitive function in animals. Psychopharmacology (Berl). 1998;138(3-4):217-30.

Levy RB, Reyes AD, Aoki C. Nicotinic and muscarinic reduction of unitary excitatory postsynaptic potentials in sensory cortex; dual intracellular recording in vitro. J Neurophysiol. 2006;95(4):2155-66.

Lidow MS, Gallager DW, Rakic P, Goldman-Rakic PS. Regional differences in the distribution of muscarinic cholinergic receptors in the macaque cerebral cortex. J Comp Neurol. 1989;289(2):247-59.

Liepelt I, Maetzler W, Blaicher HP, Gasser T, Berg D. Treatment of dementia in parkinsonian syndromes with cholinesterase inhibitors. Dement Geriatr Cogn Disord. 2007;23(6):351-67.

Lindner MD, Hogan JB, Hodges DB Jr, Orie AF, Chen P, Corsa JA, Leet JE, Gillman KW, Rose GM, Jones KM, Gribkoff VK. Donepezil primarily attenuates scopolamine-induced deficits in psychomotor function, with moderate effects on simple conditioning and attention, and small effects on working memory and spatial mapping. Psychopharmacology (Berl). 2006;188(4):629-40.

Linster C, Wyble BP, Hasselmo ME. Electrical stimulation of the horizontal limb of the diagonal band of broca modulates population EPSPs in piriform cortex. J Neurophysiol. 1999;81(6):2737-42.

Logothetis, NK, Pauls J, Augath M, Trinath T, Oeltermann A. Neurophysiological investigation of the basis of the fMRI signal. Nature. 2001;412:150-157.

Logothetis NK. The neural basis of the blood-oxygen-level-dependent functional magnetic resonance imaging signal. Philos Trans R Soc Lond B Biol Sci. 2002;357(1424):1003-37.

London ED, Ball MJ, Waller SB. Nicotinic binding sites in cerebral cortex and hippocampus in Alzheimer's dementia. Neurochem. Res. 1989. 14: 745–750.

Lorch EP, Anderson DR, Well AD. Effects of irrelevant information on speeded classification tasks: Interference is reduced by habituation. J. Exp. Psychol: Hum. Perception Performance 1984;10, 850–864.

Lundh LG, Ost LG. Explicit and implicit memory bias in social phobia. The role of subdiagnostic type. Behav Res Ther 1997;35:305–317.

Lundqvist D, Flykt A, Vhman A. The Karolinska Directed Emotional Faces. Pictoral face set available from the Department of Neurosciences, Karolinska Hospital, Stockholm, Sweden. 1998.

Machulda MM, Ward HA, Borowski B, Gunter JL, Cha RH, O'Brien PC, Petersen RC, Boeve BF, Knopman D, Tang-Wai DF, Ivnik RJ, Smith GE, Tangalos EG, Jack CR Jr. Comparison of memory fMRI response among normal, MCI, and Alzheimer's patients. Neurology. 2003;61(4):500-6.

Mandzia JL, Black SE, McAndrews MP, Grady C, Graham S. fMRI differences in encoding and retrieval of pictures due to encoding strategy in the elderly. Hum Brain Mapp. 2004;21(1):1-14.

Mandzia JL, McAndrews MP, Grady CL, Graham SJ, Black SE. Neural correlates of incidental memory in mild cognitive impairment: An fMRI study. Neurobiol Aging. 2007; doi:10.1016/j.neurobiolaging.2007.08.024.

Mark GP, Rada PV, Shors TJ. Inescapable stress enhances extracellular acetylcholine in the rat hippocampus and prefrontal cortex but not the nucleus accumbens or amygdala. Neuroscience. 1996;74(3):767-74.

Marklund P, Fransson P, Cabeza R, Larsson A, Ingvar M, Nyberg L. Unity and diversity of tonic and phasic executive control components in episodic and working memory. Neuroimage. 2007;36(4):1361-73.

Marston HM, West HL, Wilkinson LS, Everitt BJ, Robbins TW. Effects of excitotoxic lesions of the septum and vertical limb nucleus of the diagonal band of Broca on conditional visual discrimination: relationship between performance and choline acetyltransferase activity in the cingulate cortex. J Neurosci. 1994;14(4):2009-19.

Martinez A, DiRusso F, Anllo-Vento L, Sereno MI, Buxton RB, Hillyard SA. Putting spatial attention on the map: timing and localization of stimulus selection processes in striate and extrastriate visual areas. Vision Res 2001;41:1437–1457.

Mash DC, White WF, Mesulam MM.Distribution of muscarinic receptor subtypes within architectonic subregions of the primate cerebral cortex. J Comp Neurol. 1988;278(2):265-74.

Mattay VS, Callicott JH, Bertolino A, Heaton I, Frank JA, Coppola R, Berman KF, Goldberg TE, Weinberger DR. Effects of dextroamphetamine on cognitive performance and cortical activation. Neuroimage. 2000;12(3):268-75.

Matthews PM, Honey GD, Bullmore ET. Applications of fMRI in translational medicine and clinical practice. Nat Rev Neurosci. 2006; 7(9): 732-44.

Maviel T, Durkin TP. Role of central cholinergic receptor sub-types in spatial working memory: a five-arm maze task in mice provides evidence for a functional role of nicotinic receptors in mediating trace access processes. Neuroscience. 2003;120(4):1049-59.

Mazère J, Prunier C, Barret O, Guyot M, Hommet C, Guilloteau D, Dartigues JF, Auriacombe S, Fabrigoule C, Allard M. In vivo SPECT imaging of vesicular acetylcholine transporter using [(123)I]-IBVM in early Alzheimer's disease. Neuroimage. 2008;40(1):280-8.

McAlonan GM, Dawson GR, Wilkinson LO, Robbins TW, Everitt BJ. The effects of AMPA-induced lesions of the medial septum and vertical limb nucleus of the diagonal band of Broca on spatial delayed non-matching to sample and spatial learning in the water maze. Eur J Neurosci. 1995;7(5):1034-49.

McCormick DA, Prince DA. Mechanisms of action of acetylcholine in the guinea-pig cerebral cortex in vitro. J. Physiol. 1986. 375: 169–194.

McCormick DA, Prince DA. Two types of muscarinic response to acetylcholine in mammalian cortical neurons. Proc Natl Acad Sci USA 1985. 82: 6344–6348.

McGaughy J, Dalley JW, Morrison CH, Everitt BJ, Robbins TW. Selective behavioral and neurochemical effects of cholinergic lesions produced by intrabasalis infusions of 192 IgG-saporin on attentional performance in a five-choice serial reaction time task. J Neurosci. 2002;22(5):1905-13

McGaughy J, Kaiser T, Sarter M. Behavioral vigilance following infusions of 192 IgG-saporin into the basal forebrain: selectivity of the behavioral impairment and relation to cortical AChE-positive fiber density. Behav Neurosci. 1996;110(2):247-65.

McGaughy J, Sarter M. Sustained attention performance in rats with intracortical infusions of 192 IgG-saporin-induced cortical cholinergic deafferentation: effects of physostigmine and FG 7142. Behav Neurosci. 1998;112(6):1519-25.

McKeith I, Del Ser T, Spano P, Emre M, Wesnes K, Anand R, Cicin-Sain A, Ferrara R, Spiegel R. Efficacy of rivastigmine in dementia with Lewy bodies: a randomised, double-blind, placebo-controlled international study. Lancet. 2000;356(9247):2031-6.

McNamara D, Larson DM, Rapoport SI, Soncrant TT. Preferential metabolic activation of subcortical brain areas by acute administration of nicotine to rats. J Cereb Blood Flow Metab. 1990;10(1):48-56.

McQuail JA, Burk JA. Evaluation of muscarinic and nicotinic receptor antagonists on attention and working memory. Pharmacol Biochem Behav. 2006;85(4):796-803.

Mechawar N, Cozzari C, Descarries L. Cholinergic innervation in adult rat cerebral cortex: a quantitative immunocytochemical description. J Comp Neurol. 2000;428:305–318.

Mechawar N, Watkins KC, Descarries L. Ultrastructural features of the acetylcholine innervation in the developing parietal cortex of rat. J Comp Neurol. 2002;443:250-8.

Mechelli A, Price CJ, Friston KJ, Ashburner J. Voxel-based morphometry of the human brain: methods and applications. Curr Med Imaging Rev. 2005; 1:105–113.

Mentis MJ, Alexander GE, Krasuski J, Pietrini P, Furey ML, Schapiro MB, Rapoport SI. Am J Psychiatry. Increasing required neural response to expose abnormal brain function in mild versus moderate or severe Alzheimer's disease: PET study using parametric visual stimulation. 1998;155(6):785-94.

Mentis MJ, Sunderland T, Lai J, Connolly C, Krasuski J, Levine B, Friz J, Sobti S, Schapiro M, Rapoport SI. Muscarinic versus nicotinic modulation of a visual task. a pet study using drug probes. Neuropsychopharmacology. 2001;25(4):555-64.

Mesulam M, Shaw P, Mash D, Weintraub S. Cholinergic nucleus basalis tauopathy emerges early in the aging-MCI-AD continuum. Ann Neurol. 2004;55(6):815-28.

Mesulam MM. Neuroanatomy of cholinesterases in the normal human brain and in Alzheimer’s disease. In Cholinesterases and Cholinesterase Inhibitors. Ed Giacobini. 2000.

Mesulam MM. The Cholinergic Lesion of Alzheimer's Disease: Pivotal Factor or Side Show? Learn Mem. 2004. 11: 43-49.

Mesulam MM, Geula C. Acetylcholinesterase-rich neurons of the human cerebral cortex: cytoarchitectonic and ontogenetic patterns of distribution. J Comp Neurol. 1991;306(2):193-220.

Mesulam MM, Geula C. Overlap between acetylcholinesterase-rich and choline acetyltransferase-positive (cholinergic) axons in human cerebral cortex. Brain Res. 1992;577(1):112-20.

Mesulam MM, Mufson EJ. Neural inputs into the nucleus basalis of the substantia innominata (Ch4) in the rhesus monkey. Brain. 1984;107 (Pt 1):253-74.

Mesulam MM, Mufson EJ, Rogers J. Age-related shrinkage of cortically projecting cholinergic neurons: A selective effect. Ann. Neurol. 1987. 22: 31–36.

Mewaldt SP, Ghoneim MM. The effects and interactions of scopolamine, physostigmine and methamphetamine on human memory. Pharmacol Biochem Behav. 1979 ;10(2):205-10.

Miasnikov AA, Chen JC, Weinberger NM. Specific auditory memory induced by nucleus basalis stimulation depends on intrinsic acetylcholine. Neurobiol Learn Mem. 2008a;90(2):443-54.

Miettinen RA, Kalesnykas G, Koivisto EH. Estimation of the total number of cholinergic neurons containing estrogen receptor-alpha in the rat basal forebrain. J Histochem Cytochem. 2002;50(7):891-902.

Miezin FM, Maccotta L, Ollinger JM, Petersen SE, Buckner RL. Characterizing the hemodynamic response: effects of presentation rate, sampling procedure, and the possibility of ordering brain activity based on relative timing. Neuroimage. 2000;11(6 Pt 1):735-59.

Miller EK, Desimone R. Scopolamine affects short-term memory but not inferior temporal neurons. Neuroreport. 1993;4(1):81-4.

Minger SL, Esiri MM, McDonald B, Keene J, Carter J, Hope T, Francis, PT. Cholinergic deficits contribute to behavioural disturbance in patients with dementia. Neurology 2000 55: 1460–1467.

Mishima K, Iwasaki K, Tsukikawa H, Matsumoto Y, Egashira N, Abe K, Egawa T, Fujiwara M. The scopolamine-induced impairment of spatial cognition parallels the acetylcholine release in the ventral hippocampus in rats. Jpn J Pharmacol. 2000;84(2):163-73.

Mitsis GD, Iannetti GD, Smart TS, Tracey I, Wise RG. Regions of interest analysis in pharmacological fMRI: how do the definition criteria influence the inferred result? Neuroimage. 2008;40(1):121-32.

Moore, H., Sarter, M. and Bruno, J.P., Bidirectional modulation of cortical acetylcholine efflux by infusion of benzodiazepine receptor ligands into the basal forebrain, Neurosci Lett. 1995.189:31–34.

Morris JS, Buchel C, Dolan RJ. Parallel neural responses in amygdale subregions and sensory cortex during implicit fear conditioning. Neuroimage. 2001;13(6):1044-52.

Morris JS, Friston KJ, Dolan RJ. Experience-dependent modulation of tonotopic neural responses in human auditory cortex. Proc. R. Soc. Lond. B Biol. Sci. 1998;265:649–657.

Mrzljak L, Pappy M, Leranth C, Goldman-Rakic PS. Cholinergic synaptic circuitry in the macaque prefrontal cortex. J Comp Neurol. 1995;357(4):603-17.

Mufson EJ, Ginsberg SD, Ikonomovic MD, DeKosky ST. Human cholinergic basal forebrain: chemoanatomy and neurologic dysfunction. J Chem Neuroanat. 2003;26(4):233-42.

Muir JL, Everitt BJ, Robbins TW. AMPA-induced excitotoxic lesions of the basal forebrain: a significant role for the cortical cholinergic system in attentional function. J Neurosci. 1994;14(4):2313-26.

Muir JL, Everitt BJ, Robbins TW. The cerebral cortex of the rat and visual attentional function: dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. Cereb Cortex. 1996;6(3):470-81.

Muller CM, Singer W. Acetylcholine-induced inhibition in the cat visual cortex is mediated b7 a GABAergic mechanism. Brain Res. 1989;487;335–342.

Muramoto O, Sugishita M, Ando K. Cholinergic system and constructional praxis: a further study of physostigmine in Alzheimer's disease. J Neurol Neurosurg Psychiatry. 1984;47(5):485-91.

Murphy PC, Sillito PC. Cholinergic enhancement of direction selectivity in the visual cortex of the cat. Neuroscience 1991;40(1):13–20.

Nelson CL, Sarter M, Bruno JP. Prefrontal cortical modulation of acetylcholine release in posterior parietal cortex. Neuroscience. 2005;132(2):347-59.

Newhouse PA, Potter A, Singh A. Effects of nicotinic stimulation on cognitive performance. Curr Opin Pharmacol 2004;4:36–46.

Nordberg A. Functional studies of cholinergic activity in normal and Alzheimer disease states by imaging technique. Prog Brain Res. 2004;145:301-10.

Nutt JG. Effect of cholinergic agents in Huntington's disease: a reappraisal. Neurology. 1983;33(7):932-5.

Ogawa M, Fukuyama H, Ouchi Y, Yamauchi H, Matsuzaki S, Kimura J, Tsukada H. Uncoupling between cortical glucose metabolism and blood flow after ibotenate lesion of the rat basal forebrain: a PET study. Neurosci Lett. 1996;204(3):193-6.

Ogawa M, Magata Y, Ouchi Y, Fukuyama H, Yamauchi H, Kimura J, Yonekura Y, Konishi J. Scopolamine abolishes cerebral blood flow response to somatosensory stimulation in anesthetized cats: PET study. Brain Res. 1994;650(2):249-52.

Ogawa S, Tso-Ming L. Magnetic resonance imaging of blood vessels at high fields: in vivo and in vitro measurements and image simulation. Magn Reson Med. 1990;16:9–18.

Otten LJ, Henson RN, Rugg MD. State-related and item-related neural correlates of successful memory encoding. Nat Neurosci. 2002;5(12):1339-44.

Ouchi Y, Fukuyama H, Ogawa M, Yamauchi H, Kimura J, Magata Y, Yonekura Y, Konishi J. Cholinergic projection from the basal forebrain and cerebral glucose metabolism in rats: a dynamic PET study. J Cereb Blood Flow Metab. 1996;16(1):34-41.

Parasuraman R, Greenwood PM, Haxby JV, Grady CL. Visuospatial attention in dementia of the Alzheimer type. Brain. 1992;115 (Pt 3):711-33.

Parent MB, Baxter MG. Septohippocampal acetylcholine: involved in but not necessary for learning and memory? Learn Mem. 2004;11(1):9-20.

Parikh V, Kozak R, Martinez V, Sarter M. Prefrontal acetylcholine release controls cue detection on multiple timescales. Neuron. 2007;56(1):141-54.

Parikh V, Man K, Decker MW, Sarter M. Glutamatergic contributions to nicotinic acetylcholine receptor agonist-evoked cholinergic transients in the prefrontal cortex. J Neurosci. 2008;28(14):3769-80.

Parnavelas JG, Kelly W, Burnstock G. Ultrastructural localization of choline acetyltransferase in vascular endothelial cells in rat brain. Nature. 1985;316(6030):724-5.

Passetti F, Dalley JW, O'Connell MT, Everitt BJ, Robbins TW. Increased acetylcholine release in the rat medial prefrontal cortex during performance of a visual attentional task. Eur J Neurosci. 2000;12(8):3051-8.

Paterson D, Nordberg A. Neuronal nicotinic receptors in the human brain. Prog Neurobiol. 2000;61(1):75-111.

Patil MM, Hasselmo ME. Modulation of inhibitory synaptic potentials in the piriform cortex. J Neurophysiol. 1999;81(5):2103-18.

Pattinson KT, Rogers R, Mayhew SD, Tracey I, Wise RG. Pharmacological FMRI: measuring opioid effects on the BOLD response to hypercapnia. J Cereb Blood Flow Metab. 2007;27(2):414-23.

Pazzagli A, Pepeu G. Amnesic properties of scopolamine and brain acetylcholine in the rat. Int J Neuropharmacol. 1965;4(5):291-9.

Pepeu G, Giovannini MG. Changes in acetylcholine extracellular levels during cognitive processes. Learn Mem 2004. 11: 21–27.

Perlstein WM, Elbert T, Stenger VA. Dissociation in human prefrontal cortex of affective influences on working memory-related activity. Proc Natl Acad Sci U S A. 2002;99(3):1736-41.

Perry EK, Blessed G, Tomlinson BE, Perry RH, Crow TJ, Cross AJ, Dockray GJ, Dimaline R, Arregui, A. Neurochemical activities in human temporal lobe related to aging and Alzheimer-type changes. Neurobiol. Aging 1981. 2: 251–256.

Perry RJ, Hodges JR. Attention and executive deficits in Alzheimer's disease. A critical review. Brain. 1999;122 (Pt 3):383-404.

Perry RJ, Watson P, Hodges JR. The nature and staging of attention dysfunction in early (minimal and mild) Alzheimer's disease: relationship to episodic and semantic memory impairment. Neuropsychologia. 2000;38(3):252-71.

Phillips JM, McAlonan K, Robb WG, Brown VJ. Cholinergic neurotransmission influences covert orientation of visuospatial attention in the rat. Psychopharmacology (Berl). 2000;150(1):112-6.

Phillis JW, Chong GC. Acetylcholine release from the cerebral and cerebellar cortices: its role in cortical arousal. Nature. 1965.207:253–1255.

Pietrini P, Alexander GE, Furey ML, Dani A, Mentis MJ, Horwitz B, Guazzelli M, Shapiro MB, Rapoport SI. Cerebral metabolic response to passive audiovisual stimulation in patients with Alzheimer's disease and healthy volunteers assessed by PET. J Nucl Med. 2000;41(4):575-83.

Pirch JH, Turco K, Rucker HK. A role for acetylcholine in conditioning-related responses of rat frontal cortex neurons: microiontophoretic evidence. Brain Res. 1992;586(1):19-26.

Plakke B, Ng CW, Poremba A. Scopolamine impairs auditory delayed matching-to-sample performance in monkeys. Neurosci Lett. 2008;438(1):126-30.

Polli FE, Barton JJ, Cain MS, Thakkar KN, Rauch SL, Manoach DS. Rostral and dorsal anterior cingulate cortex make dissociable contributions during antisaccade error commission. Proc Natl Acad Sci USA. 2005;102:15700 –15705.

Poole NA, Agrawal N. Cholinomimetic agents and neurocognitive impairment following head injury: a systematic review. Brain Inj. 2008;22(7-8):519-34.

Price CJ, Friston KJ. Cognitive conjunction: a new approach to brain activation experiments. Neuroimage. 1997;5(4 Pt 1):261-70.

Prusky GT, Shaw C, Cynader MS. The distribution and ontogenesis of [3H]nicotine binding sites in cat visual cortex. Brain Res. 1988;467(2):161-76.

Prvulovic D, Hubl D, Sack AT, Melillo L, Maurer K, Frolich L, Lanfermann H, Zanella FE, Goebel R, Linden DE, Dierks T. Functional imaging of visuospatial processing in Alzheimer's disease. Neuroimage. 2002;17(3):1403-14.

Puce A, Allison T, McCarthy G. Electrophysiological studies of human face perception. III. Effects of top-down processing on face-specific potentials. Cereb Cortex 1999;9:445–458.

Quigley KS, Sarter MF, Hart SL, Berntson GG. Cardiovascular effects of the benzodiazepine receptor partial inverse agonist FG 7142 in rats. Behav. Brain Res. 1994; 62, 11-20.

Rasch BH, Born J, Gais S. Combined blockade of cholinergic receptors shifts the brain from stimulus encoding to memory consolidation. J Cogn Neurosci 2006;18:793– 802.

Rasmusson DD, Smith SA, Semba K. Inactivation of prefrontal cortex abolishes cortical acetylcholine release evoked by sensory or sensory pathway stimulation in the rat. Neuroscience. 2007.149(1):232-241.

Rasmusson DD. The role of acetylcholine in cortical synaptic plasticity. Behav Brain Res. 2000;115(2):205-18.

Rauch A, Rainer G, Logothetis NK. The effect of a serotonin-induced dissociation between spiking and perisynaptic activity on BOLD functional MRI. Proc Natl Acad Sci U S A. 2008;105(18):6759-64.

Rauchs G, Piolino P, Mézenge F, Landeau B, Lalevée C, Pélerin A, Viader F, de la Sayette V, Eustache F, Desgranges B. Autonoetic consciousness in Alzheimer's disease: neuropsychological and PET findings using an episodic learning and recognition task. Neurobiol Aging. 2007;28(9):1410-20.

Reber PJ, Stark CE, Squire LR. Contrasting cortical activity associated with category memory and recognition memory. Learn Mem 1998;5:420–428.

Rees G, Friston K, Koch C. A direct quantitative relationship between the functional properties of human and macaque V5. Nat Neurosci. 2000;3(7):716-23.

Rezvani AH, Bushnell PJ, Levin ED. Effects of nicotine and mecamylamine on choice accuracy in an operant visual signal detection task in female rats. Psychopharmacology (Berl). 2002;164(4):369-75.

Ricciardi E, Pietrini P, Schapiro MB, Rapoport SI, Furey ML. Cholinergic modulation of visual working memory during aging: a parametric PET study. Brain Res Bull. 2009;79(5):322-32.

Richardson RT, DeLong MR. Context-dependent responses of primate nucleus basalis neurons in a go/no-go task. J Neurosci. 1990;10(8):2528-40.

Rieck R, Carey RG. Evidence for a laminar organization of basal forebrain afferents to the visual cortex. Brain Res. 1984;297(2):374-80.

Riekkinen P Jr, Riekkinen M. THA improves word priming and clonidine enhances fluency and working memory in Alzheimer's disease. Neuropsychopharmacology 1999;20: 357–364.

Rissman J, Gazzaley A, D'Esposito M. Dynamic adjustments in prefrontal, hippocampal, and inferior temporal interactions with increasing visual working memory load. Cereb Cortex. 2008;18(7):1618-29.

Rizzo M, Anderson SW, Dawson J, Nawrot M. Vision and cognition in Alzheimer's disease. Neuropsychologia. 2000;38:1157-1169.

Robbins TW. Arousal and attention: psychopharmacological and neuropsychological studies in experimental animals. in Parsuraman, R. (Ed.), The Attentive Brain. 1998. MIT Press, Cambridge, pp. 189–220.

Robbins TW, Everitt BJ, Marston HM, Wilkinson J, Jones GH, Page KJ. Comparative effects of ibotenic acid- and quisqualic acid-induced lesions of the substantia innominata on attentional function in the rat: further implications for the role of the cholinergic neurons of the nucleus basalis in cognitive processes. Behav Brain Res. 1989;35(3):221-40.

Robbins TW, Roberts AC. Differential regulation of fronto-executive function by the monoamines and acetylcholine. Cereb Cortex. 2007;17 Suppl 1:i151-60.

Roberts MJ, Zinke W, Guo K, Robertson R, McDonald JS, Thiele A. Acetylcholine dynamically controls spatial integration in marmoset primary visual cortex. J Neurophysiol. 2005;93(4):2062-72.

Rodriguez R, Kallenbach U, Singer W, Munk MH. Short- and long-term effects of cholinergic modulation on gamma oscillations and response synchronization in the visual cortex. J Neurosci. 2004;24(46):10369-78.

Rogers SL, Farlow MR, Doody RS, Mohs R, Friedhoff LT. A 24-week, double-blind, placebo-controlled trial of donepezil in patients with Alzheimer's disease. Donepezil Study Group. Neurology. 1998;50(1):136-45.

Rombouts SA, Goekoop R, Stam CJ, Barkhof F, Scheltens P. Delayed rather than decreased BOLD response as a marker for early Alzheimer's disease. Neuroimage. 2005;26(4):1078-85.

Rombouts SA, Scheltens P, Kuijer JP, Barkhof F. Whole brain analysis of T2* weighted baseline FMRI signal in dementia. Hum Brain Mapp. 2007;28(12):1313-7.

Rombouts SARB, Barkhof F, van Meel CS, Scheltens P. Alterations in brain activation during cholinergic enhancement with rivastigmine in Alzheimer’s disease Disease. J Neurol Neurosurg Psychiatry 2002;73:665–671

Rorden C, Brett M. Stereotaxic display of brain lesions. Behav Neurol, 2000.12:191–200.

Rose RP, Moulthrop MA. Differential responsivity of verbal and visual recognition memory to physostigmine and ACTH. Biol Psychiatry. 1986;21(5-6):538-42.

Rosier AM, Cornette L, Dupont P, Bormans G, Mortelmans L, Orban GA. Regional brain activity during shape recognition impaired by a scopolamine challenge to encoding. Eur J Neurosci. 1999;11(10):3701-14.

Rotshtein P, Malach R, Hadar U, Graif M, Hendler T. Feeling or features: different sensitivity to emotion in high-order visual cortex and amygdala. Neuron. 2001;32(4):747-57.

Rowe JB, Passingham RE. Working memory for location and time: activity in prefrontal area 46 relates to selection rather than maintenance in memory. Neuroimage. 2001;14(1 Pt 1):77-86.

Rugg MD, Otten LJ, Henson RN. The neural basis of episodic memory: evidence from functional neuroimaging. Philos Trans R Soc Lond B Biol Sci. 2002;357(1424):1097-110.

Rupniak NM, Tye SJ, Brazell C, Heald A, Iversen SD, Pagella PG. Reversal of cognitive impairment by heptyl physostigmine, a long-lasting cholinesterase inhibitor, in primates. J Neurol Sci. 1992;107(2):246-9.

Rusted JM, Warburton DM. Facilitation of memory by post-trial administration of nicotine: evidence for an attentional explanation. Psychopharmacology 1992;108:452–455.

Rusted JM, Warburton DM. The effects of scopolamine on working memory in healthy young volunteers. Psychopharmacology (Berl). 1988;96(2):145-52.

Rypma B, Berger JS, Prabhakaran V, Bly BM, Kimberg DY, Biswal BB, D'Esposito M. Neural correlates of cognitive efficiency. Neuroimage. 2006;33(3):969-79.

Sahakian B, Jones G, Levy R, Gray J, Warburton D. The effects of nicotine on attention, information processing, and short-term memory in patients with dementia of the Alzheimer type. Br J Psychiatry. 1989;154:797-800.

Sahakian BJ, Coull JT. Tetrahydroaminoacridine (THA) in Alzheimer's disease: an assessment of attentional and mnemonic function using CANTAB. Acta Neurol Scand Suppl. 1993;149:29-35.

Sahakian BJ, Owen AM, Morant NJ, Eagger SA, Boddington S, Crayton L, Crockford HA, Crooks M, Hill K, Levy R. Further analysis of the cognitive effects of tetrahydroaminoacridine in Alzheimer's disease: assessment of attentional and mnemonic function using CANTAB. Psychopharmacology. 1993; 110: 395-401.

Sarter M, Bruno JP, Dudchenko P. Activating the damaged basal forebrain cholinergic system: tonic stimulation versus signal amplification. Psychopharmacology (Berl). 1990;101(1):1-17.

Sarter M, Bruno JP, Givens B. Attentional functions of cortical cholinergic inputs: what does it mean for learning and memory? Neurobiol Learn Mem. 2003;80:245-56.

Sarter M, Bruno JP. Brain Res Brain Res Rev. Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. 1997;23(1-2):28-46.

Sarter M, Gehring WJ, Kozak R. More attention must be paid: the neurobiology of attentional effort. Brain Res Rev. 2006;51(2):145-60.

Sarter M, Givens B, Bruno JP. The cognitive neuroscience of sustained attention: where top-down meets bottom-up. Brain Res Brain Res Rev. 2001;35(2):146-60.

Sarter M, Hasselmo ME, Bruno JP, Givens B. Unraveling the attentional functions of cortical cholinergic inputs: interactions between signal-driven and cognitive modulation of signal detection. Brain Res Brain Res Rev. 2005a;48(1):98-111.

Sarter M, Nelson CL, Bruno JP. Cortical cholinergic transmission and cortical information processing in schizophrenia. Schizophr Bull. 2005b;31(1):117-38.

Sarter M, Parikh V, Howe WM. Phasic acetylcholine release and the volume transmission hypothesis: time to move on. Nat Rev Neurosci. 2009;10(5):383-90.

Sato A, Sato Y, Uchida S. Activation of the intracerebral cholinergic nerve fibers originating in the basal forebrain increases regional cerebral blood flow in the rat's cortex and hippocampus. Neurosci Lett. 2004;361(1-3):90-3.

Sato A, Sato Y, Uchida S. Regulation of cerebral cortical blood flow by the basal forebrain cholinergic fibers and aging. Auton Neurosci. 2002;96(1):13-9.

Sato H, Hata Y, Masui H, Tsumoto T. A functional role of cholinergic innervation to neurons in the cat visual cortex. J Neurophysiol. 1987a; 58(4):765-80.

Sato H, Hata Y, Hagihara K, Tsumoto T. Effects of cholinergic depletion on neuron activities in the cat visual cortex. J Neurophysiol. 1987b;58(4):781-94.

Satoh K, Fibiger HC. Cholinergic neurons of the laterodorsal tegmental nucleus: efferent and afferent connections. J Comp Neurol. 1986;253(3):277-302.

Saykin AJ, Wishart HA, Rabin LA, Flashman LA, McHugh TL, Mamourian AC, Santulli RB. Cholinergic enhancement of frontal lobe activity in mild cognitive impairment. Brain. 2004;127(Pt 7):1574-83.

Scacchi R, Gambina G, Moretto G, Corbo RM. Variability of AChE, BChE, and ChAT genes in the late-onset form of Alzheimer's disease and relationships with response to treatment with Donepezil and Rivastigmine. Am J Med Genet B Neuropsychiatr Genet. 2009;150B(4):502-7.

Scali C, Giovannini MG, Bartolini L, Prosperi C, Hinz V, Schmidt B, Pepeu G. Effect of metrifonate on extracellular brain acetylcholine and object recognition in aged rats. Eur J Pharmacol. 1997a;325(2-3):173-80.

Scali C, Giovannini MG, Prosperi C, Bartolini L, Pepeu G. Tacrine administration enhances extracellular acetylcholine in vivo and restores the cognitive impairment in aged rats. Pharmacol Res. 1997b;36(6):463-9.

Schacter DL, Buckner RL. Priming and the brain. Neuron 1998;20:185–195.

Schacter DL, Buckner RL, Koutstaal W, Dale AM, Rosen BR. Late onset of anterior prefrontal activity during true and false recognition: an event-related fMRI study. Neuroimage. 1997;6(4):259-69.

Schon K, Atri A, Hasselmo ME, Tricarico MD, LoPresti ML, Stern CE. Scopolamine reduces persistent activity related to long-term encoding in the parahippocampal gyrus during delayed matching in humans. J Neurosci. 2005;25(40):9112-23.

Schretlen DJ, Buffington AL, Meyer SM, Pearlson GD. The use of word-reading to estimate "premorbid" ability in cognitive domains other than intelligence. J Int Neuropsychol Soc. 2005;11(6):784-7.

Schwartz BL, Hashtroudi S, Herting RL, Schwartz P, Deutsch SI. d-Cycloserine enhances implicit memory in Alzheimer patients. Neurology 1996;46:420–424..

Scremin OU, Sonnenschein RR, Rubinstein EH. Cholinergic cerebral vasodilatation in the rabbit: absence of concomitant metabolic activation. J Cereb Blood Flow Metab. 1982;2(2):241-7.

Selden NR, Gitelman DR, Salamon-Murayama N, Parrish TB, Mesulam MM. Trajectories of cholinergic pathways within the cerebral hemispheres of the human brain. Brain. 1998;121(12):2249-57.

Semba K. Multiple output pathways of the basal forebrain: organization, chemical heterogeneity, and roles in vigilance. Behav Brain Res. 2000;115(2):117-41.

Shimamura AP, Salmon DP, Squire LR, Butters N. Memory dysfunction and word priming in dementia and amnesia. Behav Neurosci 1987;101:347–351.
(12):2154-61.

Sillito AM, Kemp JA. Cholinergic modulation of the functional organization of the cat visual cortex. Brain Res. 1983;289(1-2):143-55.

Silver MA, Shenhav A, D'Esposito M. Cholinergic enhancement reduces spatial spread of visual responses in human early visual cortex. Neuron. 2008;60(5):904-14.

Simpson JR, Ongür D, Akbudak E, Conturo TE, Ollinger JM, Snyder AZ, Gusnard DA, Raichle ME. The emotional modulation of cognitive processing: an fMRI study. J Cogn Neurosci. 2000;12 Suppl 2:157-70.

Smiley JF, Morrell F, Mesulam MM. Cholinergic synapses in human cerebral cortex: an ultrastructural study in serial sections. Exp Neurol. 1997;144(2):361-8.

Smith AJ, Blumenfeld H, Behar KL, Rothman DL, Shulman RG, Hyder F. Cerebral energetics and spiking frequency: the neurophysiological basis of fMRI. Proc Natl Acad Sci U S A. 2002;99(16):10765-70.

Snodgrass JG, Corwin J. Pragmatics of measuring recognition memory: applications to dementia and amnesia. J Exp Psychol Gen. 1988;117(1):34-50.

Sohal VS, Hasselmo ME. A model for experience-dependent changes in the responses of inferotemporal neurons. Network. 2000;11(3):169-90.

Sotero RC, Trujillo-Barreto NJ. Biophysical model for integrating neuronal activity, EEG, fMRI and metabolism. Neuroimage. 2008;39(1):290-309.

Sperling R, Chua E, Cocchiarella A, Rand-Giovannetti E, Poldrack R, Schacter DL, Albert M. Putting names to faces: successful encoding of associative memories activates the anterior hippocampal formation. Neuroimage. 2003;20(2):1400-10.

Sperling R, Greve D, Dale A, Killiany R, Holmes J, Rosas HD, Cocchiarella A, Firth P, Rosen B, Lake S, Lange N, Routledge C, Albert M. Functional MRI detection of pharmacologically induced memory impairment. Proc Natl Acad Sci U S A. 2002;99(1):455-60.

Stefanovic B, Schwindt W, Hoehn M, Silva AC. Functional uncoupling of hemodynamic from neuronal response by inhibition of neuronal nitric oxide synthase. J Cereb Blood Flow Metab. 2007;27(4):741-54.

Stein EA, Pankiewicz J, Harsch HH, Cho JK, Fuller SA, Hoffmann RG, Hawkins M, Rao SM, Bandettini PA, Bloom AS. Nicotine-induced limbic cortical activation in the human brain: a functional MRI study. Am J Psychiatry. 1998;155(8):1009-15.

Stewart C, Burke S, Marrocco R. Cholinergic modulation of covert attention in the rat. Psychopharmacology (Berl). 2001;155(2):210-8.

Stratton L & Petrinovich L. Post-trial injections of an anticholinesterase drug and maze learning in two strains of rats. Psychopharmacoligia, 1963.5, 47–54.

Suckling J, Wink AM, Bernard FA, Barnes A, Bullmore E. Endogenous multifractal brain dynamics are modulated by age, cholinergic blockade and cognitive performance. J Neurosci Methods. 2008;174(2):292-300.

Summers WK, Majovski LV, Marsh GM, Tachiki K, Kling A. Oral tetrahydroaminoacridine in long-term treatment of senile dementia, Alzheimer type. N Engl J Med. 1986;315(20):1241-5.

Suzuki WA, Miller EK, Desimone R. Object and place memory in the macaque entorhinal cortex. J. Neurophysiol. 1997;78:1062-81.

Svensson AL, Giacobini E. Cholinesterase inhibitors do more than inhibit cholinesterase. In Cholinesterases and Cholinesterase Inhibitors. 2000;227-236. Giacobini E. Martin Dunitz.

Talairach J, Tournoux P. Co-planar Stereotactic Atlas of the Human Brain. 3-Dimensional Proportional System: An Approach to Cerebral Imaging. Georg Thieme, Stuttgart, 1988.

Tang Y, Aigner TG. Release of cerebral acetylcholine increases during visually mediated behavior in monkeys. Neuroreport. 1996;7(13):2231-5.

Tang Y, Mishkin M, Aigner TG. Effects of muscarinic blockade in perirhinal cortex during visual recognition. Proc Natl Acad Sci U S A. 1997;94(23):12667-9.

Teipel SJ, Drzezga A, Bartenstein P, Möller HJ, Schwaiger M, Hampel H. Effects of donepezil on cortical metabolic response to activation during (18)FDG-PET in Alzheimer's disease: a double-blind cross-over trial. Psychopharmacology (Berl). 2006;187(1):86-94.

Terry AV Jr, Buccafusco JJ, Jackson WJ. Scopolamine reversal of nicotine enhanced delayed matching-to-sample performance in monkeys. Pharmacol Biochem Behav. 1993;45(4):925-9.

Thiel CM, Huston JP, Schwarting RK. Cholinergic activation in frontal cortex

and nucleus accumbens related to basic behavioral manipulations: handling, and the role of post-handling experience. Brain Res. 1998;812(1-2):121-32.

Thiel CM, Henson RN, Morris JS, Friston KJ, Dolan RJ. Pharmacological modulation of behavioral and neuronal correlates of repetition priming. J Neurosci. 2001;21(17):6846-52.

Thiel CM, Henson RN, Dolan RJ. Scopolamine but not lorazepam modulates face repetition priming: a psychopharmacological fMRI study. Neuropsychopharmacology. 2002a;27:282-92.

Thiel CM, Friston KJ, Dolan RJ. Cholinergic modulation of experience-dependent plasticity in human auditory cortex. Neuron. 2002b;35(3):567-74.

Thiel CM, Bentley P, Dolan RJ. Effects of cholinergic enhancement on conditioning-related responses in human auditory cortex. Eur J Neurosci. 2002c;16(11):2199-206.

Thiel CM, Zilles K, Fink GR. Nicotine modulates reorienting of visuospatial attention and neural activity in human parietal cortex. Neuropsychopharmacology. 2005;30(4):810-20.

Thiel CM, Fink GR. Visual and auditory alertness: modality-specific and supramodal neural mechanisms and their modulation by nicotine. J Neurophysiol. 2007;97(4):2758-68.

Thiel CM, Fink GR. Effects of the cholinergic agonist nicotine on reorienting of visual spatial attention and top-down attentional control. Neuroscience. 2008;152(2):381-90.

Thienel R, Kellermann T, Schall U, Voss B, Reske M, Halfter S, Sheldrick AJ, Radenbach K, Habel U, Shah NJ, Kircher T. Muscarinic antagonist effects on executive control of attention. Int J Neuropsychopharmacol. 2009b;12(10):1307-17.

Thienel R, Voss B, Kellermann T, Reske M, Halfter S, Sheldrick AJ, Radenbach K, Habel U, Shah NJ, Schall U, Kircher T. Nicotinic antagonist effects on functional attention networks. Int J Neuropsychopharmacol. 2009a;12(10):1295-305.

Tiraboschi P, Hansen LA, Alford M, Sabbagh MN, Schoos B, Masliah E, Thal LJ, Corey-Bloom J. Cholinergic dysfunction in diseases with Lewy bodies. Neurology. 2000;54:407-11.

Tootell RB, Reppas JB, Kwong KK, Malach R, Born RT, Brady TJ, Rosen BR, Belliveau JW. J Neurosci. 1995;15(4):3215-30. Functional analysis of human MT and related visual cortical areas using magnetic resonance imaging.

Tsukada H, Kakiuchi T, Ando I, Ouchi Y. Functional activation of cerebral blood flow abolished by scopolamine is reversed by cognitive enhancers associated with cholinesterase inhibition: a positron emission tomography study in unanesthetized monkeys. J Pharmacol Exp Ther. 1997;281(3):1408-14.

Tsukada H, Nishiyama S, Fukumoto D, Ohba H, Sato K, Kakiuchi T. Effects of acute acetylcholinesterase inhibition on the cerebral cholinergic neuronal system and cognitive function: Functional imaging of the conscious monkey brain using animal PET in combination with microdialysis. Synapse. 2004;52(1):1-10.

Tsukada H, Sato K, Kakiuchi T, Nishiyama S. Age-related impairment of coupling mechanism between neuronal activation and functional cerebral blood flow response was restored by cholinesterase inhibition: PET study with microdialysis in the awake monkey brain. Brain Res. 2000;857(1-2):158-64.

Turchi J, Sarter M. Cortical acetylcholine and processing capacity: effects of cortical cholinergic deafferentation on crossmodal divided attention in rats. Brain Res Cogn Brain Res. 1997;6(2):147-58.

Turrini P, Casu MA, Wong TP, De Koninck Y, Ribeiro-da-Silva A, Cuello AC. Cholinergic nerve terminals establish classical synapses in the rat cerebral cortex: synaptic pattern and age-related atrophy. Neuroscience. 2001;105(2):277-85.

Uchida S, Hotta H. Cerebral cortical vasodilatation mediated by nicotinic cholinergic receptors: effects of old age and of chronic nicotine exposure. Biol Pharm Bull. 2009;32(3):341-4.

Uchida S, Kagitani F, Nakayama H, Sato A. Effect of stimulation of nicotinic cholinergic receptors on cortical cerebral blood flow and changes in the effect during aging in anesthetized rats. Neurosci Lett. 1997;228(3):203-6.

Umbriaco D, Garcia S, Beaulieu C, Descarries L. Relational features of acetylcholine, noradrenaline, serotonin and GABA axon terminals in the stratum radiatum of adult rat hippocampus (CA1). Hippocampus. 1995;5(6):605-20.

Umbriaco D, Watkins KC, Descarries L, Cozzari C, Hartman BK. Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections. J Comp Neurol. 1994;348(3):351-73.

van der Staay FJ, Hinz VC, Schmidt BH. Effects of metrifonate, its transformation product dichlorvos, and other organophosphorus and reference cholinesterase inhibitors on Morris water escape behavior in young-adult rats. J Pharmacol Exp Ther. 1996;278(2):697-708.

van Eijsden P, Hyder F, Rothman DL, Shulman RG. Neurophysiology of functional imaging. Neuroimage. 2009;45(4):1047-54.

Varsaldi F, Miglio G, Scordo MG, Dahl ML, Villa LM, Biolcati A, Lombardi G. Impact of the CYP2D6 polymorphism on steady-state plasma concentrations and clinical outcome of donepezil in Alzheimer's disease patients. Eur J Clin Pharmacol. 2006;62(9):721-6.

Vidailhet P, Danion JM, Chemin C, Kazes M. Lorazepam impairs both visual and auditory perceptual priming. Psychopharmacology (Berl) 1999;147:266–273.

Vinberg J, Grill-Spector K. Representation of shapes, edges, and surfaces across multiple cues in the human visual cortex. J Neurophysiol. 2008;99(3):1380-93.

Völlm BA, de Araujo IE, Cowen PJ, Rolls ET, Kringelbach ML, Smith KA, Jezzard P, Heal RJ, Matthews PM. Methamphetamine activates reward circuitry in drug naïve human subjects. Neuropsychopharmacology. 2004;29(9):1715-22.

von Engelhardt J, Eliava M, Meyer AH, Rozov A, Monyer H. Functional characterization of intrinsic cholinergic interneurons in the cortex. J Neurosci. 2007;27(21):5633-42.

Vossel S, Thiel CM, Fink GR. Behavioral and neural effects of nicotine on visuospatial attentional reorienting in non-smoking subjects. Neuropsychopharmacology. 2008;33(4):731-8.

Vossel S, Kukolja J, Thimm M, Thiel C, Fink G. The effect of nicotine on visuospatial attention in chronic spatial neglect depends upon lesion location. J Psychopharmacol. 2009 May 28. [Epub ahead of print]

Voytko ML, Olton DS, Richardson RT, Gorman LK, Tobin JR, Price DL. Basal forebrain lesions in monkeys disrupt attention but not learning and memory. J Neurosci. 1994;14(1):167-86.

Vuilleumier P, Armony JL, Driver J, Dolan RJ. Effects of attention and emotion on face processing in the human brain: an event-related fMRI study. Neuron. 2001;30(3):829-41.

Wagner AD, Koutstaal W, Schacter DL. When encoding yields remembering: insights from event-related neuroimaging. Philos Trans R Soc Lond B Biol Sci. 1999;354(1387):1307-24.

Wagner AD, Schacter DL, Rotte M, Koutstaal W, Maril A, Dale AM, Rosen BR, Buckner RL. Building memories: remembering and forgetting of verbal experiences as predicted by brain activity. Science. 1998;281(5380):1188-91.

Wainer BH, Mesulam MM. Ascending cholinergic pathways in the rat brain. In: Brain cholinergic systems. Steriade M, Biesold D, Eds. Oxford: Oxford UP.1990.65–119.

Wais PE. FMRI signals associated with memory strength in the medial temporal lobes: a meta-analysis. Neuropsychologia. 2008;46(14):3185-96.

Walla P, Püregger E, Lehrner J, Mayer D, Deecke L, Dal Bianco P. Depth of word processing in Alzheimer patients and normal controls: a magnetoencephalographic (MEG) study. J Neural Transm. 2005;112(5):713-30.

Warburton DM, Skinner A, Martin CD. Improved incidental memory with nicotine after semantic processing, but not after phonological processing. Psychopharmacology (Berl). 2001;153(2):258-63.

Watkins PC, Martin CK, Stern LD. Unconscious memory bias in depression: perceptual and conceptual processes. J Abnorm Psychol 2000;109:282–289.

Wattmo C, Hansson O, Wallin AK, Londos E, Minthon L. Predicting long-term cognitive outcome with new regression models in donepezil-treated Alzheimer patients in a naturalistic setting. Dement Geriatr Cogn Disord. 2008;26(3):203-11.

Wecker L, Mobley PL, Dettbarn WD. Central cholinergic mechanisms underlying adaptation to reduced cholinesterase activity. Biochem Pharmacol. 1977;26(7):633-7.

Weinberger NM, Ashe JH, Metherate R, McKenna TM, Diamond DM, Bakin JS, Lennartz RC, Cassady JM. Neural adaptive information processing: A preliminary model of receptive-field plasticity in auditory cortex during Pavlovian conditioning, in: Gabriel, M., Moore, J. (Eds.),

Learning and Computational Neuroscience: Foundations of Adaptive Networks. 1990. MIT Press, Cambridge, MA, pp. 91–138.

Weinberger NM. Physiological memory in primary auditory cortex: characteristics and mechanisms. Neurobiol. Learn. Mem. 1998;70, 226–251.

Weinberger NM. Auditory associative memory and representational plasticity in the primary auditory cortex. Hear Res. 2007;229(1-2):54-68.

Wesnes K, Warburton DM. Effects of scopolamine and nicotine on human rapid information processing performance. Psychopharmacology. 1984. 82:147--150.

Wesnes KA, Simpson PM, Kidd AG, An investigation of the range of cognitive impairments induced by scopolamine 0.6 mg s.c., Hum Psychopharmacol. 1988: 3:27–41.

Wetherell A. Effects of physostigmine on stimulus encoding in a memory-scanning task. Psychopharmacology (Berl). 1992;109(1-2):198-202.

Whitehouse PJ, Price DL, Clark AW, Coyle JT, DeLong MR. Alzheimer disease: evidence for selective loss of cholinergic neurons in the nucleus basalis. Ann Neurol. 1981;10(2):122-6.

Wiggs CL, Martin A. Properties and mechanisms of perceptual priming. Curr Opin Neurobiol 1998;8:227–233.

Wilkinson DG, Doody RS, Black SE, Salloway SP, Schindler RJ. Donepezil in vascular dementia: combined analysis of two large-scale clinical trials. Dement Geriatr Cogn Disord. 2005;20(6):338-44.

Williams GV, Castner SA. Under the curve: critical issues for elucidating D1 receptor function in working memory. Neuroscience. 2006;139(1):263-76.

Willson MC, Wilman AH, Bell EC, Asghar SJ, Silverstone PH. Dextroamphetamine causes a change in regional brain activity in vivo during cognitive tasks: a functional magnetic resonance imaging study of blood oxygen level-dependent response. Biol Psychiatry. 2004;56(4):284-91.

Wilson FA, Rolls ET. Neuronal responses related to the novelty and familarity of visual stimuli in the substantia innominata, diagonal band of Broca and periventricular region of the primate basal forebrain. Exp Brain Res. 1990;80(1):104-20.

Winblad B, Wimo A, Engedal K, Soininen H, Verhey F, Waldemar G, Wetterholm AL, Haglund A, Zhang R, Schindler R. 3-year study of donepezil therapy in Alzheimer's disease: effects of early and continuous therapy. Dement Geriatr Cogn Disord. 2006;21(5-6):353-63.

Wink AM, Bernard F, Salvador R, Bullmore E, Suckling J. Age and cholinergic effects on hemodynamics and functional coherence of human hippocampus. Neurobiol Aging. 2006;27(10):1395-404.

Winters BD, Bartko SJ, Saksida LM, Bussey TJ. Scopolamine infused into perirhinal cortex improves object recognition memory by blocking the acquisition of interfering object information. Learn Mem. 2007;14(9):590-6.

Winters BD, Bussey TJ. Removal of cholinergic input to perirhinal cortex disrupts object recognition but not spatial working memory in the rat. Eur J Neurosci. 2005;21(8):2263-70.

Winters BD, Saksida LM, Bussey TJ. Paradoxical facilitation of object recognition memory after infusion of scopolamine into perirhinal cortex: implications for cholinergic system function. J Neurosci. 2006;26(37):9520-9.

Witte EA, Davidson MC, Marrocco RT. Effects of altering brain cholinergic activity on covert orienting of attention: comparison of monkey and human performance. Psychopharmacology (Berl) 1997;132:324–334.

Wojciulik E, Kanwisher N, Driver J. Covert visual attention modulates face-specific activity in the human fusiform gyrus: fMRI study. J Neurophysiol.1998;79(3):1574-8.

Womelsdorf T, Fries P, Mitra PP, Desimone R. Gamma-band synchronization in visual cortex predicts speed of change detection. Nature. 2006;439(7077):733-6.

Wonnacott S, Barik J, Dickinson J, Jones IW. Nicotinic receptors modulate transmitter cross talk in the CNS: nicotinic modulation of transmitters. J Mol Neurosci. 2006;30:137--140.

Worsley KJ, Evans AC, Marrett S, Neelin P. A three-dimensional statistical analysis for CBF activation studies in human brain. J Cereb Blood Flow Metab. 1992;12(6):900-18.

Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, Evans AC. A unified statistical approach to determining significant signals in images of cerebral activation. Hum. Brain Mapp. 1996;4, 58–73.

Worsley KJ, Friston KJ. A test for a conjunction. Stat Prob Letters. 2000;47, 135-140.

Wu DM, Kawamura H, Sakagami K, Kobayashi M, Puro DG. Cholinergic regulation of pericyte-containing retinal microvessels. Am J Physiol Heart Circ Physiol. 2003;284(6):H2083-90.

Xiang Z, Huguenard JR, Prince DA. Cholinergic switching within neocortical inhibitory networks. Science. 1998;281(5379):985-8.

Yantis S, Schwarzbach J, Serences JT, Carlson RL, Steinmetz MA, Pekar JJ, Courtney SM. Transient neural activity in human parietal cortex during spatial attention shifts. Nat Neurosci. 2002;5(10):995-1002.

Ye M, Hayar A, Strotman B, Garcia-Rill E. Cholinergic modulation of fast inhibitory and excitatory transmission to pedunculopontine thalamic projecting neurons. J Neurophysiol. 2010;103(5):2417-32.

Young BJ, Otto T, Fox GD, Eichenbaum H. Memory representation within the parahippocampal region. J Neurosci. 1997;17(13):5183-95.

Young SL, Bohenek DL, Fanselow MS. Scopolamine impairs acquisition and facilitates consolidation of fear conditioning: differential effects for tone vs context conditioning. Neurobiol Learn Mem. 1995;63(2):174-80.

Yu AJ, Dayan P. Acetylcholine in cortical inference. Neural Netw 2002; 15:719–730

Yu AJ, Dayan P. Uncertainty, neuromodulation, and attention. Neuron. 2005;46:681-92.

Zaborszky L, Gaykema RP, Swanson DJ, Cullinan WE. Cortical input to the basal forebrain. Neuroscience. 1997;79(4):1051-78.

Zaborszky L, Hoemke L, Mohlberg H, Schleicher A, Amunts K, Zilles K. Stereotaxic probabilistic maps of the magnocellular cell groups in human basal forebrain. Neuroimage. 2008.42(3):1127-41.

Zaborszky L. The modular organization of brain systems. Basal forebrain: the last frontier. Prog Brain Res. 2002;136:359-72.

Zarahn E, Aguirre GK, D'Esposito M. Empirical analyses of BOLD fMRI statistics. I. Spatially unsmoothed data collected under null-hypothesis conditions. Neuroimage. 1997;5:179-197.

Zarahn E, Aguirre GK, D'Esposito M. Temporal isolation of the neural correlates of spatial mnemonic processing with fMRI. Brain Res Cogn Brain Res. 1999;7(3):255-68.

Zarow C, Lyness SA, Mortimer JA, Chui HC. Neuronal loss is greater in the locus coeruleus than nucleus basalis and substantia nigra in Alzheimer and Parkinson diseases. Arch Neurol. 2003;60(3):337-41.

Zhang H, Lin SC, Nicolelis MA. Spatiotemporal coupling between hippocampal acetylcholine release and theta oscillations in vivo. J Neurosci. 2010;30(40):13431-40.

Zheng QH, Gao M, Mock BH, et al. Synthesis and biodistribution of new radiolabeled high-affinity choline transporter inhibitors [11C]hemicholinium-3 and [18F]hemicholinium-3. Bioorg Med Chem Lett. 2007;17(8):2220-4.

Zilles K, Palomero-Gallagher N, Grefkes C, Scheperjans F, Boy C, Amunts K, Schleicher A. Architectonics of the human cerebral cortex and transmitter receptor fingerprints: reconciling functional neuroanatomy and neurochemistry. Eur Neuropsychopharmacol. 2002;12(6):587-99.

Zilles K, Wree A, Dausch N-D Anatomy of the neocortex: neurochemical organization. In: The cerebral cortex of the rat (Kolb B, Tees RC, eds). 1990: pp 113-150. Cambridge, Mass: MIT Press.

Zinke W, Roberts MJ, Guo K, McDonald JS, Robertson R, Thiele A. Cholinergic modulation of response properties and orientation tuning of neurons in primary visual cortex of anaesthetized Marmoset monkeys. Eur J Neurosci. 2006;24(1):314-28.