How is sound location represented in auditory cortex?

By Katherine Wood

Submitted in partial fulfilment for
the degree of Doctor of Philosophy

University College London

September 2015

Declaration

I, Katherine Wood confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis. It has not been previously submitted, in part or whole, to any university or institution for any degree, diploma, or other qualification. In accordance with the University College London guidelines, this thesis does not exceed 100,000 words, and it contains less than 150 figures.

Signed:………………………………………………………………………….

Date:…………………………………………………………………………….

Katherine C Wood

London

Abstract

The present work tested two competing hypotheses about how the location of sounds in space is encoded by auditory cortex. The labelled-line hypothesis says that each azimuthal location is encoded by maximal firing of a specific small and sharply tuned population of neurons. The two-channel hypothesis says that a sound location is encoded by the relative activity of two populations of neurons with broad tuning and maximal activity at ± 90°. To test these hypotheses a new behavioural task was developed in which subjects had to report the location of a target sound relative to a preceding reference. Models of the two-channel hypothesis and a modified version of the labelled-line hypothesis that accounted for better sound localisation precision at the midline, predicted best performance in the task around the midline with performance decreasing in the periphery whereas the labelled-line hypothesis predicted equal performance throughout space. Consistent with both the two-channel and modified labelled-line model, both ferret and human performance was best at the midline, highlighting the need for neural recordings in auditory cortex to distinguish between these models. The peaks of spatial receptive fields of neurons recorded from auditory cortex of ferrets performing the relative localisation task were distributed across the contralateral hemisphere, rather than clustered at 90° as predicted by the two channel model. Decoding of location from populations of neurons using two-channel or labelled-line maximum-likelihood decoders indicated that both decoders performed as well as ferrets localising sounds in the same testing chamber but that the labelled-line decoder out-performed the two-channel decoder. Finally, the necessity for an intact auditory cortex for sound localisation was confirmed after developing cortical cooling in the ferret as a method to reversibly silence areas of cortex during behaviour.

Contents

Acknowledgements

I would like to thank my supervisor, Jenny, for teaching me pretty much everything I know about auditory neuroscience and ferrets, for her patience with my many questions (some of which may have been repeated a few times!) and for her support throughout my time in the lab. I can’t really thank you enough so this is really just a tiny bit of what I am grateful for. I will miss our talks over lunches in the atrium and your home-baked goods! I would like to thank Stephen for all his help; I couldn’t have written this without your help and encouragement. I’ll miss our discussions over matlab code, Man United and other such delights! I look forward to having keyboards with all the keys… Thanks to everyone in the lab for enduring my endless requests for intricate details to be adhered to, for also enduring the pain that is cooling, for all the laughs we had and for making my whole experience of being part of our lab really enjoyable!

I would like to thank Dan for biscuits, beer and *essential* advice on what paper to print this on. Thanks to Andy for even more beer and your encouragement. Thanks to Roland for enduring my endless questioning of Jenny! Thanks to David McAlpine for advice and support during my time at the EI. Thanks to Nick Lesica for helping me with my decoders. Thanks to my fellow PhD students; Nicolas, Warren, Lorcan and Anahita, and everyone at the EI for good times in the pub and your help whenever I needed it. Thanks to my parents (Mum, Dad and Shelley) for supporting me in everything that I do (whether they think it is a good idea or not!) and for looking after me when I come home. Thank you to Rod for his support over the last two years, especially for looking after me during the write-up and for proof reading it (p.s.)! Finally, thanks to my ferrets; Sofia, Kerry, Ladybird, Junebug, Spider, Solero, Fab and Magnum, without their dedication to the cause none of this work would have been possible.

I would like to acknowledge UCL Grand Challenges for financial support of this work.

List of Tables

List of Figures

List of Abbreviations and Acronyms

2AFC	two-alternative forced choice
A1	primary auditory cortex
A2	secondary area of cat auditory cortex
AAF	anterior auditory field
AC	auditory cortex
AEG	anterior ectosylvian gyrus
AES	anterior ectosylvian sulcus
ANF	auditory nerve fibre
ANOVA	analysis of variance
AVCN	anteroventral cochlear nucleus
BBN	broadband noise
BPN	band-pass noise
DCN	dorsal cochlear nucleus
DNLL	dorsal nucleus of the lateral lemniscus
DZ	dorsal zone
GABA	gamma-Aminobutyric acid
HEM	hemispheric two-channel model
HPN	high-pass noise
HRTF	head-related transfer function
IC	inferior colliculus
ILD	interaural level difference
IPD	interaural phase difference
IR	infra-red
ITD	interaural time difference
ISD	inter-stimulus duration
LED	light emitting diode
LL	labelled-line model
LNTB	lateral nucleus of the trapezoid body
LPN	low-pass noise
LSO	lateral superior olive
MAA	minimum audible angle

MEG	medial ectosylvian gyrus
MI	mutual information
MNTB	medial nucleus of the trapezoid body
MSO	medial superior olive
NB	narrow-band
OPP	opponent two-channel model
PAF	posterior auditory field
PEG	posterior ectosylvian gyrus
PSTH	post-stimulus time histogram
RAF	rate-azimuth function
RM	repeated measures
SC	superior colliculus
SD	standard deviation
SEM	standard error of the mean
SNR	signal-to-noise ratio
SRF	spatial receptive field
SSY	suprasylvian cortex

List of Appendices

Chapter 1: Introduction

This thesis presents an investigation into the neural representation of sound location in auditory cortex. The introduction describes the processing of information prior to auditory cortex with respect to sound location and then goes on to discuss relevant work that has been performed in auditory cortex. Following the discussion of the literature, the remaining questions are outlined and the aims of this project defined.

1.1 Sound localisation

The ability to localise a sound has important survival value for both prey and predator; whether it is to pinpoint or track the source of a sound (e.g. locating prey) or to segregate important sounds from irrelevant background sounds (e.g. detecting a predator). Sound is a percept arising because of physical vibrations travelling through a medium, e.g. air. These vibrations, sound waves, enter the ear canal where they in turn vibrate the tympanic membrane causing the bones of the middle ear to vibrate. The bones of the middle ear contact the oval window which transfers the energy into the cochlea, the sensory organ of the ear. The cochlea contains the basilar membrane, along the length of which runs the organ of Corti which contains the hair cells that convert the physical vibration of a sound into a chemical signal. The hair cells synapse onto auditory nerve fibres that convey the signal to the central nervous system. The basilar membrane is tuned to different sound frequencies as a result of variation of mass and stiffness along its length from base to apex. The basal end encodes high sound frequencies and the apical end, low frequencies. The basilar membrane thus performs frequency decomposition since different sound frequencies entering the cochlea will vibrate the basilar membrane maximally at different points. Sensory hair cells positioned along the basilar membrane are thus tuned to different sound frequencies according to their location. Sound is therefore initially represented in the periphery according to its frequency. This is quite unlike other sensory systems such as vision and touch where the sensory neurons have *spatial* receptive fields and form a topographic representation of the external world. Consequently, the brain must compute the spatial location of sound from cues it has available to it. This thesis will investigate how the brain achieves this, primarily focusing on the coding of sound location in auditory cortex both in humans, and an animal model (the ferret) in which it is possible to directly correlate behaviour and neural firing. Before considering the experiments performed in this thesis I will review our current understanding of how the location of a sound source is computed by the brain.

1.2 Spatial location cues

There are two main types of cue which provide information about sound location; monaural, caused by interaction of sound with the external ear, head and body and binaural cues, which result from the physical separation of the ears. At the end of the 19th Century, Lord Rayleigh investigated localisation abilities of human listeners and described the use of binaural cues for localising stimuli. Using a pair of mistuned tuning forks he showed that human listeners were sensitive to the ongoing phase of low frequency sounds and differences in the timing of sounds arriving at each ear. He also demonstrated that for high frequency stimuli the difference in sound pressure level at each ear could be used to localise stimuli (Rayleigh, 1907). This understanding of using phase differences for localising low frequency sounds and level difference for high frequencies became known as Rayleigh’s Duplex Theory and was further corroborated in later experiments (e.g. Stevens and Newman, 1936). When testing localisation of tones by humans, Stevens and Newman (1936) showed high accuracy for tones with frequencies below 1.5 kHz and above 5 kHz with a less accurate performance between these frequencies, which suggested a ‘transition’ between two localisation mechanisms.

Lord Rayleigh also appreciated that listeners could not distinguish whether a pure tone stimulus was in front of or behind the listener but that for sounds ‘of character’ (i.e. with a larger bandwidth than a tone) this front-back discrimination was possible. Subsequent work has revealed that high frequency sounds interact with the external folds of the ear leading to modification in the spectra of the sound source. Since the modification at each will be dependent on the shape of the ear in question, the changes are termed monaural cues since only one ear is necessary for them to occur. These spectral changes, largely generated by interactions with the pinna and concha of the external ear, are characterised by the head-related transfer functions (HRTFs), which describe the space-dependent frequency filtering. Characteristic ‘notches’ in the spectrum of sounds entering the ears give cues as to the location of the sound source. The frequency and magnitude of the notch varies with sound source location in azimuth and, in particular, elevation, providing the brain with monaural cues which help to locate sounds in space and disambiguate front-back discriminations (Parsons et al., 1999; Wightman and Kistler, 1999; Grothe et al., 2010; Schnupp et al., 2011). Since these spectral cues are imposed by the external ear, there is ambiguity regarding the origin of the spectral features; the brain does not ‘know’ if the notches are part of the sound or if they are imposed by the filtering of the ear. Indeed, the perceived location of a narrowband sound alters with changes in the centre frequency (Musicant and Butler, 1985). Thus in order to provide reliable spatial cues, the spectrum of sounds must be relatively flat, familiar to the listener or comparable at the two ears (Schnupp et al., 2011).

In humans, this direction-dependent filtering of incoming sounds similarly imposes a pattern of peaks and notches on the spectra of sounds above ~4 kHz (Middlebrooks, 2015). The importance of spectral cues to human localisation has been demonstrated by filling the ears of listeners with moulds and observing the resulting decrease in localization ability, especially in the vertical plane (Musicant and Butler, 1984). Although spectral cues do vary with azimuth, when judging the location of a sound in the horizontal plane, binaural cues dominate perceived location (Macpherson and Middlebrooks, 2002). Humans and animals can learn over time to localise sounds accurately with ‘new’ spectral cues, e.g. with moulds in the ear. Upon removal of the moulds spatial hearing returns to normal, and, with replacement of the moulds after some time, localisation is as good as was learned with the moulds originally (reviewed by Carlile, 2014). This suggests that spectral cues must be learned and are specific to each individual but with general characteristics which are similar between listeners owing to similarities of ear shape.

As discovered by Rayleigh (1907), binaural cues depend on having two ears and being able to compare the sound waveform at each ear. The geometry of the head and physical separation of the ears create differences in the timing, angle and loudness of a sound arriving at each ear. The difference in the time of arrival of a sound source at the two ears is called the interaural time difference (ITD). ITDs for different frequencies vary consistently across space, with the maximum time difference occurring when the relative distance from the sound source to each ear is greatest (King et al., 2001). The maximum ITD physiologically available to humans is around 700 µs. Animals with smaller heads will experience a smaller range of ITDs, because their ears are closer together. The ITD of stimuli for human listeners can be calculated by assuming the head with a rigid sphere with ears at antipodal points on it (the Woodworth formula) and this method has been empirically shown to estimate well the ITDs caused by clicks and is physically valid when the wavelength of the stimulus is much shorter than the radius of the head, roughly >4 kHz in air (Woodworth and Schlosberg, 1962; also see discussion in Aaronson and Hartmann, 2014). However, for stimuli with frequencies below this the estimation is poor. Kuhn (1977) empirically showed that for stimuli below 500 Hz, ITD can be calculated from the low-frequency limit formula originally described by Lord Rayleigh. For sounds above this frequency simple corrections can be made to the Woodworth formula, for sounds <2 kHz a factor of 3 is required, while for sounds higher in frequency a factor of 2 is required (Kuhn, 1977).

An interaural level difference (ILD) results from the difference in intensity of a sound arriving at each ear. ILDs change considerably with wavelength because sounds with long wavelength (low frequency) propagate around the head with little interference whereas those with higher frequencies are diffracted by the head and torso to the opposite side (which is located in the ‘acoustic shadow’), resulting in an intensity/level difference between the two ears. In humans, ILDs only become reliable localisation cues at frequencies above 5 kHz (Middlebrooks and Green, 1991). However, significant ILDs can occur for low frequency sounds located in the near field (Coleman, 1963) and ITD sensitivity can be conveyed by the envelope of complex high frequency sounds (Bernstein, 1985). Thus the duplex theory may describe the frequency dependence of the binaural cues rather than describe two distinct channels (Grothe et al. 2010). The relative weighting of the cues for forming a perception of space can change depending on the reliability of the cue for a given task (Van Wanrooij and Van Opstal, 2007). ILDs and spectral cues experienced by each listener will vary depending upon their external ear and head/torso characteristics; they can be estimated by empirically measuring the ILDs and spectral cues experienced by listeners by placing microphones inside the ear and recording the transformation of sounds presented from known locations, these are the head-related transfer functions (e.g. Shaw and Vaillancourt, 1985).

When investigating the neural mechanisms underlying sound localisation in mammals it is important to consider how the use of binaural cues may have evolved. It has been shown that mammalian tympanic hearing evolved separately from, and later than, tympanic hearing in sauropsids (including birds and reptiles) and Anura (frogs) (reviewed in Grothe and Pecka, 2014). At the time when tympanic hearing arose in sauropsids, these animals were very large and therefore able to take advantage of large interaural time differences and their middle ear bones were of a size and mass appropriate to take advantage of low frequency sounds. Mammalian ancestors on the other hand, were very small when the first tympanic ears arose. Because of their size they would have experienced very small ITDs whereas at higher frequencies they experienced very large differences in the level of sound between the two ears. Their middle-ear was very small with a low mass more suited to high frequency sound conduction. Low frequency hearing has since evolved in some mammals and its encoding is likely to have been shaped by what was already present for extracting binaural cues i.e. ILD extraction (Grothe et al., 2010; Grothe and Pecka, 2014). Indeed it has been found that birds encode sound location in a different way from mammals (Schnupp and

Carr, 2009) in that they appear to have neurons with sharp tuning for ITDs within the physiological range of ITDs that they experience (Knudsen and Konishi, 1978), whereas in mammals, tuning for ITDs appears to be broad with best ITDs that are not within the physiological range (McAlpine et al., 2001; Brand et al., 2002), although this finding has recently been questioned (Franken et al., 2015).

1.3 Brainstem processing

I will now discuss how these cues are extracted and processed in the brainstem. Auditory nerve fibres (ANFs) receive input from sensory hair cells in the cochlea and carry it to the cochlear nucleus in the brain. The ANFs of mammals can phase-lock their firing to the fine structure of simple periodic stimuli (pure tones) up to ~3 kHz whereas in the barn owl phase-locking can occur up to 9 kHz (Koppl, 1997; reviewed in McAlpine, 2005). Upon entering the cochlear nucleus the ANFs divide into ascending and descending branches. The ascending branch has strong connections with spherical and globular bushy cells in the antero-ventral cochlear nucleus (AVCN). Bushy cells are specialised for extracting binaural cues; they can also phase-lock to the fine structure of sound and to the envelope of high-frequency sounds. The bushy cells target the superior olive where the comparisons underlying binaural processing occur; outputs from here target the lateral lemniscus and the inferior colliculus (IC). The descending branch of the ANFs carries information to the dorsal cochlear nucleus (DCN) which is involved in extracting spectral cues and from here projections go to the ipsilateral lemniscus or directly to the contralateral IC (Grothe et al., 2010, Schnupp et al., 2010). See Figure 1.1 for a simplified diagram of connections in mammalian ascending auditory pathway.

1.3.1 Spectral cues

The neurons of the DCN appear to be specialised for the processing of spectral cues and are exquisitely sensitive to spectral notches/troughs in the spectra of incoming wide-band sounds containing high frequencies (Nelken and Young, 1994; reviewed by Grothe et al., 2010). Notches such as these

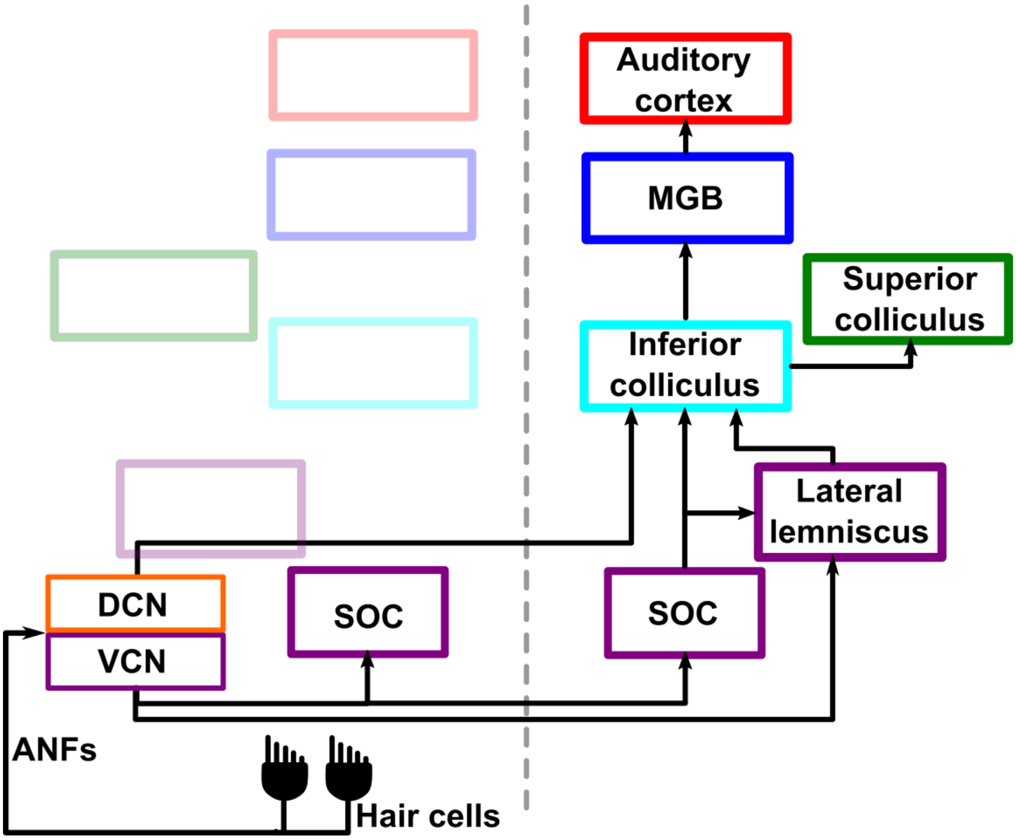


Figure 1.1- Simplified ascending auditory pathway up to auditory cortex for one hemisphere. ANF: Auditory Nerve Fibre, DCN: Dorsal Cochlear Nucleus, VCN: Ventral Cochlear nucleus, SOC: Superior Olivary Complex (contains Lateral and Medial Superior Olive (LSO, MSO), MGB: Medial Geniculate Body. Adapted from Grothe et al. (2010).

are introduced by interaction of the sound with the external ear and pinna (hence why high notches are only introduced in sounds containing energy at high frequencies) and vary in their frequency location in the spectrum according to the azimuth and elevation of the stimulus (Musicant, 1990). These frequency notches feature prominently in cat head-related transfer functions (HRTFs) (Rice et al., 1992), where their frequency location varies systematically with horizontal and vertical angle of the sound source. The location of the notches depends upon the azimuth and elevation of the sound. For example, for a sound of a 15° azimuth, a change in elevation of the stimulus from -15° (below the cat) to +30° shifts the frequency location of the first visible spectral notch from approximately 10 kHz to approximately 12.5 kHz. Combining this information from both sides of the brainstem could be sufficient to localise broadband sounds in the frontal hemisphere in the cat (May, 2000). Although humans also have a similar, if less well-defined, relationship of notch location and sound location (Musicant, 1990), work has shown that spectral cues, such as notches, are mainly used to determine the elevation of a stimulus and binaural cues are used to determine azimuthal location (Macpherson and Middlebrooks, 2002). Cells in the IC that receive a direct projection from contralateral DCN show excitatory responses selective for the centre frequencies of these introduced notches (Nelken and Young, 1994; Grothe et al., 2010; Middlebrooks, 2015).

1.3.2 Interaural level difference

The firing rates of ANFs increase with increasing sound level, therefore ILDs will reach the brain as a difference in firing rates from the left and right ears. ILD processing requires comparisons of the mean firing rates of these high frequency nerve fibres, essentially subtracting one side from the other. This comparison occurs in the lateral superior olive (LSO). Neurons in the LSO are biased toward high frequencies despite being tonotopically organised. They are excited by the ipsilateral ear and inhibited by the contralateral ear and are hence known as ‘IE’ neurons. Excitation comes directly from spherical bushy cells in the ipsilateral AVCN. Glycinergic inhibition originates in the contralateral medial nucleus of the trapezoid body (MNTB), which in turn receives its input from the contralateral AVCN (Schnupp et al., 2010). A sound from directly in front would not cause a strong response in an IE neuron because the sound intensity at each ear would be equal. However, if the sound moves to the ipsilateral side sound intensity in the that side will be higher than the contralateral side, thereby decreasing inhibition from the contralateral side and increasing excitation on the ipsilateral side, which causes the LSO neuron to respond more strongly, and vice-versa if the sound moves contralaterally (Figure 1.2). In this way a rate-code is established for sound source location. This rate code would be relatively insensitive to changes in overall intensity since activity will increase by the same amount in both sides cancelling out any increase in excitatory/ inhibitory input. Most LSO neurons will be completely inhibited when an ILD occurs favouring the contralateral ear and are fully activated when the ILD favours the ipsilateral ear, their ILD-spike rate functions therefore look sigmoidal which generates high sensitivity for small changes in ILD along the slope of the function (Grothe et al., 2010). The peak and slopes of these LSO spatial tuning functions are highly plastic and are affected by preceding activity levels; thus representations of spatial locations change in accordance with the context in which they are presented (Grothe et al., 2010). LSO IE neurons send excitatory projections to the contralateral IC. There is also a smaller glycinergic inhibitory projection direct to the ipsilateral IC from the LSO and a contralateral projection to the Dorsal Nucleus of the Lateral Lemniscus (DNLL), which in turn sends GABAergic bilateral inhibitory projections to the IC (Glendenning et al., 1992).

1.3.3 Interaural time difference

Initially, temporal features are encoded by the phase-locked firing of the ANFs, which are tuned to relatively narrow frequency bands according to the location of their inputs along the length of the basilar membrane. ITDs are often extremely small and any jitter in the phase-locked spike trains could be very bad for signal quality. Excitatory projections from the cochlea via ANFs to the AVCN bushy cells operate via an unusually large and temporally precise synapse called the endbulb of Held. At these synapses, a single

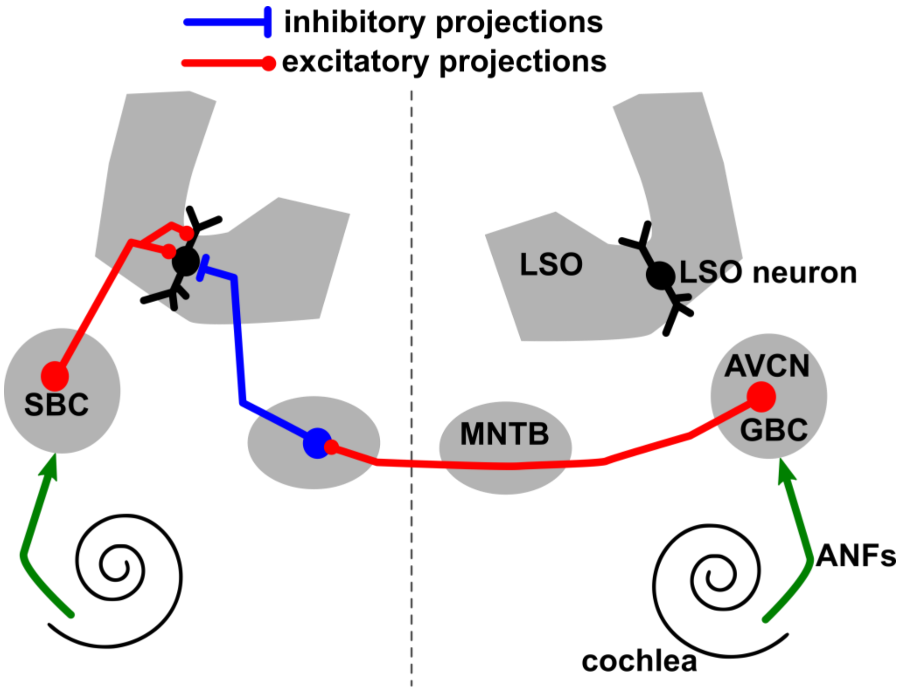


Figure 1.2 – ILD processing in the brainstem. LSO neurons receive excitatory input from spherical bushy cells (SBC) in the ipsilateral Antero-Ventral Cochlear Nucleus (AVCN) and glycinergic inhibitory inputs from the ipsilateral Medial Nucleus of the Trapezoid Body (MNTB) which itself is innervated by globular bushy cells (GBC) from the contralateral AVCN. The AVCN receives its input from the Auditory Nerve Fibres (ANF). Adapted from Grothe and Pecka (2014).

presynaptic spike is enough to trigger a spike in the post-synaptic bushy cell ensuring that no spikes are lost and the phase-locking to the fine structure is maintained. The result is that spherical bushy cells actually fire with a greater temporal precision than their inputs (Joris et al., 1998).

The question remains of how interaural differences are translated to a spatial location by the brain. In the case of ITDs, for many years one model has dominated the thinking of how this calculation occurs, the Jeffress model (Jeffress, 1948). The Jeffress model proposes that a topographic map of space is formed by a population of neurons acting as coincidence detectors that fire only when they receive simultaneous input from both ears. In this model, axons from each AVCN vary in length or conduction velocity so that they form ‘delay lines’ to their targets, neurons in the medial superior olive (MSO). Only when inputs from each AVCN coincide are the MSO neurons activated, in this manner the activity of neurons in the MSO varies systematically with spatial location, thereby forming a ‘place code’, or topographic map (Figure 1.3). Such a topographic map has been identified in the barn owl brain stem auditory nucleus (Nucleus Laminaris), where axonal delay lines and coincidence detectors have been observed (Carr and Konishi, 1990). However in mammals, differences in the encoding of ITDs appear to contradict a topographic representation of ITDs in the brainstem of mammals.

Ipsilateral and contralateral spherical bushy cells send excitatory projections to the medial superior olive cells (and cells receiving these inputs are hence known as ‘EE’ neurons). Best frequencies of MSO neurons are biased toward low frequencies (unlike the LSO which is biased to high frequencies). As in the Jeffress model, MSO neurons receive excitatory input from both ears but are a noisier form of coincidence detector than that proposed by Jeffress, firing when the phases of the volleys of inputs they receive from each ear overlap, they thus act as ‘cross-correlators’ (Yin and Chan, 1990). Contradicting the Jeffress model, MSO neurons also receive glycinergic inhibitory inputs from the ipsilateral lateral nucleus of the trapezoid body (LNTB) as well as inhibition from the ipsilateral MNTB via the contralateral

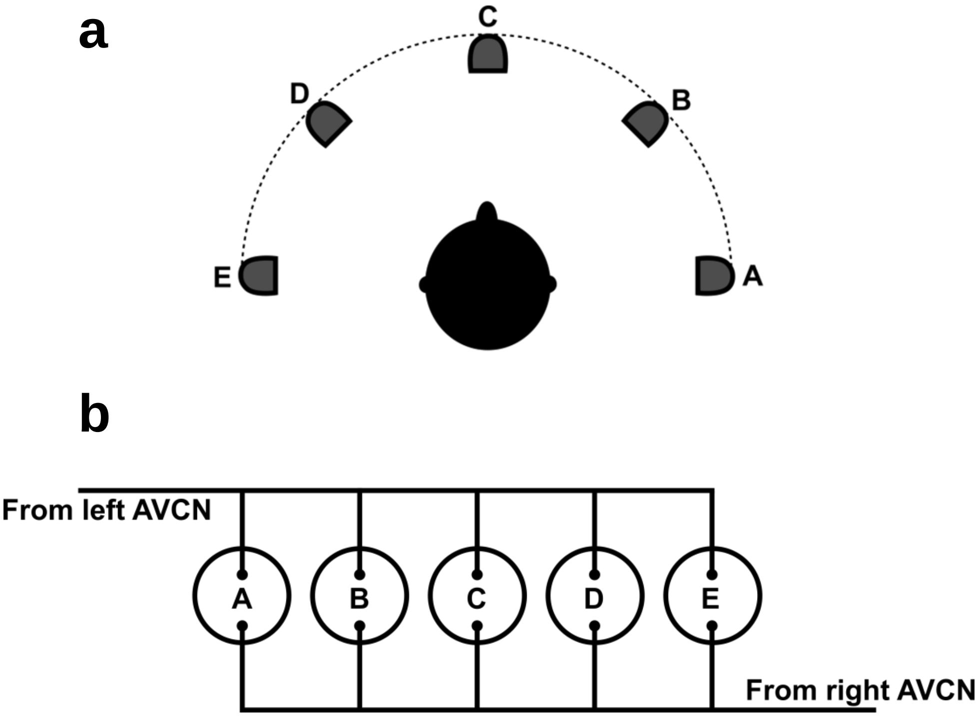


Figure 1.3 – The Jeffress model. In [a] a person is shown surrounded by 5 speakers in azimuth. They can receive auditory input from 5 locations, indicated by the speakers labelled A-E. [b] represents 5 neurons in the MSO that receive input from the left and right AVCNs. The delay of the neural input is indicated by the length of the connection from each AVCN to each neuron and the pattern of delays mean that each neuron indicated would be tuned to the location as labelled A-E. For example, if a sound were to presented from speaker A, the sound waves arrive at the right ear first thus action potentials from the right AVCN would be fired before those in the left. Therefore, those from the right must be delayed longer than those on the left in order to coincide at one of the MSO neurons (labelled A).

globular bushy cells (Figure 1.4). The main purpose of the inhibition from the MNTB appears to be to tune the best delays of binaural excitation by modulation of the time window for integration of binaural inputs (Pecka et al., 2008). The ipsilateral source of inhibition via the LNTB has also been shown to modulate the timing of binaural coincidence (Grothe and Pecka, 2014). Often the best ITDs of MSO neurons fall outside of the physiological range of the animal which is again inconsistent with a topographic encoding of space where the peak firing rates should be distributed across the physiological range, not outside it. The results of the peak locations being located outside the physiological range is that the slopes of the functions actually fall across the midline where perception of changes in ITD is maximal (Mills, 1958). This is consistent with a rate code of ITDs. However, other studies have suggested that in the gerbil, there are ITDs within the physiological range; van der Heijden (2013) found that 57% of the units they recorded from the gerbil MSO had a best ITD within the physiological range (also Franken et al., 2015).

Preceding activity can affect the ITD tuning of MSO neurons, specifically with a laterally placed adapting sound causing a shift in the best delay towards the adapted side. This would shift the steepest point of the ITD function slope away from the midline. This shift, measured in gerbil MSO and caused by $GABA_B$-receptor mediated feedback loop, can predict changes in perception of sound location resulting from lateral adapters in humans (Stange et al., 2013). Assuming a two-channel-like model where the slope of the ITD functions recorded from neurons in MSO crosses the midline and the peaks of the functions lie outside of the physiological range, an adaptor caused the crossover point of the two slopes for left and right hemisphere MSO (which are contralaterally tuned) shifted towards the lateral adapter. This predicts that the perceived midline (predicted to be the cross-over point) would shift towards the adapted side. Stange et al. (2013) showed that humans reported the perceived location of a test tone as more peripheral in the direction of the adapter compared with no adapter, consistent with a shift in the perceived midline towards the adapter.

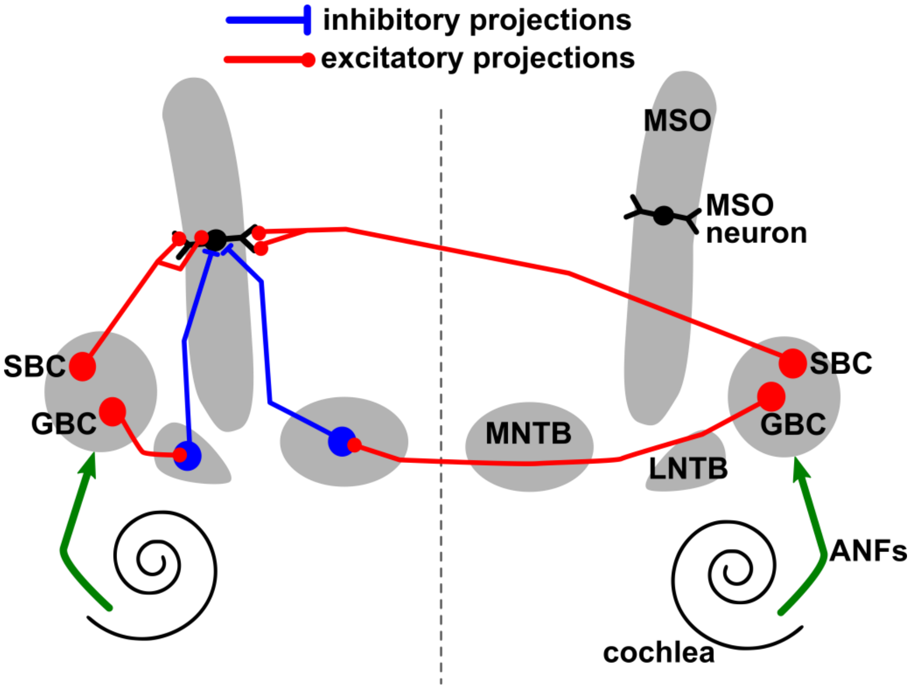


Figure 1.4 – ITD processing in the brainstem. Medial Superior Olive (MSO) neurons receive excitatory input from the ipsilateral and contralateral spherical bushy cells (SBC) in the AVCN. They also receive glycinergic inhibitory input from the ipsilateral Lateral Nucleus of the Trapezoid Body (LNTB), which is innervated by the ipsilateral globular bushy cells (GBC) of the Antero-Ventral Cochlear Nucleus (AVCN). The MSO neurons also receive inhibition from the ipsilateral Medial Nucleus of the Trapezoid Body (MNTB) which is innervated by the contralateral GBCs. Adapted from Grothe and Pecka (2014).

From an evolutionary perspective, ITD sensitivity is likely to have been favoured in response to increased body and head size of mammals resulting in larger interaural distance and larger larynx. It has been shown that low frequency LSO neurons are sensitive to changes in ITD, indicating that the precision of the LSO processing is good enough for detection of ITDs. In light of this, Grothe et al. (2014) suggest that ITD processing is likely to be a refined version of the LSO/ILD circuitry and therefore encoded more like a rate code than a topographic encoding. Furthermore, mammals with good low frequency hearing usually possess a large low-frequency area of the LSO as well as a well-developed MSO (Grothe et al., 2010) which potentially contributes to the spatial localisation of auditory stimuli. The output of the MSO to the midbrain is mainly excitatory and ipsilateral to the inferior colliculus (IC) (McAlpine, 2005, Schnupp et al., 2010).

1.3.4 Inferior colliculus

Pathways from the LSO, MSO and DCN converge at the IC (reviewed by Schnupp et al., 2011). Excitatory projections from the MSO and the LSO are kept separate while the LSO and DCN projections converge providing the basis for combining two forms of localisation cues, ILD and spectral cues. Ipsilateral inhibitory projections from the LSO overlap with the excitatory projections from the MSO, thus it is not surprising that IC neurons are generally sensitive to more than one localisation cue. Chase and Young (2008) discovered that different coding mechanisms were used to represent the different aural cues. They showed that localisation cues converged on individual cells in the IC to different degrees and information about the different cues was contained in varying amounts in the spike rate, latency of first spike and on-going spike timing, thus providing a way of combining information about location from different sources whilst keeping independent representations (Chase and Young, 2008). This convergence occurs when different localisation cues appear within the frequency response area of the IC neuron in question (Slee and Young, 2011). Neurons in the IC show sensitivity to sound features not found at earlier stages of the processing hierarchy; for example, neurons in the IC are sensitive to simulated stimulus motion created by varying the phase of the stimulus (Spitzer and Semple, 1993; McAlpine et al., 2000). McAlpine et al. (2000) argued that this directional sensitivity arose as a result of the response history of the IC neuron in question; thus, motion sensitivity in the IC may be the result of a non-specific adaptation mechanism. In a further study using free-field apparent motion they concluded that, despite the receptive fields of IC neurons shifting toward the direction of motion, there was no evidence of selectivity for motion direction or velocity (Ingham et al., 2001). However, direction selectivity has been found in the barn owl External Nucleus of the inferior colliculus, in the nucleus laminaris (Wang et al., 2014), so it is possible that somewhere in the mammalian auditory pathway selectivity to direction of motion will arise. Thus the IC is a key processing nucleus for sound localisation and dynamic localisation cues. Neurons from the IC project to the auditory cortex via the MGB, an important relay centre that sends projections to many areas of auditory cortex and receives feedback from the auditory cortex. The IC also sends projections to the superior colliculus where a topographically organised map of auditory space has been identified (Palmer and King, 1982). The map of space here lines up with topographic maps of space representing the visual and somatosensory fields. The SC is involved in reflexive movement of the head and eyes to objects of interest (Lomber et al., 2001).

1.4 Labelled-line and two-channel models

The topographic model, as originally proposed by Jeffress (1948) states that an internal map of sound location in space is formed in the brain, this type of map has not been observed in mammals except in the SC of cats, ferrets and guinea pigs (Palmer and King, 1982; Middlebrooks and Knudsen, 1984; King and Hutchings, 1987). The Jeffress model reduces the tuning functions of individual neurons to the location of their peaks within the map. The present study tests the labelled-line model which takes advantage of the both the shapes of individual tuning functions and the distribution of peaks across the population. In this model, each azimuth is represented by the pattern of activity across the population. For any source location, the pattern of activity across the population of neurons will be distinct from the patterns of activity evoked by sources at other locations due to heterogeneous tuning. The model assumes then that at some higher level there is a layer of neurons that can assimilate this information and provide a representation of the azimuth experienced. In a labelled-line code multiple neurons with broad tuning like those that would be observed with the two-channel model would be useless whereas the tuning heterogeneity found in a labelled-line code would degrade a population rate-based code.

Despite the discovery of a Jeffress-like coding strategy in the Barn Owl midbrain, little evidence has been found for a similar strategy in the mammalian midbrain (reviewed by Grothe et al., 2010). McAlpine et al. (2001) showed that neurons in the guinea pig IC have maximum firing rates at ITDs too large to be physiologically relevant to a guinea pig; these neurons are tuned to ITD locations that do not exist. They also found that the best ITD tuning varies with each neurons' preferred sound frequency; the lower the characteristic frequency the larger the best ITD. This is hard to reconcile with the idea that ITDs are represented in a place code since ITDs should vary across rather than with the tonotopic axis (McAlpine et al., 2001). They therefore proposed a new model, where sound location in the IC is indicated by comparing the activity of two broadly tuned populations of neurons on each side of the brain, the hemispheric two-channel model.

Neurons in the MSO, which project to the IC, can phase-lock to the fine structure of low frequency sounds up to ~1.5 kHz, so the longer period of lower frequency sounds will result in binaural cross-correlation at larger ITDs, thus explaining why best ITD varies with the tonotopic axis. This means that ITD tuning curves become broader at lower frequencies. However, barn owl auditory neurons can phase lock up to 9 kHz and interaural cross-correlation of such short periods in very high frequencies would lead to steep functions, with sharp peaks that lie within the physiological range of these birds thus making place coding efficient. A model which takes this information into account and is based on the principle that the most accurate coding would be the best found that the optimal coding strategy depended upon head size and sound frequency (Harper and McAlpine, 2004). For small head sizes and low frequency sounds, the optimal coding strategy tended towards two subpopulations of neurons tuned to ITDs outside the hearing range of the animal (in this case, cat and gerbil) with the slopes crossing the midline. When the method was applied to the barn owl, it resulted in a homogeneous distribution of neurons tuned to ITDs within the physiological range above 3 kHz. These results are consistent with experimental observations in gerbils and owls (Knudsen and Konishi, 1978; McAlpine et al., 2001; Brand et al., 2002; Konishi, 2003). In a follow up paper comparing the model to neural data they showed that the coding of spatial location may be more diverse than that postulated either by the topographic or by the two-channel model and that multiple codes are used within the same species depending on the stimulus frequencies (Harper et al., 2014). For mammals with small heads, distributions of best inter-aural phase differences displayed characteristics consistent with the two-channel model for coding of ITDs.

A key point of the two-channel coding strategy is that the steepest points of the functions lie across the midline, so a small change in location would result in a large change in the firing-rate of the two channels. This point of highest sensitivity corresponds to the highest point of perceptual sensitivity for a change in location of a sound (Mills, 1958). Dahmen et al. (2010) showed that changes in the cross-over point of broadly tuned neurons in the IC (in anaesthetised ferrets) could account for shifts in spatial perception of ILDs in human listeners. However, the precise mechanism of encoding of sound location in IC is still a matter for debate; Day & Delgutte (2013) found that location of sounds by ITD could better be decoded using a ‘pattern’ decoder than a decoder based on the two-channel model using spike rates recorded from awake rabbit inferior colliculus neurons. The pattern decoder takes into account the variability in the spatial tuning of each individual unit, unlike the two-channel model which uses the mean firing rate on each side of the brain. However, their findings were inconsistent when the models were compared with inactivation studies; Jenkins and Masterton (1982) showed that lesion of one IC resulted in deficits in localisation in contralateral space. Day and Delgutte found that the two-channel model was consistent with in that performance of the model decreased contralaterally when one channel was removed but with the labelled line decoder, all of space was still well represented when using neurons from one IC inconsistent with the inactivation studies. They suggest that coding of space ipsilateral to the lesion may be less reliable when other dimensions of the sounds are changing. Further work has indicated that the pattern decoder was also advantageous in more demanding listening conditions, such as with background noise (Goodman et al., 2013). Studies in anaesthetised gerbil IC have found that a two-channel decoder performs as well as a labelled-line decoder (Belliveau et al., 2014).

Studies in auditory cortex have found that the steepest point of rate-azimuth functions and ILD-rate functions falls on the midline, suggesting that this two-channel theory may be applicable in the auditory cortex (Stecker et al., 2005b; Campbell et al., 2006).

Although a topographic map of sound location is formed in the external nucleus of the inferior colliculus of barn owls (Knudsen and Konishi, 1978). It is also noted by Konishi (Konishi, 2003) that lesion of the map of space in the barn owl renders the animal incapable of localising sounds in the contralateral hemifield, however after some time, they are again able to localise sounds using an alternative pathway which appears to involve a pathway from the core of the IC to prefrontal areas via the thalamus where no map of space has been observed, thus the same animal can use a mapped or unmapped neural system to localise sounds, although ITDs are isomophically mapped in the nucleus laminaris (Carr and Konishi, 1990) and project topographically to the IC of the barn owl.

In summary, there is much evidence that isomorphic maps of auditory space do form in birds such as the barn owl and the chicken (Knudsen and Konishi, 1978; reviewed by Grothe et al., 2010) but that these maps are not strictly necessary for sound localisation (Knudsen et al., 1993). In the alternative pathway used by the barn owl to localise sound, neural tuning is within the physiological range but not organised in an isomorphic map. In mammals, no isomorphic maps of auditory space have yet been observed and tuning has often been observed that is broad and for ITDs, best delays have been observed outside of the physiological range (reviewed by Grothe et al., 2010) which led to the postulation of the two-channel model for coding of ITDs. More recent work however has revealed tuning to ITDs in mammals can occurs within the physiological range but not organised isomorphically, such tuning would degrade a two-channel model encoding of space (van der Heijden et al., 2013; Franken et al., 2015). Sharp but non-isomorphically organised tuning of neurons could be taken advantage of by a labelled-line type code and it has indeed been found that labelled-line decoders can report sound locations accurately (Day and Delgutte, 2013). If sound location can be decoded from neural responses in the IC, then what role does auditory cortex play? The next section will describe the role of auditory cortex in sound localisation as far as it is currently known.

1.5 Auditory cortex

The primate auditory cortex comprises multiple sound responsive regions which can be loosely grouped into three main areas; the core, the belt, which surrounds the core, and lateral to the belt, the parabelt (Figure 1.5). The core contains three tonotopically organised fields, which are characteristic of primary auditory cortex. Connectivity studies have shown that these regions are serially connected to their neighbours but not to further away regions, i.e. there is no connection from the core directly to the parabelt (Hackett, 2011). Core fields tend to respond well to pure tones and noise whereas the belt and parabelt areas tend to respond better to more complex stimuli. This is consistent with arrangements in the visual and somatosensory cortex, where the receptive fields of cortical neurons increase in complexity as one progresses from core to belt areas, demonstrating serial processing of information (Recanzone and Cohen, 2010). Areas of auditory cortex in other mammals such as the cat and ferret are thought to correspond to the core and second-order belt areas (core areas are highlighted in Figure 1.5; Bizley et al., 2005; Schnupp et al., 2011).

Projections from the parabelt to higher order cortical areas define the auditory dorsal and ventral processing streams. The dorsal stream projects

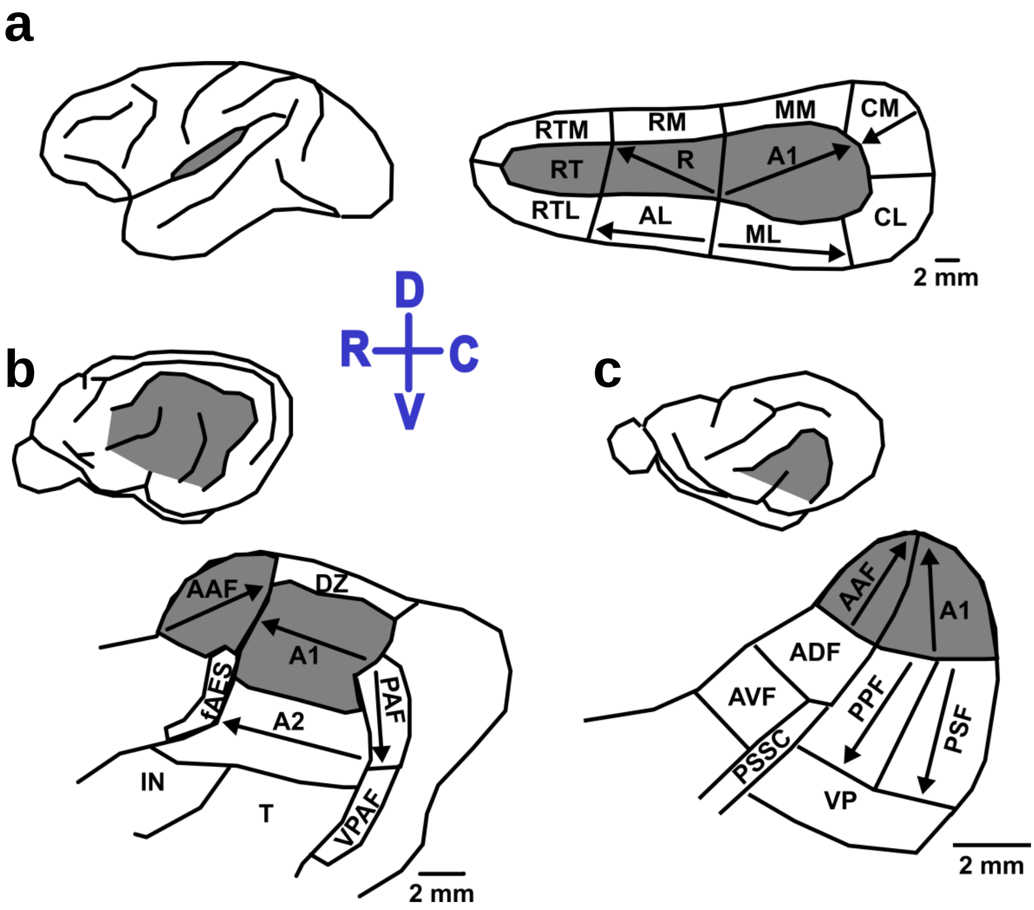


Figure 1.5 – Auditory Cortex in the Macaque, cat and ferret. [a] Macaque auditory cortex. The inset shows the location of the auditory cortex, and the schematic illustrates the location of identified auditory fields with core areas shaded in grey. The black arrows represent the tonotopic axis of fields with tonotopicity with the arrows pointing from low to high frequency. The blue letters in the middle represent anatomical direction (R = Rostral, C= Caudal, D = Dorsal and V = Ventral). [b] Cat auditory cortex. [c] Ferret auditory cortex. The scale bars each indicate 2 mm. A1: primary auditory cortex, R: rostral field, RT: rostral temporal field, CM: caudomedial belt, CL: caudolateral belt, ML: mediolateral belt, AL: anterolateral belt, MM: mediomedial belt, RM: rostromedial belt; RTM: rostrotemporal medial belt, AAF: anterior auditory field, PAF: posterior auditory field, VPAF: ventral posterior auditory field, A2: secondary auditory area, fAES: auditory field of the anterior ectosylvian sulcus, PSSC: pseudosylvian sulcal cortex, INS: insular, T: temporal region, PPF: posterior pseudosylvian field, PSF: posterior suprasylvian field, ADF: anterior dorsal field, AVF: anterior ventral field, VP: ventral posterior field (Adapted from Bizley and Walker, 2010).

to the parietal lobe and the ventral stream to the temporal lobe and prefrontal cortex. These parietal and prefrontal areas overlap with the visual dorsal (spatial/where) and ventral (non-spatial/what) processing streams respectively (Romanski et al., 1999), suggesting that auditory cortical processing could be segregated in the same way (Rauschecker and Tian, 2000). Further evidence for ‘what’ and ‘where’ processing streams came from Tian et al. (2001), who recorded neuronal activity from the lateral belt of anaesthetised macaques while they were being stimulated with species-specific vocalisations from different azimuths. They found neurons in the anterior belt were more specific for the type of call than those in the caudal belt, which showed greater spatial selectivity.

There is also evidence from cat and human studies to support these what and where streams (Clarke et al., 2000; Alain et al., 2001; Arnott et al., 2004; Lomber and Malhotra, 2008). A meta-analysis of fMRI studies on patients found that the majority of studies showed posterior activation in the auditory cortical areas when patients were performing a spatial task and anterior activation in non-spatial tasks (Arnott et al., 2004). However, recent work suggests that things may not be so simple, evidence for what and where streams may be an epiphenomenon caused by simplistic behavioural task designs. Michalka et al. (2015) show that spatial and temporal short term memory tasks recruit visual and auditory attention networks in the frontal lobe independently of the sensory modality of the task. There is also evidence from physiological studies in mammals that spatial information is distributed across all areas of auditory cortex (Harrington et al., 2008; Bizley et al., 2009).

1.5.1 Auditory cortex is necessary for sound localisation

Lesion studies in cats and primates have shown that the auditory cortex is necessary for sound localisation (Jenkins and Masterton, 1982; Heffner and Heffner, 1990). Heffner & Heffner (1990) tested the ability of Japanese macaques to perform a sound localisation task before and after bilateral ablation of the auditory cortex. They found the monkeys had deficits in localisation of sounds post-ablation (Heffner and Heffner, 1990). Lesions in cat and ferret auditory cortex also cause sound localisation deficits (Jenkins and Merzenich, 1984; Kavanagh and Kelly, 1987; Nodal et al., 2010).

Other studies have helped to reveal the relative involvement in sound localisation of different cortical areas. Some of the most compelling evidence for a spatial processing pathway comes from a series of studies by Lomber and colleagues who reversibly deactivated distinct sections of the cat auditory cortex with a cryoloop (Lomber and Payne, 1999; Malhotra et al., 2004, 2008; Lomber et al., 2007b; Malhotra and Lomber, 2007; Lomber and Malhotra, 2008). The cryoloop can be used to reversibly inactivate areas of brain through cooling the area down. Below 20°C neurons can no longer initiate action potentials effectively, thus the area of brain where neurons are cooled to below 20°C is inactivated. Upon warming, the area returns to normal function (Lomber and Payne, 1999; Coomber et al., 2011). In one study, they unilaterally inactivated thirteen separate areas of the auditory cortex finding that deactivation of A1 and dorsal zone (DZ), posterior auditory field (PAF) or anterior ectosylvian sulcus (AES) resulted in profound deficits in sound localisation ability in the contralateral field. Bilateral deactivation of the same areas caused bilateral deficits, although animals maintained the ability to distinguish sounds from the left and right (Lomber et al., 2007b). These studies indicate that although A1 is necessary for sound localisation, it is not sufficient; other areas of auditory cortex are also required. The role of other cortical areas in sound localisation is further supported by sharper spatial tuning seen in PAF and DZ of cat auditory cortex (Stecker et al., 2003, 2005a; Harrington et al., 2008).

Studies investigating the role of auditory cortex in sound localisation in the ferret have assessed the contribution of auditory cortex to both reflexive sound orienting behaviour and approach-to-target behaviour, which is thought to require an accurate perception of the location of the sound source in the ‘external world’. It appears that primary auditory cortex is not necessary for head orienting localisation responses but is necessary for approach-to-target localisation in ferrets whereas larger areas of cortex, including secondary areas, appear necessary for both behaviours (Smith et al., 2004; Nodal et al., 2010, 2012). Projections from auditory cortex to the superior colliculus (SC) are mainly ipsilateral and from secondary areas (Bajo et al., 2010; Chabot et al., 2013) and the SC is necessary for acoustic head-orienting responses (Lomber et al., 2001). This suggests that some sound location information may feed back from the auditory cortex into the orienting response driven by the SC. Interestingly, deactivation of the SC contralateral to an extensive unilateral lesion of auditory cortex can restore orienting localisation ability in space contralateral to the cortical lesion (Lomber et al., 2007a). Auditory cortex is also necessary for plasticity of sound localisation ability; feedback connections form AC to IC are necessary for learning new localisation cues (caused by the presence of an earplug) but not for localising locations already learned (Bajo et al., 2007).

There are also lesion studies to suggest that cortex is necessary for spatial perception of sound in humans (Clarke et al., 2000; Zatorre and Penhune, 2001). Zatorre and Penhune (2001) showed that, for humans, there appears to be a deficit only for right sided ablations and little change in localisation for left-sided ablations of auditory cortex. Some patients with larger left hemisphere lesions showed a bilateral localisation deficit. This highlights the difficulty of pinpointing exact lesion locations and differences in the way the brains of individual patients have compensated (or not) for lesions, so making studies of patients difficult to interpret. A recent MEG study indicated the opposite finding to that of Zatorre and Penhune (2001); that the left side of auditory cortex appears to encode all of auditory space whereas the right side encodes mainly contralateral space (Salminen et al., 2010a). The reality is probably something much more complicated. It is important to remember that areas of the auditory cortex may only be part of the processing required to perform a sound localisation. Auditory cortex sends projections both onward to prefrontal or parietal cortex where spatial location may in fact be decoded (Romanski et al., 1999) and back down to sub-cortical processing areas (Bajo et al., 2007, 2010). So the cortex appears *necessary* for sound source localisation but how do neurons there encode locations of sounds in space?

1.5.2 What features of neural firing contain information about spatial location?

There appears to be no Jeffress-like place map of auditory space in the mammalian subcortical auditory pathway (except for in the SC) and the search for one in the auditory cortex has also yielded little evidence. Similar to the mammalian IC, spatial tuning in mammalian auditory cortex is very broad, often encompassing an entire hemifield. Broad spatial receptive fields have been demonstrated in neurons in A1, PAF, AAF, AES and DZ in anaesthetised cats (Middlebrooks and Pettigrew, 1981; Middlebrooks et al., 1994; Brugge et al., 2001; Stecker et al., 2003, 2005a; Harrington et al., 2008), A1 of anaesthetised ferrets (Mrsic-Flogel et al., 2005; King et al., 2007) and A1, CM and CL fields of primates (Recanzone et al., 2000; Woods et al., 2006). In keeping with unilateral inactivation studies, the broad tuning of units in auditory cortex tends to towards the contralateral hemisphere, although some units are found tuned to the ipsilateral hemisphere (Stecker et al., 2005b). Typically spatial receptive fields increase in size with an increase in intensity (Middlebrooks and Pettigrew, 1981; Mrsic-Flogel et al., 2005; Woods et al., 2006), which is important in view of human ability to localise a sound being relatively invariant to changes in level (Macpherson and Middlebrooks, 2000).

Spike rate is commonly used to estimate the spatial tuning of neurons (e.g. Woods et al., 2006) however, information about sound location is carried not only in spike rate but also in the latency and pattern of firing (Middlebrooks et al., 1994, 1998; Brugge et al., 1996, 2001; Furukawa and Middlebrooks, 2002; Stecker and Middlebrooks, 2003; Nelken et al., 2005). Middlebrooks et al. (1994) trained an artificial neural network to classify spike patterns of single neurons in cat AES in response to varying sound location and found that spike patterns carried more information than spike counts alone. Furthermore, Nelken et al. (2005) found that spike counts and mean response times in ferret A1 essentially carried all the information about the stimulus location and together carried as much as the ‘full spike pattern’. Thus both spike count and temporal information could be important for perception of sound location (Middlebrooks et al., 1994, Nelken et al., 2005).

On the other hand, a study of macaque auditory cortical neurons indicates that spike rate alone contains enough information to account for human psychophysical results in sound localisation (Miller and Recanzone, 2009).

Several studies in cats and non-human primates have shown that there is more information contained in firing patterns of non-primary areas of auditory cortex than in A1 (Stecker et al., 2003, 2005a; Woods et al., 2006; Harrington et al., 2008). For example, Stecker et al. (2003) found that neurons in the PAF of cats have spike latencies longer and more strongly modulated by stimulus azimuth than neurons in A1. Neurons in the PAF contain more information about the stimulus azimuth than A1 and this information is contained in the pattern of the spikes. They also show that neurons in the PAF are more tolerant to changes in intensity than those in A1, particularly when considered in ensembles of neurons as opposed to individually.

Until recently, the majority of studies in this area have been on anaesthetised animals, and thus results should be interpreted carefully. In awake cats increased intensity tolerance has been demonstrated in A1 neurons (Mickey and Middlebrooks, 2003). Spatial tuning was also sharper in awake cats but still spanned close to a hemifield. Studies in anaesthetised animals have found that first-spike latency is important for transmission of information about sound location (Brugge et al., 1996; Furukawa and Middlebrooks, 2002), however, in the absence of anaesthesia spike rate appeared to contribute more information than first spike latency but the temporal pattern was still important since only 50% of the information about sound location remained after spike timing was removed (Mickey and Middlebrooks, 2003) .

A study by Lee and Middlebrooks (2011) revealed that active listening (during a non-spatial task) and an active localisation task sharpened the spatial tuning of neurons in A1 compared with passive listening. They used an elegant behavioural paradigm which enabled them to compare neural responses to different sound locations while the cat was attending to the location, attending to sound timbre but not to the location, and in an idle condition where the cat was not performing any task (Lee and Middlebrooks, 2011). In the ‘not-attending to the location’ task they found neurons were more sharply tuned than those in the idle condition and the spatial tuning sharpened further when the cats had to attend to the location of the sound in order to complete the task correctly, in particular they had to listen for a deviation of the sound from the azimuthal plane to a location above this. Perhaps the azimuthal spatial tuning would have sharpened even further if the animals had had to pay attention to the azimuth of the sound rather than just detect a change in elevation. A further study with the same behavioural task but comparing recordings from A1, PAF and DZ of cat auditory cortex revealed that area DZ contained many more units sensitive to central areas of space whereas PAF units had more evenly distributed best azimuths. A1 was shown to be somewhere between these two. All three areas showed significant sharpening of spatial tuning during the localisation task (Lee and Middlebrooks, 2013). Several lines of evidence pointed to inhibitory mechanisms being the source of the sharpening of the spatial receptive fields; the sharpening of tuning resulted mainly from a decrease in response to the least preferred locations in the localisation task compared with the passive conditions, in units that showed only offset responses, firing rates increased in the offset responses and decreased in the offset responses during transition from idle to localisation task, and the spike latency was longer during the localisation task than in the idle condition (Lee and Middlebrooks, 2011). It has been shown that increases in inhibition correlate with improvements in behavioural performance (Witte and Kipke, 2005) or increase of task difficulty (Atiani et al., 2009) through enhancement of the representation of the stimulus compared with suppression of responses to surrounding or distractor stimuli. These changes may be driven by top down control through attentional mechanisms from pre-frontal cortex (Zikopoulos and Barbas, 2006) which projects to the thalamic reticular nucleus. This nucleus has been shown to modulate neurons in the MGB (Cotillon-Williams et al., 2008) via inhibitory GABAergic projections. Perhaps if the task were made more difficult the sharpening of the spatial receptive fields would be greater.

Indeed, it has recently been shown that if two ongoing streams of sounds with interleaving sound bursts are presented simultaneously from two different locations the spatial tuning of units in A1 of anaesthetised cats can become markedly sharper (Middlebrooks and Bremen, 2013). Although in anaesthetised cats, this study suggests that a more difficult task, in which more than one stimulus is presented simultaneously, spatial receptive fields of auditory cortical neurons do become even sharper, and approach perceptual levels of humans in discrimination of discrete auditory stimuli/streams (Middlebrooks and Onsan, 2012). A model of segregation of the two streams of sound incorporating the single sound source spatial receptive fields of neurons predicted a forward-suppression inhibitory mechanism as the source of the sharpening of the spatial receptive fields. This forward suppression was found not to be a result of adaptation caused by previous discharge in the neurons and therefore must have arisen in a sub-cortical pathway or from outside of cortex (Middlebrooks and Bremen, 2013). The surround suppression observed by Lee and Middlebrooks (2011) is reminiscent of that observed in the external nucleus of the barn owl where it sharpens spatial tuning of units there (Wang et al., 2014). This type of surround suppression is also consistent with the narrowing of the spatial receptive fields seen in Middlebrooks and Bremen (2013). There is evidence that suggests GABAergic inhibition mediates surround suppression in sensory cortex (reviewed by Wang et al., 2014). Thus there are some similarities between mechanisms observed in the barn owl external IC, where an isomorphic map of space has been observed, and AC of mammals. Although a map of space has not been observed in the AC, Middlebrooks and Bremen (2013) observed groups of neurons tuned to similar locations much like that observed in the internal nucleus of the barn owl IC (Konishi, 2003).

1.5.3 Auditory cortex coding model

Since, generally, the tuning of individual neurons is too broad to account for behavioural performance in sound localisation tasks, ensembles/populations of neurons may be required to determine spatial location of sounds.

Populations of neurons in cat PAF contain more information than populations of neurons in A1 about the location of the stimulus, and the amount of information in populations of PAF neurons increases more quickly than in A1 as the population size increases (Stecker et al., 2003). Populations of neurons in non-human primates contain enough information to account for sound localisation ability of human listeners (Miller and Recanzone, 2009). Populations of neurons are also more tolerant of changes in intensity (Stecker et al., 2003, 2005b; Miller and Recanzone, 2009), which is in keeping with the accuracy of human sound localisation over a wide range of intensities remaining relatively constant, at least with short stimuli (Macpherson and Middlebrooks, 2000). These findings raise the question of how populations of neurons encode auditory space?

The generally broad spatial tuning in auditory cortex in response to broadband sounds led Stecker et al. (2005b) to adapt the model proposed for coding in the IC by McAlpine et al. (2001) such that it was applicable to the auditory cortex; they proposed that; *“auditory space is encoded specifically by differences in the activity of two broad spatial channels corresponding to subpopulations of contralateral and ipsilateral units within each hemisphere (i.e. a left-right opponent process).”* In this opponent channel model, coding should be robust in any case where both channels exhibit similar sensitivity to a ‘nuisance dimension’ (e.g. level, frequency etc.). They demonstrated using a simple population model that the difference between the two channels can encode space more accurately than either channel alone with changing intensity levels (Stecker et al., 2005b). However, this work was performed in anaesthetised cats and, as note above, it has been shown that spatial receptive fields of auditory cortical neurons often exhibit sharper tuning than that seen in anaesthetised animals (Mickey and Middlebrooks, 2003; Lee and Middlebrooks, 2011). Even sharper tuning can be observed with two sounds are presented from different locations with different rhythms (Middlebrooks and Bremen, 2013). Recent work in the anaesthetised gerbil provides evidence for a two-channel hemispheric encoding of ITDs at the level of the IC but a labelled-line-like encoding of space in auditory cortex suggesting that there is a transformation of encoding of auditory space from brain-stem to cortex (Belliveau et al., 2014).

Despite observed sharpening of spatial tuning of units in auditory cortex during a sound localisation task, the tuning still remains quite broad. This broadness of tuning however does not exclude a sharp population tuning for sound location/direction. In Macaques, the tuning of individual neurons in M1 is very broad for direction of motion of the monkey's arm (Georgopoulos et al., 1986) but sharp and accurate tuning for direction of motion of the arm can be observed in the response of populations of neurons using a population vector model. Moreover, a population vector model performed well in determining the direction of motion of visual stimuli (Steinmetz et al., 1987). However, population vectors have not fared well in determining the location of sounds in space (Day and Delgutte, 2013).

Investigations into the representation of auditory space in the auditory cortex of humans have concluded a hemispheric two-channel like encoding of ITDs (Magezi and Krumbholz, 2010; Salminen et al., 2010b; Briley et al., 2013). Salminen et al. (2009) also showed data consistent with the hemispheric two-channel coding for ILDs in humans. Thus both forms of interaural cue could be coded for in the same way. This would seem to be advantageous since in 'real-world' listening often all types of spatial cues are available to determine spatial location (Salminen et al., 2009). Follow-up work from the same group indicated that indeed, there is evidence to suggest that encoding of space in human auditory cortex is independent of the cue type provided to perform the localisation (Salminen et al., 2015b). The two-channel code could allow other stimulus features, such as pitch or loudness, to be encoded by the same population of neurons without the need for maps upon maps, allowing efficient combining of spatial and non-spatial information. Bizley et al. (2009) showed that the majority of neurons in ferret A1 were modulated by combinations of two or more of pitch, timbre and spatial location cues, perhaps supporting this multi-feature coding in individual neurons.

It has been posited that there may be a third spatial channel tuned to the midline. Using a psychophysical adaptation paradigm, Dingle and colleagues

(2010) showed that lateralised adaptors caused a shift in perceived targets towards the midline, consistent with a midline channel. With only two channels one might expect to see no change in the perceived location of target sounds as both channels would be adapted by the same amount (Dingle et al., 2010). Similar results have been found with stimuli providing only ILD cues (Dingle et al., 2012). These results tie in with findings that around 10% of spatially tuned neurons are tuned to midline locations (Stecker et al., 2005b) and there may even be a specialised brain area to encode central locations (Lee and Middlebrooks, 2013). However, a recent study that adapted the midline found that this improved listeners ability to detect changes in location in the midline area providing support for a two-channel model; if there was a midline channel it would have been adapted by the adaptor and one might expect a decrease in ability since the sensitivity of the channel would have been reduced (Maier et al., 2012).

1.6 This body of work

From the introduction above we can see that there are a number of unanswered questions relating to how auditory cortex represents the location of a sound source and how that pertains to the perception of the location. Some evidence has been provided for a labelled-line encoding of space in auditory cortex of anaesthetised gerbils (Belliveau et al., 2014) and, prior to cortex, in the IC of awake rabbits (Day and Delgutte, 2013) and in the MSO of gerbils (van der Heijden et al., 2013; Franken et al., 2015). A dramatic sharpening of spatial tuning is seen in auditory cortical neurons of anaesthetised cats when more than one sound is presented (Middlebrooks and Bremen, 2013), pushing evidence away from a two-channel encoding of auditory space in cortex. Evidence for the two-channel model encoding of auditory space in *auditory cortex* is provided by experiments in anaesthetised animals, where spatial tuning is broad and often contralateral (Stecker et al., 2005b). Spatial tuning properties of cortical neurons have been investigated in awake animals performing only reduced forms of localisation behaviour (Recanzone et al., 2000; Lee and Middlebrooks, 2011). As far as the author is aware, there is not any published work in which the encoding of auditory space in AC has been investigated in an animal performing a sound localisation task where a comparison of the azimuthal location of the sounds is necessary to perform the task.

This thesis investigates how auditory spatial location is encoded in auditory cortex, specifically whether encoding is in the form of a two-channel code or a labelled-line code. Coding is investigated in humans and in an animal model, the ferret, where it is possible to directly correlate behaviour and neural firing patterns in a task where judging azimuthal location is necessary to perform the task. The work takes advantage of different predictions of patterns of results from the different models of auditory space discussed; if the neural code for sound location in auditory cortex is like the hemispheric two-channel model then the spatial receptive fields (SRFs) of neurons would be broadly and contralaterally tuned with the peak at 90°. If the opponent two-channel code were used then the SRFs would again be broad and tuned to 90° but neurons tuned to ipsilateral and contralateral space would be expected within each hemisphere. If the coding is more like the labelled-line model, then we would expect to see units with sharp tuning representing all of azimuthal space. The SRFs of neurons will be investigated by recording from A1 of ferrets performing a localisation task. The models will be further tested with population decoders and results compared with performance in an absolute sound localisation task. Results from psychophysical experiments will also be compared to predictions about perception based on the models.

The work will be divided into four further sections. Chapter 2 presents results from an investigation into the relative sound localisation ability of human subjects. Results are interpreted by drawing on predictions made by models of coding of auditory space. Chapter 3 presents behavioural findings from ferrets trained on a very similar relative localisation task to the human study. Ferrets were used since, like humans, they have good low frequency hearing and they vocalise, they are capable of learning complex listening tasks (Yin et al., 2010; Bizley et al., 2015). This allows training in a relatively abstract task such as the one developed in this thesis and makes findings more relevant for understanding how the human brain would perform a similar computation, although it is worth mentioning that it is likely that ferrets low frequency hearing is obtained through wider receptive fields in auditory nerve fibres than those found in humans (Sumner and Palmer, 2012). Chapter 4 describes analysis of neural responses simultaneously recorded with the ferret behaviour. Chapter 5 presents ferret behavioural findings in an absolute sound localisation task with inactivation of A1 by cooling. Here ferrets learned two auditory tasks, one spatial and one non-spatial. They performed these tasks while A1 was inactivated by a cooling loop. The experiment is in part a proof of concept of cooling in awake-behaving ferrets since inactivation of A1 provides a clear prediction for effect on sound localisation and provides some limited evidence for effects of inactivating A1 on a non-spatial discrimination task.

In summary the guiding hypothesis is to determine how the location of sounds in space are encoded in auditory cortex. The aims of the work are; (1) to develop a behavioural task that requires discrimination of azimuthal location, (2) explore spatial tuning properties of individual units in AC of an animal performing a location discrimination task, (3) explore models of encoding of auditory space using populations of neurons recorded from AC, (4) to see if there is any information about the direction of the relative location of sounds presented in azimuth and (5) to develop the method of cooling in ferrets.

Chapter 2: Relative sound localisation ability of humans

2.1 Introduction

This work has been published in the Journal of the Acoustical Society of America: Relative sound localisation abilities in human listeners, Wood and Bizley (2015).

The aim of this chapter is to develop a two-alternative forced choice sound localisation task that can provide a means of investigating the coding of auditory space in auditory cortex. Psychophysical investigations of sound localization abilities generally fall into one of two classes: Absolute localisation studies determine the accuracy with which human listeners can localise the source of a sound, generally by requiring subjects to indicate the perceived origin of the source (Stevens and Newman, 1936; Makous and Middlebrooks, 1990; Carlile et al., 1999). In contrast, other studies seek to determine the spatial resolution of the subject by measuring the minimum discriminable difference in source location that a listener can reliably discern; results generate what is termed the minimum audible angle (MAA) (Mills, 1958). MAA tasks are well suited to standard psychophysical techniques, such as two-alternative forced choice (2AFC) procedures. This is advantageous if one wants to combine behavioural investigations with neuronal recordings, as established methods facilitate ‘neurometric’ approaches (Parker and Newsome, 1998). However, measurement of the MAA can be time consuming, especially if one is interested in exploring how spatial resolution varies throughout space. In contrast, an absolute localisation task allows relatively rapid assessment of localisation abilities throughout auditory space. However, because an absolute localisation task has many response options (i.e. at least as many as there are source locations), analysis of simultaneously recorded neural activity is considerably more complicated. A modified form of the MAA task is developed in this chapter in which the respondent must report the relative location of a target sound relative to a reference sound, left or right. The reference stimulus was presented from varying locations throughout frontal azimuth and the target was presented 15° to the left or right of this reference. Signal detection theory was used to estimate sensitivity (*d'*) thus enabling measurement of spatial localisation abilities throughout azimuth.

Very few studies have investigated the ability of either human or non-human listeners to judge the relative location of two sequential sources outside of the MAA context (Recanzone et al., 1998; Maddox et al., 2014). Determining the relative location of two sound sources, or the direction of movement of a single source, is an ethologically relevant task. For example, the relative location of two voices could help a person pick out a voice in a crowded room, or for a wild animal, being able to follow the direction of a moving sound, be it prey or predator, could be important for survival. Real-world hearing frequently entails listening in noisy environments composed of multiple sound sources. Therefore movement discrimination is distinct from, but closely related to, relative sound localisation - especially at adverse signal-to-noise ratios where the target sound may only be intermittently audible. The target and reference were always separated by a fixed interval of 15° and both were embedded in a continuously varying noisy background which was independently generated for each of the 18 speakers in the testing arena. Signal to noise ratios (SNRs) of 0 to +6 dB were tested, this ensured that subjects could both detect and segregate the sources in order to determine their relative location.

As discussed in the Introduction, the spatial location of a sound source must be computed centrally using sound location cues, including binaural cues that can be extracted by comparing the signal at the two ears; i.e. interaural timing differences (ITDs) and interaural level differences (ILDs), as well as monaural or spectral cues, which arise as a result of interaction of sound waves with the torso, head and with the folds of the external ear (Middlebrooks and Green, 1991). While cues for sound localisation are extracted in the brainstem (reviewed in Grothe et al., 2010), auditory cortex is required for accurate sound localisation performance (Neff et al., 1956; Jenkins and Merzenich, 1984; Kavanagh and Kelly, 1987; Heffner and

Heffner, 1990; Zatorre and Penhune, 2001; Malhotra et al., 2004, 2008; Malhotra and Lomber, 2007). However, how neurons in auditory cortex encode sound location remains a controversial subject. Several models have been proposed that account for the neural basis of sound localisation (Jeffress, 1948; Stern and Shear, 1996; Harper and McAlpine, 2004; Stecker et al., 2005b; Dingle et al., 2013).

The labelled-line model posits that space is represented by a number of neural channels, each of which is tuned to a particular region of space. Together, these spatial channels encompass and encode all of auditory space. The Jeffress model specifically says that the channels should form an isomorphic map of auditory space (Jeffress, 1948) whereas the labelled-line model effectively removes the need for an isomorphic map but neurons are still sharply tuned to particular locations in space. Modified versions of the labelled-line model include a greater number and/or more tightly tuned channels near the midline in order to account for the superior spatial resolution observed there (Stern and Shear, 1996) and the decline in localisation ability that occurs away from the midline (Middlebrooks and Green, 1991). In contrast, the two-channel or opponent channel model (Stecker et al., 2005b), proposes that two broadly tuned channels exist to represent azimuth. This model was first proposed for the encoding of ITD in small mammals (McAlpine et al., 2001) and was adapted to the auditory cortex following the observation that neural tuning in auditory cortex was typically broad and contralateral (Stecker et al., 2005b). Whilst such a model is likely an over-simplification, recent human imaging studies have suggested that both sound localisation cues and auditory space might be represented in human auditory cortex by this kind of 'hemifield code' (Salminen et al., 2009, 2010b; Magezi and Krumbholz, 2010; Briley et al., 2013).

The predictions of the three different models of neural activity described above create directly testable hypotheses and brain imaging studies have attempted to disambiguate these models by testing the response elicited by a change in location of a sound. Magezi et al. (2010) found that sounds moving away from the midline have a greater increase in neural activity than sounds moving towards the midline, which show a smaller increase in neural activity consistent with a ‘hemifield code’ (Salminen et al., 2009, Magezi and Krumbholz, 2010). However, it is not known whether there is a psychophysical correlate of this neural phenomenon; if an outwards moving sound elicits a greater increase in neural activity it seems plausible that discrimination might be enhanced for outwards versus inwards moving sounds. In the present work, the models are used to make predictions about the performance of subjects in determining the location of the target relative to the reference sound. The two channel model predicts that there will be better performance around the midline compared with the periphery. The labelled-line model predicts that there will be no difference in performance across space, while the modified version of the labelled-line model made similar a prediction to the two-channel model.

A topographic map of auditory space as described by Jeffress (1948) has been discovered in the IC of the barn owl (Knudsen and Konishi, 1978). Here it has been shown that spatial tuning of neurons is made sharper by surround inhibition but in order to report direction of movement of sounds, changes in the location of sound must be tracked over time, this can be achieved by adaptation or forward suppression (reviewed in Wang et al., 2014). If the spatial receptive fields of neurons are asymmetric then neurons will more strongly adapt for sounds moving in one direction over another which gives rise to direction selectivity. In the barn owl, spatial receptive fields are often asymmetric and the shapes of the spatial receptive fields could predict the direction sensitivity of individual neurons (Wang and Peña, 2013). In this study where auditory cortex is not being directly investigated, the models used to predict performance do not incorporate any form of adaptation. With an adaptation term, a difference would be seen in the discrimination of stimuli moving towards or away from the midline in the two-channel model. Neurons would respond more strongly if the sound were moving into the receptive field (i.e. outward) since the channel in that hemisphere would be less adapted by a more central first stimulus.

Previous work on absolute sound localisation has shown that gazing towards a visual stimulus can alter sound localisation abilities, for short periods of gaze time sound localisation is biased away from the point of gaze (Lewald and Ehrenstein, 1996) and for longer periods of time, sound localisation is biased towards the point of gaze (Razavi et al., 2007). However, it is not clear that this would necessarily affect the accuracy of comparing the location of two sounds. In another study looking at acuity of localisation cue discrimination (Maddox et al., 2014), a short gaze cue that informed subjects about the location of the sound they were about to listen to improved performance in an auditory relative localisation task. Thus the influence of gaze location was investigated by asking subjects to fixate at different locations whilst keeping their heads fixed at the midline.

The main aim of this chapter is to establish the localisation task developed for investigating the coding of auditory space in auditory cortex. The task was developed in human listeners in order to gauge how difficult the task was and to characterise the basic responses in humans in order to relate the findings to the ferret data discussed later. Thus, the relative sound-localisation abilities of subjects were first measured at different supra-detection-threshold signal-to-noise ratios in order to assess the effect of SNR on the discrimination. Since ITDs and ILDs are initially processed separately in the auditory system (Grothe et al., 2010), in order to understand the role of each cue in the discrimination task, band-pass noise stimuli in which localisation cues were dominantly ITDs or ILDs were used to compare performance when listeners were tested with broadband stimuli, in which all localisation cues were present. The sensitivity of subjects at each location was assessed according to the changes in binaural cues that occurred. Models based on the two-channel and labelled-line codes were used to predict performance in this task, and these predictions compared with the observed data. Finally, the influence of gaze direction was estimated by measuring performance while subjects fixated at ±30° while maintaining a constant head position in order to control for the location of the gaze during experiments 1 and 2.

2.2 Methods

2.2.1 Participants

This experiment received ethical approval from the University College London (UCL) Research Ethics Committee (3865/001). 20 normal hearing adults between the ages of 18 and 35 participated (13 female and 7 male), all but one participant was right-handed. 8 subjects (5 female and 3 male) took part in Experiment 1 and 16 subjects (11 female and 5 male) took part in Experiment 2, 4 of whom were subsequently excluded for poor performance, (see below for details). 4 participants (3 female and one male) took part in both Experiments. All participants had no reported hearing problems or neurological disorders.

2.2.2 Testing chamber

For testing, subjects sat in the middle of an anechoic chamber (3.6 x 3.6 x 3.3m (width x depth x height)) with sound attenuating foam triangles on all surfaces (24 cm triangular depth and total depth of 35 cm) and a suspended floor) surrounded by a ring of 18 speakers (122 cm from the centre of the subject’s head and level with the ears) arranged at 15° intervals from -127.5° to +127.5° (Figure 2.1 [a]). The subject’s head was maintained in a stationary position in the centre of the speaker ring throughout testing with the aid of a chin rest. Subjects were asked to fixate on a cross located at 0° azimuth, unless otherwise instructed.

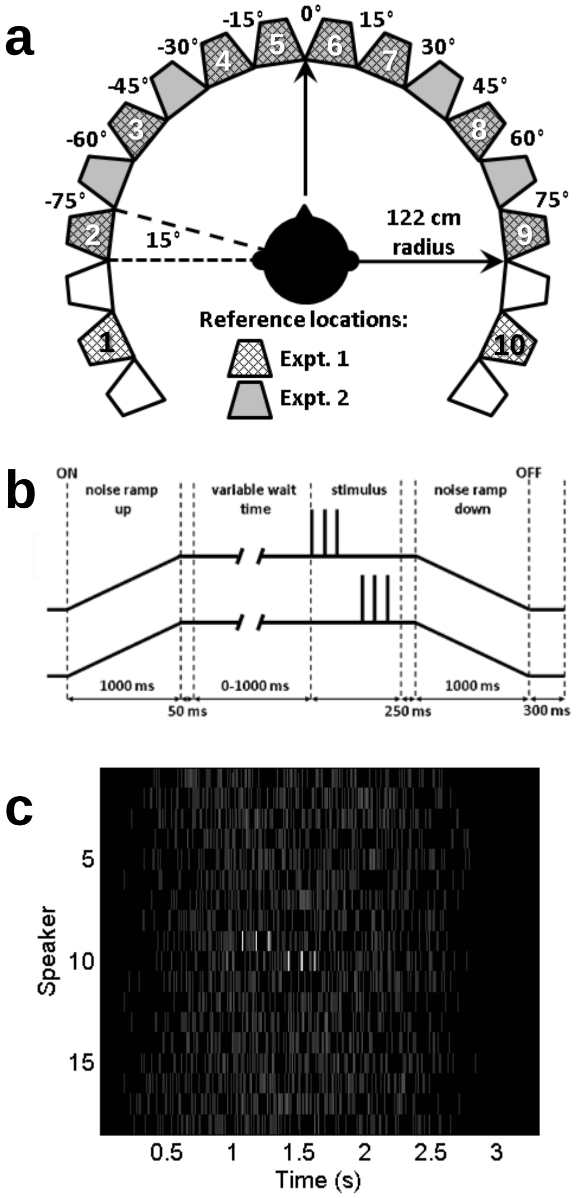


Figure 2.1 – Human psychophysics experimental setup and stimuli. [a] Speaker Arrangement: The subject's head was positioned in the centre of a ring of 18 speakers, each separated by 15°. Hatched-numbered speakers were reference locations in Experiment 1, grey speakers were reference locations in Experiment 2. Mean stimulus locations for Experiment 2 are labelled. [b] Schematic of the stimulus showing the reference and target speakers: The background noise (independently generated for each speaker) is ramped up to full intensity over 1000 ms. The reference stimulus starts between 50 and 1050 ms after this. The reference stimulus is presented from a pseudo-randomly selected speaker from those defined in the different experiments. The target is presented from a speaker 15° to the left or right of the reference speaker. The noisy background continues for a further 250 ms after the stimulus presentation before being ramped down to zero over 1000 ms. [c] Example stimulus. This diagram shows all speakers in an example stimulus: The reference stimulus comes from speaker 9 and the target from speaker 10; all speakers presented independently generated noise. Lighter colours indicate a greater intensity. Reference and target stimuli have been shown at a higher SNR than was used in testing for visualisation purposes.

2.2.3 Stimuli

All stimuli were generated and presented at a sampling frequency of 48 kHz. In the broadband noise (BBN) conditions, 3 pulses of white noise were presented from a reference speaker, followed by 3 pulses of white noise from a target speaker. Noise pulses were 15 ms in duration which included cosine ramping with 5 ms duration at the beginning and end of each pulse. Pulses were presented at a rate of 10 Hz with 130 ms delay between the end of the final reference pulse and the first target pulse in order to aid perceptual segregation of the reference and the target. Preliminary work showed that a delay of this order helped listeners to segregate the reference and the target such that they were perceived as separate sound sources within the noisy background. The sequence of reference and target pulses occurred at an unpredictable interval from trial onset (see Figure 2.1 [b]). The pulses were embedded in a noisy background generated by presenting white noise whose amplitude was varied every 15 ms with amplitude values drawn from a distribution whose mean and variance could be controlled, this control over the noise statistics being the main rationale for using such a background noise (Raposo et al., 2012), however, this type of statistical manipulation was not performed in the present study and the noise values were drawn from a Gaussian distribution with a mean and standard deviation of 1. The reference and target pulses were also 15ms in duration and were superimposed onto this background of on-going amplitude changes (see Figure 2.1 [c]) where the high amplitude white noise pulses are visible for the reference (speaker 9) and target (speaker 10) locations).

Each noise source was generated independently for each speaker on every trial while the overall level of noise was simultaneously ramped on and off with a linear ramp over 1 s for all 18 noise sources according to the schematic in Figure 2.1 [b]. The reference and target pulses could occur any time between 50 and 1050 ms after the noise levels reached their maximum (i.e. 1050–2050 ms after trial onset). In these experiments the mean noise level when all speakers were presenting the background noise was 63 dB sound pressure level (SPL, calibrated using a CEL-450 sound level meter; Casella CEL Inc., NY, USA). Stimuli in the low-pass noise (LPN) and band-pass noise (BPN) conditions were also brief noise pulses but were filtered at so they contained power below 1 kHz and between 3 and 5 kHz, respectively. Except for threshold measurements (see below), the target speaker was always 15° to the left or right of the reference speaker and subjects were oriented such that their head faced a fixation point located at 0° (see Figure 2.1 [a] for speaker locations and fixation point).

Stimuli were presented by Canton Plus XS.2 speakers (Computers Unlimited, London) via a MOTU 24 I/O analogue device (MOTU, Cambridge, MA) and two Knoll MA1250 amplifiers (Knoll Systems, Point Roberts, WA). The individual speakers were matched for level using a CEL-450 sound level meter and the spectral outputs were checked using a Brüel and Kjær 4191 condenser microphone placed at the centre of the chamber where the subject's head would be during the presentation of a stimulus. The microphone signal was passed to a Tucker Davis Technologies System 3 RP2.1 signal processor via a Brüel and Kjær 3110–003 measuring amplifier. All speakers were matched in their spectral output which was flat from 400 Hz to 800 Hz, with a smooth, uncorrected 1.2 dB/octave drop off from 400 Hz to 10 Hz, and a smooth uncorrected drop off of 1.8 dB/octave from 800 Hz to 25 kHz. The MOTU device was controlled by MATLAB (MathWorks, Natick, MA) using the Psychophysics Toolbox extension (Brainard, 1997; Kleiner et al., 2007).

2.2.4 Threshold estimation

In order to determine the SNR at which subjects were able to reliably detect the pulse train within the noise, they first performed a threshold test. In this task subjects were oriented to face a speaker at the frontal midline (0° azimuth). The reference sound was always presented from this speaker, and the target was presented from a speaker at either -90° or +90°. Subjects reported the direction in which the stimulus moved using the left and right arrows on a keyboard to indicate -90° and +90°, respectively. Stimuli were presented at 10 different SNRs by varying the signal attenuation in 1 dB steps over a 10 dB range. Subjects performed 10 trials for each direction and SNR combination, presented pseudo-randomly, over a single testing block.

Percentage correct lateralisation scores were fit using binomial logistic regression and the threshold value, selected to be 95% correct, was extracted from the fitted function. Since a 180° difference in location is well above localisation threshold (Mills, 1958), it follows that a failure to localise a sound accurately in this condition was because the subject was unable to detect the sound in the noise and hence a correct lateralisation response was used to determine detection threshold. Indeed, pilot studies demonstrated that the threshold for a yes/no detection task at 90° was within 0.1 dB of the threshold estimated using the left-right choice. A threshold value of 95% was taken because the aim was to present stimuli at a level that was clearly audible, but difficult enough to be challenging for the subsequent relative localisation task.

Difficulty was matched across subjects and task conditions by determining individual threshold values for each subject and in each task condition. The resulting threshold value determined three SNRs for Experiment 1; a 'low' SNR which was equal to the 95%-correct threshold (mean SNR ± standard deviation; -6.8 ± 1 dB, n=8), a 'medium' and a 'high' SNR, equivalent to the threshold value +3 and +6 dB, respectively. For Experiment 2, a single SNR was chosen, intermediate to the low and medium SNRs in Experiment 1; defined as the 95%-correct point +1.5 dB. The SNR of the thresholds of each subject taking part in Experiment 2 ranged from -9.4 to -7.4 dB (mean ± standard deviation = -8.3 ± 0.7 dB, n=12, Table 2.1). 4 subjects were excluded from Experiment 2 as their detection thresholds were more than 3 standard deviations from the group mean. Threshold estimations were performed for each testing condition. An example threshold test of a single participant is shown in Figure 2.2 [a]: at the lowest SNR tested (-18 dB) the subject is at 58% correct (chance being 50%) indicating that they could barely discriminate the direction the signal moved. This subject's 'low' SNR threshold was defined as -14 dB since this was the point at which the fitted function crossed the 95% correct point.

Stimulus	Mean Threshold SNR (dB)	Standard deviation (dB)	Range (dB)	
			min	max
Broad-band	-8.3	0.8	-9.4	-7.4
Low-pass filtered	-13.9	1.3	-16	-12
Band-pass filtered	-12.5	1.2	-15.5	-10.5

Table 2.I – Threshold testing results for Experiment 2.

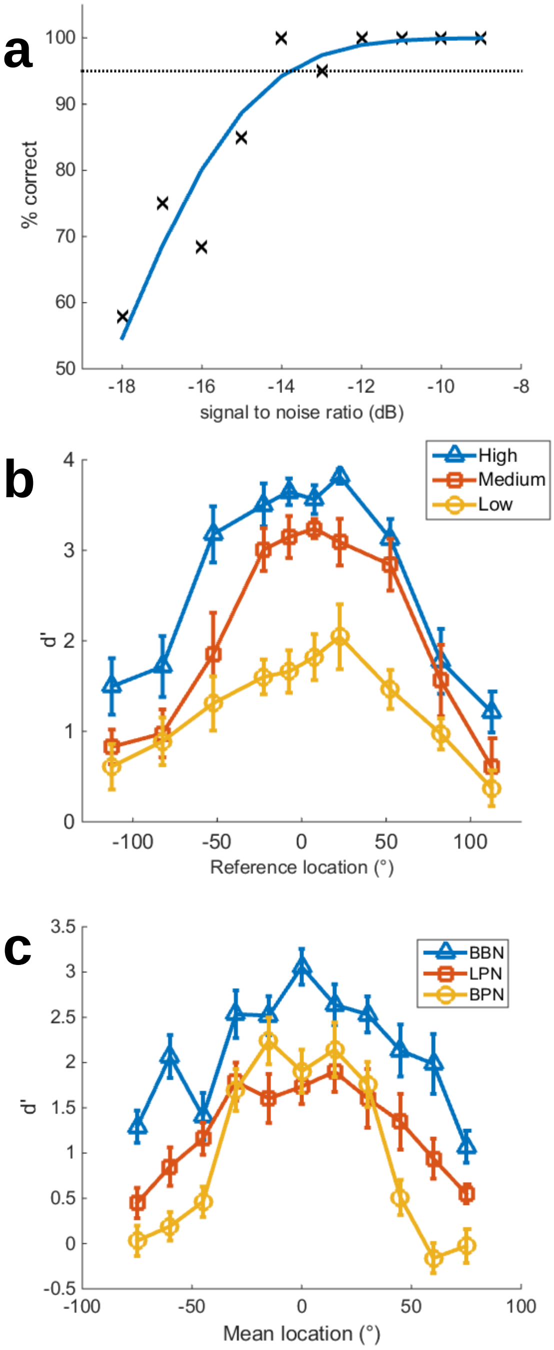


Figure 2.2 – Relative localisation threshold, and discrimination results with varying SNR and spectral band. Performance improved with higher SNRs with BBN. Performance was best with BBN and decreased when binaural cues were restricted to either ITDs or ILDs. [a] Example threshold from a single participant: The dotted black line indicates the 95% correct mark. The solid line is the binomial fit. A person's threshold was taken as the 95% correct point of the binomial fit. [b] Effect of SNR: Mean *d*' of all subjects showing discriminability of the direction of the target sounds relative to the reference at three different SNRs, which were specific to each participant. Low was defined as their 95% threshold SNR, with medium and high being the threshold plus 3 and 6 dB, respectively. [c] Mean *d*' of the mean stimulus location for all participants in Experiment 2 in each condition; BBN, low-pass filtered (<1 kHz, LPN) and band-pass filtered (3–5 kHz, BPN). These experiments were all performed at the subject's 95% threshold plus 1.5 dB.

2.2.5 Testing

During testing, on each trial the reference sound was presented from one of the speakers in the ring (speaker selected pseudo-randomly from the set of speakers used in that experiment, see Methods sections 2.2.6-8 for speakers used) and the target was presented from an adjacent speaker, either to the left or right (a 15° change in location). The participants were instructed to report which way the target had moved relative to the reference using the left and right arrows on a keyboard. Each trial began automatically 1 second after the subject made a response in the preceding trial. Testing runs were divided into blocks lasting approximately 5 minutes. At the end of each block the subject could take a break and choose when to initiate the next block.

2.2.6 Experiment 1: Effect of SNR on relative sound localisation

In this task, BBN pulses were presented to the participants at the three individually determined SNRs (see section 2.2.4 Threshold estimation). The reference locations were -112.5°, -82.5°, -52.5°, -22.5°, -7.5°, 7.5°, 22.5°, 52.5°, 82.5° and 112.5°, and targets were the speakers to the right and left of these locations (e.g. -127.5° and -97.5° for a reference of -112.5°, see Figure 2.1 [a]). Subjects performed 20 trials for each direction / SNR combination across 3 testing runs, each divided into 5 blocks of approximately 6 minutes. 8 subjects completed Experiment 1. Of these, 2 subjects performed 3 testing runs with a mix of all 3 SNRs and 6 performed 2 runs with a mix of the low and medium SNRs and 1 run with the high SNR only.

2.2.7 Experiment 2: Effect of spectral band on relative sound localisation

BBN pulses were presented to the participants at a single SNR (95% + 1.5 dB) determined by the threshold testing and intermediary to the ‘low’ and ‘medium’ SNR in Experiment 1. In this Experiment, reference locations were restricted to the frontal hemifield but tested all possible speaker positions within it. The reference positions were therefore: -97.5°, -82.5°, -67.5°, -52.5°, -37.5°, -22.5°, -7.5°, 7.5°, 22.5°, 37.5°, 52.5°, 67.5°, 82.5° and 97.5°. In the low-pass noise (LPN) condition, the white noise pulses were low-pass filtered (<1 kHz, implemented in MATLAB, low-pass finite-duration impulse response (FIR) filter, 70 dB attenuation at 1.2 kHz) while in the BPN condition, the white noise pulses were band-pass filtered (3-5 kHz, implemented in MATLAB with a band-pass FIR filter, 70 dB attenuation at 2.6/5.4 kHz). Threshold estimates were made for each stimulus type (BBN, LPN, BPN) for each subject immediately before testing the relevant stimulus type. Subjects performed a total of 480 trials (20 trials per direction per reference location) in 1 testing run divided into 5 blocks of approximately 6 minutes each for each stimulus condition.

2.2.8 Experiment 3: Ruling out effects of gaze location

Since eye position was not specifically controlled, it was merely requested that listeners focus on a cross located at the midline and level with the eyes, subjects were asked to focus their gaze on points 30° to the left or right of the midline while maintaining a 0° azimuth head position and perform the same task as in Experiment 2 with BBN stimuli. During this experiment, eye position was monitored and trials where the eyes were not on the fixation point were excluded and repeated. This experiment thus acted as a control for eye position. Subjects performed 20 trials at each speaker location (10 left moving, 10 right moving) at each eye position, a total of 720 trials in 1 testing run divided in 6 blocks of approximately 7 minutes each.

2.2.9 Modelling localisation performance

Three simple models were created; a two-channel model, a labelled-line model and a modified labelled-line model. In each case the model was used to predict the performance that an observer would make in the relative localisation task. The two-channel model (McAlpine et al., 2001; Stecker et al., 2005b) was estimated by modelling two spatial channels as cumulative Gaussians with a mean of 0° and standard deviation of 46°, as found by Briley et al. (2013) (Figure 2.3 [a]). The peak of this model occurs at 90° reflecting the fact that the largest interaural time difference cue values occur at this point. Changing the standard deviation effectively altered the slope of

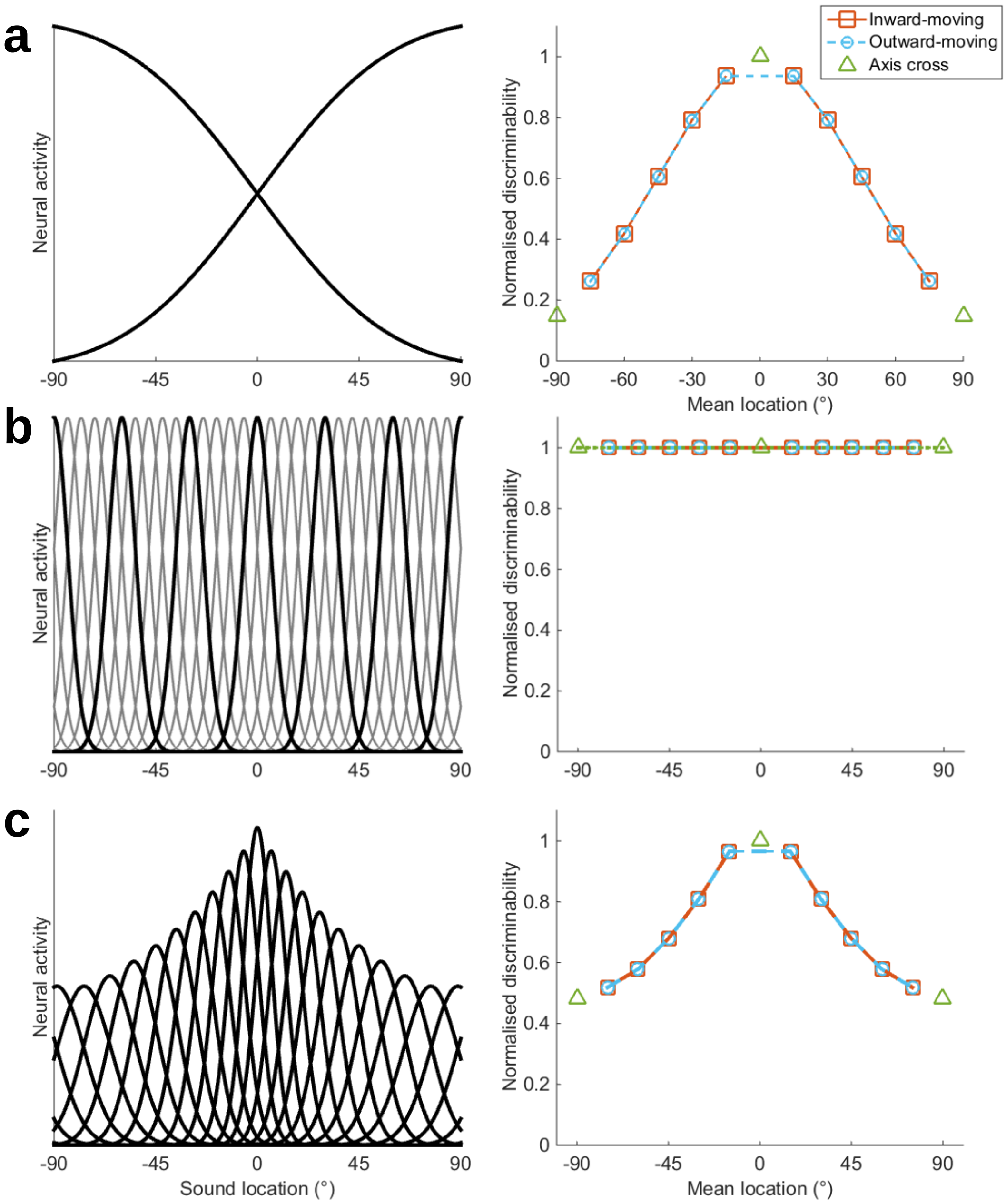


Figure 2.3 – Models of sound location coding and prediction of performance. The left-hand column shows representations of the models in terms of neural activity that would be expected for a given sound source location. The two-channel model [a] is represented by two Gaussians with means of -90° and +90° and standard deviation of 46°. The labelled-line model [b] is represented by multiple Gaussian curves located 6° apart with a standard deviation of 6°. The modified labelled-line model [c] is represented by multiple Gaussian curves, with the midline represented by more and more narrowly tuned channels, the narrowest being 6° and the broadest, 12°. In [b] the channels are shown in grey with every tenth channel in black for visualisation purposes. The right-hand column shows the normalised discriminability (where 0 is chance and 1 is maximum performance) of the direction of the stimulus at the mean stimulus location based on the models. In the two-channel model [a] discriminability is calculated as the change in the ratio of activity of each channel between the reference and target stimuli. In labelled-line [b] and modified labelled-line [c] discriminability is estimated by calculating the difference in Euclidean distance between the peak population activity generated by the reference and target sounds. The two-channel model and the modified labelled-line model both predict performance to decrease towards the periphery and to be best at the midline. The labelled-line model predicts equal performance throughout space. None of the models predict a difference for inward or outward-moving stimuli.

the two channels and the extent to which tuning overlapped across the midline. To determine predictions of performance, the model of neural tuning was convolved with a representation of the stimulus based upon the actual sound level (the SNR value was selected as the across-subject mean BBN threshold of -8.3 dB SPL from Experiment 2). From this, the amount of neural activity was estimated as the ratio of the area under the resulting activity pattern of the two channels and the resulting change in activity between the reference and target was used as a measure of discriminability. For the two-channel model, performance was best around the midline and decreased towards the periphery. Without an adaptation term, there was no difference between inward and outward moving stimuli however, were an adaptation term to be added, a difference would be seen in the discrimination depending on the direction of the stimuli. Neurons would respond more strongly if the sound were moving into the receptive field (i.e. outward) since the channel in that hemisphere would be less adapted by a more central first stimulus.

For the labelled-line model, tuning functions were constructed as a series of Gaussians with a standard deviation of 6° spread across 360° of azimuth with 50% overlap between adjacent channels (Figure 2.3 [b]). The width of 6° was chosen based upon Carlile et al. (2014). The modelled neural channels were convolved with the acoustic stimulus as described for the two-channel model above to determine the activity elicited in each channel. The activity elicited by the reference and target sounds were therefore described by two vectors, each representing the activity elicited in each channel. Discriminability was calculated as the Euclidean distance between the two population vectors: a large value indicates that the two sounds activate different patterns of activity across the neural population. This model predicted that performance would be equal across space.

The modified labelled-line model used a similar approach but rather than channels of equal width and spacing, channels increased in width from 6° to 12° from the midline to 72°. A 50% overlap was maintained so that as channels became more narrowly tuned they were also more closely spaced (Figure 2.3 [c]). Again, the choice of channel widths was estimated from

Carlile et al. (2014). This model predicted similar findings to the two-channel model, that there would be best performance around the midline and worse performance in the periphery.

2.2.10 Analysis

Overall performance was assessed by calculating sensitivity index (d') for subjects' ability to discriminate whether a target sound moved left or right at each reference or target speaker location and bias was calculated by estimating the criterion (Green and Swets, 1974). Using d' as a sensitivity index implies the subject is using a model with two possible stimulus classes represented by normal distributions with different means. The distance between these distributions determines a subject's sensitivity (estimated d') in the task. The subject is assumed to decide which class has occurred by comparing each observation with an adjustable criterion. The location of this criterion with no bias would be in the middle of the two stimulus class means, whereas, any bias would be indicated by the criterion shifting closer to one or the other mean thus increasing the likelihood of a response for that stimulus class (Macmillan and Creelman, 2004). The formula used to calculate d' in this case was (Stanislaw and Todorov, 1999):

$$d' = Z\,(\text{hit rate}) - Z\,(\text{false alarm rate})$$

Where,

$$\text{Hit rate} = \text{hits} / (\text{hits} + \text{misses})$$

$$\text{False alarm rate} = \text{false alarms} / (\text{false alarms} + \text{correct rejections})$$

A hit was defined as a response left when the stimulus direction was left, a miss was a response right when the stimulus direction was left, a false alarm was a response left when the stimulus moved right and a correct rejection was defined as a response right when the stimulus moved right. The response bias was calculated as follows:

$$\text{Bias} = -\,(Z\,(\text{hit rate}) + Z\,(\text{false alarm rate})) / 2$$

Data were further analysed by separating trials into those where the target sound moved towards the midline and those where it moved away from the midline and calculated % correct performance for each SNR with respect to the reference location in Experiment 1. In Experiment 2, data were considered relative to the mean location of the reference and target location rather than either in isolation as this meant that inward and outward-moving sounds at this location elicited equivalent changes in localisation cues. This was not possible in Experiment 1 because the fixed set of reference locations and their respective targets did not make a full set of overlapping reference-target pairs. Statistical analyses were performed in SPSS (IBM SPSS, NY, USA) and are described at the relevant sections in the text. For repeated measures ANOVAs, Greenhouse-Geisser sphericity corrections were used for when Mauchly's test for sphericity was significant. For comparison of the data to the predicted spectral cues available, we estimated the ITD of the stimuli using the formula for low frequency sounds described by Kuhn (1977) :

$$ITD = 3 * r/c * (\sin \theta)$$

Where r is the radius of the head (8.75 cm was used here), c is the speed of sound (343 ms^{-1}) and θ is the angle of incidence of the sound in radians. For estimation of ILDs available in the stimuli we used the information provided by Shaw and Vaillancourt (1985).

2.3 Results

Participants performed a single interval two-alternative forced choice task where they were asked to report whether a target sound was presented to the left or right of a preceding reference. The reference and target stimuli each consisted of three 15 ms pulses of noise presented in a background of noise generated and presented independently from each of the 18 speakers in the ring. Prior to completing the main Experiments, each participant performed a threshold task to establish the signal-to-noise ratios over which testing took place.

2.3.1 Thresholds

Each subject completed a threshold task to determine their individual threshold for detecting the stimuli embedded in the background noise. Subjects performed a modified task whereby reference sounds were presented from a speaker at 0°, and target sounds from ±90°. Since a location shift of this magnitude was well above perceptual threshold, it follows that if the subject could correctly discriminate the relative location, then the target was audible above the noise. Figure 2.2 [a] shows an example of a threshold for a single participant. At high SNRs (-9 to -12 dB) the participant is able to identify correctly the direction the target had moved, but as the SNR decreased, performance decreased towards chance (50%). The 95% correct threshold was at a SNR of -14 dB. Each participant performed an independent threshold experiment for the BBN (Experiments 1-3), low-pass and band-pass filtered stimuli (Experiment 2). Table 2.1 shows the summary of threshold values for participants.

2.3.2 Experiment 1: Effect of SNR

Experiment 1 aimed to determine how SNR influenced spatial sensitivity assessed with signal detection theory. Figure 2.2 [b] plots the across-subject discriminability index (mean d' ± SEM) for discrimination of the direction of the target at the three SNRs. Sensitivity (d') values are higher for judgments made in frontal space than in the periphery. Subjects' best performance was at the highest SNR followed by the medium SNR and then lowest SNR. A two-way repeated measures (RM) ANOVA (dependent variable: d', independent variables: reference location and SNR) revealed a main effect of reference location ($F_{(3.32,36.54)} = 26.31$, $\eta^2_p = 0.705$, $p < 0.001$, Greenhouse-Geisser corrected for sphericity) and SNR ($F_{(2,22)} = 15.06$, $\eta^2_p = 0.578$, $p < 0.001$) and a significant interaction between these factors ($F_{(18,1986)} = 2.55$, $\eta^2_p = 0.188$, $p = 0.001$). *Post-hoc* pairwise comparisons (Bonferroni's multiple comparison test, $p < 0.05$) showed that subjects tended to be worse at the peripheral speakers than the central speakers (speaker 1 was significantly different from speakers 3-8, speakers 2 and 10 from speakers 4-8, speaker 3 from speakers 1 and 5-7, speakers 4, 5 and 8 from speakers 1, 2, and 10, speakers 6-7 from speakers 1-3, and 10, see Figure 2.1 [a] for speaker locations) and that performance at each SNR was different from the other two. While Experiment 1 demonstrated a clear effect of SNR and reference speaker location on performance, some subjects were confused at the most lateral speaker locations (which were behind them) and the speaker selection did not allow testing of left-right discriminations across pairs of speakers with equal changes in localisation cues. In Experiment 2, testing was therefore restricted to frontal space (-82.5° to +82.5°, Figure 2.1 [a]), and all possible reference-target speaker pairs were tested thus allowing comparison of left-right discriminations with equal but opposite changes in localisation cues.

2.3.3 Experiment 2: Effect of spectral band

In Experiment 2, all speaker locations in frontal space were tested using three types of acoustic stimulus; broadband noise (BBN, as in Experiment 1) and two types of narrow-band stimulus designed to restrict the dominant sound localisation cues to either ITDs (low-pass filtered noise <1 kHz, LPN) or ILDs excluding spectral cues (band-pass filtered noise 3-5 kHz, BPN). Figure 2.2 [c] shows the effects of varying the spectral band on sensitivity measures, plotting data according to the mean reference-target location such that left and rightwards moving stimuli elicited changes in localisation cues that were identical in magnitude. Qualitatively, it is clear that performance is best in the BBN condition relative to LPN and BPN. Generally, performance is better centrally than peripherally, although the decrement in peripheral performance is particularly marked in the BPN condition. A two-way RM ANOVA (independent variables - mean location and task condition, and dependent variable *d'*) revealed main effects of spectral band condition ($F_{(2,22)} = 15.74$, $\eta^2_p = 0.589$, $p < 0.001$) and mean speaker location ($F_{(10,110)} = 41.94$, $\eta^2_p = 0.792$, $p < 0.001$) and the task condition showed an interaction with mean location ($F_{(20,220)} = 4.38$, $\eta^2_p = 0.285$, $p < 0.001$). *Post–hoc* pairwise comparisons (Bonferroni's multiple comparison test, $p < 0.05$) revealed that the BBN condition was significantly different from the LPN and BPN conditions but the BPN and LPN conditions were not different from each other. *Post-hoc* analysis of mean stimulus location revealed that the main differences were between peripherally located stimuli and those located around the midline (mean locations -75° to -45° *vs.* -30° to 30°, -30° *vs.* -75° to -45° & 60° to 75°, -15° to 30° *vs.* -75° to -45° & 45° to 75°, 45° *vs.* -15° to 30° & 60° to 75°, 60° *vs.* -30° to 30°, and, 75° *vs*. -30° to 45°, see Figure 2.1 [a] for mean stimulus locations).

As well as exploring sensitivity the bias was also estimated (Figure 2.4) for performance in each of the three conditions. A positive bias value indicates subjects were more likely to report that the target was to the right of the reference, and a negative value indicates subjects were more likely to report that the target was to the left of the reference. A two-way RM ANOVA examining the influence of stimulus condition and speaker location on bias showed an interaction between speaker location and condition ($F_{(20,220)} = 3.03$, $\eta^2_p = 0.216$, $p < 0.001$) but no main effect of speaker location or condition, indicative of conditions having different patterns of bias; for example the BPN condition shows a bias favouring the target on the side peripheral to the reference. However, analysis to determine whether the bias was significantly different from zero (*t*-tests, *p*-values Bonferroni corrected for multiple comparisons) suggest that the across-subject bias is relatively modest; only in the BPN case was any bias value significantly non-zero (mean location 75°, $p = 0.0013$).

2.3.4 Models

Previous neuroimaging studies have measured the change in neural activity elicited by a change in sound source location following a brief adapting stimulus in order to compare two-channel and labelled-line models of sound localisation and have demonstrated that predictions generated from a two-channel model best match the observed data (Salminen et al., 2009, 2010b; Magezi and Krumbholz, 2010; Briley et al., 2013). In order to compare the observed behavioural performance to that predicted by different models of auditory space, relative localisation abilities were modelled using three different approaches: a two-channel model, with two channels broadly tuned to ipsilateral and contralateral space, a labelled-line model with equally

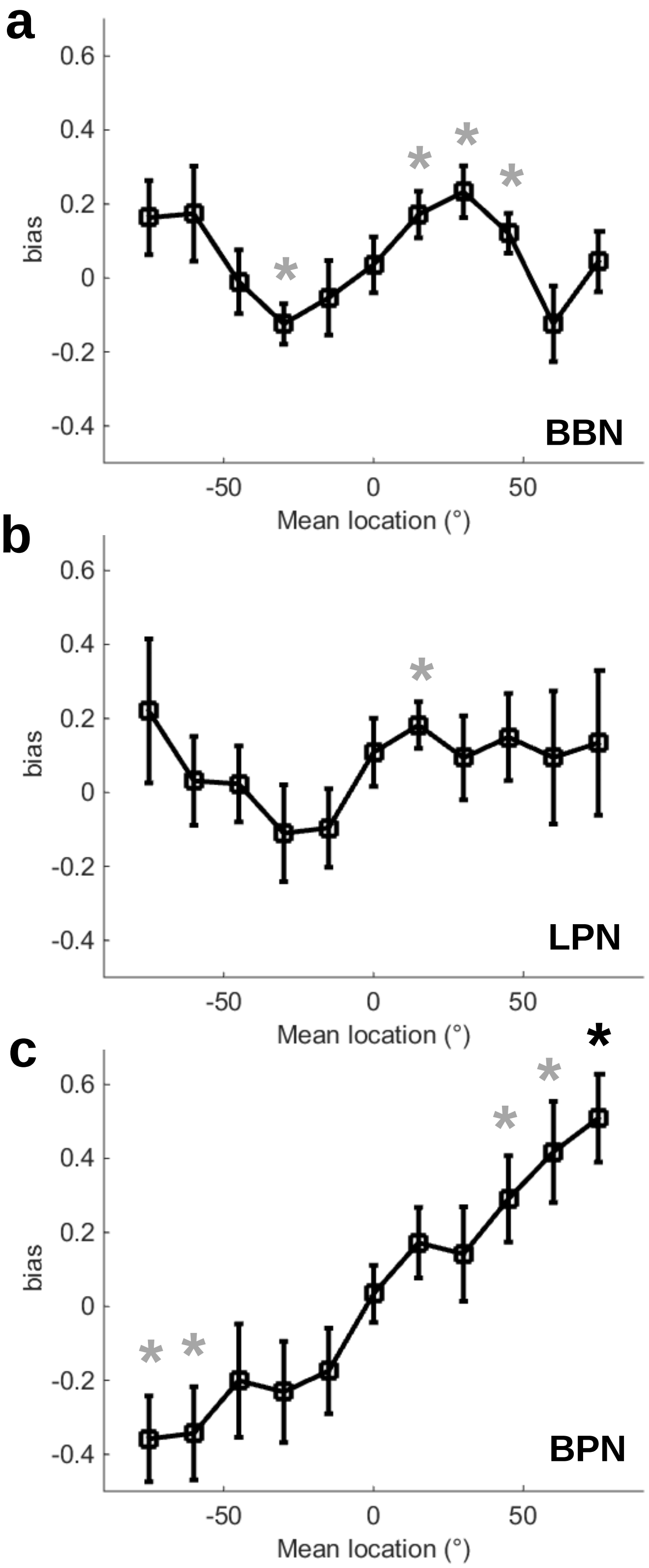


Figure 2.4 – Bias at the mean stimulus location across spectral band conditions. Bias was relatively small in the BBN and LPN conditions but varied systematically in the PBN condition where subjects were more inclined to report the stimulus moving away from the midline. Mean bias ±SEM of all participants at the mean locations of the stimuli in the BBN [a], LPN [b], and BPN [c] conditions. Grey asterisks indicate $p < 0.05$ in a *t*-test to check for difference from zero. The black asterisk in [c] indicates significance in the *t*-test after Bonferroni correction ($p < 0.0045$).

spaced equal-width channels spanning all of auditory space, and a modified labelled-line model, with channels that were both narrower and more closely spaced near the midline (see methods, Figure 2.3 [a-c] first column). For each model a representation of the stimulus, including the background noise, was convolved with the spatial channels and the discriminability of the reference and target sounds was estimated, computing measures for inward and outward changes in spatial location separately, throughout frontal space (see Methods Section 2.2.9). The models, and the resulting normalised discriminability measures (where 0 is equal to chance and 1 to maximum performance), are plotted in Figure 2.3, second column. Note that the models are only intended to provide a qualitative impression of the characteristics one might observe as the measures of 'discriminability' are not necessarily equivalent across models.

The models produce different predicted patterns of discriminability: First, in the two-channel model (Figure 2.3 [a]), performance is best around the midline and worse in the periphery. Second, in the labelled-line model (Figure 3 [b]); performance does not change across auditory space. Finally, in the modified labelled-line model (Figure 2.3 [c]), again performance is best at the midline, with a drop in performance peripherally. The models generated a testable prediction relating to whether there was a change in performance across space (as in the two-channel and modified labelled-line model).

Since the models did not contain an adaptation component there was no difference for inward or outward moving stimuli, however, as discussed in the Introduction, an adaptation component in the two-channel model would introduce better discrimination for outward moving stimuli. Thus, the data from Experiment 2 were analysed according to whether the target sound moved towards or away from the midline to address the hypotheses above. Each point in Figure 2.5 represents a pair of reference-target sounds that share the same mean location (and therefore localisation cues) and differ only in the direction of movement. Figure 2.5 shows the resulting mean (± SEM) performance scores of all participants in each of the three spectral

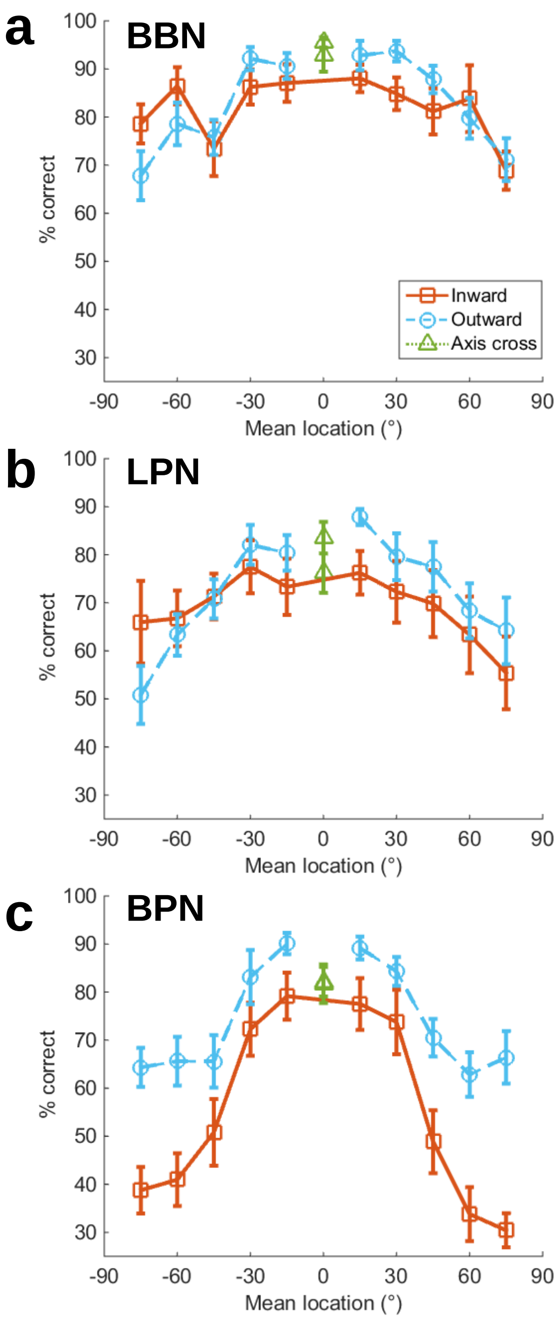


Figure 2.5 – Performance by direction relative to midline. Performance in all conditions was better at the midline than the periphery however, only the LPN condition did not show any difference for inward or outward moving stimuli. [a] Mean percent correct of all participants separated into the direction of the target relative to the reference in the broadband (BBN) condition. Circles/dashed lines show targets that moved away from the midline (0º) relative to the reference location, squares/solid lines show sounds that moved toward the midline relative to the reference and triangles show targets that crossed the midline. [b] Mean percent correct for all participants in the low-pass (LPN) condition. [c] Mean percent correct for all participants in the band-pass (BPN) condition.

band conditions. Two-way RM ANOVAs (dependent variable: % correct; independent variables: speaker location and direction of target stimulus) on each condition revealed main effects of speaker location in all conditions but only in the BPN condition was there a significant effect of direction of target (Table 2.2). There was also an interaction between direction and speaker location in the BBN condition ($F_{(4.11,45.25)} = 2.57$, $\eta^2_p = 0.189$, $p = 0.049$, Greenhouse-Geisser corrected for sphericity). The LPN condition is consistent with the two-channel model and the modified labelled-line model, in that there is a significant effect of location, but not a statistically significant difference between inward and outward-moving sounds. None of the models is consistent with the BBN condition, where there is a significant location-direction interaction or the BPN condition where there is a significant main effect of direction but showing higher performance for outward-moving sounds, similar to what may be expected if a neural adaptation term were to be introduced to the two-channel model.

2.3.5 Assessing the relationship between performance and binaural cue values

Since a 15° shift in azimuth does not produce an equal change in localisation cues across all spatial locations, the change in ITD and ILD cues that would be elicited for each reference-target pair were estimated. The estimated cue values were then used to analyse the relative localisation abilities according to the magnitude of the change in ITD or ILD each stimulus pair produced. ITD values were estimated using a spherical head of diameter 18 cm (Rayleigh, 1907) and ILD values were estimated using data from Shaw & Vaillancourt (1985) weighted to reflect the spectra of the speakers and the bandwidth of the stimuli used in the present study. Figure 2.6 [a] shows the resulting ITD and ILD values for the range of space tested. Figures 2.6 [b] converts these values into the change in cue value between reference and target sounds at each mean location. Figures 2.6 [c & d] plot sensitivity (d') measures from Experiment 2 according to the change in ITD and ILD values respectively in each of the narrow-band (NB) conditions. For the LPN condition this shows that d' decreases for smaller changes in ITD, and that performance is well fit with a linear regression line ($R^2 = 0.95$, $p < 0.0001$).

Independent variable	Degrees of freedom	Total degrees of freedom	Task condition	F	p	η^2_p	Post hoc pairwise comparisons (Tukey-Kramer, p<0.05)
Speaker location	9	99	BBN	9.98	< 0.001†	0.476	-75° vs -60°, -30° and 30° -60° vs -75° & 75° -45° vs -30°, -15°, 15° & 30° -30° and 30° vs -75°, -45° & 75° -15° and 15° vs -45° & 75° 75° vs -60°, -30°, -15°, 15° & 30°
			LPN	10.97	< 0.001	0.499	-75° vs -30°, -15° & 15° -60° vs -15° & 15° -45° vs -30° -30° vs -75°, -45° & 75° -15° vs -75° & -60° 15° vs -75°, -60°, 60° & -75° 60° vs 15° 75° vs -30° & 15°
			BPN	37.42	< 0.001	0.773	-75° to -45° & 45° to 75° vs -30°, -15°, 15° & 30°
Direction Target Moved	1	11	BBN	0.2	0.663†	0.018	
			LPN	0.21	0.657†	0.019	
			BPN	8.66	0.013†	0.440	

Table 2.2 – *Post-hoc* analysis of ANOVA exploring relationship between per cent correct and speaker location and direction of target stimulus. *Post hoc* analysis on significant differences between the mean stimulus locations, see Figure 2.1 (a) for locations, of a two-way repeated measures ANOVA on individual bandwidth conditions with dependent variable percent correct and independent variables speaker location and direction the target moved. † Greenhouse-Geisser correction for sphericity. BBN: broad-band noise, LPN: low-pass noise, BPN: band-pass noise.

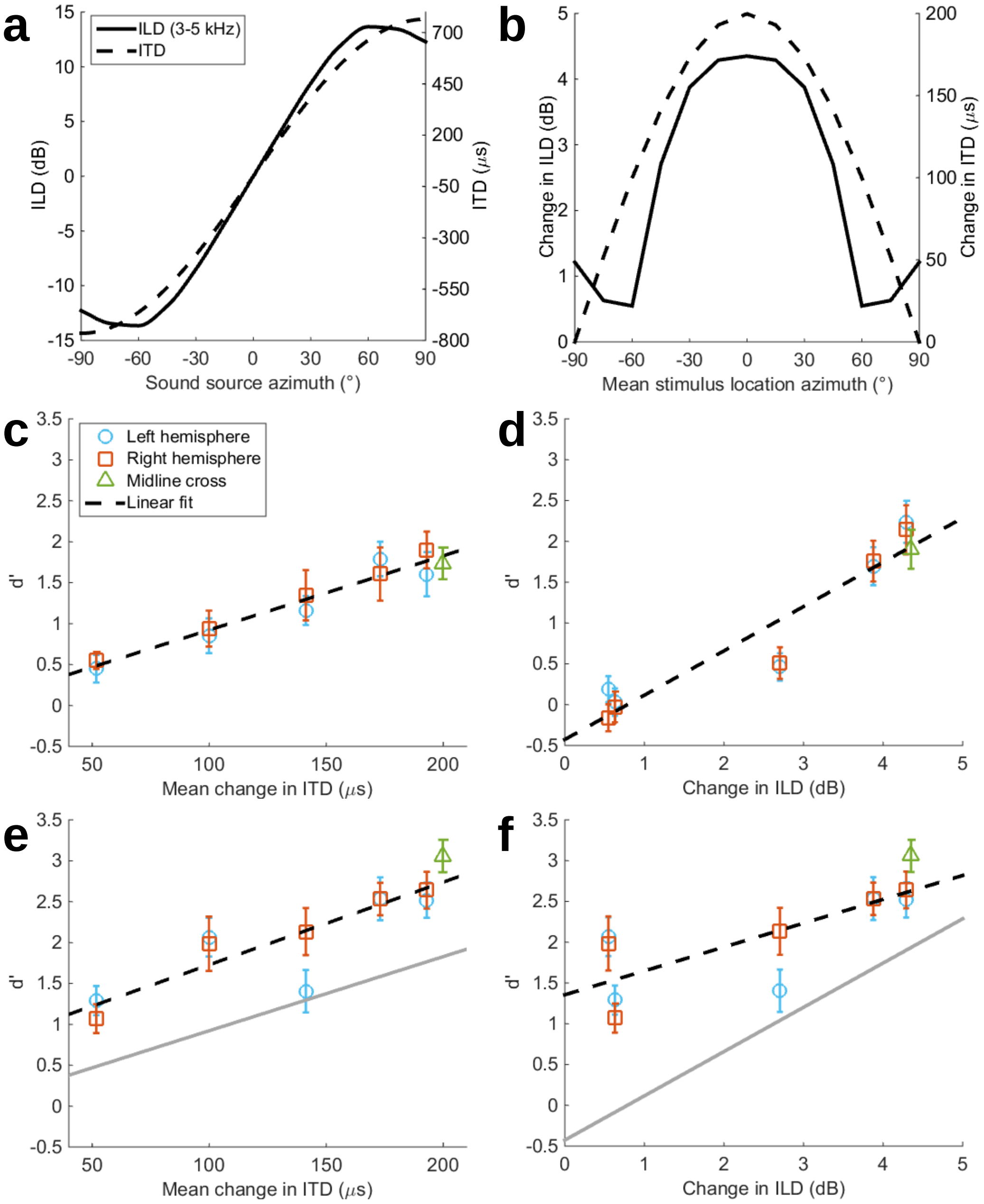


Figure 2.6 – Performance by change in the 'real-world' binaural cues. [a] Changes in ITD and ILD cues as sound source azimuth varies. The dashed line shows changing ITD cues and the solid line shows changing ILD cues (ILD values calculated using data from Shaw and Vaillancourt (1985)). [b] Shows the change in ILD and ITD cues at the mean stimulus locations. [c] Shows the mean *d'* values from the LPN condition plotted as a function of the change in ITD elicited by the stimuli. The dashed line shows a linear fit of the data. [d] Shows the mean *d'* values from the BPN condition plotted as a function of the change in ILD (frequency weighted to reflect the band-pass filter of 3–5 kHz). The dashed line shows a linear fit of the data. [e] Shows the mean *d'* values from the BBN condition plotted as a function of the change in ITD. The dashed line shows a linear fit of this data. The grey solid line shows the linear fit of the LPN data from [c] for comparison. [f] Shows the mean *d'* values from the BBN condition plotted as a function of the change in ILD (frequency weighted to reflect the broad-band stimulus presented at 48 kHz). The dashed line shows a linear fit of this data. The grey solid line shows the linear fit of the BPN data from [d] for comparison.

The BPN data show that performance also declines with decreasing ILD change. Regression analysis was also used to yield a linear fit of these data, but the fit was slightly less good ($R^2 = 0.90$, $p < 0.0001$) possibly due to a floor effect in performance at small changes in ILD. The resulting regression coefficients were used to compare performance in the BBN condition to that in both NB conditions. Figures 2.6 [e & f] show the discriminability index with change in ITD and ILD for the BBN condition. Performance in the BBN condition is higher than performance in either of the spectrally restricted cases and is less well fit with a linear regression line (ITD: $R^2 = 0.77$, $p < 0.001$, ILD: $R^2 = 0.69$, $p < 0.01$) in both cases. While performance in the BBN case is superior to either NB case, the slopes of the regression lines in each condition are very similar when comparing the BBN and NB conditions (NB ITD: 0.0091 d'µs^{-1} and BBN ITD: 0.0101 d'µs^{-1}, NB ILD: 0.5427 d'dB^{-1} and BBN ILD: 0.4214 d'dB^{-1}). The decrease in performance from BBN to NB is more marked in the BPN condition (~2 d') than in the LPN condition (~0.5 d').

2.3.6 Experiment 3: Ruling out effects of gaze location

Although subjects were asked to fixate on an ‘X’ located at the midline, gaze was not explicitly monitored in Experiments 1 and 2. Thus, Experiment 3 aimed to test whether the gaze direction affected the discrimination ability of the subjects. Discriminability from the BBN condition (Experiment 2), where gaze was fixed at 0° (but not monitored), were compared with discriminability when gaze was at 30° to the left or 30° right whilst maintaining the head fixed at 0° azimuth. Figure 2.7 shows the discriminability at each fixation point, with central fixation in grey for comparison. A two-way RM ANOVA (dependent variable d' and independent variables mean speaker location and direction of gaze (central, left or right)) showed a main effect of speaker location ($F_{(3.39,37.31)} = 11.69$, $\eta^2_p = 0.515$, $p < 0.001$, Greenhouse-Geisser corrected for sphericity) but not direction of gaze ($F_{(1.23,13.48)} = 0.32$, $\eta^2_p = 0.003$, $p = 0.9$, Greenhouse-Geisser corrected for sphericity).

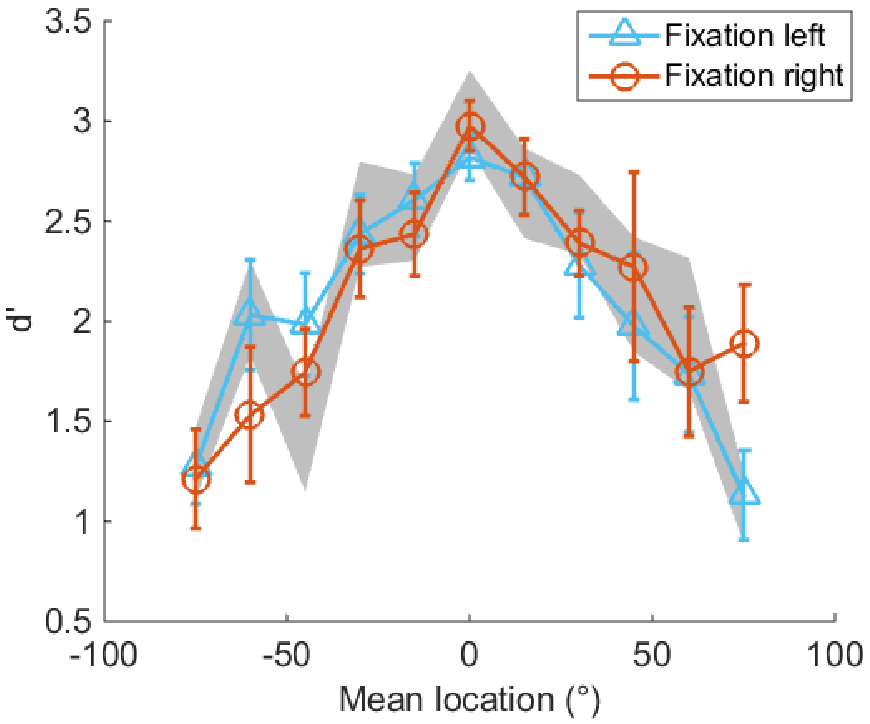


Figure 2.7 – Effect of eye position on relative localisation performance. Direction of gaze did not affect performance in the task with BBN stimuli. Gaze fixation 30° to the left or right of the midline made no difference to the discriminability compared with fixation at 0°. Mean *d*ˈ ± SEM of all participants in the broadband condition with a fixation of 30° left (blue triangles) or 30° right (orange circles). The *d*ˈ ± SEM from the central fixation point (0°) is shown for comparison (grey filled area).

2.4 Discussion

The goal of this study was to develop a two-alternative forced choice task based on the classic MAA paradigm to allow the measurement of spatial acuity throughout auditory space. A relative localisation task was developed that facilitated measurement of spatial resolution at fixed 15° intervals throughout auditory space by requiring human listeners to discriminate the relative location of two sequentially presented sound sources. In order to simulate more real-world listening conditions stimuli were presented in the presence of multiple independent noise sources. Experiment 1 demonstrated that decreasing the SNR impaired performance throughout auditory space. Experiment 2 tested the ability of listeners to perform this task with band-pass stimuli in order to investigate the contribution of binaural cues to the discrimination and compared performance to broadband stimuli containing ITD, ILD and spectral cues. Subjects were able to perform the relative localisation task at a high level of accuracy across the frontal hemifield in the broadband condition with performance reduced relative to this in the low-pass and band-pass conditions. Predictions generated from models of three common theories of how auditory space is encoded by the brain showed that the low-pass data were compatible with two-channel and modified labelled-line models but that data from the band-pass and broadband conditions were incompatible with any of the model predictions. The differences in discrimination abilities observed across space were well described by the underlying acoustic cues available to listeners. Experiment 3 determined that eye/gaze fixation position did not impact upon behavioural performance in this task.

Auditory performance in a variety of tasks declines with decreasing SNR with single masker noise sources (Good and Gilkey, 1996; Lorenzi et al., 1999) and multiple uncorrelated noise sources (Lingner et al., 2012). Experiment 1, which tested ability in the relative localisation task across three SNRs (all of which were above subjects' detection thresholds), demonstrated that listeners were less able to perform this task at adverse SNRs, consistent with results obtained in an absolute localisation task (Good and Gilkey, 1996). There was an interaction between the SNR and the performance across auditory space, indicating that increasing the SNR improved performance differently throughout space – this may partly be explained by ceiling effects in the highest SNR and/or floor effects in the lowest SNR.

When stimuli were presented at equivalent audibility but band-pass filtered, designed to restrict localisation cues to predominantly ITD or ILD cues, subjects could still perform the task but showed weaker performance in each narrow-band condition compared with the broadband stimuli, notably in the band-pass condition, consistent with absolute localisation studies (Carlile et al., 1999; Freigang et al., 2014). This finding is also consistent with data from Recanzone et al. (1998), who measured the ability of listeners to detect changes in source location and demonstrated that performance declined when subjects were given spectrally limited *vs.* broadband noise stimuli.

The data from Experiment 2 also demonstrate that listeners were substantially more biased in the BPN condition than in the other two stimulus conditions. This bias could be a ‘response bias’ which shifted the decision criterion in the direction of the hemisphere in which the sound was presented (Hartmann and Rakerd, 1989).

In order to exclude monaural spectral cues, the BPN stimuli were highly restricted in their spectral band, with the consequence that the spectral bandwidth differed between the LPN and BPN conditions, potentially accounting for some of the observed decrement in performance between BPN and the other conditions. The spectral band chosen also limited listeners to relatively small ILD cues (Figure 2.6 [a & b]) with which to perform the task and it has been previously shown that performance is poor for localising pure tones in the region of 3-5 kHz (Stevens and Newman, 1936). Listeners may also have been able to utilise envelope ITDs in the BPN condition (Bernstein and Trahiotis, 1994). Future experiments are necessary to explore the contribution of spectral bandwidth, as well as both envelope and temporal fine structure cues, to performance in this task. Performance was best in the broadband condition, when both binaural and monaural spectral cues were available, although it is likely that subjects relied on binaural cues to perform the task even when spectral cues were available since spectral cues contribute little information when normal binaural cues are available in an absolute localisation task (Macpherson and Middlebrooks, 2002).

Analysis of the underlying cues available to listeners in the band-pass conditions allowed us to compare performance in the task with estimates of available binaural cues. For pairs of speakers at peripheral locations, the change in the available ILD cue was <1 dB and since Mills (1960) reported a just noticeable difference of approximately 1.6 dB ILD about the midline for pure tones of 3-5 kHz, it is perhaps unsurprising that subjects performed poorly at these locations in the BPN condition. In contrast to the limited availability of ILD cues at peripheral locations, ITD cues did not decline as sharply in the periphery and behavioural performance reflected this. For tones of 1 kHz or less, presented in silence at 75° azimuth, the MAA corresponds to an ITD change of approximately 70 μs (Mills, 1958). In the present study, the most peripheral locations the change in ITD corresponded to only ~86 μs, a value fractionally higher than the measured corresponding MAA. Contrary to the Mills study, the current task required that listeners report the direction of the stimulus movement and not just report a change in location; a higher minimum audible angle may be required for reporting the direction of the movement.

In Experiment 2, the slopes of the regression lines estimated from the available cues in the BBN case were broadly similar to those in the spectrally restricted cases (Figure 2.6), however, the intercept was higher in the broadband case than both LPN (BBN: 0.0157 *d'*, LPN: 0.7182 *d'*) and BPN (BBN: 1.38 *d'*, BPN: -0.43 *d'*) cases. This suggests that listeners integrate the available binaural and spectral cues in the BBN condition to allow better relative localisation than either cue alone, just as they do during absolute localisation studies (Hebrank and Wright, 1974; Macpherson and Middlebrooks, 2002) although, previous studies have shown a role for spectral cues in absolute localisation studies (Musicant and Butler, 1985; Yost and Zhong, 2014)

An open question is how these cues are integrated to form a perception of auditory space within the brain. Three simple models were developed where auditory space was represented as a two-channel model, a labelled-line model or a modified labelled-line model, based on recent non-behavioural imaging studies that tested brain responses to shifts in sound source locations (Salminen et al., 2009, 2010b; Magezi and Krumbholz, 2010), and predictions were generated for psychophysical performance in this task. Specifically, the two-channel and modified labelled-line model predicted that performance should be better around the midline than in the periphery, whereas performance should be equal throughout space for the labelled-line model. Statistical analysis of the behavioural data demonstrated that in all three-cue conditions (LPN, BPN and BBN) performance varied throughout space, and that midline performance was superior to that in the periphery. Additionally, in the LPN condition where ITDs are the dominant localisation cue, performance was consistent with the two-channel (McAlpine et al., 2001) and modified-labelled line models.

As mentioned in the Introduction, changes in the location of sound must be tracked over time to provide information about direction of motion; this can be achieved by adaptation or forward suppression. In the barn owl, spatial receptive fields are often asymmetric and the shapes of the spatial receptive fields can predict the direction sensitivity of individual neurons. Furthermore the more peripheral the cells were tuned, the more they preferred sounds moving toward the midline (Wang and Peña, 2013). Directional sensitivity in the barn owl can also result from surround inhibition on a population scale since the areas of interest to the barn owl (locations in front and below it) are overrepresented, much like that described in the modified labelled-line model, and thus stronger surround inhibition is provided by central locations, this results in preference for sounds approaching the front/midline in the barn owl (reviewed in Wang et al., 2014). This type of direction selectivity requires a topographic map of space the like of which has not been found in auditory cortex of mammals. However, direction selectivity of the adaptation type could be found in mammalian auditory cortex where receptive fields are often complex and asymmetric (e.g. Mrsic-Flogel et al., 2005). The fact that there are more neurons preferring inward-moving stimuli may predict that owls would be better a discriminating stimuli moving toward the midline, there is currently no data to confirm or falsify this prediction.

In contrast, the data for the BBN and BPN conditions were not satisfactorily explained by any of the models. That the predictions made by the two-channel model and the modified labelled-line model cannot be distinguished highlights the need for invasive physiology experiments to directly measure spatial receptive fields. If a two-channel model were used in auditory cortex then we would expect to see broad tuning with peaks at ±90° and the steepest slopes across the midline whereas for a modified labelled-line model sharper spatial receptive fields covering all of space would be expected.

While recent neuroimaging studies have lent support to a two-channel model of sound location in human auditory cortex (Salminen et al., 2009, 2010b; Magezi and Krumbholz, 2010; Briley et al., 2013), alternative models of the neural representation of sound location propose that space may be represented by a three-channel model (Dingle et al., 2010, 2012, 2013) or that an optimal model would change according to both frequency and head size such that, for humans, coding is predicted to be two-channel at low frequencies and labelled-line at higher frequencies (Harper et al., 2014). Recent physiological findings from auditory cortex are also consistent with a labelled-line code for sound localisation cues (Belliveau et al., 2014; Moshitch and Nelken, 2014). There is also conflicting evidence from the gerbil where neurons with ITD tuning inside the physiological range have been identified (van der Heijden et al., 2013; Franken et al., 2015), contrary to two-channel coding predicted by optimal coding (Harper and McAlpine, 2004). It may also be the case that different localisation-based tasks tap into different levels of the auditory brain in which different coding schemes may operate. For example a recent behavioural study using multiple auditory objects to probe the representation of auditory space is consistent with there being multiple, narrowly tuned, spatial channels (Carlile et al., 2014), while neurophysiological studies support a coding transformation for ITDs from two-channel to labelled-line from midbrain to cortex (Belliveau et al., 2014).

Experiment 3 explored whether eye position influenced performance in the relative localisation task by asking subjects to fixate 30° to the left or 30° to the right of the midline while maintaining a central head-position. We found that gaze location had no effect on the discriminability of left and right moving sounds for our subjects, indicating that the superior performance at the midline in Experiments 1 and 2 is relative to head position rather than eye position or attentional focus, or some combination of the these factors. This is in contrast to previous work on absolute sound localisation, which has shown that gazing towards a visual stimulus can alter sound localisation abilities, for short periods of time sound localisation is biased away from the point of gaze (Lewald and Ehrenstein, 1996) and for longer periods of time, sound localisation is biased towards the point of gaze (Razavi et al., 2007). However, it is not clear that this would necessarily affect the accuracy of comparing the location of two sounds. In another study looking at acuity of localisation cue discrimination (Maddox et al., 2014), a short gaze cue that informed subjects about the location of the sound they were about to listen to improved performance in an auditory relative localisation task. Our results do not show a difference in performance but this could be because our subjects had their gaze fixed for minutes at a time in one location, which in itself offered no information about the likely origin of the upcoming sound. When Maddox et al. (2014) used uninformative cues there was no improvement in performance. Thus, the present data are consistent with auditory space being represented relative to the orientation of the head, rather than the direction of gaze.

Individual thresholds were measured for each signal type using a modified version of the task which required that listeners report whether a target sound originated from ±90° left or right of the midline. Signals at 90° eccentricity presented in noise will be more audible than those presented at the midline due to a combination of the better ear effect (Zurek, 1992) and spatial release from masking (Blauert, 1997). Pilot experiments demonstrated that 95% detection thresholds were on average 0.4 dB lower at +90° than at 0°. If audibility was limiting performance at central locations we might predict that localisation performance would also decrease towards the midline whereas the data in Experiment 1-3 suggest the opposite. Nevertheless, it is possible that at the lowest signal-to-noise ratio, where performance at the midline is substantially poorer than the medium and high SNRs, audibility differences might be imposing a limit on performance.

In conclusion, a two-alternative forced choice localisation task that provides a rapid way of assessing spatial sensitivity throughout auditory space has been developed. Rather than collecting thresholds for spatial discrimination at multiple locations, or requiring that subjects make some sort of absolute localisation judgment listeners were tested in a task that measured localisation abilities at fixed 15° intervals in the frontal hemisphere. Such a test provides a robust, sensitive and flexible method that could prove useful both in clinical settings for examining the precision of localisation in hearing impaired listeners and for testing in animal models; advances have been made towards this step (Bizley et al., 2016; under revision). For invasive neurophysiological studies that must necessarily be performed in animal models, this task represents an ideal way to explore the neuronal correlates of sound localisation in animals actively engaged in a localisation task. The first stimulus allows spatial receptive fields to be explored and predictions about the spatial receptive fields that would be present in each model, as mentioned above, can be investigated. Unlike an approach-to-target task this paradigm reduces the response options to two, thus allowing more powerful neurometric analysis. Chapter 3 will present performance data from ferrets trained in a very similar relative localisation task.

Chapter 3: Relative sound localisation ability of ferrets

3.1 Introduction

Chapter 2 developed a novel relative localisation task and presented data from human listeners performing the task, in this chapter performance of ferrets in a very similar task is presented. To briefly summarise the results from chapter 2, in which subjects had to discriminate the direction of movement of a target stimulus relative to a reference stimulus in the azimuthal plane, performance was best around the midline and worse in the periphery (Wood and Bizley, 2015). Performance decreased when sounds were spectrally restricted to make ITDs or ILDs the dominant spatial cue, this deficit in performance was partially explained by the binaural cues available in each condition. Performance in the low pass condition, where ITDs were the dominant spatial cue, was consistent with performance prediction by a two-channel model. The fact that the models inadequately described the results (except for the ITD condition) highlights the need for invasive neurophysiological studies that must necessarily be performed in animal models. This two-alternative forced choice (2AFC) task is an ideal way to explore the neuronal correlates of sound localisation in animals actively engaged in a localisation task, since unlike an approach-to-target task, this paradigm reduces the response options to two, thus allowing more powerful neurometric analysis, which is advantageous if one wants to combine behavioural investigations with neuronal recordings (Parker and Newsome, 1998). As mentioned in the introduction, there is a lack of studies presenting neurophysiological data from auditory cortex of animals actively engaged in discrimination of sounds in azimuth. In this chapter psychophysical performance of ferrets trained in a relative localisation task is presented. I demonstrate both that ferrets can learn to perform the relative localisation task and that their performance with broadband noise stimuli is similar to humans'.

To the author’s knowledge, no behavioural work in the ferret measuring any form of relative location where discrimination of the location of sounds in azimuth must be performed has been published. Previous studies in mammals have merely required reporting of a change in location (Recanzone et al., 2000; Lee and Middlebrooks, 2011), in the present task the animal must report the direction of the change in location. Ferrets are excellent models for behavioural investigations and are capable of performing complex behavioural tasks such approach-to-target sound localisation (Parsons et al., 1999), discrimination of pitch, where they are able to report the direction of change in pitch in two sequentially presented sound bursts (Walker et al., 2009; Yin et al., 2010), as well as absolute pitch discrimination (Fritz et al., 2005a), click-rate discrimination (Fritz et al., 2005b), gap detection in noise (Kelly et al., 1996; Gold et al., 2015), timbre discrimination (Bizley et al., 2013a; Town et al., 2015) and speech discrimination (Bizley et al., 2015). Here ferrets are trained to perform the relative localisation task developed in chapter 2 in which they must report whether a target stimulus originated from the left or right of a preceding reference stimulus in the azimuthal plane in a two-alternative forced choice (2AFC) design.

Since it was found that humans weighted ITD cues higher than other cues in a very similar task (chapter 2), testing in the ferrets was performed with different band-pass stimuli in order to limit the localisation cues available. Three band-pass stimuli were tested; low-pass, where only ITDs were available to the ferret, narrow band-pass, where it has been shown that ferrets use ILDs to perform localisation of stimuli (Keating et al., 2014) and high-pass, where there are no ITDs available to the ferret. Performance in these three conditions was compared to the performance in the broadband condition where all localisation cues were available.

It has previously been shown that addition of a diffuse background noise reduces the size of the spatial receptive fields (SRFs) of cat primary cortical neurons caused by a reduction in firing rate (Brugge et al., 1998). This reduction in SRF size could be mirrored by reducing the intensity of the signal alone and thus the noise served as a masker and reduced the detection threshold of the individual units. The slope of the SRFs, which is thought to convey information about the location of sounds, was maintained in the presence of the background noise. Similar findings were also observed in a secondary area of cat auditory cortex by Furukawa and Middlebrooks (2001), who tested the ability of an artificial neural network to determine spatial location using the firing rates of neurons in the presence of a background noise and in silence and found that it did well in both cases. This is consistent with studies that show human localisation is relatively insensitive to a continuous background masker (Good and Gilkey, 1996). Furukawa and Middlebrooks also found a second class of neuron whose firing rates were maintained in the presence of noise or even facilitated, the mechanism of this is suggested to be a reduced inhibitory input from pre-cortical areas. The effects of a background masker on the relative localisation task were investigated by presenting background noise from a speaker located directly above the ferret while it performed the task.

Ferrets were also tested with an increased duration (100 ms) between the first and second sounds to investigate whether having to ‘remember’ the location of the first sound for longer affected performance. Ferrets have previously been tested with go-no go tasks that require comparison of two sounds presented sequentially (e.g. Yin et al., 2010) and thus necessarily the ferrets must use a form of working memory in order to compare the two stimuli. However, working memory has not been explicitly extensively studied in ferrets.

3.2 Methods

3.2.1 Animals

All animal procedures were approved by the local ethical review committee and performed under license from the UK Home Office in accordance with the Animal (Scientific Procedures) Act 1986. Four adult, female, pigmented ferrets (*Mustela putorius*) were used in this study (F1301, F1302, F1310, F1313), housed in groups of between two and eight with free access to high-protein food pellets and water. All animals received regular otoscopic examinations throughout the experiment, to ensure that both ears were clean and disease free.

3.2.2 Training/testing runs

Animals were water restricted during training/testing runs and had free access to water during rest periods. The weight of the animals was monitored throughout training and testing to ensure that they maintained their body weight within 85% of its starting level (typical weight losses were much smaller than this). During training and testing, the amount of water they received daily as positive reinforcement was monitored and if necessary supplemented with wet mash (ground dry food mixed with water) to ensure they received 60 ml/kg of water per day. A training run usually consisted of 5 days of training, followed by two days of rest with free access to water on the home cage. Ferrets were trained in two sessions per day during a training run. Ferrets were given extended periods of rest (normally 1 week) to ensure that they were not under water deprivation for more than 50% of the time (measured in days).

3.2.3 Stimuli

All stimuli were generated and presented at a sampling frequency of 48 kHz. Sound stimuli were presented from thirteen loud speakers (Visaton SC 5.9) positioned in a semicircle of 24.1 cm radius around one end of the testing chamber (See Figure 3.1), speakers were evenly positioned from -90° to 90° at 15° intervals approximately at the height of the ferret’s head when at the central start spout. Speakers were calibrated to produce a flat response from 200 Hz to 25 kHz when measured in an anechoic environment using a microphone (Brüel and Kjær 4191 condenser microphone). The microphone signal was passed to a TDT System 3 RX8 signal processor via a Brüel and Kjær 3110–003 measuring amplifier. Golay codes were presented through the speakers and the spectrum was analysed and an inverse filter was constructed to flatten the spectrum (Zhou, 1992). All sounds were presented

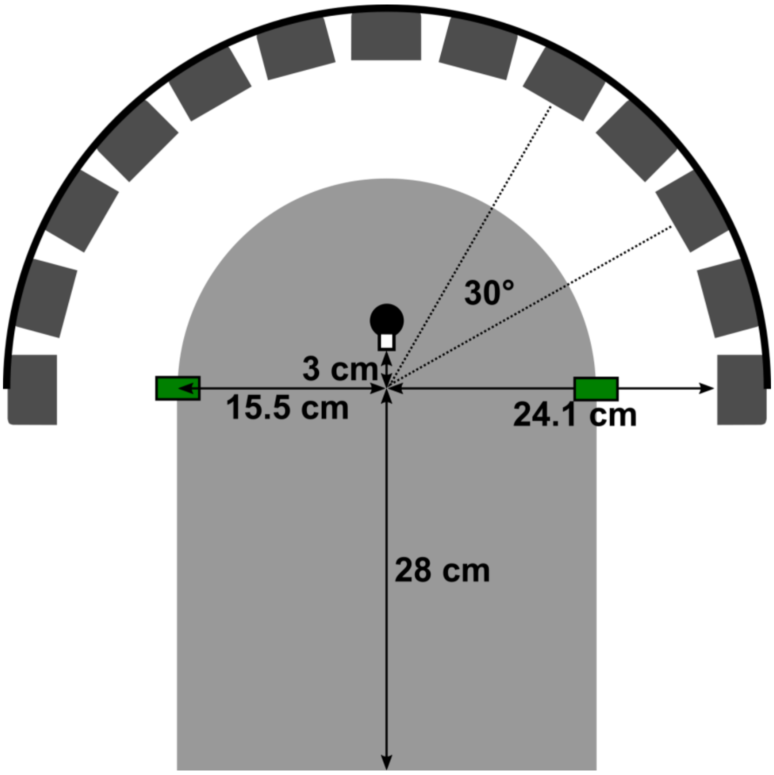


Figure 3.1 – Relative localisation testing chamber. The dimensions of the chamber are indicated. Speaker locations are shown by dark grey rectangles and are positioned 24.1 cm from the centre of the chamber. The response spouts are indicated by the green rectangles and the start spout is represented by the white square in the middle of the arena.

low pass filtered below 22 kHz (finite-duration impulse response (FIR) filter <22 kHz, 70 dB attenuation at 22.2 kHz) and with the inverse filters applied.

All the speakers were matched for level using a microphone positioned upright at the level of the ferret head in the centre of the semi-circle; correcting attenuations were applied to the stimuli before presentation, stimuli in all conditions were presented as standard at 61 dB SPL unless the testing required otherwise. A light emitting diode (LED) was also mounted outside the chamber, behind the plastic mesh that enclosed the chamber, approximately 15 cm from the floor of the chamber and flashed (at 3 Hz) to indicate the availability of a trial to the ferret. The LED was continually illuminated whenever the animal successfully made contact with the IR sensor within the central start spout. The training and testing of the ferrets (once sounds were being presented) was fully automated with IR spout input transferred via 2 TDT system III SA8s into a custom-written circuit running in Open Project (TDT Software) which also communicated with MATLAB. Stimuli were generated in MATLAB and were presented automatically when a trial was triggered via two TDT system III RX8 processors. Water rewards were given by custom-built devices controlling solenoids which opened tubing feeding water to the response and start spouts. Ferrets always received a water reward for correct responses from the response spout and received a reward from the start spout 5% of the time. In training, stimuli were two 200 or 150 ms broadband noise bursts, including a 5 ms cosine envelope at onset and offset, presented sequentially from two speakers separated by 30° with a 20 ms silent gap between them. The first stimulus is the reference sound and the second stimulus is the target sound, the location of which must be reported relative to the reference sound. Two sets of speakers were tested, -90° to 90° at 30° intervals or -75° to 75° at 30° intervals. During training, the level of the stimuli varied between 61 dB and 55 dB SPL.

Two major changes were made to make the task suitable for ferrets and neural recordings: The stimulus was changed from 15 ms pulses to continuous bursts of noise (150 ms or 200 ms in duration) and the angle of separation between the reference and the target stimuli was changed from 15° to 30°. Many previous studies have looked at encoding of the spatial location of noise bursts (e.g. Stecker et al., 2005b) and thus the change in type of stimulus from pulses to continuous noise bursts allowed easier comparison between the data of the present study and others. The angle separation was changed because the minimum audible angle for ferrets at 45° (for noise bursts; Parsons et al., 1999) was much larger than that for humans at the same angle (low frequency tones; Mills, 1958). Thus it appeared that discrimination in the ferret at peripheral locations may not be possible at 15° separations.

3.2.4 Behavioural paradigm

The ferrets were trained to perform a relative localisation task similar to the task performed by humans (see chapter 2). Ferrets were trained to report the location of a target sound relative to a preceding reference sound presented from a location ±30° from the target sound (or two preceding reference sounds presented from locations 30° and 60° from the target sound), effectively reporting the direction the sounds moved, by responding to two response spouts indicating left and right respectively.

During training, animals were initially rewarded if they approached either of the left or right response spouts or start spout (see Figure 3.1). After the ferret was comfortable with receiving water from all the spouts it approached (typically 3-4 sessions), a new contingency was introduced such that the ferret had to approach the start spout and remain there for a variable hold time (usually 500-2500 ms) before it was rewarded and was then able to respond to one of the response spouts in order to receive a further reward. Following acquisition of this basic task structure, sounds were presented after the ferret activated the start spout for the duration of the hold time. This variable hold time ensured that ferrets kept their heads still at the centre of the testing chamber during the stimulus presentation period therefore ensuring good control of the spatial location of the stimuli relative to the ferret. Ferrets were initially trained with 3 sounds where the first sound was just to the left or right of the midline (i.e. ±30° or ±15° depending on the ferret), with the second and third sounds 30° and 60° lateral to the first sound respectively (i.e. locations of ±60° & ±90° or ±45° & ±75° depending on ferret). At this stage, ferrets making an incorrect response did not terminate the trial and the sound repeated (with a 500 ms silent gap between each cycle of the three sounds) until they approached the correct response spout, where they received a water reward. Once the ferret had performed a few (typically 3-4) sessions the task was modified so that now a response to the incorrect spout terminated the trial. This was indicated by the presentation of a short duration tone (5 kHz) and followed by a time out during which a trial could not be initiated. This time out was initially very short (1 second) and was increased over several weeks to be approximately 7 seconds.

At this stage of training the ferret can essentially perform a lateralisation task as, for example, all of the sounds that move left are presented on the left. To train the animal on the full relative localisation task required gradually introducing sound locations throughout the frontal hemisphere so that the ferret had to make a genuine relative localisation judgment that was invariant to the starting or finishing location of the sound sequence. Therefore from this point, the per cent correct values of each training session were monitored and when ~70% correct was reached for a given set of stimuli the training was increased in complexity by adding in additional reference starting points. The final stage of training was to reduce the number of sounds presented to two (a single reference and a target) and train with all possible speaker locations (-90° to 90° in 30° steps for F1301 and F1302 and -75° to 75° in 30° steps for F1310 and F1313). During training, the level of the stimuli presented varied. Once testing commenced, stimuli were presented at a single level (except in specific testing sessions designed to explore level invariance) in order to facilitate neurometric analysis by maximising the number of trials obtained for any given stimulus.

Once training was completed and ferrets were able to perform the task with two stimuli (i.e. one reference and one target location) at a good overall level

(~70%) they were then able to be tested, this usually took around 9-10 weeks going through all the stages of training with weeks not training as specified by the project licence. Testing was similar to training in that there were two testing sessions per day and a testing run usually consisted of 5 consecutive days of testing followed by two days of rest. At the start of every testing run ferrets were trained for one day (two sessions) on the level-varying BBN stimuli.

Ferrets were tested in 6 different conditions that they were not trained in specifically: Broadband noise at a single sound level, low pass noise (LPN) where the stimuli were low pass filtered less than 1 kHz using MATLAB (FIR filter <1 kHz, 70 dB attenuation at 1.2 kHz), band pass noise (BPN) where stimuli were band pass filtered at sixth octaves about 15 kHz (FIR filter sixth octave around 15 kHz, 60 dB attenuation at 7.5 kHz and 68 dB attenuation at 21 kHz), high-pass noise (HPN) where stimuli were high pass filtered above 3 kHz (FIR filter >3 kHz, 70 dB attenuation at 2 kHz), longer interval of 100 ms with broadband stimuli and sound level-varying broadband stimuli in a noisy background. The noisy background consisted of 15 ms tokens of amplitude modulated broadband noise bursts presented from a single speaker located directly above the animal when it has its head at the start spout. All stimuli were presented at 61 dB SPL for single level stimuli. In the background noise condition, stimuli were presented at varying levels from 55 dB to 61 dB SPL in 3 dB steps, the average level of the noise was 55 dB SPL at the level of the ferret head. When comparing the performance with the background noise to without, the level varying training stimuli were used since in the standard BBN testing, the level was not roved. Only *training* sessions where the ferrets' performance exceeded chance performance were included in the comparison (binomial test, $p > 0.05$). The low-pass stimuli were designed to present the ferret with ITD cues only, the band-pass stimuli to provide ILD cues, at this narrowband frequency, ferrets rely on ILD cues to localise sounds (Keating et al., 2014) and the high-pass stimuli to exclude ITD cues. With the ferret at a good level of performance, each different testing paradigm was tested in two sessions on the same day over a two week period (so one day of testing in every 10 for each type of test).

Ferrets were trained on a certain set of speakers (references at either -90° to +90° in 30° steps or -75° to +75° in 30° steps) with level-varying stimuli and new broadband noise burst generated for each trial. During testing, the stimuli they were tested on did not change in level and ferrets were occasionally tested with alternative sets of speakers and in some cases always tested on speakers that they were not trained on. For example, the majority of the training was performed with reference locations at -75° to +75° in 30° steps, in testing, specifically for the broadband noise stimuli and the band-pass noise stimuli, speakers at -30°, 0° and 30° were included and these were not specifically trained on. Thus, it is unlikely that ferrets were able to learn speaker-specific responding. Furthermore, the number of speaker-specific combinations that would have to be learnt would be prohibitive for example in the -75° to +75° in 30° steps, the ferret would have to learn 10 different combinations and with differing band-pass stimuli. Also, speakers were matched by calibration such that they were spectrally matched.

3.3 Results

Ferrets performed a single interval 2AFC task where they were asked to report whether a target sound was presented to the left or right of a preceding reference. During testing, the reference and target stimuli, separated by 20 ms of silence, were 150 ms or 200 ms duration and consisted of either broadband noise (BBN) bursts, low-pass filtered (<1 kHz) noise (LPN) bursts, band-pass filtered (1/6 octave about 15 kHz) noise (BPN) bursts, high-pass filtered (>2 kHz) noise (HPN) bursts (all Experiment 1), BBN bursts separated by 100 ms (Experiment 2) or level varying BBN bursts presented in a noisy background (Experiment 3).

3.3.1 Experiment 1: Effect of spectral band

Figure 3.2 shows the performance of the ferrets when all binaural and monaural cues are available (BBN) and the effects of limiting the spectrum of the stimuli with low-pass noise (LPN) designed to restrict available cues to ITDs, band-pass noise (BPN) designed to restrict available cues to ILDs and

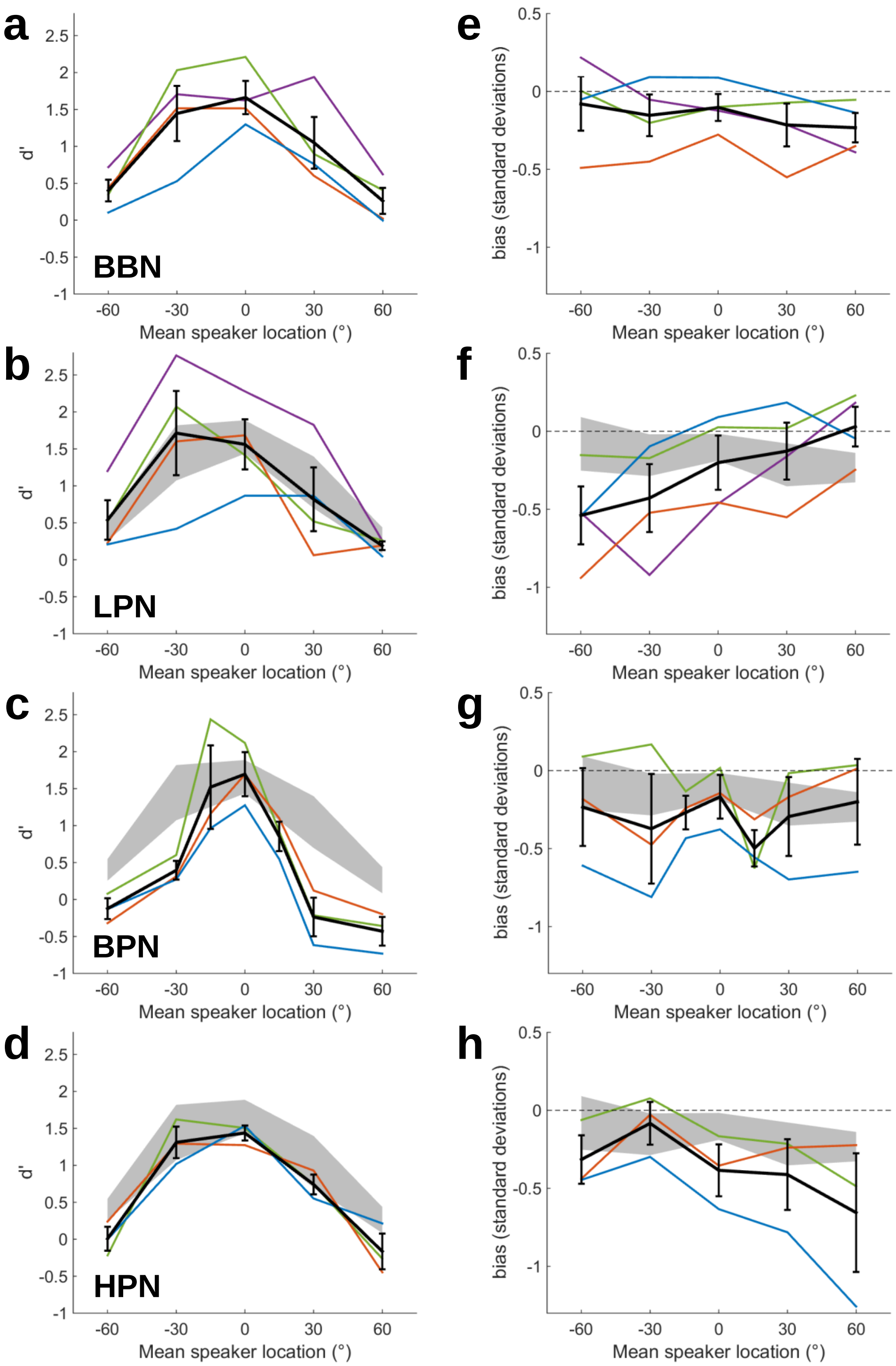


Figure 3.2 – Effect of limiting the spectral band on sensitivity and bias in the relative localisation. Performance was decreased compared with the BBN condition in the BPN condition but not the LPN or HPN conditions**.** The left column show dˈ values for each band pass condition; Broadband noise (BBN, [a]), low-pass noise (LPN, [b]), band-pass noise (BPN, [c]) and high-pass noise (HPN, [d]). The right column shows the corresponding bias in each condition; BBN [e], LPN [f], BPN [g] and HPN [h]. Black lines indicate the mean sensitivity of all ferrets ± standard error of the mean (SEM) and thin coloured lines indicate individual ferrets sensitivity and bias. Grey fill shows mean ±SEM BBN sensitivity and bias for comparison.

high-pass noise (HPN) designed to exclude ITD cues. The data are plotted according to the mean location of the reference and target so that performance is compared across equivalent changes in sound localisation cue. In general, the ferrets perform better at more central locations than at locations more peripheral. The decrement in the periphery is more marked in the BPN case than in the other cases. Two-way RM ANOVAs between the broadband condition and each of the narrow-band conditions (independent variables: mean location and band-pass condition and dependent variable: d') revealed a main effect of location in all comparisons (LPN: $F_{(4,12)} = 13.01$, $p < 0.001$, BPN: $F_{(4,8)} = 51.09$, $p < 0.001$, HPN: $F_{(4,8)} = 20.88$, $p < 0.001$), and a difference between BBN and BPN conditions ($F_{(1,2)} = 60.44$, $p = 0.016$) but not BBN and the other conditions (LPN: $F_{(1,3)} = 0.00$, $p = 0.995$, HPN: ($F_{(1,2)} =0.96$, $p = 0.430$) and no interactions. *Post hoc* pairwise comparisons (Bonferroni's multiple comparison test, $p < 0.05$) of the locations revealed that in the BBN and LPN conditions, the performance at the midline was significantly better than performance in the two most peripheral locations.

The bias was also estimated for each condition and each ferret (Figure 3.2, right-hand column), a negative value indicates that the ferrets were more likely to report that the target was to the left of the reference and a positive value indicates that the ferret was more likely to report that the target was to the right of the reference stimulus. Two-way RM ANOVAs between the broadband condition and each of the narrow-band conditions (independent variables: mean location and band-pass condition and dependent variable: bias) revealed no effect of condition or speaker location in any comparisons but did show interactions in the BBN *vs.* LPN and HPN cases (LPN: $F_{(4,12)} = 5.82$, $p = 0.008$, HPN: $F_{(4,8)} = 4.39$, $p = 0.036$) indicating that the distribution of bias across the sound source locations differed between the two stimulus conditions.

In order to compare the performance of the ferrets to the models described in the previous chapter (in Section 2.3), the data were reanalysed according to whether the target moved away from the midline or towards the midline.

Each point in Figure 3.3 shows the mean performance (± SEM) of the ferrets in each band pass condition at each paired inward *versus* outward moving stimuli. Two-way RM ANOVAs in each stimulus condition (independent variables: mean location and direction stimulus moved (inward *vs.* outward) and dependent variable: % correct) revealed a main effect of the mean stimulus locations in the BBN ($F_{(3,9)} = 13.15$, $p = 0.001$), BPN ($F_{(5,10)} = 17.15$, $p < 0.001$) and HPN ($F_{(3,6)} = 16.38$, $p = 0.003$) but not the LPN $\mathrm{F}_{(1.5,4.6)} = 5.66$, $p = 0.061$, Greenhouse-Geisser corrected for sphericity) conditions. *Post hoc* pairwise comparisons (Bonferroni's multiple comparison test, $p < 0.05$) of the mean stimulus locations did not reveal any to be significantly different from the other in the BBN and HPN conditions, however, qualitatively, performance is worst in the most peripheral locations. In the BPN condition, 15° was found to be significantly different from 30° and 60°. Only the LPN condition showed a main effect of inward *versus* outward moving stimuli (LPN: $F_{(1,3)} = 30.56$, $p = 0.012$, BBN: $F_{(1,3)} = 2.20$, $p = 0.234$, BPN: $F_{(1,2)} = 0.12$, $p = 0.767$, HPN: $F_{(1,2)} = 3.40$, $p = 0.207$), reflecting the large bias to report left for stimuli in the left hemifield (see Figure 3.2 [f]). Only the HPN condition showed any interaction between the stimulus locations and the direction the stimulus moved, again this reflected the bias of the ferrets to report left (Figure 3.2 [h]; HPN: $F_{(3,6)} = 5.75$, $p = 0.034$, BBN: $F_{(3,9)} = 2.17$, $p = 0.162$, LPN: $F_{(3,9)} = 3.49$, $p = 0.063$, BPN: $F_{(5,10)} = 2.90$, $p = 0.071$).

3.3.2 Assessing the relationship between performance and binaural cue values

Since a 30° change in azimuth does not elicit an equal change in localisation cues across space, the data were compared to estimated changes in ILD and ITD for each pair of reference and target locations tested. ITDs were estimated using a model described in Schnupp et al. (2003) and ILDs were obtained from Direction Transfer Functions used for constructing virtual acoustic space (Mrsic-Flogel et al. 2005). Figures 3.4 [a] and 3.5 [a & d] shows the estimated changes in ITD (mean of 3 of the ferrets in this study) or ILD (15 kHz band-pass filter and >2 kHz high-pass filter) for the mean speaker-pair locations tested. Figures 3.4 [b] and 3.5 [b & e] plot the

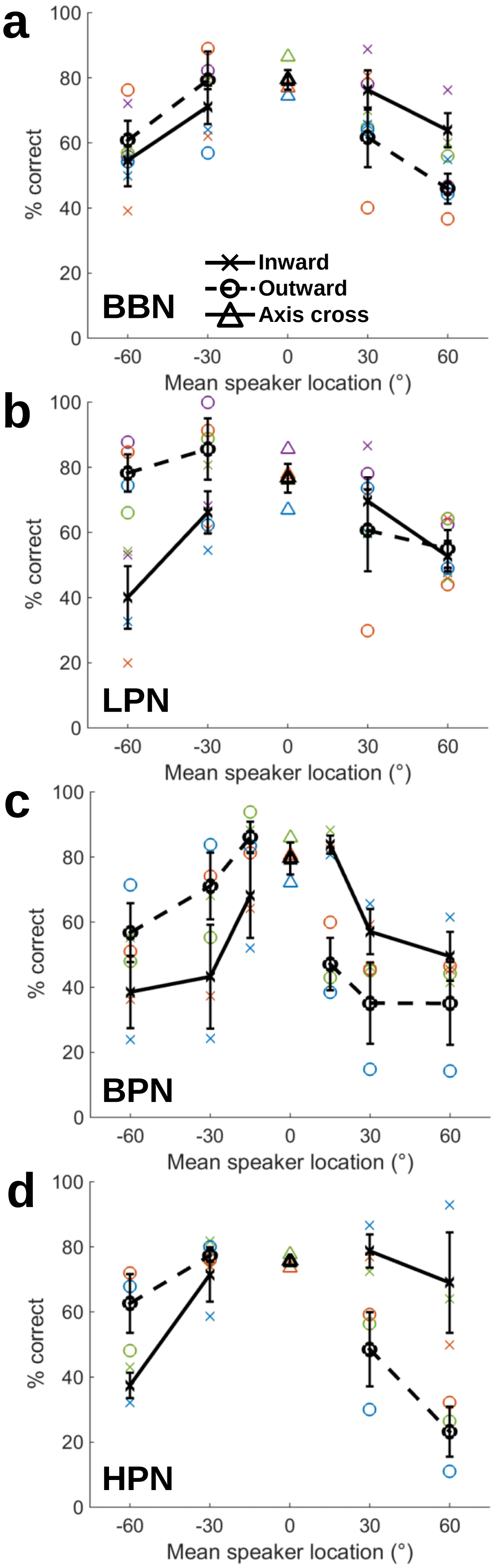


Figure 3.3 – Performance for inward and outward moving stimuli in the band-pass conditions. Performance in all conditions was best around the midline and decreased in the periphery. The bias of the ferrets is evident in the inward vs. outward moving stimuli, ferrets tended to be biased to the left. The graphs show the mean performance ±SEM in each condition; BBN [a], LPN [b], BPN [c] and HPN [d]. Individual ferret performance are indicated by the coloured symbols. Data are separated into inward moving (toward the midline, crosses and solid line), outward moving (away from the midline, circles and dashed line) and comparisons across the midline (triangles).

sensitivity (*d'*) of the animals in the LPN, BPN and HPN conditions (from Figure 3.2) as a function of the change in ITD or ILD. For the LPN condition, as the ITD increases, performance also increases however the performance is not well fit with a linear regression line ($R^2 = 0.71$, $p = 0.075$), possibly due to the bias to respond to the left (see Figure 3.2 [f]). The BPN data show an increase in performance with increasing changes in ILD and performance is well fit with a linear regression ($R^2 = 0.86$, $p = 0.003$). For the HPN condition, there is also an increase in performance as the change in ILD increases, again performance was well fit by a linear regression ($R^2 = 0.91$, $p = 0.011$). The regression fits were used to compare the data to performance in the BBN condition, Figures 3.4 [c] and 3.5 [c & f] plot the performance of ferrets in the BBN condition as a function of the change in ITD and ILD respectively. The black lines indicate the linear regression lines from the BBN conditions and the grey lines plot the linear regression from the narrow-band conditions for comparison. Unlike in the LPN condition, the BBN condition data were better fit by a linear regression for the ∆ITD (Figure 3.5 [c], $R^2 = 0.93$, $p = 0.008$) and were also well fit for the ∆ILD (Figure 3.5 [f], $R^2 = 0.74$, $p = 0.014$). While it is problematic to interpret slopes with non-significant regressions (LPN condition), the performance in the BBN condition is very similar to that of the LPN condition, with the two linear regression parameters very similar (slope, intercept: LPN ∆ITD: 0.043 $d'\ \mu s^{-1}$, -2.62 *d'* and BBN ∆ITD: 0.047 $d'\ \mu s^{-1}$, -2.92 *d'*). However, performance in the BBN condition when compared across changes in ILD is quite different to performance in either the BPN and HPN conditions. The slopes and intercepts are different, possibly due to floor performance effects at the smallest ILD changes (slope, intercept: BPN ∆ILD: 0.97 $d'\ dB^{-1}$, -4.56 *d'*, HPN ∆ILD: 0.33 $d'\ dB^{-1}$, -0.65 *d'*, BBN ∆ILD: 1.17 $d'\ dB^{-1}$, -1.31 *d'*). In summary, it appears that when ITDs are available, ferrets are using them to perform the task.

3.3.3 Experiment 2: Effect of increased interval between reference and target stimuli

Figure 3.6 shows the sensitivity (*d'*) of the ferrets with a 100 ms silent gap between the reference and target (black line) rather than the standard 20 ms

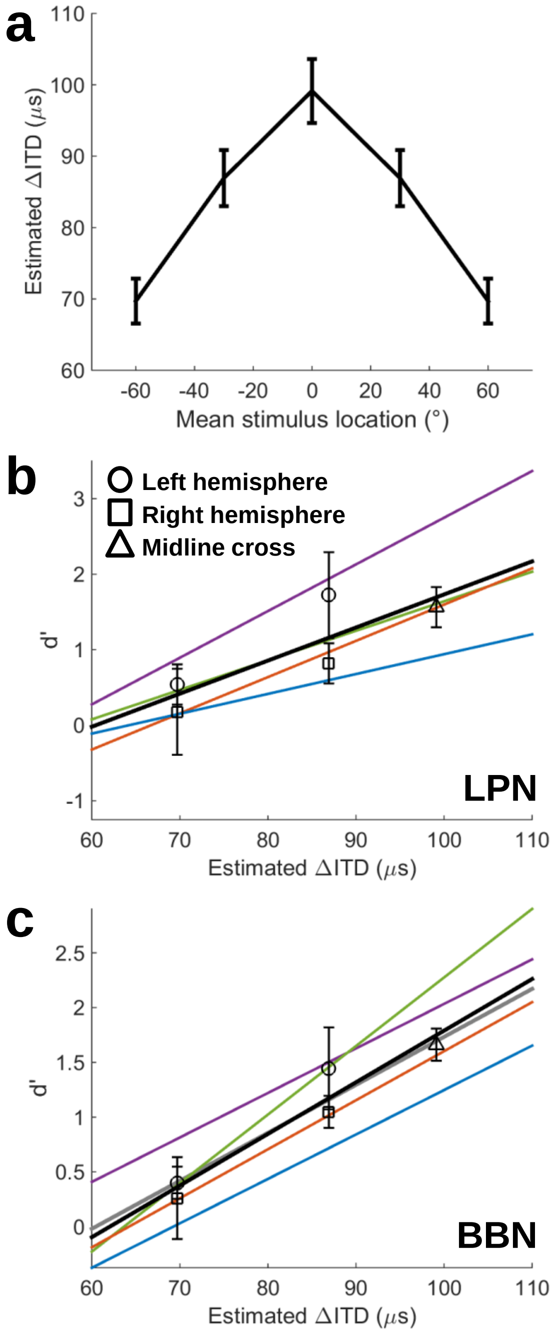


Figure 3.4 – Comparison of behavioural sensitivity with available ITDs. [a] shows the estimated change in ITD at each mean location. [b] shows the mean sensitivity ±SEM at each of the locations (black symbols) to the low-pass stimuli by the change in ITD between the reference and target locations. The black line indicates the fit of a linear regression to the mean data. The coloured lines indicate the fit to the data of each ferret. [c] shows the mean sensitivity ±SEM at each of the locations (black symbols) to the broadband stimuli by the change in ITD between the reference and target locations. Again the black line represents the linear fit of the mean data and coloured line represent the mean fits to the data of each ferret. The grey line shows the mean fit from the LPN stimuli for comparison.

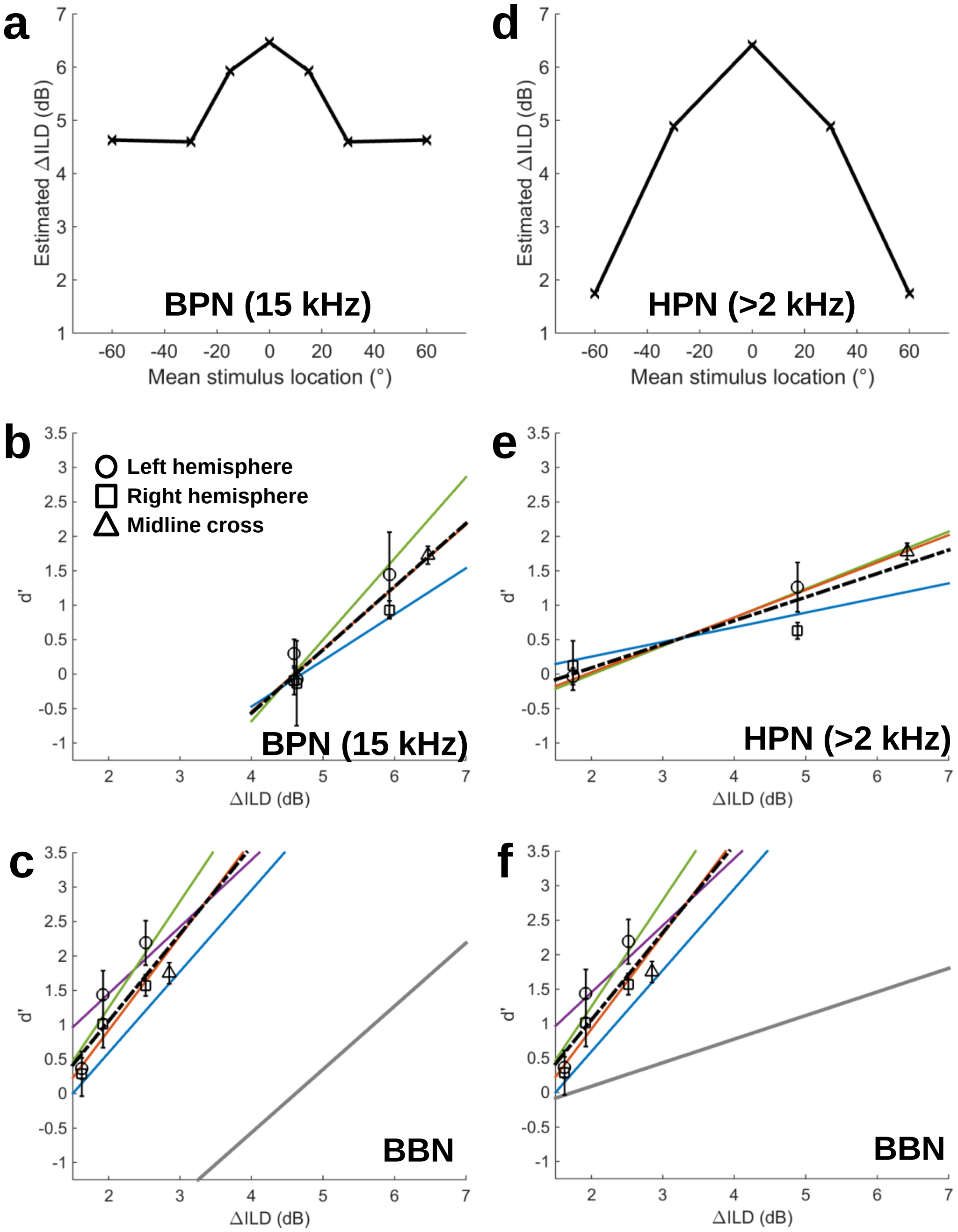


Figure 3.5 – Comparison of behavioural sensitivity with available ILDs. [a] and [d] shows the estimated mean change in ILD across frequencies at each mean location for the band-pass and the high-pass stimuli. [b] and [e] show the mean sensitivity ±SEM at each of the locations (black symbols) to the band-pass and high-pass stimuli by the change in ILD between the reference and target locations. The black line indicates the fit of a linear regression to the mean data. The coloured lines indicate the fit to the data of each ferret. [c] and [f] show the mean sensitivity ±SEM at each of the locations (black symbols) to the broad-band stimuli by the mean change in ILD across frequencies of the broad-band sounds between the reference and target locations. Again the black line represents the linear fit of the mean data and coloured line represent the mean fits to the data of each ferret. The grey line shows the mean fit from the BPN or HPN stimuli respectively for comparison.

gap (grey filled area), and the bias of the ferrets in this task. Qualitatively, performance is close to that observed in the BBN condition with a slightly lower performance overall. However, a two-way RM ANOVA (independent variables: mean location and testing condition and dependent variable: *d'*) revealed a main effect of location ($F_{(4,12)} = 10.66$, $p = 0.001$) but not testing condition ($F_{(1,3)} = 9.12$, $p = 0.057$) nor any interaction ($F_{(4,12)} = 1.05$, $p = 0.421$). *Post hoc* analysis of mean stimulus location revealed that 0° was significantly different than ±60°, reflecting the observation that performance is better around the midline compared to the periphery (Bonferroni's multiple comparison test, $p < 0.05$).

3.3.4 Experiment 3: Effect of a noisy background

Background noise was presented from a speaker located directly above the ferret while it performed the relative localisation task with the varying intensity BBN stimuli (see Methods 3.2.3). The effect of SNR (SNRs of stimuli to noise were 0 – +6 dB) on performance and the effect of having a distractor stimulus during the discrimination was investigated. Figure 3.7 [a & b] plot the performance (*d'*) and bias respectively in the noisy background condition with the level varying training stimuli presented in silence. Performance in the noisy background was not significantly different from performance in silence. A two-way RM ANOVA (independent variables: mean location and testing condition and dependent variable: *d'*) revealed a main effect of location ($F_{(4,8)} = 15.90$, $p = 0.001$) but not testing condition ($F_{(1,2)} = 1.91$, $p = 0.301$) or any interaction ($F_{(4,8)} = 2.80$, $p = 0.105$). Performance did not change over the different SNRs. It appears that ferrets can maintain their performance over this range of levels in silence or with a background noise/distractor.

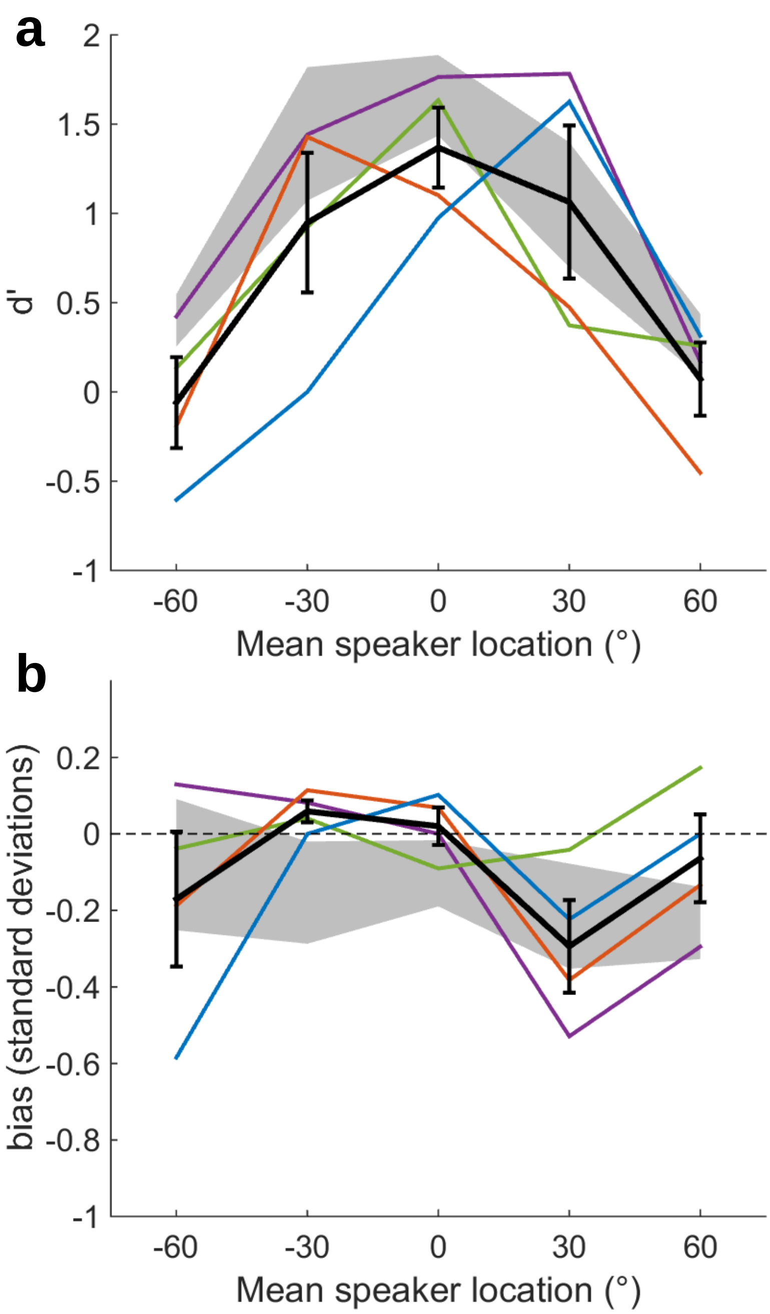


Figure 3.6 – Behavioural performance with an increased inter-stimulus interval. Performance was not affected by a longer interval between the reference and target stimuli. [a] shows the mean sensitivity ±SEM to direction the stimulus with an increased inter-stimulus interval (100 ms) moved of the ferrets at each mean speaker location (black line). The sensitivity of each ferret is indicted by the different coloured lines and the mean performance with inter-stimulus interval of 20 ms is shown for comparison (grey fill). [b] shows the same as a but for the bias of the ferrets.

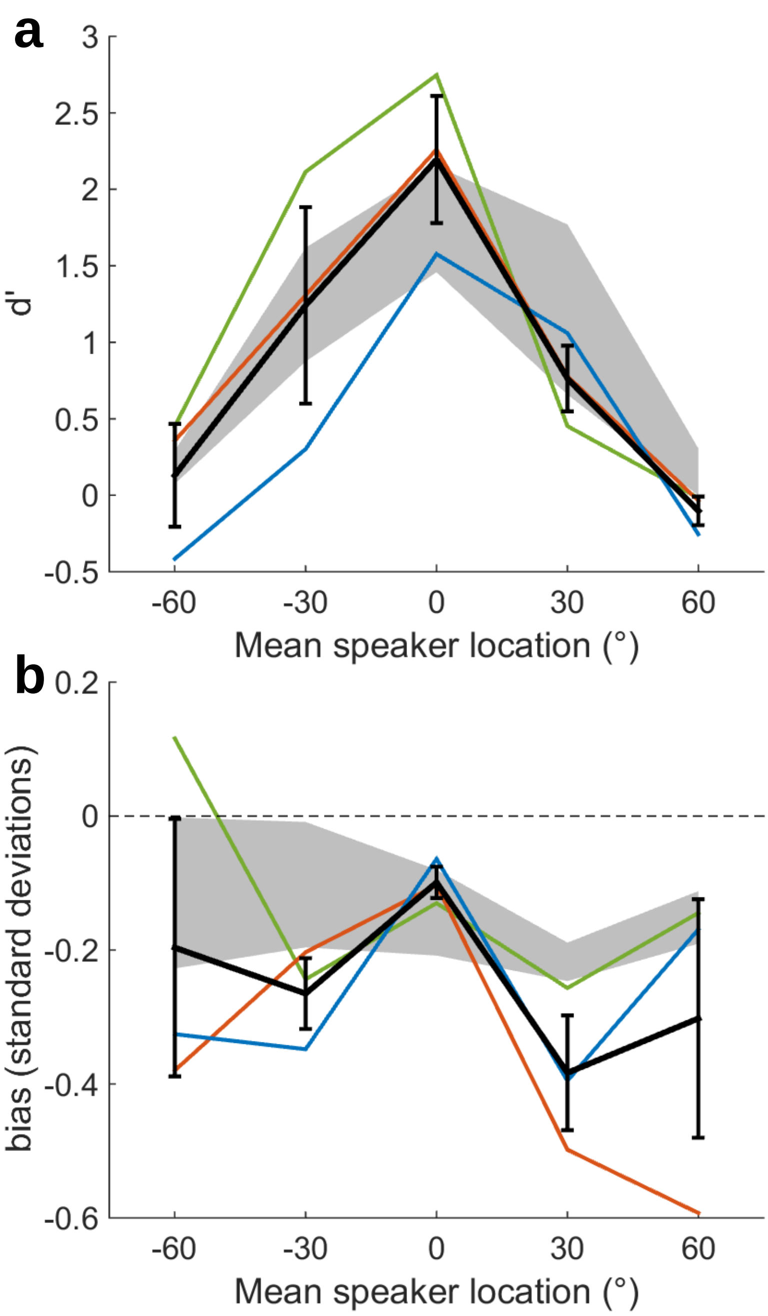


Figure 3.7 – Behavioural performance with a noisy background. Performance was not affected by addition of a background noise. [a] shows the mean sensitivity ±SEM to direction the stimulus with a noisy background moved of the ferrets at each mean speaker location (black line). The sensitivity of each ferret is indicted by the different coloured lines and the mean performance with level-varying BBN stimuli presented in silence is shown for comparison (grey fill). [b] shows the same as a but for the bias of the ferrets.

3.4 Discussion

The goal of this chapter was to establish whether the relative localisation task developed in humans in Chapter 2 was suitable for psychophysical testing of ferrets. The aim was to develop a 2AFC localisation task to allow measurement of spatial acuity throughout auditory space that could subsequently be combined with neurophysiological investigation. A 2AFC task was chosen in order to provide scope for more powerful neurometric analysis rather than using an approach-to-target localisation task where there are several response options. Experiment 1 investigated the ability of the ferrets to perform the task under different spectral band conditions and four conditions were tested: A low-pass noise condition (<1 kHz) designed to limit available localisation cues to ITDs, a band-pass noise condition (1/6 octave filter, centre frequency 15 kHz) designed to limit available localisation cues to ILDs, a high-pass noise condition where ITD cues are absent and a broadband noise condition in which all cues are available. In general performance was best around the midline and decreased towards the periphery. Ferrets performed equally well in the LPN, HPN and BBN conditions and worse in the periphery the BPN condition. Experiment 2 explored the effect of increasing the inter-stimulus interval between the two sounds. Performance with the longer interval (100 ms) was similar to the performance in the standard interval (20 ms) task. Experiment 3 investigated the effect of a background noise, designed to provide a distractor signal rather than a masking signal. Performance in this task was again very similar to the BBN in silence condition.

The decline in performance for more peripheral locations is consistent with available localisation cues (Figures 3.3 and 3.4) and in keeping with previous work showing that in an approach-to-target sound localisation task, performance is worse in the periphery (Parsons et al., 1999). Parsons et al. (1999) showed that the minimum audible angle (MAA) of ferrets at the midline was 10 ±4° for a 100 ms broadband stimulus, whilst at 45°, the MAA was 24 ±15°. Thus it is possible that the very peripheral stimuli, i.e. those further peripheral than 45° were not discriminable by the ferrets. Parsons et al. (1999) also showed that ferrets could perform the localisation task required for the MAA determination after pinnae removal suggesting that they relied on binaural cues to perform the task. Thus it seems likely that ferrets rely on binaural cues to perform the present relative localisation task.

Performance of the ferrets was significantly worse in the band-pass condition than in the BBN condition; while performance across the midline was maintained; at peripheral locations it was reduced when compared with the BBN. It has been shown that ferrets use ILDs to localise stimuli at this frequency (Keating et al., 2014), and when the band-pass stimuli are compared to the estimated available ILD cues, it is clear that the stimuli elicited relatively large changes in ILD. ILD changes were greatest across the midline (~6.5 dB) and lowest in the periphery (~4.6 dB). Performance differences were also reflected in the slopes and intercepts of linear regressions of the performance as functions of change in ILD (slope, intercept: BPN: 0.97d' dB^{-1}, -4.45 d' and BBN: 1.17 d' dB^{-1}, -1.31 d'). The smallest detectable ILD by a ferret is approximately 1.3 dB for flat-envelope broadband stimuli when discriminating a 200 ms noise burst (Keating et al., 2013b); this is relatively consistent with the smallest detectable ILD of humans which is roughly 0.5-1 dB over a wide range of frequencies at the midline (Mills, 1960). Thus it is perhaps surprising that performance was as weak in this condition, since the estimated changes are well above threshold the animal should be able to detect changes in the location of the stimuli well. However, it is possible that the minimum detectable change in ILD could be larger in central locations than at peripheral locations. Trained ferrets were able to localise sounds with the same narrowband filtered noise as used here (Keating et al., 2013a), two key differences in the present study are that the ferrets were only tested with these stimuli and not trained and that ferrets had to report changes in direction here, rather than localise the stimuli as in the Keating et al. study. It is possible that after training with these stimuli, ferrets may be able to perform the task as well as the other stimulus conditions.

Another possible explanation for the poor performance in the BPN condition is interference of or reliance on usual spectral cues. It has been shown that in narrowband conditions localisation can be influenced by spectral cues at the centre frequency of the sound (Musicant and Butler, 1984). Interaural spectral differences in the ferret at 15 kHz are fairly limited and do not vary much with locations more peripheral than 30° (Carlile and King, 1994), however, where they do vary appears to match quite well with the locations of good performance by the ferrets in the present study. It has been shown that upon unilateral occlusion of one ear, ferrets initially reweight spectral cues higher than the now unreliable ILD cues (Kacelnik et al., 2006). Although the reliability of the ILDs in the present study was not manipulated, the change from broadband training to testing in a narrow frequency band could mean that the ferrets relied on spectral cues to perform the task in the BPN condition.

In the low-pass condition, which was designed to limit available binaural cues to ITDs only, performance was not different than the BBN condition. This was reflected in the function of sensitivity by change in ITD where the two slopes of the curves were very similar (LPN: 0.043 $d'\,\mu s^{-1}$ and BBN: 0.047 $d'\,\mu s^{-1}$) as were the y-intercepts (LPN: -2.62 d' and BBN: -2.92 d'). All the changes of ITD experienced by the ferrets were well above their minimum ITD detection level at the midline of 23 μs (Keating et al., 2013b), with the minimum change being tested being ~70 μs. However, performance for ITD changes of this magnitude was less than 1 d'. In humans, ITD sensitivity of pure tones of frequency less than 1kHz is good (Mills, 1958), however, it has been shown that humans have difficulty actually localising tones of these frequencies in the periphery (reviewed by Moore et al., 2008), suggesting that it is not necessarily the case that detecting the change of location is the same as localising stimuli. When the performance of ferrets in this task is compared with that of human performance (presented in Chapter 2, Figure 2.6), it is clear that the ferrets' performance dropped off more quickly with smaller changes in ITD and ILD.

In order to compare the data to models generated in chapter 2 (Section 2.2.9), the data were re-analysed according to whether the stimuli moved towards or away from the midline. However, since the number of test locations in the ferrets (only 4 locations where there was a change in location toward or away from the midline were tested) and the number of ferrets tested was more restricted than in the human testing, some caution should be taken before putting too much emphasis on these results. The number of locations tested was more restricted in the ferret in order to ensure there were enough trials at each location for the neural analysis. The ferrets also tended to be biased towards responding in one direction for error trials thus artificially increasing performance in this direction, this is evident in all the conditions and reflects the bias shown by the ferrets (Figure 3.2 [e-h]). For example, inspection of the bias in the LPN condition (Figure 3.2 [f]) reveals little bias in the right hand side of space and large left-sided bias in the left side of space. This is reflected in the % correct data (Figure 3.5 [b]); there is little difference in the in *vs.* out performance on the right hand side of space but a large difference in the left hand side of space, with performance for sounds moving away from the midline (i.e. left) better than towards.

The increase in the inter-stimulus interval increases the length of time that the ferret must retain information about the location of the reference sound in order to compare the target location to it (from 20 ms to 100 ms). There was no difference in the performance of ferrets in this task compared to the standard interval task (with a 20 ms interval). It has been shown in anaesthetised and awake cats that the responses of most auditory cortical neurons to a second sound presented up to 10 ms after a preceding sound were suppressed compared with the first sound (Mickey and Middlebrooks, 2005). This suppression was found regardless of the locations of the two stimuli or the intensity of the stimuli. Even at inter-stimulus durations (ISDs) of up to 20 ms the firing patterns of cortical neurons varied in response to the 2nd sound indicating that there was sensitivity to the preceding stimulus even when no suppression to the second stimuli was observed. This phenomenon is reflected in localisation behaviour of stimuli with short ISDs and is known in the literature as the precedence effect (Litovsky et al., 1999). The suppression of responses to the lagging sound with short ISDs correspond well with localisation behaviour in the cat, after approximately 10 ms the cats perceived two separate sounds each of which could be localised and the IC neurons no longer showed a suppression effect (Tollin et al., 2004). A recent study shows that ferrets experience the precedence effect in a similar manner to humans and that by the time the ISD is 20 ms (the ISD in the present study) their behaviour is consistent with no precedence effect (Tolnai et al., 2014). Thus, at the ISDs in the present study, it is not expected that there would be any perceptual problem with precedence effect, as was observed.

Adding in a noisy background to the task did not change the performance. The background noise was presented from above the ferrets and thus would have provided an equal level and timing of noise at each ear of the ferret. No attempt was made to measure any kind of threshold for detection in the noise but sounds were presented at 3 different SNRs, 0, 3 and 6 dB. There was no difference in performance across the different SNRs. Lingner et al. (2012) compared the performance of gerbils and humans in a localisation task of low-passed noise stimuli in two different types of spatially presented low-passed noise, a correlated noise, where the same noise was presented from all speakers being tested and an uncorrelated noise where independently generated noise was presented from all the speakers. Performance in humans increased when the noise was correlated whereas performance in the gerbil decreased. This discrepancy could be explained by the size of the head and the broader width of the low frequency auditory filters of the gerbil compared to humans. Gerbils required positive SNRs to localise the stimuli in the correlated (8.5 dB) and uncorrelated (0.9 dB) noisy backgrounds whereas humans could localise at negative SNRs in both conditions (correlated: - 8.7 dB; uncorrelated: - 4.7dB). Ferrets also have broad low frequency auditory filters and small heads so it may have been expected that the performance in the noise would have decreased compared with stimuli presented in silence, the fact that it did not maybe because the SNRs tested were too high (Lingner et al., 2012).

This chapter has shown that ferrets can judge the direction a sound has moved in the azimuthal plane. Performance only decreased when they were limited to a narrow-band noise centred at 15 kHz, where ferrets rely on ILDs to perform localisation of these stimuli (Keating et al., 2013a, 2014). Since performance was not different in the HPN condition compared with the BBN condition, ferrets may use spectral cues in combination with ILDs to perform the task in the absence of ITDs. Ferrets performed equally well in the LPN and BBN conditions; this was different to performance of humans in the task whose performance dropped when only ITDs were available to perform the azimuthal judgement. In order to assess the contributions of ITD and ILD further, ferrets would have to be trained with headphones and sounds presented in virtual auditory space (Mrsic-Flogel et al., 2005). Ferrets' performance remained the same in the presence of a noisy background and when the gap between the stimuli was made longer. The results still leave in question the mechanism of how perception of auditory space is represented in auditory cortex, whilst results from human psychophysical and imaging studies suggest a two-channel model like encoding (Salminen et al., 2009; Magezi and Krumbholz, 2010), other studies suggest a labelled-line encoding (Carlile et al., 2014). In order to investigate the neural mechanisms of how perception of auditory space is encoded in auditory cortex, electrophysiological recordings from A1 of auditory cortex while ferrets perform this task must be performed. The next chapter presents neural data recorded from A1 of ferrets performing the task presented here.

Chapter 4: Neural correlates of relative sound localisation

4.1 Introduction

The previous chapter showed that ferrets were able to perform a relative localisation task in which they had to report the direction a sound stimulus had moved. The behavioural data were difficult to compare with the models of sound location encoding in the brain detailed in chapter 2 (Section 2.2.9) owing to bias in the ferret responses which confounded the interpretation of performance for inward *vs.* outward moving stimuli. Thus, in order to test the models of sound location encoding, recordings were made from auditory cortex of ferrets performing the task. This allowed direct correlation of neural firing patterns with the stimuli presented and with the perception of the ferret.

It is known that information about sound location is represented in auditory cortex (Nelken et al., 2005; Miller and Recanzone, 2009; Walker et al., 2011) and that primary auditory cortex (A1) is necessary to perform an approach-to-target sound localisation task (e.g. Malhotra et al., 2004, also see Chapter 5), therefore it was decided to record from A1 while the ferrets performed the relative localisation task. It is conceivable that auditory cortex could represent information relevant to this task in two ways: Firstly by encoding the absolute spatial location of both reference and target sounds with some other neural population performing the comparison and encoding of the direction judgment. Secondly, neuronal responses could explicitly encode the direction the stimulus has moved or the *relative* location of the second sound. These two functions could be performed simultaneously within A1, potentially in different neurons or cell layers, or, if there is no evidence for direction encoding in A1, we could conclude some higher order brain area is performing this function. In the first case we would predict that there would be information in the firing patterns (e.g. many previous studies have found information in the firing rate (Stecker et al., 2005b) or latency to first spike (Brugge et al., 1996)) of units about the location of both the reference and target sounds. In the second case, we would predict that there would be information about the direction the target moved, or the relative location of the target (i.e. the target location in the context of the preceding stimulus). Indeed it has been shown that encoding of auditory space is context dependent in awake macaque AC (Malone et al., 2002). The hypotheses were tested by investigating how well neural units and populations of units encoded the location of the reference, the location of the target or the direction the stimuli moved.

Since the direction of a stimulus movement is a relatively abstract concept it may well be that, if it is encoded at all, it is encoded as perceived direction, thus it may be better to decode the behavioural choice of the ferret rather than the actual direction of the stimulus. In order to do this one must correlate the firing pattern or spike count with either the stimulus direction or the choice direction of the ferrets and compare the two in order to generate a choice probability. There are currently no published studies that report neurometric analyses in auditory cortex looking for correlates of perception in a spatial task. Choice probabilities have been shown in auditory cortex in non-spatial tasks, for example Niwa et al. (2013) show choice probabilities in macaque core and belt auditory cortex for an amplitude-modulation detection task and Bizley et al. (2013b) show choice probabilities in the auditory cortex of ferrets in a pitch discrimination task. However, Tsunada et al. (2011) find that auditory cortex represents speech categories, but not choice, in a more complex task task where macaques must report whether a speech token was the same or different to a preceding speech token. Where previous work has shown that information about the choice an animal makes, it is generally encoded over long time windows (i.e. with a coarse time resolution) (Bizley et al., 2013b; Niwa et al., 2013) and such information can be found in the auditory cortex of ferrets (Bizley et al., 2013b). In this chapter, whether there is any information in AC about spatial decisions is investigated by decoding the choice the animal made (i.e. the perception) from the firing patterns individual units.

Previous studies in monkeys have shown that the spatial tuning of individual cortical neurons to sound location or interaural phase differences is not able to account for behavioural performance in sound localisation tasks (Recanzone et al., 2000; Scott et al., 2009). However, small ensembles of neurons using the full spike pattern (cat: Stecker et al., 2003), the firing rate (macaque: Miller and Recanzone, 2009; gerbil: Belliveau et al., 2014) or spike latencies (cat: Reale et al., 2003) are able to improve neural localisation to a level that can account for behavioural localisation. In some studies of neurons recorded from auditory cortex of *anaesthetised* animals attempts have been made to compare the different models of auditory space encoding using populations of neurons (Stecker et al., 2005b; Belliveau et al., 2014; Keating et al., 2015). Stecker et al. (2005b) proposed the opponent two-channel model in which two broadly tuned channels exist within each hemisphere and the location of a sound in space is given by the difference in activity of the two channels, they showed that this model was able to localise sounds accurately even when the intensity of the sounds were changing. Keating et al. (2015) used a very similar model to Stecker and colleagues (2005b) where firing rates from auditory cortex of anaesthetised ferrets were compared in two broadly tuned spatial channels, either grouped by hemisphere or by best azimuth, in order to determine the spatial location of narrowband stimuli. Finally, Belliveau et al. (2014) compared the hemispheric two-channel model with a labelled-line model using maximum likelihood decoders. A maximum likelihood decoder does not constrain the model to any one particular calculation (i.e. the difference in firing rate between two channels) and thus may convey an advantage over the models in the two preceding studies. They found that the hemispheric two-channel model did not perform as well as a labelled-line model in decoding spatial location of stimuli but that the two-channel model, as assessed from recordings made in auditory cortex of anaesthetised gerbils, was still able to account for the ability of gerbils to localise auditory stimuli. The recordings here were made from naïve animals and compared with behavioural data collected from a different set of trained gerbils (Lesica et al., 2010). In the present study the different models of sound location encoding are compared using maximum likelihood decoders of the firing rates of populations of neurons, similar to those in Belliveau et al. (2014).

Previous studies on the coding of sound location in auditory cortex in *behaving* animals have focused on a form of azimuthal *detection* task as opposed to a *discrimination* task like the one in the present study. Lee and Middlebrooks (2011) found that spatial tuning in auditory units narrowed when cats were performing a localisation task compared with passively listening to the same stimuli. In the localisation task target sounds originated from speakers elevated above the horizontal plane, while reference sounds were presented from azimuthal locations around the cat and used to measure the spatial receptive fields. Since the cat had to detect when a sound deviated from the horizontal plane, a form of go-no go detection task, the cat does not need to discriminate different azimuthal locations. In another study in awake macaques (Recanzone et al., 2000), units in the core auditory cortex were recorded from while the monkeys performed a go-no go detection task, in which they had to release a lever when a repeating stimulus deviated from the 90° location to somewhere in the frontal field. Again, it is debatable whether or not the monkeys must discriminate different location or merely detect a change in stimulus. In the present study, ferrets must also detect a movement of an auditory stimulus, but crucially they must report the direction that stimulus has moved rather than whether or not it has moved, thus they must listen to the location of both stimuli and discriminate between them. One study has recorded from macaque auditory cortex whilst the monkeys were performing a task where discrimination of sounds in azimuth was necessary (Benson et al., 1981). In this study, neural firing patterns when the monkey was detecting the presence of a sound were compared with those when the monkey was performing an absolute sound localisation task. It was reported that out of 196 units, only 16 had different response rates for the two tasks. This difference was typically an increase in firing rate for a particular location in space. None of these studies attempted to test models of two-channel or labelled-line encoding of auditory space. The present study investigates the models of encoding of sound location using firing patterns of populations of units recorded from AC of ferrets performing a sound location discrimination task.

In chapter 3, it was shown that the ferrets performed equally well in the BBN condition as in the LPN, where ITD cues were available, and also the HPN condition, where ILD and spectral cues were available but their behaviour worsened when they were constrained to a narrow band of noise (1/6th Octave wide about 15 kHz), in which ferrets rely on ILD information to localise sounds in azimuth (Keating et al., 2014). The changes in ILD were quite large even for sounds in the periphery so it is surprising that the ferrets performed badly in this task, this could be owing to the fact that the ferrets were not trained with these stimuli. By contrast, the human psychophysics results in chapter 2 suggest that humans weighted ITDs and ILDs strongly since performance decreased in both conditions compared to when either were present alone. Although the models were very simplistic, the data from testing relative localisation of ITDs fit the two-channel model and the modified labelled-line model whereas testing of narrow-band stimuli (where ILDs were the dominant cue) and broadband stimuli did not fit with any of the models. That these two models could not be distinguished highlights the need for invasive neurophysiology to directly measure neural firing and spatial receptive fields. If the two channel coding is present in auditory cortex then we would expect to find broad tuning curves with peaks at ±90°, in the case of the labelled line, then we would expect narrower tuning curves with distribution either evenly across all of space or more units tuned to the midline as in the modified labelled-line model. As previously noted in Section 1.4, Harper et al. (2014) suggest that the optimal code for ITDs varies with head size and sound frequency which might account for the differences between the way in which ferrets and humans represent auditory space and consequently perform this task. Their model predicts for macaques, a two-channel model is only optimal below ~209 Hz whereas for a cat, it remains optimal up to 500 Hz. Therefore, possible differences between the encoding of auditory space in different spectral bands were investigated by comparing neural responses to band-pass stimuli.

In summary, the aims of the present chapter are (1) to measure the amount of information about the location of the stimuli in the firing patterns and rates of units in A1 of ferrets and to assess the spatial tuning properties of individual units with regard to the different models (as mentioned above), (2) to test whether there is any representation of the direction of the stimulus or choice of the ferret in A1 units, by comparing the amount of information present in the firing patterns and rate of units about the direction of the stimuli or the choice that the ferret made, (3) to investigate the models of spatial location using populations of neurons and (4) to test for differences in the encoding spatial location when different localisation cues are available by band-pass filtering the stimuli. These aims will be addressed by recording activity of units in auditory cortex in both hemispheres during behaviour and analysing spike patterns and local field potentials.

4.2 Methods

4.2.1 Animals

All animal procedures were approved by the local ethical review committee and performed under license from the UK Home Office in accordance with the Animal (Scientific Procedures) Act 1986. Four adult, female, pigmented ferrets (*Mustela putorius*) were used in this study (F1301, F1302, F1310, F1313). All animals received regular otoscopic examinations before the experiment, to ensure that both ears were clean and disease free.

4.2.2 Behavioural procedures

As described in Chapter 3, section 3.2.2.

4.2.3 Electrode construction

The electrode arrays were constructed using Warp16 electrode arrays (Neuralynx Inc., Bozeman, MT), and comprised 16 (in a 4 x 4 configuration) individually moveable, high impedance (~2 MΩ), tungsten electrodes. Each array weighs approximately 0.8 g. Guide tubes into which electrodes were placed were approximately 800 μm apart from the centres. Insulation was removed from the section of electrode that made contact with guide tube (~12 mm from the tip) using a hot soldering iron to melt the insulation and forceps to scrape the electrode clean. Electrodes were then inserted tail-end into the guide tube of the Warp16 under a microscope until the tip just disappeared from the end of the guide tube. The electrode was then moved a further 3 mm exposing the de-insulated part, and a 30° bend in the electrode was made before the electrode was trimmed approximately 3 mm from the bend and pushed back into the guide tube. All the guide tubes were filled with electrodes and the bottom of the Warp16 drive was then covered in Silastic (QWIK-SIL, WPI, Sarasota, FL) to prevent fluid wicking up the guide tubes after implantation, thus protecting the array from shorting. To prevent the Silastic from filling the guide tubes, a small drop of triple antibiotic ointment was applied to the end of each guide tube. Wires were soldered to the two ground contact points on the array. The base of the connector was strengthened with epoxy-resin (adapted from Eliades and Wang, 2008).

4.2.4 Electrode implantation

Surgical procedures are detailed in Appendix 7.3 and are described here briefly. Anaesthesia was induced by a single dose of a mixture of medetomidine (Domitor; 0.022 mg/kg/h; Pfizer) and ketamine (Ketaset; 5 mg/kg/h; Fort Dodge Animal Health). The ferret was intubated, placed on a ventilator (683 small animal ventilator; Harvard Apparatus) and ventilated with oxygen and isoflurane (1-3.5%) to maintain anaesthesia throughout the surgery. Further doses of ketamine were given during surgery as necessary. A local anaesthetic/analgesic (Marcaine, 0.5%) was injected under the skin where incisions were to be made. An incision was made along the midline of the ferret’s head and the connecting tissue cut to free the skin from the underlying muscle. The posterior 2/3 of the left temporal muscle was removed exposing the dorsal and lateral parts of the skull. Two anchor/ground screw holes were drilled into the posterior medial part of the skull and self-tapping bone screws inserted. A craniotomy was made over auditory cortex. The pre-assembled electrode array was put in place covering A1 using a micromanipulator so that the bottom of the array was in contact with the dura. The array was then retracted, the craniotomy filled with Silastic and the array replaced before the Silastic set. The ground wires of each implant were wound around each other and wound around the ground screws. The protective cap screw was secured in place around the array with dental cement. A metal bar with two nuts was placed in the centre of the head to provide a head-fixing device for electrode movement. Further analgesia (Marcaine, 0.5%) was injected around the wound margin before the ferret was allowed to recover from the surgery. Post-operatively ferrets were given pain relief (buprenorphine, 0.01-0.03 mg/kg) for 3-5 days post-surgery and prophylactic antibiotics (Amoxycare LA, 15 mg/kg) and anti-inflammatories (Loxicam, 0.05 mg/kg) for 5 days post-surgery.

4.2.5 Electrode moving

Electrodes were initially inserted into the brain approximately 1 week after surgery. The ferret was sedated with medetomodine (Domitor 0.022 mg/kg) and placed inside a plastic tube containing a heat pad. The temperature probe was placed between the ferret and the heat pad and the temperature was maintained at 38°C. The ferret’s head was held fixed using the nuts implanted during surgery. During the initial electrode insertion, the impedance of the electrodes was monitored (using an Omega-Z-Tip Impedance Meter, World Precision Instruments, UK) as they were pushed down into the brain using a Manual Cyborg Electrode Pusher (Neuralynx Inc., Bozeman, MT). The pusher probe guide tube was lowered around the electrode guide tube on the implant, and a probe wire was pushed down into the guide tube using a micro-manipulator (Harvard Instruments, USA). The electrode was pushed down until a drop in the impedance of the electrode was observed indicating the electrode had left the Silastic into which the array was implanted; the electrode was then pushed down a further 100-150 µm. All further movements were measured from this point which was defined as the surface of the brain. The electrodes were moved whenever the ferret had completed all required behavioural testing, they were then moved 50-150 µm and behavioural testing at this depth was commenced. In this manner over the course of 1-2 years recordings were made from each cortical layer in each ferret. The ferret completed the testing when the electrodes had moved a depth that exceeded the estimate for the depth of auditory cortex (2 mm). The location and final depths of the electrodes were later investigated by histology. These data, combined with estimates of frequency tuning made at each site enabled an estimate of the location of each electrode in auditory cortex.

4.2.6 Stimuli

Stimuli were presented as described in the previous psychophysics section (Section 3.2.3). Briefly, two 150 or 200 ms noise bursts separated by 20 ms were presented from two different locations in frontal 180° of space separated by 30°. The ferret was required to report the location of the second sound (the target) relative to the first sound (the reference) for which they received a water reward for correct judgements and a time out of 7 seconds for incorrect responses. To assess the contribution of different spatial cues, low-pass noise (LPN, <1 kHz), band-pass noise (BPN, 1/6th octave wide about 15 kHz) and high-pass noise (HPN, >2 kHz) were also tested. All stimuli were matched for level and each trial was presented at a single level of 61 dB SPL. Behavioural testing (chapter 3) also included BBN stimuli presented in a noisy background and with a longer duration interval between the two stimuli, in the present chapter, analysis of neural recordings from the spectral band varying stimuli is presented.

4.2.7 Neuronal Recording

Cables were plugged into the heads of the ferrets and secured there by attaching them to custom made posts which screwed onto the protective caps. The cables were supported at the top of the testing chamber allowing the ferret free movement within the chamber. Voltage signals were recorded, amplified up to 20,000 times and digitised at 25 kHz (using a PZ5 amplifier, Tucker-Davis Technologies, USA). Data acquisition was performed using Tucker-Davis Technologies System 3 multichannel recording systems, together with desktop computers running OpenProject software (Tucker-Davis Technologies, Alachua, USA) and custom scripts written in MATLAB (MathWorks Inc., Natick, USA).

4.2.8 Frequency tuning of units

To determine the Frequency Response Areas (FRAs) of any units being recorded from, ferrets were placed in an alternative testing arena (see Figure 5.1 [b]) with speakers located on the left and right (24 cm from the ferret head) at head height of a central spout. Ferrets were provided with a constant stream of water from the central spout while sounds were presented to them, in a ‘passive’ listening condition. The ferrets did not have to perform a task during an FRA recording session. Speakers were matched for level against each other and for presentation of all frequencies in an anechoic environment using a Brüel and Kjær 4191 condenser microphone attached to a Brüel and Kjær 3110–003 measuring amplifier. Sounds of varying frequency (150 Hz to 20 kHz at 1/3 octave intervals) at varying level (0 dB to 70 dB SPL) were presented to the ferrets while their head was at the central spout and recordings from auditory cortex were made.

4.2.9 Data Analysis

4.2.9.1 Spike sorting

The raw broadband voltage trace was filtered using an elliptical filter with bandwidth 300-5000 Hz (Matlab). The resulting filtered trace was processed to remove noise correlated across channels using methods described in Musial et al. (2002). Spikes were detected using an amplitude threshold set automatically using methods described in Quiroga et al. (2004). Spikes were detected and clustered into "units" using algorithms adopted from Wave_Clus (Quiroga et al., 2004). Clusters were manually checked post-sorting and assigned as multi-unit or single-unit. Recording sessions performed at the same depth and within 3 days were combined and spike-sorted as if they were a single recording session. The spike shapes, rasters and PSTHs of these sessions were then checked manually for how well the recordings combined and were rejected if there was any inconsistency, and were then spike sorted individually. Table 4.1 shows the number of recordings and units (single and multi) for each ferret. Since the majority of the units were multi-units, only multi-units have been included in the present analysis and will be referred to from now on as units. Since multiple

		number of multi-units					number of single-units				
	ferret	F1301	F1302	F1310	F1313	Total	F1301	F1302	F1310	F1313	Total
BBN	Number of recordings	125	466	134	50	**775**	8	11	5	0	**24**
	Number of unique sites	77	184	51	39	**351**	8	9	3	0	**20**
LPN	Number of recordings	77	246	50	36	**409**	4	10	2	3	**19**
	Number of unique sites	59	148	28	27	**262**	3	10	1	3	**17**
BPN	Number of recordings	0	121	30	41	**192**	0	2	0	0	**2**
	Number of unique sites	0	69	18	16	**103**	0	1	0	0	**1**
HPN	Number of recordings	0	56	17	20	**93**	0	0	1	0	**1**
	Number of unique sites	0	33	17	11	**61**	0	0	1	0	**1**
Total	Number of recordings	**202**	**889**	**231**	**147**	**1469**	**12**	**23**	**8**	**3**	**46**
	Number of unique sites	**136**	**434**	**114**	**93**	**777**	**11**	**20**	**5**	**3**	**39**

Table 4.1 – Number of multi-unit and single unit recordings from each ferret in each spectral condition. A recording was defined as a single or multiple combined testing sessions at a particular depth and location in each ferret. Multiple recordings were made at each site and each depth and if these could not be combined (see Methods 4.2.9.1) then they were referred to as different recordings. In order to not to include the same units multiple times in the analysis, the ‘best’ recordings were selected according to defined criteria (depending on analysis type). The number of unique sites represents the number of recordings with unique depths and locations in each ferret.

recordings were taken at the same location, if the recordings could not be combined then the ‘best’ recording session for the particular location and depth was taken so as not to include the same unit in the analyses multiple times. The ‘best’ recording refers either to the recording with the highest number of trials or the recording with the best MI, which units are chosen is described in each analysis.

4.2.9.2 Spatial tuning features

Rate azimuth functions were defined by calculating the mean spike count at each location across the presentation of the reference or target sound. A unit was defined as tuned to the reference or target if it had significantly different firing rates at one or more locations (Kruskal-Wallis test, $p < 0.05$). The preferred azimuth of each unit was given by its centroid or peak firing rate. The centroid was calculated in a similar way to Middlebrooks and Bremen (2013): The peak rate range of one or more contiguous stimulus locations that elicited spike rates within 75% of the unit’s maximum rate plus one location on either side of that range. The locations within the peak range were treated as vectors weighted by their corresponding spike rates. A vector sum was performed, and the direction of the resultant vector was taken as the centroid. The breadth of spatial tuning of a unit was represented by the width of its equivalent rectangular receptive field (ERRF, Lee and Middlebrooks, 2011), which corresponds to the width of a rectangle with a total area the same as the area under the rate-azimuth function and height equal to the peak firing rate. Modulation depth was defined as the percentage change in firing rate between the peak and the minimum firing rate of the spatial receptive field. Differences between the distributions of centroid, ERRF widths and modulation depths in each of the bandwidth conditions tested (BBN, LPN, BPN and HPN) were tested with a two-sample Kolmogorov-Smirnov test, with the p value (0.05/4) adjusted for multiple comparisons (Bonferroni corrected).

4.2.9.3 Spike pattern decoding of individual units

Post-Stimulus Time Histograms (PSTHs) of the spike pattern on each trial were made with 15 ms, 50 ms or 150 ms bins across the reference or target sound presentation. PSTHs were also made with the same bin widths of the mean spike count across each variable being tested (e.g. at each reference spatial location). For each single trial the Euclidean distance between its firing rate and each of the mean PSTHs (means were calculated excluding the trial being tested) were calculated and the trial was classified as being the result of the condition with the lowest Euclidean distance (e.g. classified as being evoked by a particular reference spatial location). Mutual information (MI) was calculated between the classified responses and the stimulus to quantify how well the decoder had performed. This process was performed 100 times with random sampling with replacement of the single trials. The MI was defined as the mean MI. To test for significance, Monte Carlo simulations (250 repeats without resampling) were performed. The MI was deemed significant if the mean of the MI distribution was higher than 4 standard deviations of the Monte Carlo shuffle simulation. Neuronal responses were decoded according to (A) reference location, (B) target location, (C) the direction of target movement, (D) the left/right choice of the ferret or (E) the target location in the context of the relative location of the reference (i.e. the direction the stimulus moved).

4.2.9.4 Population decoding

A Bayesian maximum likelihood decoder was implemented to test different models of location coding and direction of sound movement. Bayes rule states that the probability of a stimulus given firing rate *x* is:

$$p(stim \mid firing\ rate_x) = p(stim) * p(firing\ rate_x \mid stim) / p(firing\ rate_x)$$

For the location decoding, three models were tested, the labelled-line or labelled-line model, the two-channel hemispheric model and the two-channel opponent model. For the labelled-line model, the probability of a given firing rate in each neuron in the population given stimulus *Y* was calculated as follows:

$$p(firing\ rate_n|Y) = p(firing\ rate_i|Y) * p(firing\ rate_{ii}|Y) * \ldots p(firing\ rate_n|Y)$$

For the two-channel hemispheric model, two populations of neurons were defined by the hemisphere of the brain they were recorded from or for the two-channel opponent model by hemisphere of their best azimuth (the location of the peak firing rate). The likelihood term was calculated using the mean firing rate across all neurons in the population at each location. The probability of given firing rates in the 'left' or 'right' populations given stimulus Y was calculated as:

$$p(\text{firing rate}_{left}, \text{firing rate}_{right}|Y) = p(\text{firing rate}_{left}|Y) * p(\text{firing rate}_{right}|Y)$$

The models were tested for populations increasing in size from 1 to 50 units. For each unit of the population, the mean spike count and standard deviation of spike count at each azimuth was calculated. These values were used to simulate responses by sampling from a Gaussian distribution with a mean and variance from those calculated for each cell. This method was used to increase the number of trials at each location (Belliveau et al., 2014) since these recordings were made simultaneously with behaviour and populations were constructed from non-simultaneous recordings where the ferret may have performed different trial numbers. Any unit recordings with fewer than 7 trials at any location were excluded from the population testing. Units were selected from recordings where testing locations were from -75° to 75° in 30° steps. Units were required to be tuned to the reference location or target location (depending on whether reference or target location was being tested) and the best units were selected by choosing those with the best significant MI about reference or target location or randomly if there was no significant MI. If there were fewer than 50 units recorded from a side or fewer than 50 units with best azimuths in a side, a random sample of cells from the contralateral side were selected and their response data flipped about the midline to make up the population to 50. Units for the population testing were selected from the all available units with replacement.

4.3 Results

4.3.1 Spatial tuning across spectral band

As discussed in the introduction (Section 4.1), one can hypothesise two ways as to how auditory cortex may encode direction of the stimuli; firstly by encoding the absolute spatial location of both reference and target sounds with some other population of neurons (in A1 or elsewhere) performing the comparison and encoding the direction judgment. Secondly, neuronal responses could explicitly encode the direction the stimulus has moved or the relative location of the second sound. In the first case we would predict that there would be information in the firing patterns of units about the location of both the reference and target sounds. Table 4.2 shows the number of units with significant spatial tuning to the reference, target stimuli (irrespective of the relative location of the reference), to the location of both reference and target stimuli and to the targets on the left and right of the reference (Kruskall-Wallis $p < 0.05$). To investigate the nature of the spatial tuning, spatial receptive fields were constructed from the firing rate to stimuli at different locations in space and their characteristics were quantified in three ways, the centroid, which gives an estimate of the best location for that unit, the ERRF width, a measure of how broad the spatial tuning is and the modulation depth, how much the firing rate is modulated by the location of the stimuli. Figure 4.1 shows example firing patterns of tuned units from each of the spectral band conditions. The top row shows the raster plot of the unit ordered first by reference location (as indicated by the coloured backgrounds) with trials within each reference location ordered according to the target location. The middle row shows the post-stimulus time histogram (PSTH) of the response. The bottom row shows the rate-azimuth function of the unit with the green box showing the ERRF, indicating the relative width of tuning, and the star plotting the centroid of the unit. All of these units show contralateral spatial tuning, as do the majority of the units that were recorded from (Figure 4.2).

If restricting the spectral band so that a single set of localisation cues was present in the stimulus altered the encoding method for how auditory space was represented (for example from a two-channel to a labelled-line), we might predict measuring spatial tuning functions with different spectral bands would reveal differences in the characteristics of the tuned neural population. Contrary to this, the majority of units showed contralateral spatial tuning with most centroids located between -45° and -30° (Figure 4.2 [a]), and Kolgomorov-Smirnoff tests ($p > 0.0083$, Bonferroni corrected for multiple

		% of total							
		All recordings				Unique sites			
		BBN	LPN	BPN	HPN	BBN	LPN	BPN	HPN
Kruskal-Wallis Test ($p < 0.05$)	Reference	50	47	42	43	45	48	38	44
	Target	45	40	36	39	44	40	33	43
	Both Ref & Tar	37	30	29	34	35	31	27	36
	Left target	31	29	28	24	33	31	27	30
	Right target	29	29	28	31	29	29	26	36
Significant location MI	Reference	56	49	56	46	53	51	57	52
	Target	52	44	48	47	53	45	55	48
	Both Ref & Tar	39	29	43	31	41	31	44	36

Table 4.2 – Percentage of multi-units conveying spatial tuning. Top panel: Percentage of units with a firing rate significantly modulated by reference location, target location, both reference and target location, or the direction in which the target moved. Bottom panel: % of units whose spike pattern conveys significant (permutation test, $p<0.05$, see methods) information at any decoding time-scale for reference, target or both reference and target locations.

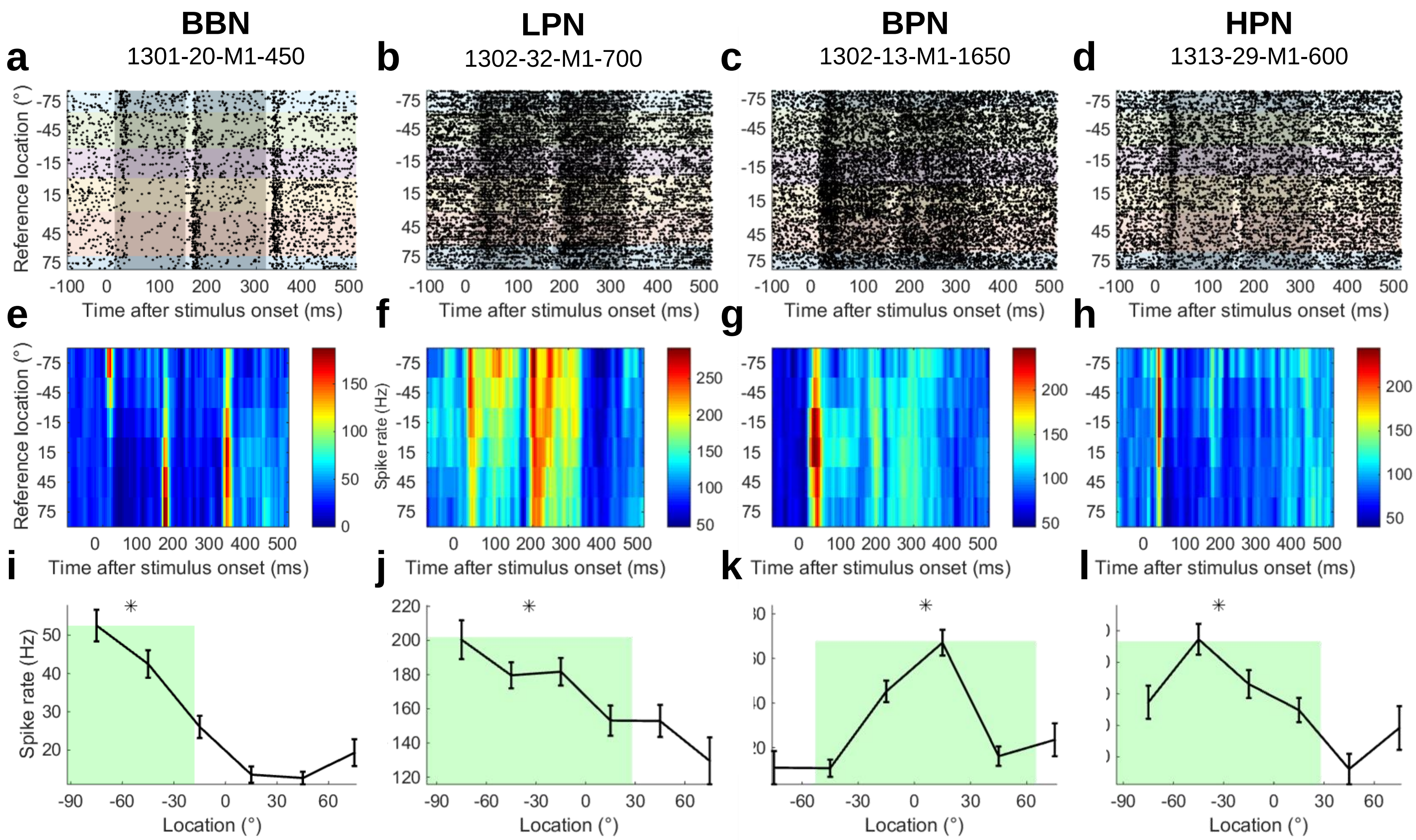


Figure 4.1 – Example response properties of units from each spectral band condition. The top row shows raster plots [a-d] for 4 different units in response to broad-band (BBN), low-pass (LPN), band-pass (BPN) and high-pass (HPN) stimuli. The raster plots are ordered by reference stimulus location as indicated by the coloured horizontal bars. The vertical grey bars indicate when the stimuli were presented. The second row [e-h] shows for each raster plot above a Post-Stimulus Time Histogram (PSTH) in 5 ms bins (smoothed with 3-point hanning window) at each reference location. The bottom row [i-l] shows the rate-azimuth function for each unit, it plots the mean firing rate (± standard error of the mean) during presentation of the reference stimuli at each location, negative locations represent contralateral tuning. The green box shows the equivalent rectangular receptive field (ERRF) and the black stars show the location of the centroid.

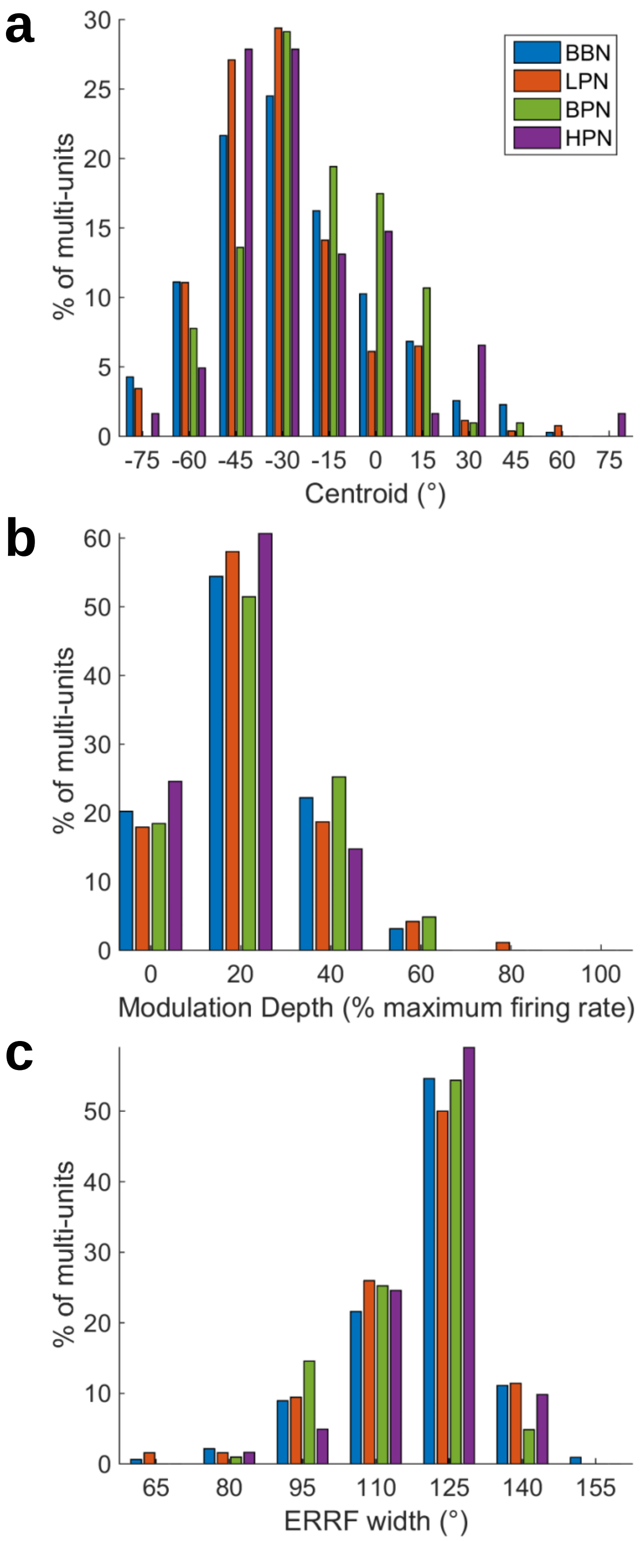


Figure 4.2 – Tuning characteristics of multi-unit responses to different spectral band conditions. No difference in the centroid location, modulation depth or ERRF width was observed between the different stimulus condition. [a] Shows the distribution of the centroids in 15° bins in each band-pass condition of unique units (recordings with most trials were used where there were multiple recordings). Negative locations represent contralateral space. The modulation depth distributions in 20% bins are shown in [b] and [c] shows the equivalent rectangular receptive field width distributions in 15° bins. Kolgomorov-Smirnoff tests ($p < 0.05$, Bonferroni corrected) revealed no significant differences between the distributions in any of the spectral conditions.

comparisons) revealed no significant differences between the distributions in any of the spectral conditions in any of the tuning characteristics quantified.

While the tuning properties of cells did not vary with stimulus condition, a variety of tuning functions were observed. Figure 4.3 illustrates the spatial tuning properties of units tuned to the reference location in response to BBN. Figure 4.3 [a] shows the normalised spatial firing rates of all units tuned to the reference location. While the centroids were located between 45° and 30° in the contralateral hemisphere, there is a fairly even spread of maximum firing rates from -45° to 45° with more units tuned to -75° and 75° than to intermediary locations. The relatively high number of units with maximal spike rates to the extreme locations could be due to the fact that some of these units are actually tuned to more peripheral locations that are not tested. Figure 4.3 [b] shows the spread of the maximum firing rates across space for units from each hemisphere. As was demonstrated in Figure 4.2 [a], the majority of units have their best azimuths in contralateral space, but there is a roughly equivalent number of units with maximal firing rates at each location from ±45°. In order to assess how spatial tuning in single units might relate to the representation of auditory space in the neural population, average rate-azimuth functions were created according to two different two-channel models and a labelled-line model. Figure 4.3 [c] shows the mean normalised firing rates of units according to two two-channel models of how azimuthal space may be encoded, the first being the hemispheric model (McAlpine et al., 2001) indicated by average activity across all neurons recorded in the left and right hemispheres and the second being the opponent channel model (Stecker et al., 2005b) constructed by averaging the responses of all neurons with best azimuths in left and right space (irrespective of the hemisphere in which they were recorded). Since the majority of units are tuned to contralateral space, there is little difference between these two models and we cannot differentiate between them. For comparison, a labelled-line model was also constructed, creating spatial ‘channels’ by averaging the responses of all neurons with a best azimuth at each of the tested locations (Figure 4.3 [d]). When modelled in this way, the data resemble a labelled-line type tuning where each location in space is

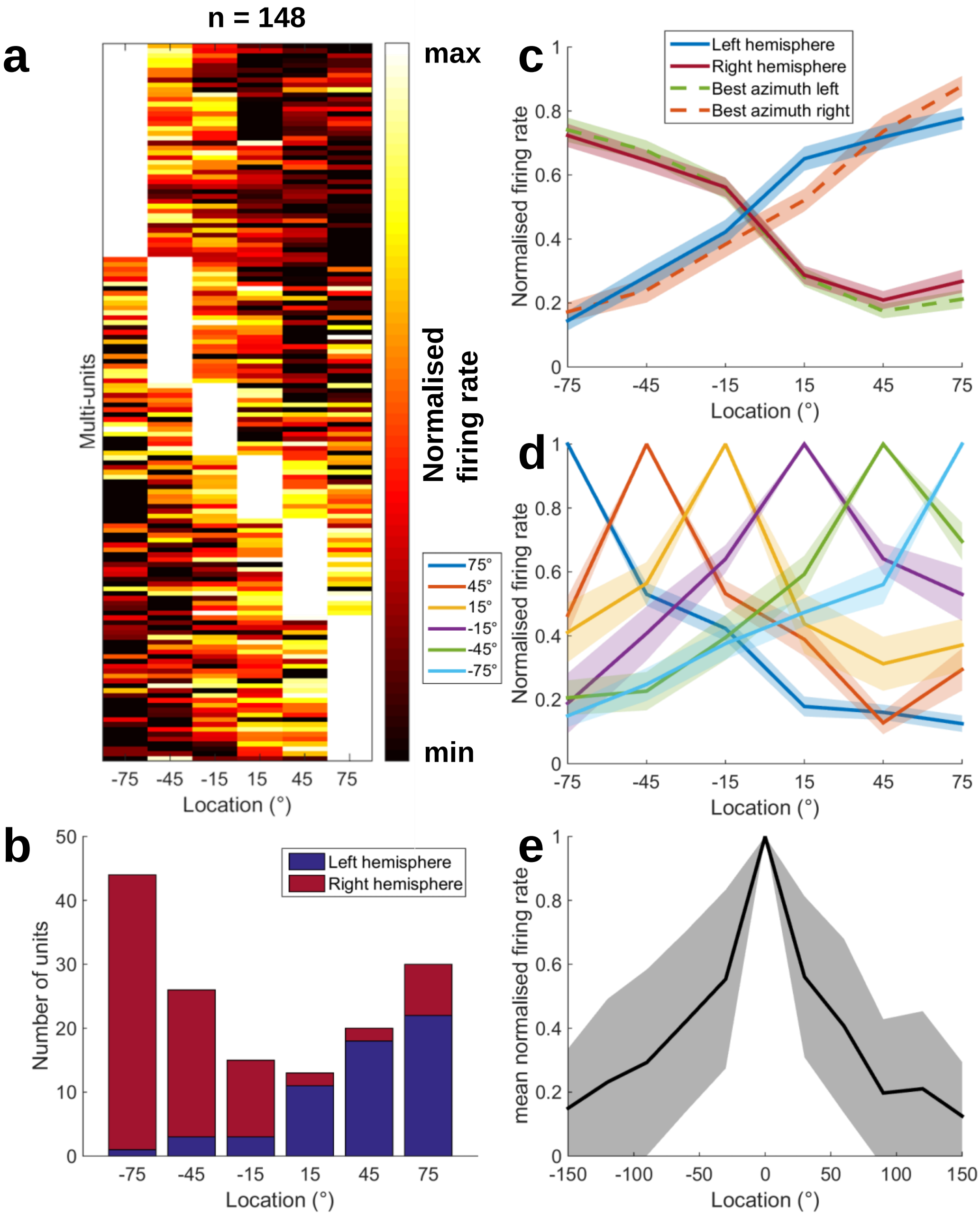


Figure 4.3 – Tuning properties of multi-units with significant reference location tuning in response to broad-band stimuli. Spatial receptive fields for the reference location are narrower than would be expected for a purely two-channel encoding of space. [a] shows the normalised reference location spatial tuning curves of all units responsive to broad-band stimuli ordered by best azimuth. A histogram of the number units by hemisphere with best azimuth at each reference location tested are shown in [b]. [c] Shows the mean ±SEM normalised spatial tuning curves of units in each hemisphere (solid blue (left) and red (right) lines) and those with best azimuths in the left (dashed purple line) or right hemispheres (dashed orange line). [d] Shows the mean ±SEM normalised spatial tuning curves of units with best azimuths at each reference location. [e] Shows the mean ±SEM normalised spatial tuning curves of all units with the peak firing rate aligned at zero. Unique units were selected by choosing units with the best MI for reference location. Here negative locations represent the left side of space and positive the right side of space.

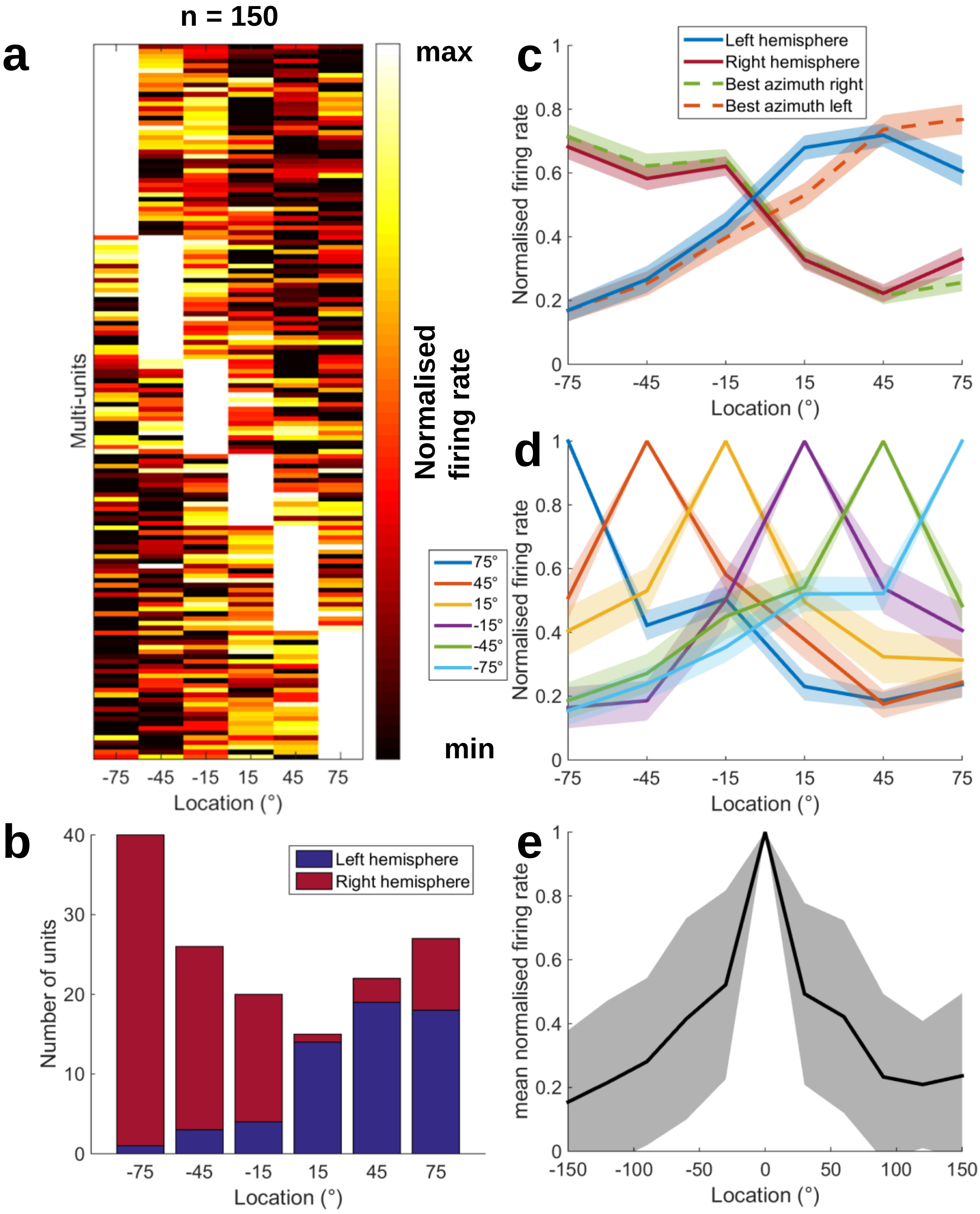


Figure 4.4 – Tuning properties of multi-units with significant target location tuning in response to broad-band stimuli. Spatial receptive fields for the target location are narrower than would be expected for a purely two-channel encoding of space. [a] shows the normalised target location spatial tuning curves of all units responsive to broad-band stimuli ordered by best azimuth. A histogram of the number units by hemisphere with best azimuth at each reference location tested are shown in [b]. [c] Shows the mean ±SEM normalised spatial tuning curves of units in each hemisphere (solid blue (left) and red (right) lines) and those with best azimuths in the left (dashed purple line) or right hemispheres (dashed orange line). [d] Shows the mean ±SEM normalised spatial tuning curves of MUs with best azimuths at each target location. [e] Shows the mean ±SEM normalised spatial tuning curves of all units with the peak firing rate aligned at zero. Unique units were selected by choosing units with the best MI for target location. Here negative locations represent the left side of space and positive the right side of space.

represented by a distinct neuronal sub-population. In a final attempt to disambiguate these competing models of auditory space, the spatial tuning functions for all cells were aligned by centring at the maximum firing rate (Figure 4.3 [e]). I hypothesised that if the spatial receptive fields of the units were consistent with a two-channel model, where (in the present study) all the peak firing rates occur at ±75°, then one would expect a broad spatial tuning function with a relatively shallow slope either side of the peak, much like that observed in the mean of all units in one hemisphere (Figure 4.3 [c]), and the steepest part of the curve would be 75°away from the peak, corresponding to the midline location. Instead, what is observed is that the steepest part of the slope occurs immediately adjacent to the peak, a finding that is more consistent with a labelled-line model than a two-channel model. Figure 4.4 shows that the spatial tuning observed in response to the target sound is virtually indistinguishable from that observed in response to the reference sound.

4.3.2 Decoding spatial location from individual units

In order to directly compare how much information is available about the spatial location of both reference and target sound sources and sounds that differ in their spectral band a Euclidean distance classifier was used to decode the spatial location of sound sources from the neuronal responses elicited. Sound location was decoded from the spike patterns of individual units by classifying single trial PSTHs (spike counts binned at 15, 50 or 150 ms resolution) to a series of templates generated from the mean response to each stimulus class. The performance of the classifier was assessed by calculating the Mutual Information (MI) from the resulting confusion matrix of actual and decoded stimulus locations (see Methods Section 4.2.9.3). To determine whether the classifier performed better than chance, a 250 iteration Monte Carlo permutation test was performed by decoding the responses after first shuffling the relationship between neural response and stimulus parameter without replacement.

To illustrate the decoding process, Figure 4.5 shows four example units with significant MI for the reference location [a & b] and the target location [c & d] across the different time resolutions. The left-hand images show the PSTHs of the units at the same time resolution as the decoder. The confusion matrices on the right hand side indicate the classification of the stimuli by the Euclidean distance classifier. A perfect classification would be indicated by a diagonal line of equality and no classifications deviating from this line. The amount of MI between the stimulus and the classification is indicated above the confusion matrix. The unit in Figure 4.5 [a] contains information about the location of the reference sound: It is clear in this unit that the classifier performs poorly within each hemisphere but rarely misclassifies the hemisphere from which the sound originated. Figures 4.5 [b-d] show three further examples of units with significant MI about the location of the stimuli at different time resolutions and in response to different stimuli as indicated on the left-hand side.

To test the hypothesis that neuronal responses to the BPN stimuli contained less information about auditory space, the proportion of neurons containing significant information about auditory space was compared for each stimulus condition and each temporal resolution. Figures 4.6 [a & d] present the percentage of unique units with significant MI in each spectral band condition for the reference and the target locations at the three different time resolutions. In order to investigate whether the temporal resolution with which responses were decoded or the spectral band influenced the proportion of units containing information about sound source location binomial logistic regression was performed. The contribution of these factors was assessed by comparing models containing either temporal resolution or spectral band as predictors and comparing the resulting deviance measures to those obtained with the constant model. The regression analysis on the reference location data revealed that the model fits were significantly improved with bin size and stimulus condition predictors compared with a constant model and that models that had both factors were better than those with each predictor, indicating a significant main effect of these two factors (Analysis of deviance, X^2 distribution, $p < 0.025$; see Appendix 7.4 for full statistical description). Subsequent *post-hoc* testing (two-proportion Z-test with Bonferroni correction for multiple comparisons) within bin size

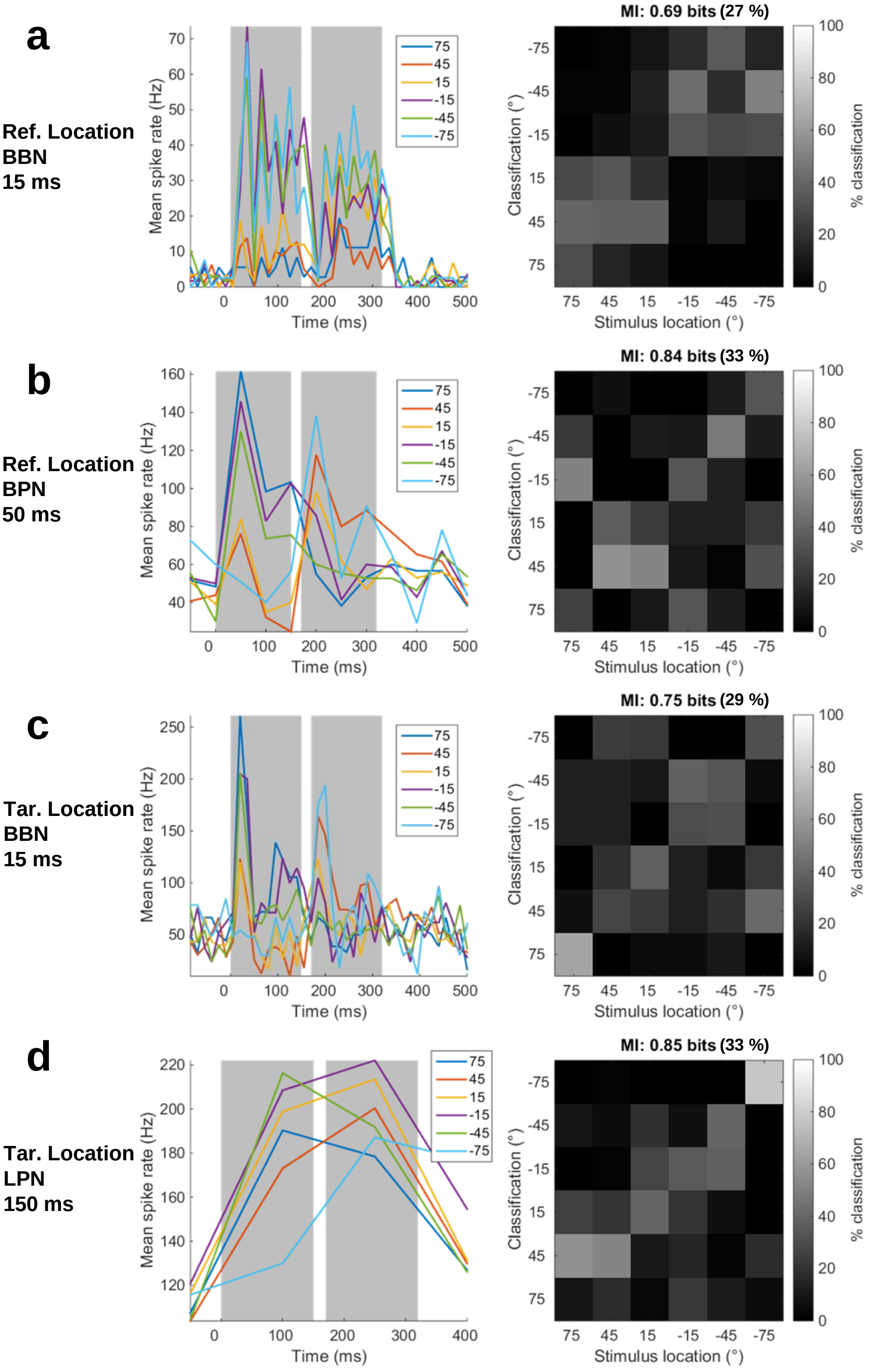

a
Ref. Location
BBN
15 ms
MI: 0.69 bits (27 %)
b
Ref. Location
BPN
50 ms
MI: 0.84 bits (33 %)
c
Tar. Location
BBN
15 ms
MI: 0.75 bits (29 %)
d
Tar. Location
LPN
150 ms
MI: 0.85 bits (33 %)
Mean spike rate (Hz)
Time (ms)
75
45
15
-15
-45
-75
Classification (°)
Stimulus location (°)
% classification

Figure 4.5 – Example multi-units with significant MI about stimulus location. Unit spiking activity was binned to form PSTHs. A Euclidean distance classifier was then used to decode single trial responses and the resulting decoder performance was quantified by estimating the Mutual Information (MI) of the confusion matrix. [a-d] Show four example units. The first column shows the PSTH at the temporal resolution for which the MI is significant (assessed by a permutation test, p<0.05). The right column shows the confusion matrix from the Euclidean distance decoder and the resulting MI in each case is indicated above the confusion matrix, with the % maximum MI in brackets. Negative locations indicate the left side of space while positive locations indicate the right side of space. Neural activity was binned at either 15 ms [a, c], 50 ms [b] or 150 ms (rate code, [d]) over a 150 ms time window beginning at the onset of the reference sound [a and b] or target sound [c and d]. [a] and [c] are in response to BBN stimuli, [b] to BPN stimuli and [d] to LPN stimuli.

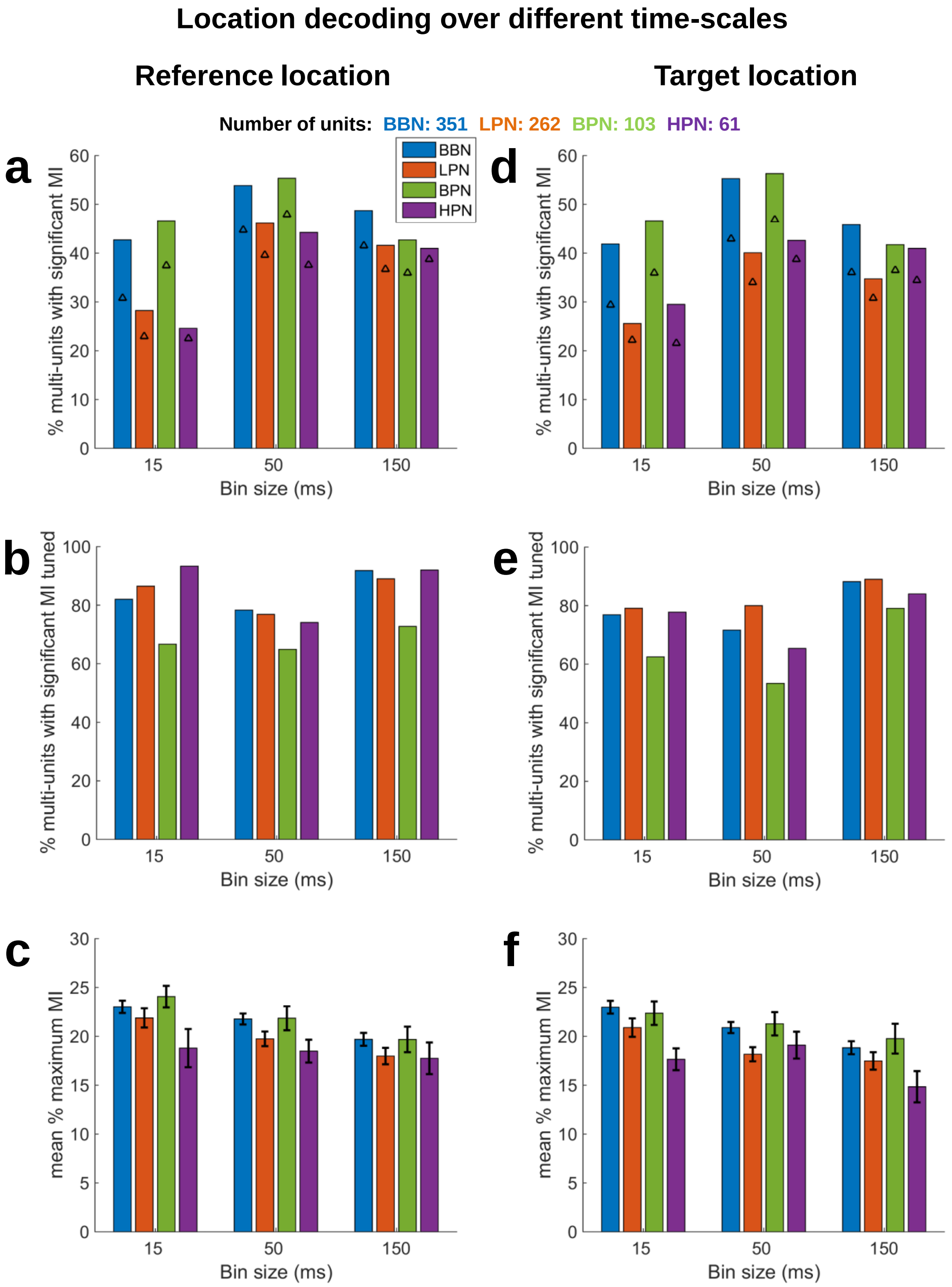


Figure 4.6 – Location decoding over different time-scales. The number of units with information about the reference and target location was highest at 50 ms resolution [a] Shows the % of units with significant MI about the reference location at three different decoding bin windows. [b] Shows the % of units with significant MI (from [a]) that also show spatial tuning for the reference location (p<0.05 Kruskal-Wallis test). [c] Shows the mean % of maximum MI of the units with significant information about the reference location at each bin width. [d-f] Show the same as [a-c] for the target location, [e] shows those units with spatial tuning for the target location. Results are for unique units selected by choosing the recording with the best MI for either reference [a-c] or target location [d-f]. Black open triangles indicate the % of the total number of unit recordings with significant MI.

($p < 0.0167$) revealed that 50 ms resolution contained a higher proportion of units with MI than the 15 or 150 ms bins, and 150 ms had a higher proportion of units than 15 ms. Testing within stimulus condition ($p < 0.0083$) revealed that the LPN stimulus contained a lower proportion of units with a significant amount of information about auditory space than the BBN and BPN conditions and that BBN proportion was higher than HPN.

For the target location data, again, binomial logistic regression was performed and revealed that the fits were significantly improved with bin size and stimulus condition predictors compared with a constant model or with models of each predictor, indicating a significant main effect of these two factors (Analysis of deviance, X^2 distribution, $p < 0.0083$). Subsequent *post-hoc* testing (two-proportion *Z*-test with Bonferroni correction for multiple comparisons) revealed the same effects as in the reference location data. In summary, more units had significant MI in a coarse pattern (50 ms resolution) than a fine temporal pattern (15 ms resolution) or rate code. While stimulus condition influences the proportion of neurons with significant MI about auditory space, the fact that there was no interaction suggests that the coding pattern does not change between the different stimulus conditions.

In order to relate the spike rate based spatial tuning (i.e. significant modulation by spatial location, Krusal-Wallis $p < 0.05$) and spatial location decoding (Figures 4.6 [a & d]), the proportion of informative units (those with significant MI) that were also significantly tuned to space was calculated. Across all units roughly 44% were spatially tuned to the reference location and 40% to the target location (Table 4.2). However, in the subset of units that were informative about space using the Euclidean classifier, closer to 80% of units were spatially tuned to the reference location and 75% to the target location. Figure 4.6 [b & e] show the proportion of units with significant information about the reference or target locations that also showed significant spatial tuning to either the reference or the target locations (i.e. spike rate modulated by spatial location, Kruskal-Wallis, $p < 0.05$). In all cases, the units show a higher proportion of tuning than the general population of recorded units. This suggests that both modulation of spike rate and spike pattern can be used to show a unit is tuned to auditory space

To further test the hypothesis that there might be less information about the spatial location of BPN stimuli, the amount of spatial information (expressed as the % of the maximum available, i.e. $\log_2$ of the number of variables, e.g. for 6 locations the maximum MI would be $\log_2(6) = 2.585$ bits) the amount of information available in significantly informative units was compared across temporal resolutions and spectral bands. Figures 4.6 [c & f] show the average amount of information about reference and target locations conveyed by the significantly informative neurons. Consistent with the results reported above, both temporal resolution and spectral band influenced the amount of spatial information available. Two-way ANOVAs were performed on the % maximum MI values. Both the data for reference location and target location showed main effects ($p < 0.05$, see appendix 7.4 for detailed results of statistical analysis) of bin size and stimulus condition and no interaction between them. For both the reference and target location *post-hoc* testing (Tukey-Kramer, $p < 0.05$) showed that the 15 ms bin had higher % maximum MI than the 150 ms bin, and that HPN was lower than BPN and BBN and BPN was higher than LPN. The results suggest that a pattern type code contains more information about the stimulus location than a rate code. Although there were significant differences in the amount of MI between the stimulus conditions, the differences are very small, and did not match the pattern of results predicted by the ferret psychophysics.

To explore whether rate information was encoded early or later in the stimulus presentation window, the data were analysed by spike rate in 50 ms time windows (0-50, 50-100, 100-150 ms) over the reference or target stimulus, Figures 4.7 [a & c] show the resulting % of units that had significant MI about the location of the reference and the target stimuli over the moving 50 ms time window. Comparison with the rate code over 150 ms (Figure 4.6 [a & d]) demonstrates that fewer units were informative. Binomial logistic regression was performed to determine the effect of the time of the analysis window and the stimulus type. For the target location (Figure 4.7 [c]) the fits

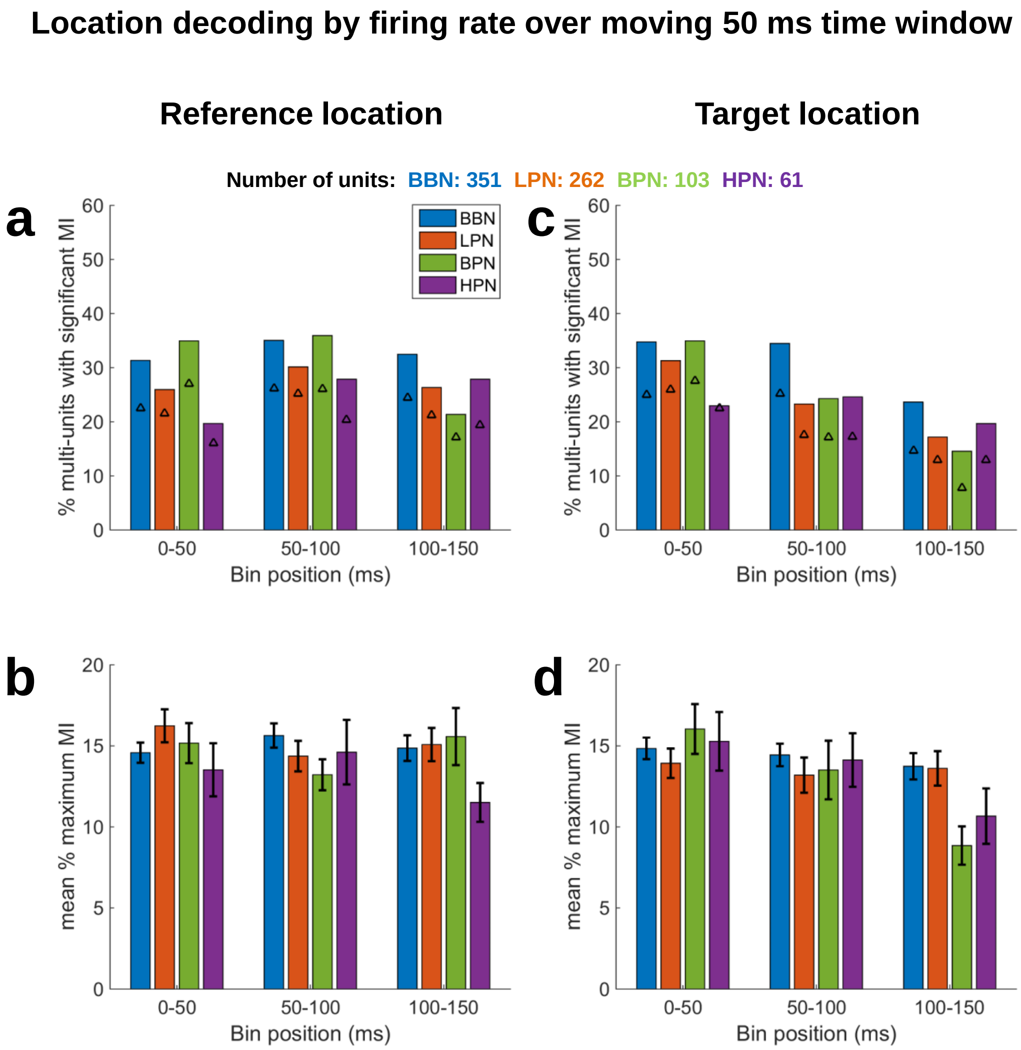
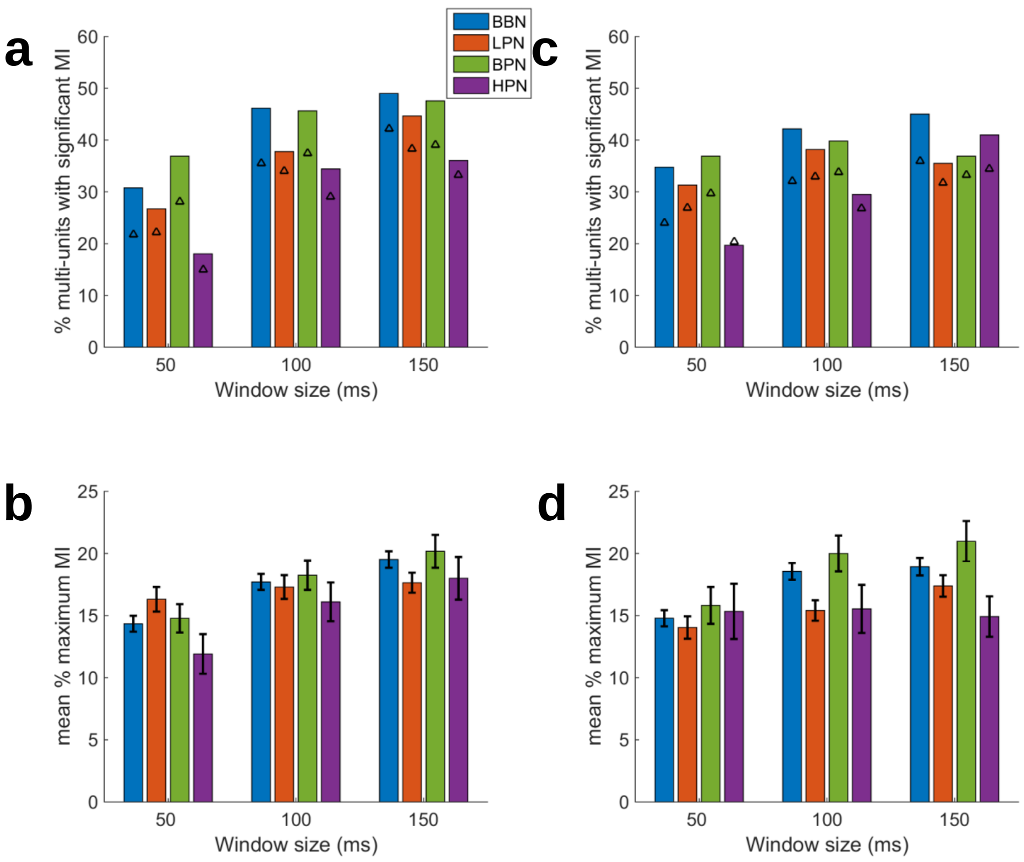


Figure 4.7 – Decoding location by firing rate in moving 50 ms time window. The amount of information about the reference location in a moving 50 ms time window was the same throughout the stimulus presentation, whereas for the target location, there was more information at the beginning of the stimulus than at the end [a] Shows the % of units with significant MI about the reference location at three moving 50 ms decoding windows. [b] Shows the mean % of maximum MI of the units with significant information from [a]. [c & d] Show the same as [a & b] for the target location. Results are for unique units selected by choosing the recording with the best MI. Black open triangles indicate the % of the total number of unit recordings with significant MI.

were significantly improved by including an analysis window predictor ($p < 0.001$) but not a stimulus condition predictor ($p = 0.905$, Analysis of deviance, X^2 distribution) and *post-hoc* analysis (two-proportion Z-test) revealed that the last analysis window had significantly fewer units than the first two analysis windows. Consistent with this, a 2-way ANOVA on the % of maximum MI (Figure 4.7 [d]) revealed a main effect of analysis window ($p < 0.05$, see appendix 7.4 for detailed results of statistical analysis) but not stimulus condition or any interaction. *Post-hoc* testing (Tukey-Kramer, $p < 0.05$) again showed that the last analysis window was significantly less informative than the first two analysis windows. For the reference location there was no main effect of window location or stimulus condition since neither factor significantly improved the model fit compared with a constant model (Analysis of deviance, X^2 distribution, $p > 0.025$). A 2-way ANOVA on the % of maximum MI also revealed no effect of analysis window or stimulus condition ($p > 0.05$). In summary, rate decoding of the reference location revealed that information about the location was spread evenly across the stimulus duration and not in the onset as previously found in anaesthetised ferrets, however when decoding the target location it was found that there was more information in the first window (0-50 ms) than in the last window (100-150 ms).

In order to see whether the same information was present in each of the 50 ms time bins or different information was present in each of the times bins, the decoding was repeated using increasingly long analysis windows. Figures 4.8 [a & c] show the % of units with significant MI in the firing rate about reference and target locations for time windows of 50,100 and 150 ms. It is clear that there are fewer informative units over shorter time windows than the full time window of 150 ms for both target and reference locations. Comparison of the data revealed a main effect of bin size and stimulus condition (Analysis of deviance on binomial logistic regressions, X^2 distribution, $p < 0.025$, see appendix 7.4 for detailed statistics) in both reference and target locations. *Post-hoc* analysis (two-proportion Z-test with Bonferroni correction, bin size: $p < 0.0167$, stimulus: $p < 0.0083$) showed that the 50 ms bin contained fewer units than the 100 and 150 ms bin sizes and



Location decoding by firing rate over increasing duration time windows

Reference location **Target location**

Number of units: BBN: 351 LPN: 262 BPN: 103 HPN: 61

Figure 4.8 – Decoding location by firing rate in increasing duration time windows. More information about the location of the stimuli was available as the stimulus was presented [a] Shows the % of units with significant MI about the reference location in firing rate at three increasing duration decoding windows (50, 100 and 150 ms). [c] Shows the mean % of maximum MI of the unitss with significant information from [a]. [c & d] Show the same as [a & b] for the target location. Results are for unique units selected by choosing the recording with the best MI. Black open triangles indicate the % of the total number of unit recordings with significant MI.

that BBN contained more units with MI than HPN and LPN conditions for both reference and target locations. Additionally for the reference location, BPN had more units than HPN, a similar pattern to that seen for pattern decoding (Figure 4.6 [a & d]). As the number of units with information increased across bin size, so did the amount of information contained about the reference and target locations (Figures 4.8 [b & d], 2-way ANOVA, $p < 0.05$) with the 50 ms bin containing less information than the 100 or 150 ms bins (Tukey-Kramer, $p < 0.05$). For the target location, LPN contained less information than the BBN and BPN conditions. These results suggest that there is information about the location of the stimuli in the firing rate across the whole window rather than only in the onset. For the reference location, the fact that there is no change in the amount of information in the moving window but the amount of information increases as the window size increases suggests that there is information accumulating over the duration of the encoding window. The same is suggested about the encoding of location in the target window despite there being a drop in the amount of information towards end of the stimulus in the moving window.

4.3.3 Decoding of stimulus direction or choice of the ferret from individual units

As discussed in the introduction, there is the possibility that auditory cortex may directly encode direction as opposed to or as well as the individual locations of the two stimuli. In order to investigate whether the relative location was encoded directly, the direction of the stimulus was decoded from the spike patterns of individual units in each condition. Figure 4.9 [a] shows the proportion of units with significant information about the direction of the stimulus in each condition during the target presentation. There were very few units in any of the conditions that have information about the direction of the stimuli – so few that it is difficult to make any comparisons between bin sizes or stimulus conditions. Generally it appears that being tuned to the target location makes little difference to whether there is any information about the direction (Figure 4.9 [b]) and around 7% of the maximum MI can be decoded (Figure 4.9 [c]). Since direction of the stimulus movement is a relatively abstract concept it may well be that, if it is encoded

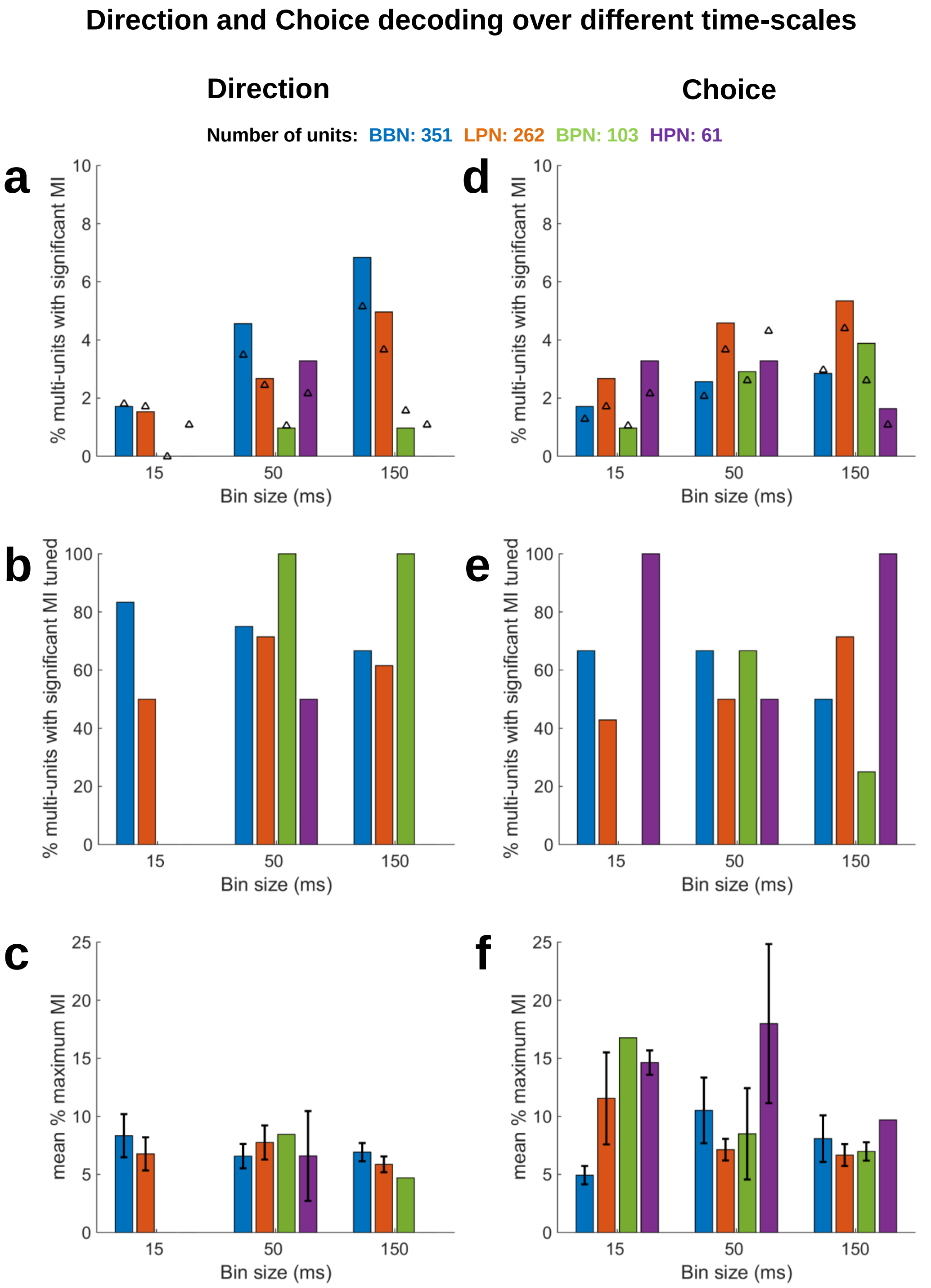


Figure 4.9 – Decoding of the direction of the stimulus and ferret choice over different time-scales during the target stimulus window. Very few units contained any information about the direction the stimulus or the choice of the ferret [a] Shows the % of units with significant MI about the direction the stimulus moved at three different decoding resolutions. [b] Shows the % of units with significant MI (from [a]) that also show spatial tuning for the target location (p<0.05 Kruskal-Wallis test). [c] Shows the mean % of maximum MI of the units with significant information from [a]. [d-f] Show the same as [a-c] for the choice the ferret made. Results are for unique units selected by choosing the recording with the best MI. Black open triangles indicate the % of the total number of unit recordings with significant MI.

at all, it is encoded as perceived direction, thus it may be better to decode the behavioural choice of the ferret which reflects the perceived direction. However, this does not appear to be the case either since the decoding of choice of the ferret is little better than decoding of direction. Figure 4.9 [d] shows the proportion of units with significant MI about the choice the ferret made, as in the decoding of direction there are very few units with significant information. The tuning of the units (Figure 4.9 [e]) does not seem to be very important since the average tuning is roughly similar to that of all units (Table 4.2). Figure 4.9 [f] indicates the mean % maximum MI of the units with significant information. When compared with those units that were informative about direction it appears that slightly more information can be extracted about the perception of the ferret than for the actual stimulus direction; around 10% more. Since the numbers of units were so low, no choice probability was calculated.

As mentioned in the introduction to this chapter, there is also the possibility that the encoding of direction is not explicitly different to the encoding of the target location. Figure 4.10 [a] shows the proportion of units with significant MI about the target location in the context of the relative reference location (i.e. the direction). Binomial logistic regression revealed main effects of bin size and stimulus condition (Analysis of deviance, X^2 distribution, $p < 0.025$; see appendix 7.4 for detailed statistics). *Post-hoc* analysis (two-proportion *Z*-test) revealed that the rate decoder (150 ms bin) found fewer units with MI than the fine pattern (15 ms) and coarse pattern (50 ms) decoders and that the HPN stimulus condition contained fewer significant units than the other conditions. When considering the amount of information encoded by the units, a two-way ANOVA showed main effects of bin size and stimulus condition ($p < 0.05$). *Post*-hoc analysis (Tukey-Kramer, $p < 0.05$) revealed that the rate (150 ms bin size) decoder contained fewer significant units than the pattern (15 and 50 ms bin sizes) decoders and that the BPN condition contained more MI than the other conditions, although the difference between the groups is quite small (4.9 – 7.9 % difference in maximum MI). This suggests that the pattern of firing may be important for conveying information about the target location in the context of direction. The % of the

Joint target location-direction decoding over different time-scales

Number of units: BBN: 351 LPN: 262 BPN: 103 HPN: 61

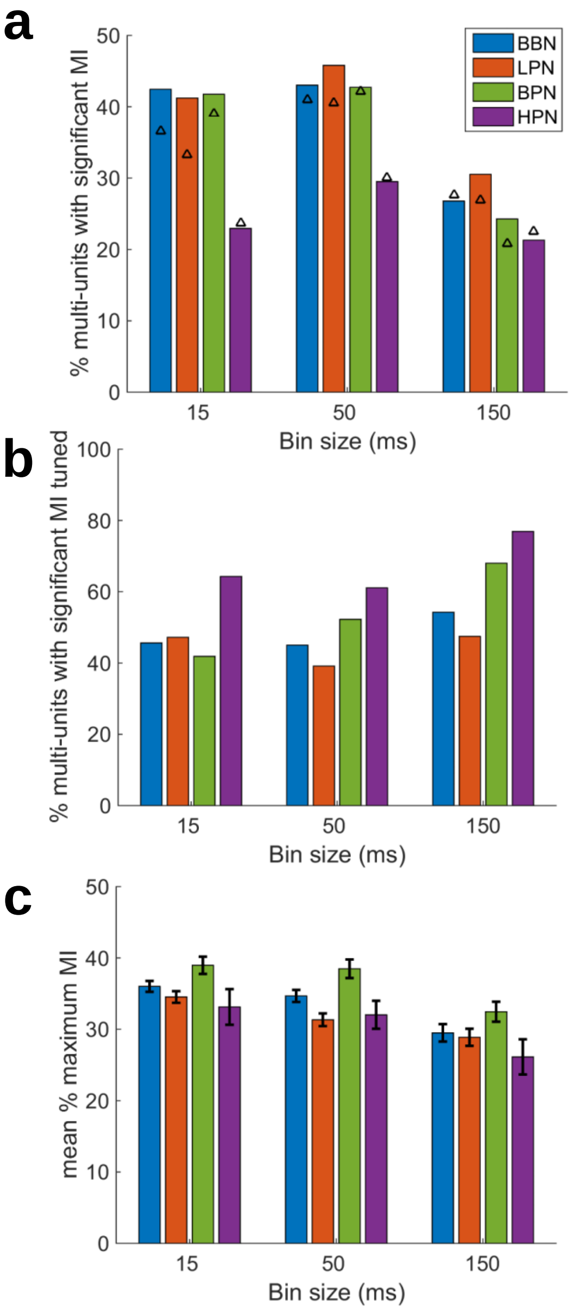


Figure 4.10 – Decoding target location in context of the previous stimulus over different time-scales. Information about the direction of the stimulus at each location was best encoded the fine (15 ms) and coarse (50 ms) firing patterns of the units. [a] Shows the % of units with significant MI about the target location with the added context of whether it was to the left or right of the reference sound at different decoding time resolutions. [b] Shows the % of units with significant MI (from [a]) that also show spatial tuning for the target location (p<0.05 Kruskal-Wallis test). [c] Shows the mean MI of the units with significant information from [a] at each bin width. Results are for unique units selected by choosing the recording with the best MI. Black open triangles indicate the % of the total number of unit recordings with significant MI.

units with significant information about the location of the target in the context of direction that are tuned (Figure 4.10 [b]) is close to the % tuning of all units (Kruskal-Wallis, $p < 0.05$) implying that being tuned to auditory space is not necessarily important for conveying information about the location of the target in the context of the direction of the stimulus.

When the proportion of units with significant MI about the target location in the context of the direction was compared with the proportion of units with significant MI about direction or target alone (binomial logistic regression, see appendix 7.4 for statistics details), there was a main effect of condition and decoded parameter ($p < 0.0167$) but not bin size. *Post-hoc* analysis (two-proportion *Z*-test), revealed that there were more significant units in the target location-context parameter than direction or target alone (Figure 4.10 [c], $p < 0.0167$). The target parameter also had more significant units than the direction parameter. As previously shown in the location decoders, BBN contained more significant units that LPN or HPN conditions and BPN contained more significant units that HPN ($p < 0.0083$). A three-way ANOVA revealed main effects of bin size and decoded parameter on the amount of MI contained in the significant units ($p < 0.05$) but not of the stimulus condition. There was also an interaction between the bin size and the decoded parameter indicating that the distribution of MI was different between the bin sizes in the different decoded parameters. *Post-hoc* analysis (Tukey-Kramer, $p < 0.05$) revealed that the direction and target parameters contained more information than either of the parameters alone; there was also more information in the target parameter than the direction parameter. The rate code (150 ms bin size) contained less information than the pattern decoders (15 and 50 ms bin sizes), suggesting that the majority of information was in the pattern of the spikes rather than overall firing rate.

4.3.4 Population decoding of spatial location

Figures 4.3 [a] and 4.4 [a], which show the normalised spatial receptive fields of neurons in response to the BBN reference and target stimuli, indicate not only that there is an over-representation of best azimuths in the most extreme locations tested, ±75°, but that there is also a substantial number of units with best azimuths not at the extremes. In a purely two-channel-like encoding of auditory space, all best azimuths would be expected at the most peripheral locations (±90°). In a labelled line code we would expect to see an over-representation in the ±75° units because those tuned to locations more peripheral than this would appear tuned to ±75°. Thus the distributions obtained appear somewhat between a purely two-channel encoding and a purely labelled-line encoding (where the distribution of best azimuths would be equal across space). There is the possibility that location can be encoded quite precisely by broadly tuned spatial receptive fields (Georgopoulos et al., 1986), however performance of a vector-encoding of auditory locations in space was poor (Day and Delgutte, 2013).

In order to explore these models of encoding of auditory space further, decoders were constructed using populations of units. The performance of a labelled-line and a two-channel decoder in localising the reference and target stimuli were tested using a maximum likelihood approach to decode location from populations of units. The decoded location was determined by comparing single trial population responses to the joint distributions of spike rates in each hemisphere (hemispheric two-channel model), in two populations of units tuned to left or right space (opponent two-channel model) or in individual cells (labelled-line model) in a method very similar to that used by Belliveau et al. (2014). Figure 4.11 shows the % correct of the population decoders of reference location as the number of units in the population increases for [a] BBN stimuli and [b] LPN stimuli, where only ITDs are available. It is clear that the labelled-line (LL) decoder performs better than the hemispheric (HEM) or opponent (OPP) two-channel models, whose performance is similar at decoding the reference location for both BBN and LPN stimuli (median performance: BBN – LL: 89.8 %, HEM: 58.1 %, OPP: 66.3 %. LPN – LL: 86.7 %, HEM: 53.2 %, OPP: 57.9 %). Generally performance is worse when decoding the target location (Figure 4.12) in all decoders but still the labelled-line decoder out-performs the hemispheric and opponent two-channel models (BBN – LL: 78.5 %, HEM: 43.1 %, OPP: 44.7 %. LPN – LL: 80.0 %, HEM: 51.9 %, OPP: 53.4 %). Generally, the performance with low-pass stimuli differs less between decoding of the

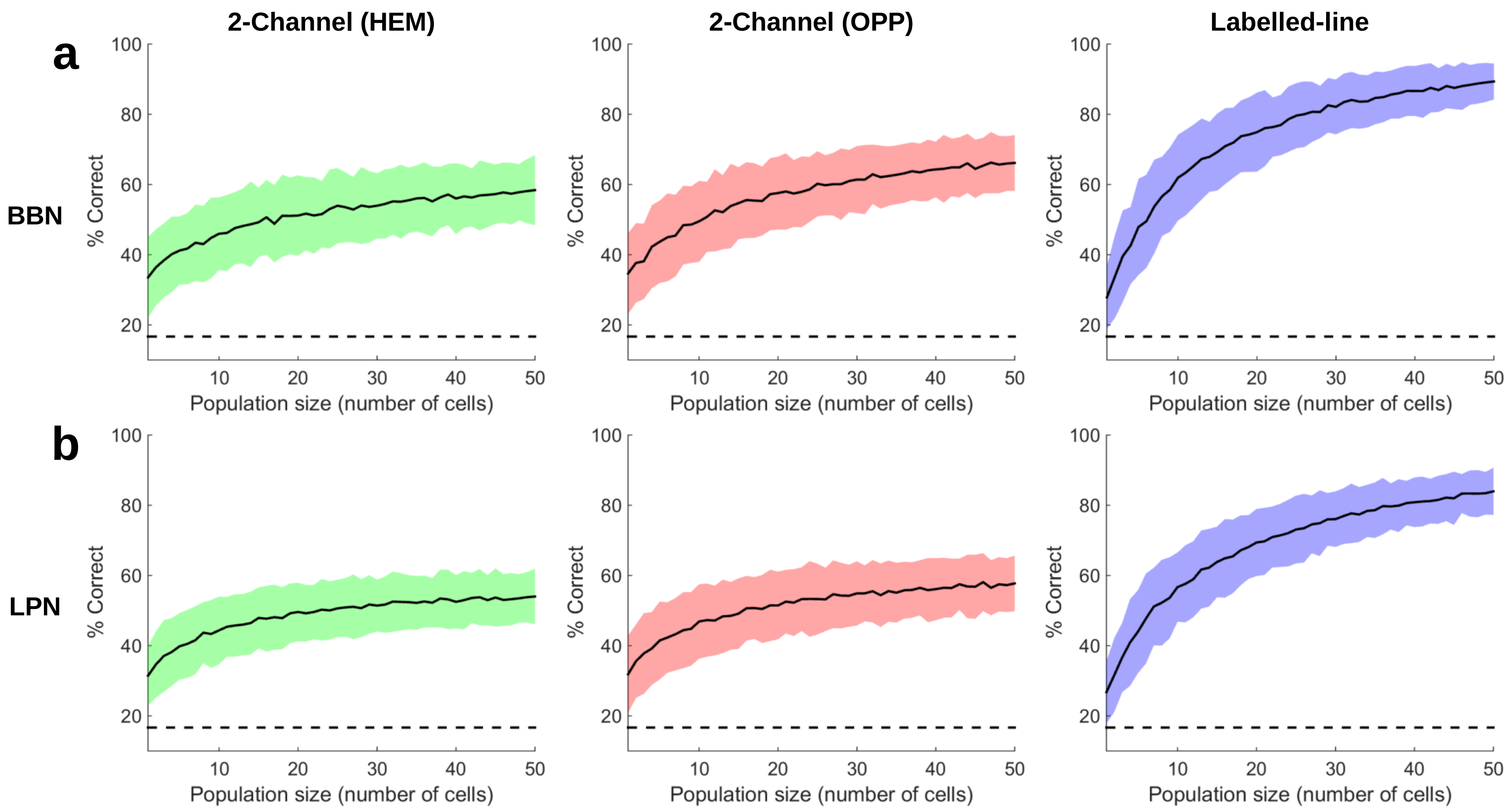


Figure 4.11 –Reference location population decoder performance. Localisation ability of the decoders improved as more units were included. The labelled-line decoder performed the best. This figure illustrates the % correct (±2 x standard deviation) achieved by the hemispheric two-channel (1st column), the opponent two-channel (2nd column) and the labelled-line (3rd column) population Bayesian decoder with increasing population size for the BBN [a] and LPN [b] conditions. The dotted line on each figure represents chance performance.

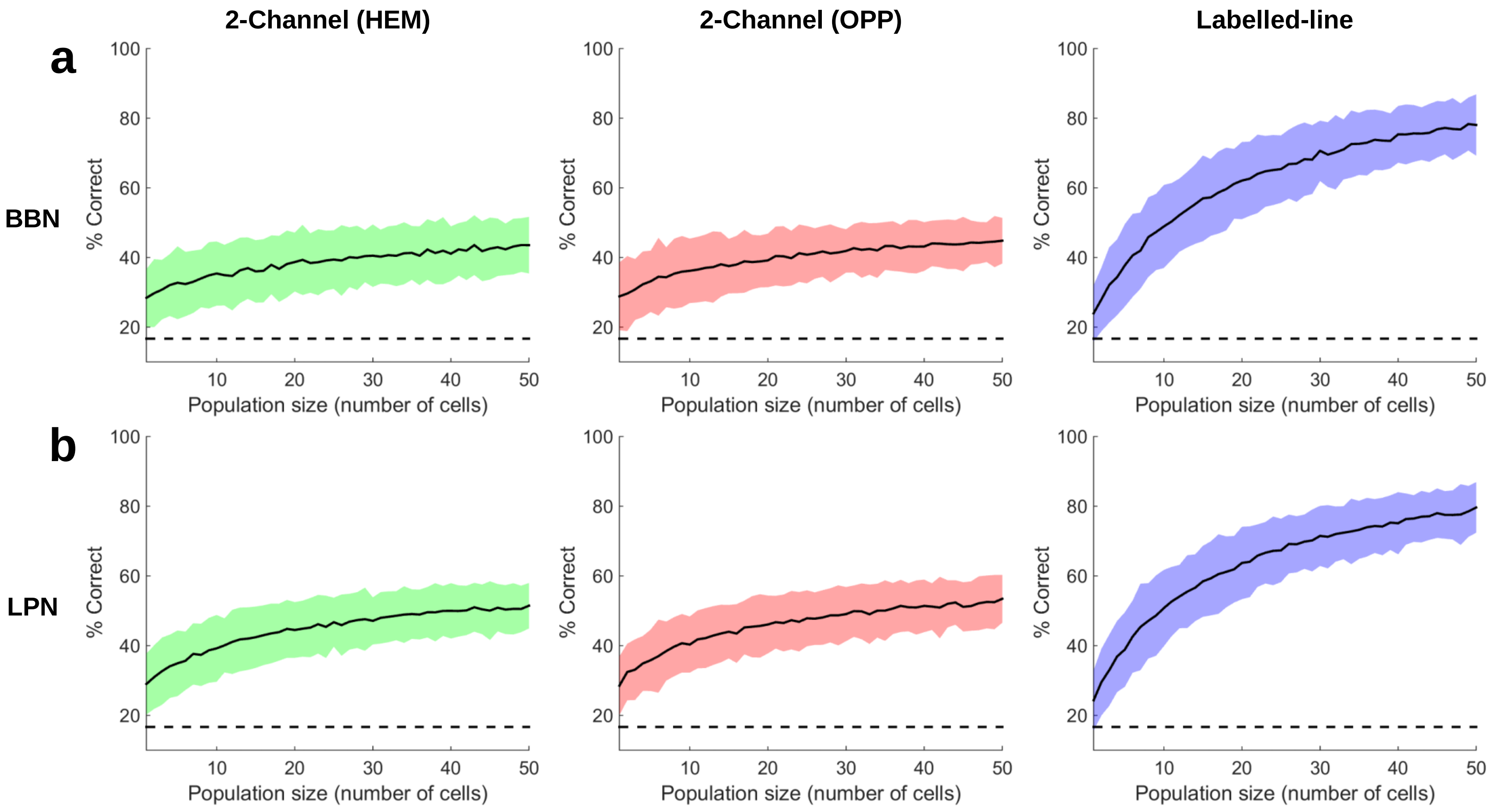


Figure 4.12 –Target location population decoder performance. This figure illustrates the % correct (±2 x standard deviation) achieved by the hemispheric two-channel (1st column), the opponent two-channel (2nd column) and the labelled-line (3rd column) population Bayesian decoder with increasing population size for the BBN [a] and LPN [b] conditions. The dotted line on each figure represents chance performance.

reference and the target (mean performance decrease across decoders: 4.2 %) than the BBN (16 % decrease). This suggests encoding of the target location in the LPN more robust than in the BBN condition however, encoding of the reference location in the LPN condition was lower than in the BBN condition so it could also reflect generally slightly worse encoding in the LPN condition.

Since unilateral inactivation of auditory cortex results in a contralateral deficit in localisation ability of carnivores and primates (see Introduction Section 1.5.1), each of the decoders was tested with only units from one hemisphere. Mean performance of the decoder decreased in both sides of space but slightly more in the contralateral (HEM: -17.1%, OPP: -25.4 %, LL: -7.8%) side of space than ipsilateral (HEM: -10.4%, OPP: -18.3 %, LL: -6.6%), there was also a greater decrease overall in the two-channel decoder.

4.4 Discussion

Responses of units in A1 were recorded while ferrets performed a novel localisation task that involved discriminating the location of a target sound relative to a preceding reference sound presented in azimuth at 30° separations. The centroids of the units were mostly contralateral, consistent with many previous studies (Benson et al., 1981; Recanzone et al., 2000; Stecker et al., 2005b; King et al., 2007). While the spatial tuning of the units appeared to be generally broad, there was a distribution of best azimuths throughout the contralateral hemisphere. This is in contrast with findings in the gerbil where units from one hemisphere responsive to ITDs were found to have best azimuths throughout ipsilateral and contralateral space (Belliveau et al., 2014). Investigation of the tuning properties of populations of individual units showed that a coarse two-channel code could exist simultaneously with a labelled-line type code, corroborating findings in anaesthetised gerbil (Belliveau et al., 2014), although, the distribution of the tuning curves, with more units tuned peripherally than centrally, meant that the coarse two-channel-like population receptive fields (Figures 4.3 and 4.4) necessarily occurred. It was found that individual units contained information about the location of sounds in their firing patterns and firing rate, consistent with a previous study in the awake cat which showed that ~50% of all transmitted information was found in the spike pattern from a spike rate-pattern decoder (Mickey and Middlebrooks, 2003). Information about stimulus location in the spike rate of the units accumulated over the duration of the stimuli, as evidenced by an increase in information over increasing duration time windows contrasting with no change in the amount of information over a sliding 50 ms time window. Few units were found whose firing patterns contained information about the direction of the stimuli or the choice the ferret made during the behavioural task. However, information about the direction of the stimuli was observed when investigating the target location in the context of the direction of the preceding reference. This finding suggests that, like in the IC of anaesthetised cat and gerbil (Spitzer and Semple, 1993) and auditory cortex of awake monkeys (Malone et al., 2002), the instantaneous firing rate of units in ferret auditory cortex contain information not only about the location of the current stimulus but also the preceding stimulus (i.e. information about the relative location). Finally, the firing rate of populations of neurons in a labelled-line decoder was better able to localise stimuli than a two-channel decoder, similar to findings in anaesthetised gerbil auditory cortex (Belliveau et al., 2014).

Around 40-50% of sound-driven units recorded from A1 of ferret showed modulation in their firing rate by the location of stimuli and the vast majority of those significantly tuned were tuned to contralateral space. These values being slightly lower than those found in the awake cat cortex (~75%; Mickey and Middlebrooks, 2003). However, this difference might in part relate to the way in which spatial tuning was defined in these studies: In both cases spatial tuning was determined by whether the spatial receptive field showed a significant modulation of firing rate with location. Mickey and Middlebrooks (2003) defined the modulation depth relative to the spontaneous firing rate, whereas the present study only looked at modulation depth as a function of the firing rate during stimulus presentation. Since some sound locations can actually cause a decrease in firing compared with the spontaneous firing rate, calculating SRFs relative to spontaneous rate is potentially a more sensitive measure, perhaps accounting for the lower numbers found.

Assessment of the spatial tuning properties of individual units by three parameters; centroid, tuning width and modulation depth, revealed that there was no difference in the distribution of these parameters between the different stimulus conditions, where binaural cues were limited by band pass filtering. This is consistent with a study by Salminen et al. (2015b) who showed in an magneto-encephalography study in humans that auditory cortex represented location independently of the binaural cue type. The centroids of the units were mostly contralateral and the tuning generally broad, consistent with many previous studies (e.g. Benson et al., 1981; Recanzone et al., 2000; Stecker et al., 2005b; King et al., 2007).

The majority of centroids in the present study were centred at 30°-45° contralaterally and most units had tuning widths of ~125°. These values are both smaller than those observed in the awake cat (Mickey and Middlebrooks, 2003). Both studies estimated tuning widths by calculating ERRF widths. In the awake cat, tuning widths were often a whole hemifield, and often contralateral, whereas in the present study, only frontal space was investigated and this may account for the narrower tuning width and more medial centroid locations than in the cat. The centroid distribution in the present study was very similar to that observed in awake, head restrained ferrets, where the majority are found in the anterior quadrant however, most units had centroids more medial to those observed in the head restrained ferret (King et al., 2007). This could be because of the head restraint or the fact that in the present study, ferrets were performing a localisation task. Another, possibly more likely, reason for more medial centroids is that in the present study, sounds were only presented in the frontal hemifield and the nature of the centroid calculation means that it would be biased more medially without more peripheral testing.

As well as significant modulation of spike rate by spatial location, the amount of information about spatial location in the spiking patterns of individual units was investigated using a spike pattern decoder based on Euclidean distance and assessing the success of the decoder by calculating the MI between the actual locations and the classified locations. This revealed that a large proportion of units (~40%) also contained information about the location of the stimuli and that of these a large percentage (~80%) were also significantly modulated by spatial location. In general, more units contained information about location of the reference or target in a coarse pattern decoder (at 50 ms resolution) than in the fine pattern decoder (15 ms resolution) and in the rate decoder (150 ms resolution). Although, of those units that contained information, those with information in the fine pattern contained more information than those in the rate code. That information is found in the pattern and the firing rate is consistent with a previous study in the awake cat which found that ~50% of all transmitted information by a pattern-rate decoder was found in the spike pattern (Mickey and Middlebrooks, 2003).

In the present study it was found that the amount of information in the spike rate about the location of a sound accumulated over the presentation of the stimuli, as evidenced by an increase in information over increasing time windows contrasting with no change in the amount of information over a sliding, fixed-duration time window. These findings contrast with results from a study in the anaesthetised ferret, where it was shown that more information about the location of a sound was encoded early in the response than over the whole stimulus window (Walker et al., 2011). In that study the sounds presented to the naïve ferrets were low frequency harmonic sounds (artificial vowels) and the animals were anaesthetised. In the present study, stimuli were spectrally restricted white noise where the only thing relevant to performance of the task was the spatial location, thus the coding strategy of the brain for auditory space may have adapted such that as much information as possible about the spatial location was represented. The conscious state of the animal may also have played a part in this difference. In the present study, the LPN stimuli are nearest (in frequency) to the vowels presented in Walker et al. (2011) and they too show that information about location is sustained throughout the response. Work in the marmoset has shown that when driven by their preferred stimuli cortical neurons can respond with sustained firing (Wang et al., 2005), thus it could be that the information over the whole window is more reliable. It has also been shown that receptive fields of units in auditory cortex can rapidly adapt to the behavioural task (Fritz et al., 2003). It could also be a difference between the encoding in anaesthetised cortex and cortex in a behaving ferret, during anaesthesia, the inhibitory state of the cortex is affected. Effects on inhibition are variable depending on the anaesthesia used, but sustained responses and offset responses can be diminished (Wang, 2007).

With electrophysiological recordings obtained in the present study, three models of auditory space were tested using the neural firing patterns observed while ferrets performed the relative localisation task. It was found that a coarse two-channel code (whether hemispheric or opponent) could co-exist with a labelled-line representation of auditory space since both types of spatial tuning were evident (Figures 4.3 and 4.4, although the two-channel like spatial receptive fields are only present when looking at the mean of the population of units) and performance of location decoders using populations of neurons both performed well above chance. The labelled-line decoder performed much better than the two-channel decoder, as has been previously observed in anesthetised gerbil AC (Belliveau et al., 2014). A modified labelled-line encoding was ruled out since there were not more units tuned to the midline compared with the periphery. Like in the gerbil though, the two-channel decoder performed as well as and the labelled-line decoder performed better than ferrets performing an absolute localisation in the same testing chamber. For stimuli of 150-200 ms duration, ferrets performed at ~45% within ±60° of the midline (see Chapter 5), however another ferret study found localisation performance of greater than 70% for stimuli of this duration within ±60° of the midline (Nodal et al., 2008).

When the hemispheric two-channel model and the labelled-line model were tested with only units recorded from one hemisphere (in a mock inactivation experiment), performance of the decoders decreased in both sides of space, and decreased slightly more in the side contralateral to the mock inactivation. Although only a relatively small difference between the ipsilateral and contralateral mock inactivation, these findings are compatible with unilateral inactivation studies in carnivores and primates that show contralateral deficits in sound localisation ability. It has been suggested that multiple models of encoding sound location could be used by the brain depending upon the frequency of the sounds presented (Harper et al., 2014). That indications of a two-channel type model and labelled-line model co-exist may support these findings.

When investigating the encoding of the direction the sound moved in individual units, very few units were found to contain information either in their firing pattern or spike rate. However, direction information could be extracted when it formed the context for decoding of the target location. In a study by Malone and colleagues (2002) on the sensitivity of auditory cortex neurons in the awake macaque to dynamic interaural phase differences (IPDs), neural firing rate was found to be sensitive to stimulus events in the recent past, much like has been observed in the IC (Spitzer and Semple, 1998; McAlpine et al., 2000; Ingham et al., 2001). In these studies and in the present study, the stimulus and discharge history cannot effectively be dissociated, this is indicated by the finding that around 40% of all units (in response to BBN, LPN and BPN, Figure 4.10) carry information about the target location in the context of the direction the stimulus moved, more than direction or target alone, and those units carry more information than direction or target alone. This could be indicative of a multiplexed coding of direction and sound location which has been found in the coding of other features of sounds, for example, pitch and timbre; Walker and colleagues (2011) showed that the majority of neurons in ferret auditory cortex were sensitive to more than one feature of a sound (pitch, azimuth or timbre) and for example, representation of sound periodicity persisted over longer durations than timbre responses. If direction and location were multiplexed rather than convolved, then they should be able to be ‘read out’ independently of the other over different timescales and/or windows. It does appear that more units with information about the direction at each location contained that information mainly in a fine pattern (15 ms decoding resolution) code whereas more units contained information about the location alone in the coarse pattern (50 ms decoding resolution) and the rate, this could allow the two different parameters to be ‘read out’ differently, for example by higher neurons acting at different integration speeds. If the two things were convolved, then the direction could not be ‘read out’ separately from the location.

It has been suggested that the adaptation observed in dynamic IPD studies could be a more general property of encoding of sounds that change over time (Malone and Semple, 2001). More units were found to carry information in the fine and coarse pattern decoders than a rate decoder, indicating that changes to the firing patterns caused by the recent stimuli can happen on a short time-scale. Little difference was found between the different stimulus conditions in this encoding of location in context, a result which supports a recent study by Salminen et al. (2015b) that found that the representation of auditory space in auditory cortex is independent of the type of binaural cue present in the stimulus. It is possible that higher cortical regions could extract the direction information in order for the ferret to perform the task.

There is also the possibility that the few units that were found to contain information about the direction form a specialised class of neuron whose responses are important for direction of stimulus motion. There are many ways in which this possibility can be further investigated: The location of these units (and others that contain information about location and or choice) can be investigated in terms of depth in cortex (different layers of cortex could perform different functions in the processing of the sounds). This type of analysis cannot be performed until histology is complete (see Figure 4.13 for an example of the placement and depth of an electrode) and the depth of recording confirmed.

At present none of the models has been successfully adapted for use in decoding the direction a sound has moved. This is one major avenue of work that can be followed up. It was shown that information about the direction was encoded implicitly with information about the target location; it may be possible that some higher brain area can extract this information. The majority of the units and of the information contained by them was found in the pattern of the firing thus it may be useful to pursue a more temporal

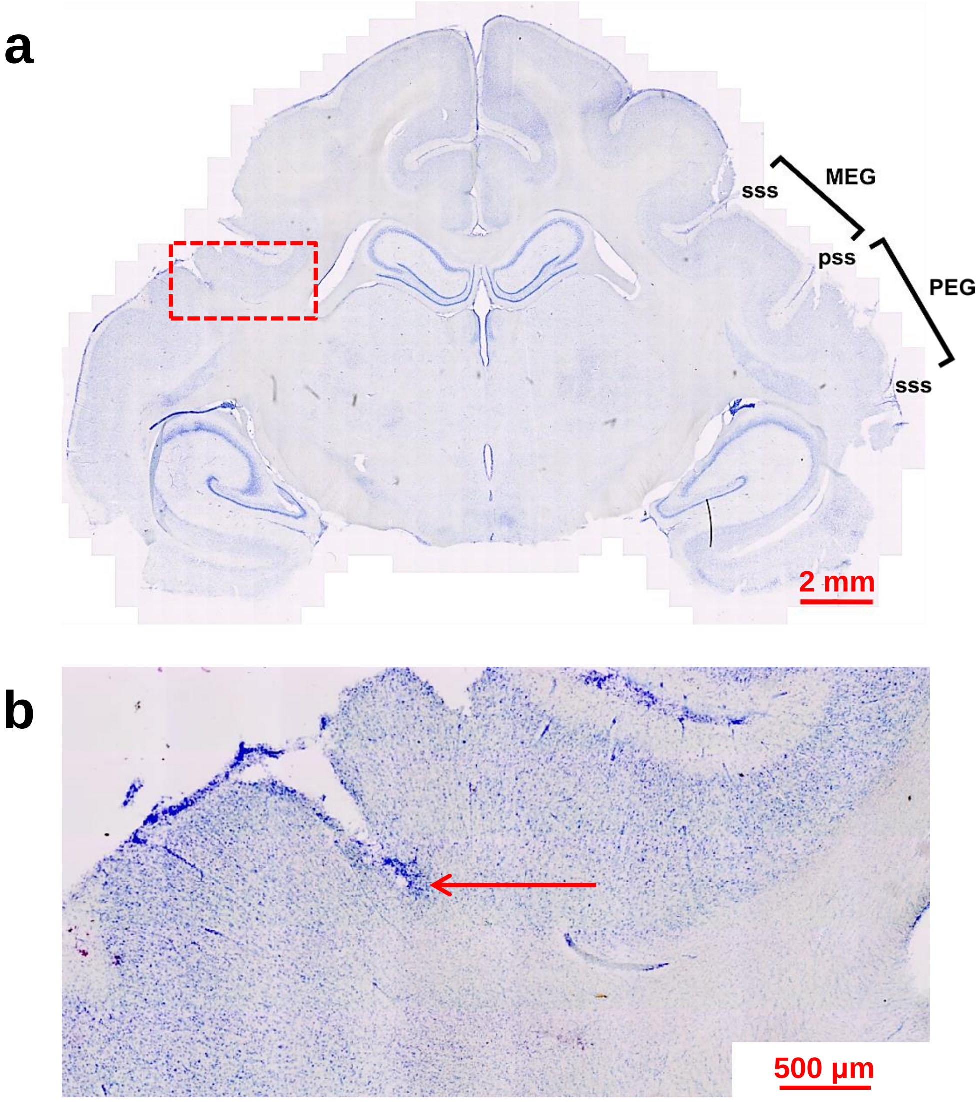


Figure 4.13 – Histological verification of electrode placement. [a] shows an image of a whole brain coronal section Nissl stained. The medial ectosylvian gyrus (MEG) and posterior ectosylvian gyrus (PEG) are indicated on the right, as are the supra-sylvian sulcus (sss) and pseudo-sylvian sulcus (pss). The red dashed box indicates the area blown up in [b]. The red arrow in [b] indicates the location of one of the electrode tracks from F1301.

based decoder than the rate decoding that is performed with the location population decoders.

Results from chapter 3 showed that ferrets performed equally well with the high-pass and low-pass stimuli as with the broadband stimuli. However, their performance decreased when they were limited to mainly ILDs with the band-pass stimuli. It was expected that there would be less information about the spatial location of band-pass stimuli than the other stimuli. In order to investigate this spatial location was decoded from units in response to the different stimuli. Fewer units contained information about the LPN stimuli than the BBN, but there was no significant difference between the proportion of units informative about the BPN and those informative about the BBN. Neither were there fewer units with information about the target location in the context of the direction the stimulus moved in the BPN condition compared with the BBN condition. There is also a possibility of oversampling of high frequency units which would be more likely to respond well to the higher frequency stimuli (i.e. the BPN and HPN) and possibly encode more information about the location of these stimuli. The frequency tuning of units has been investigated by a passive presentation of tones presented at varying frequency and level which will allow analysis of the frequency response area of each units thus identifying its characteristic frequency. The frequency tuning of units has yet to be related to the responses of the units or the type/amount of information they contain but this work is ongoing. There is clear evidence that units containing information about the BPN stimuli exist and they contain at least as much information as do units containing information about the other stimuli thus it seems likely that the ferret isn’t using these units to complete the task, possibly because of the way the animals were trained.

It has been shown in anaesthetised and awake cats that the responses of most auditory cortical neurons to a second sound presented up to 10 ms after a preceding sound were suppressed compared with the first sound (Mickey and Middlebrooks, 2005). This suppression was found regardless of the locations of the two stimuli or the intensity of the stimuli. Even at inter-stimulus durations (ISDs) of up to 20 ms the firing patterns of cortical neurons varied in response to the 2nd sound indicating that there was sensitivity to the preceding stimulus even when no suppression to the second stimuli was observed. This phenomenon is reflected in localisation behaviour of stimuli with short ISDs and is known in the literature as the precedence effect (Litovsky et al., 1999). The suppression of responses to the lagging sound with short ISDs correspond well with behaviour in the cat (Tollin et al., 2004). A recent study shows that ferrets experience the precedence effect in a similar manner to humans and that by the time the ISD is 20 ms (the ISD in the present study) their behaviour is consistent with no precedence effect (Tolnai et al., 2014). Thus, at the ISDs in the present study, it is not expected that there would be any perceptual problem with precedence effect. However, in awake rabbit auditory cortex some units were found that showed suppression to the lagging sound by ISDs of up to 300 ms (Fitzpatrick et al., 1999) thus it is possible that the precedence effect, in terms of suppression of firing rates of second stimuli, is a more general mechanism connected with the presentation of sequential stimuli. As discussed in Chapter 3, ferrets also performed the relative localisation task with an ISD of 100 ms and recordings were also made at this ISD. There is therefore an opportunity to look for changes in the modulation of the response to the lagging sound (target) at two different ISDs. It may be interesting to consider changes to this modulation which may be dependent upon the relative locations of the two stimuli.

In summary, the present work has demonstrated that two-channel model and labelled-line model decoders can perform well in determining the location of a stimulus using firing patterns of populations of units recorded from AC and can perform as well as ferrets in the same chamber (see Chapter 5). However, the labelled-line model performs much better than the two-channel model and predictions about the spatial receptive fields shapes of the units in each case favour the labelled-line model in that units are more narrowly tuned than would be found in with a two-channel model. Individual units carried information about the location of the stimuli and their firing pattern was found to be influenced by the preceding stimuli such that their present firing patterns reflected not only the current location of the stimulus but the relative location of the preceding stimulus.

Chapter 5: Effects of inactivation of primary auditory cortex on absolute sound localisation

5.1 Introduction

This chapter aims to establish the method of reversible inactivation of brain areas in the awake, behaving ferret by cooling (Lomber and Payne, 1999). This technique can be used to investigate the involvement of specific brain areas in auditory processing, or indeed any other form of task and processing, and has been used in awake, behaving cats to study the brain areas necessary for a variety of tasks including sound localisation tasks and auditory pattern discrimination tasks (Lomber and Malhotra, 2008). Adaptations of the technique have been employed in smaller mammals during *in vivo* electrophysiological recordings in anesthetised animals (Nakamoto et al., 2008, 2010; Coomber et al., 2011). However, to date the use of cooling during behavioural testing has been restricted to larger animals (for example, cats: Lomber and Malhotra, 2008; and primates: Plakke et al., 2015). Since cooling has not been performed in ferrets before a behavioural paradigm was used that provides clear predictions of the effect of inactivating cortex by cooling on performance. Thus, we trained ferrets in an approach-to-target localisation task and reversibly deactivated primary auditory cortex by cooling whilst the ferret performed the task. A deficit in performance in the hemisphere contralateral to cooling and in both hemispheres with bilateral cooling would provide evidence for efficacy of the cooling method since previous studies have demonstrated that both permanent (Kavanagh and Kelly, 1987; Nodal et al., 2010) and reversible (Smith et al., 2004; Nodal et al., 2012) lesions of auditory cortex in the ferret result in a sound localisation deficit.

Given recent advances in optogenetic methods (Packer et al., 2013) for reversibly silencing brain areas the choice of cooling may seem an ‘outdated’ method. However, there are many hindrances to implementing optogenetics in ferrets: Firstly, the sheer volume of cortex that must be inactivated, the majority of behavioural studies with optogenetics are performed in small mammals such as the rat and mouse, it has been very difficult to achieve behavioural effects in larger mammals such as primates (Diester et al., 2011), although it has been shown possible (Cavanaugh et al., 2012). Inactivation of a large site, such as ferret A1, would require multiple injections of viral constructs and multiple optical fibres. Secondly, one of the major advantages of the technique in mice and rats has been the use of transgenic animals where it is possible to direct expression of channel-rhodopsin (which activates neurons when activated by light) to inhibitory neurons by cre-recombinase methods, the promoters required for targeting inhibitory neurons such as parvalbumin positive or somatostatin positive neurons are too large to package into viral vectors (Zalocusky and Deisseroth, 2013) and activation of inhibitory neurons has been shown to be more effective than inactivation of excitatory neurons with an inhibitory opsin (e.g. ArchT). As yet, there are no genetically modified ferrets and thus viral mediated optogenetics limits inactivation to directing ArchT to excitatory neurons via the CaMKII promoter (Bajo et al., 2013). Brain areas of the ferret have also been reversibly inactivated by pharmacological methods, for example, by implantation of a sustained release polymer, Elvax, that is loaded with an agonist (muscimol) of inhibitory receptors ($GABA_A$) (Smith et al., 2004; Bizley et al., 2007), the disadvantage of this method compared with cooling is that the inactivation is sustained for a period of 3-4 weeks and over time the efficacy of inactivation decreases and the ferret adapts to the inactivation (Smith et al., 2004). With cooling, effects are acute (control data can be collected on the same day) and immediate; adaptation to the inactivation is unlikely to occur over the short time periods tested. Thus, at present, for the ferret, cooling offers an important method of reversible inactivation of brain areas.

The role of auditory cortex in spatial listening, as tested with an approach-to-target localisation, has been consistently demonstrated across species and inactivation methods (Heffner, 1978, 1997; Thompson and Cortez, 1983; Jenkins and Merzenich, 1984; Kavanagh and Kelly, 1987; Stepien et al., 1990; Heffner and Heffner, 1990; Beitel and Kaas, 1993; Zatorre and

Penhune, 2001; Smith et al., 2004; Malhotra et al., 2004, 2008; Malhotra and Lomber, 2007; Lomber and Malhotra, 2008; Nodal et al., 2010, 2012). Bilateral lesions of A1 in ferrets disrupts their ability to perform an approach-to-target localisation task to stimuli <500 ms long on both sides of space, whereas (untrained) head-orientation responses to the target are maintained (Nodal et al., 2010). More extensive lesions, including parts of the anterior and/or posterior ectosylvian gyrus cause more substantial deficits in approach-to-target ability and also disrupt the accuracy of head-orienting movements. Furthermore, ferrets with both types of lesions lose the ability to relearn how to localise sounds when they had sound localisation cues disrupted by the insertion of an ear plug, unlike non-lesioned animals, which can relearn to localise with altered aural cues (Nodal et al., 2010). The dependence of non-spatial listening on auditory cortex in the ferret is unknown, and in general, the role of specific auditory cortical fields in non-spatial tasks is much less well studied than spatial processing tasks.

In this chapter A1 is reversibly inactivated using a chronically implanted cooling loop (Lomber and Payne, 1999) in ferrets trained on two different tasks; a non-spatial discrimination task and an approach-to-target sound localisation task. Since it is well-established that unilateral inactivation of A1 causes an approach-to-target localisation deficit in the contralateral hemisphere, the localisation results were used to confirm functionality of the cooling loops and provide evidence that the method of cooling with chronically implanted cryoloops is viable in the ferret. Two ferrets were also tested in a pulse-rate discrimination task in which they had to report whether a pulse-rate was fast or slow.

5.2 Methods

5.2.1 Subjects

Six adult pigmented ferrets (female) took part in these experiments, three were trained in an absolute approach-to-target sound localisation task, two of these were also trained in a pulse-rate discrimination task and the fourth ferret was trained only in the pulse-rate discrimination task (see Table 5.1).

Ferret:	F1202*	F1204	F1303†	F1311
Sound localisation training	✓	✓		✓
Sound localisation cooling	✓	✓		✓
LED localisation training	✓	✓		✓
LED localisation cooling	✓‡	✓		✓
Pulse-rate discrimination training	✓		✓	
Pulse-rate discrimination cooling	✓‡		✓	

Table 5.1 – Subjects' training. Indicates which ferrets were trained and/or tested in the approach-to-target localisation task and the pulse-rate discrimination task. * The right loop became blocked in this ferret, ‡ indicates where only unilateral cooling was performed. † This ferret was euthanased for health reasons unrelated to the behavioural training.

The remaining two ferrets were part of *in vivo* anaesthetised experiments in which auditory cortex or suprasylvian cortex (SSY) was cooled and temperatures and neuronal activity of surrounding cortex measured. Ferrets were housed in groups of two to eight, with free access to high-protein food pellets and water bottles. On the day before training, water bottles were removed from the ferrets' home cages and they were replaced on the last day of a training run. Training runs lasted for five days or less, with at least two days between each run. On training days, ferrets received drinking water as positive reinforcement while performing a sound localisation or pulse-rate discrimination task. Water consumption during training was measured, and was supplemented as wet food in home cages at the end of the day to ensure that each ferret received at least 60 ml of water per kilogram of body weight daily. Regular otoscopic examinations were carried out to ensure that both ears of the animals were clean and healthy. All experimental procedures were approved by the local ethical review committee and were carried out under licence from the UK Home Office, in accordance with the Animals (Scientific Procedures) Act 1986.

5.2.2 Approach-to-target localisation task

5.2.2.1 Testing apparatus

Ferrets were trained in a custom built sound attenuating chamber (Zephyr Products Ltd, Suffolk, UK) measuring 90 cm by 89.5 cm by 75 cm (height x width x depth) with the inner walls coated with sound attenuating foam (45 mm). Inside this chamber a custom built testing apparatus was assembled, essentially a box with a semi-circular end (50 cm x 31 cm x 28 cm (height x width x depth) for box and semi-circular part semicircle of radius 15.5 cm and height of 50 cm, see Figure 5.1 [a]). This testing apparatus contained a semi-circular array of 7 speakers at 30° intervals (-90° to 90° about 0°) situated 24.1 cm from the centre of the semicircle. There were 7 response spouts located in front of each speaker (15.5 cm from the centre of the semicircle). A 'start spout' was located 3 cm inside the semicircle such that when a ferret initiated a trial by holding its head at this spout, the centre of its head was in the centre of the semicircle and its

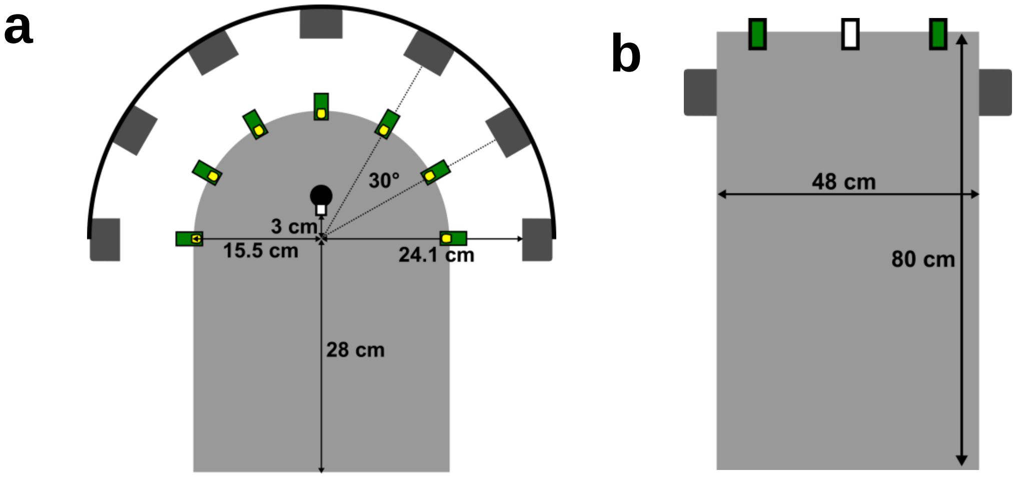


Figure 5.1 – Localisation and pulse-rate discrimination testing chambers. [a] shows the dimensions of the testing chamber and locations of speakers and LEDs for the localisation task. The dark grey squares indicate the locations of the speakers. The green rectangles represent the location of the response spouts. The yellow circles located on top of the spouts represent the LEDs. The central spout is represented by the white square in the middle of the chamber. [b] shows the dimensions of the testing chamber for the pulse-rate discrimination task.

interaural axis was in line with the -90° and 90° speakers. A light emitting diode (LED) was also mounted outside the chamber, behind the plastic mesh that enclosed the chamber, approximately 15 cm from the floor of the chamber and flashed (at 3 Hz) to indicate the availability of a trial to the ferret. The LED was continually illuminated whenever the animal successfully made contact with the start spout.

In the case of two ferrets (F1202 and F1204) LEDs were positioned at the same distance as the speakers and were located just above the speakers. For the third ferret (F1311) the LEDs were positioned at the same distance as the response spouts positioned just above them (10 cm from the floor of the chamber and 5 cm, above the response spouts). The custom-made spouts also contained infra-red (IR) sensors which fed back to a water spout operating system allowing us to detect the presence of the ferret at each water spout and to reward the ferret if necessary. Sound stimuli were delivered via seven loudspeakers (Visaton FRS SC 5.9) which were mounted on bars in a semicircle around the outside of the chamber approximately 5 cm from the floor. The behavioural task, data acquisition, and stimulus generation were all automated using custom software running on personal computers, which communicated with TDT RX8 real-time signal processors (Tucker-Davis Technologies, Alachua, FL).

Speakers were calibrated to produce a flat response from 200 Hz to 25 kHz when measured in an anechoic environment using a microphone (Brüel and Kjær 4191 condenser microphone). The microphone signal was passed to a TDT System 3 RX8 signal processor via a Brüel and Kjær 3110–003 measuring amplifier. Golay codes were presented through the speakers and the spectrum was analysed and an inverse filter was constructed to flatten the spectrum (Zhou, 1992). All sounds were presented with the inverse filters applied. All the speakers were matched for level using a microphone positioned upright at the level of the ferret head in the centre of the semi-circle; correcting attenuations were applied to the stimuli before presentation. All sounds were presented low pass filtered below 22 kHz (finite-duration impulse response (FIR) filter <22 kHz, 70 dB attenuation at 22.2 kHz).

5.2.2.2 Stimuli

The training and testing of the ferrets (once sounds were being presented) was fully automated with IR spout input transferred via 2 TDT system III SA8s into a custom-written circuit running in Open Project (TDT Software) which also communicated with MATLAB. Stimuli were generated in MATLAB and were presented automatically when a trial was triggered via two TDT system III RX8 processors. Water rewards were given by custom-built devices controlling solenoids which opened tubing, feeding water to the response and start spouts. Ferrets always received a water reward for correct responses from the response spout and received a reward from the start spout 5% of the time. All stimuli were generated and presented at a sampling frequency of 48 kHz. Sound stimuli were white noise bursts of differing durations (500 ms, 250 ms or 100 ms) cosine ramped with 5-ms duration at the onset and offset. Fresh noise bursts were generated on each trial and were presented from one pseudo-randomly selected speaker of the seven speaker locations (±90° at 30° intervals, see Figure 5.1 [a]). Sound stimuli were presented at 3 different levels; 66, 61.5 and 57 dB SPL. The 500 ms duration and possibly the 250 ms duration stimuli are within the amount of time it takes for a natural head movement in response to a sound made by ferrets (~200 ms, Nodal et al., 2008), the head movement allows the ferret to obtain dynamic cues as to the location of the sound. In the present study, the animal must maintain contact with the centre spout while sounds are presented thus preventing the natural head movement associated with sound localisation, thus although sounds are long enough to be closed loop, the ferrets would not be able to respond until the sound has been fully presented.

Light stimuli were presented by LEDs positioned at the same azimuthal locations as the speakers (±90° at 30° intervals, Figure 5.1 [a]). The duration of the LED stimuli was adjusted for each ferret in an attempt to match performance with one of the sound durations. For one ferret (F1202) light and sound stimuli were equal durations of 500 ms, and for the second ferret taking part in this experiment (F1311) sound stimuli were 250 ms and light stimuli were 750 ms.

5.2.2.3 Training

Training runs were 5 days long and two training sessions were run each day (one each in the morning and afternoon). Ferrets were trained on an approach-to-target localisation task using similar methods to (Parsons et al., 1999). Briefly, the ferret was first trained to lick the start spout and then approach the target sound, in this initial phase, the ferret could not activate spouts that were not at the target location, i.e. the trial lasted until the ferret made a correct judgment. The ferret started with a long sound duration of 1000 ms, which is easy to localise and the sound was looped with a 500 ms gap. Once the ferret was accustomed to the nature of the task (identified by regular returning to the start spout after receiving water from target locations), incorrect responses led to the termination of the trial initially with no punitive timeout for an incorrect response, and stimuli were only presented once to the ferret. If the ferret responded incorrectly, it received a ‘correction trial’ where it heard the same stimulus again and once more if another incorrect response was made. After three incorrect responses, the ferret then heard an ‘easy’ trial, where the sound was repeated until the ferret made the response. Once the ferret reached 70% correct or more at this stage the duration of the stimulus was reduced to the next shortest (500, 250 and 100 ms were tested).

Two ferrets (F1202 & F1204) were trained to localise LED light sources located at the same positions as the speakers after they had completed the testing with the auditory stimuli. Initially light stimuli were presented at the same time as sounds from the matched speaker locations using stimuli that were 1000 ms in duration. Over several training sessions, the intensity of the matched location noise stimuli was reduced and ultimately removed completely, such that the ferret was localising the light source. Once the peripheral noise stimulus had been removed the ferrets appeared reluctant to move away from the start spout before hearing an acoustic stimulus thus, a noise stimulus of the same duration as the LED was presented from above the ferret, where it offered no localisation cues. One ferret (F1204), could not reach a consistent level of performance on visual trials during the limited testing time available for a single animal (under UK Home Office restrictions) and thus was not tested on this task (or the pulse rate discrimination) with cooling. One ferret (F1311) was trained simultaneously on auditory and visual trials (randomly interspersed) using the methods described for sound localisation above. For this ferret, LED stimuli were placed at the same distance as the reward spouts as opposed to where the speakers were positioned (see Figure 5.1 [a]). This animal required no additional acoustic stimulus from above.

5.2.3 Pulse-rate discrimination

5.2.3.1 Testing apparatus

Training took place in a custom-built sound attenuating chamber (IAC acoustics, UK) whose walls were covered in sound attenuating foam. Inside this was a customised wire cage (80 x 48 x 60 cm, length x width x height) in which the ferrets were trained (Figure 5.1 [b]). The floor of the cage was made from plastic with an additional plastic skirting into which three spouts were inserted. Each spout contained an infra-red sensor (OB710, TT electronics, UK) that detected nose-pokes and an open-ended tube through which water could be delivered. Sound stimuli were presented through two loud speakers (Visaton FRS 8) positioned on the left and right sides of the head at equal distance and approximate head height. These speakers produced a flat response (62 dB) from 200 Hz to 20 kHz with an uncorrected 20 dB drop-off from 200 to 20 Hz when measured in an anechoic environment using a microphone positioned at a height and distance equivalent to that of the ferrets in the testing chamber. The behavioural task, data acquisition, and stimulus generation were all automated using custom software running on personal computers, which communicated with TDT RZ6 and RZ2 signal processors (Tucker-Davis Technologies, Alachua, FL).

5.2.3.2 Stimuli

Ferrets were required to discriminate between a slow pulse-rate (of 7 Hz) and a fast pulse-rate (of 23 Hz), the pulses consisted of 15 ms bursts of amplitude modulated white noise cosine ramped with 5-ms duration at the onset and offset.

5.2.3.3 Training

Two ferrets (F1202 and F1303) were trained to associate the right spout with the fast pulse-rate and the left spout with the slow pulse-rate and another ferret was trained on the opposite contingency (F1204). Again, ferrets were initially rewarded for approaching either the left or right response spouts or the central start spout (see Figure 5.1 [b]). Once the ferret became accustomed to receiving water from the spouts (typically 3-4 sessions), a new contingent was introduced where the ferret had to approach the start spout and remain there for a variable hold time (500-1000 ms) before it was rewarded and was then able to respond to one of the response spouts for a further reward. Following acquisition of this basic task, sounds were presented after the ferret activated the start spout for the duration of the hold time, these sounds repeated until the ferret approached the correct spout associated with the sound. At this stage, making an incorrect response did not terminate the trial and the sound repeated with a 500 ms gap between each presentation until the ferret approached the correct response spout, where they received a water reward. Once the ferret had performed a few (typically 3-4) sessions the task was modified so that now a response to the incorrect spout terminated the trial. This was indicated by the presentation of a short noise burst (500 ms) and followed by a time out during which a trial could not be initiated. This time out was initially very short (1 second) and was increased over several weeks to be approximately 10 seconds.

Once ferrets reached 70% correct in the basic discrimination task, i.e. 7 Hz *vs.* 23 Hz pulse-rates they were tested with variable pulse-rates of 7, 10, 13, 15, 17, 20 and 23 Hz. For the 15 Hz rate, the ferret was rewarded for whichever side it approached, since this was the middle of the extreme values learned during training. For stimuli less than 15 Hz, they were rewarded if they approached the spout associated with the slow pulse-rate and for stimuli greater than 15 Hz, they were rewarded if they approached the spout associated with the fast pulse-rate. 30% of all trials were the original trained stimuli.

5.2.4 Cryoloop Implantation

The cryoloop implant was a modified, miniaturised version of the cryoloop developed by Lomber & Payne (1999). For surgical procedures, see appendix 7.3. The cryoloop was constructed from 23 gauge stainless steel tubing, this was bent to form a loop shape approximately the size of primary auditory cortex (Figure 5.2 [a]). A micro-thermocouple, made from twisting together 30 AWG gauge (0.254 mm) PFA insulated copper and constantan wire (Omega Engineering Limited, Manchester, UK), was soldered to the base of the loop and secured with an epoxy adhesive. The thermocouple wire was soldered to a modified, female thermocouple connector (RS components Ltd, UK) and again secured with an epoxy adhesive. The loop was placed such that it was directly in contact with primary auditory cortex during surgery (Figure 5.2 [c]) and secured in place with dental acrylic (See Figure 5.2 [b & d]).

5.2.5 Cooling apparatus

The cooling apparatus was set up as in Figure 5.3 [a]. Ethanol from a reservoir was pumped around a tubing system by two FMI QV drive pumps (Fluid Metering, Inc., NY, USA) controlled by two V300 variable speed controllers (Fluid Metering, Inc., NY, USA). The reservoir was located outside of the chamber and the pumps next to it, FEP tubing (Adtech Polymer Engineering Ltd, UK) of inner diameter (i.d.) 1.1 mm drew ethanol from the reservoir into each pump. The pumps were connected to 0.8 mm i.d. FEP tubing approximately 2 m long which was in turn connected to 0.5 mm i.d. PTFE tubing (Adtech Polymer Engineering Ltd, UK) approximately 2 m long via a two-way connector (Diba Fluid Intelligence, Cambridge, UK). Approximately 1 m of this tubing is coiled up and placed inside a Dewar flask (Nalgene 4150-1000 Dewar Flask, NY, USA) containing a dry ice-100% ethanol mix. The tubing leaving the Dewar flask was insulated with silicon tubing and was designed to be as short as possible, as it was carrying the cooled ethanol to the cryoloop. This tubing descended into the chamber where it was connected to ~5 cm of 0.5 mm i.d. PTFE tubing via a two-way connector. The end of this short piece of tubing was pushed around the

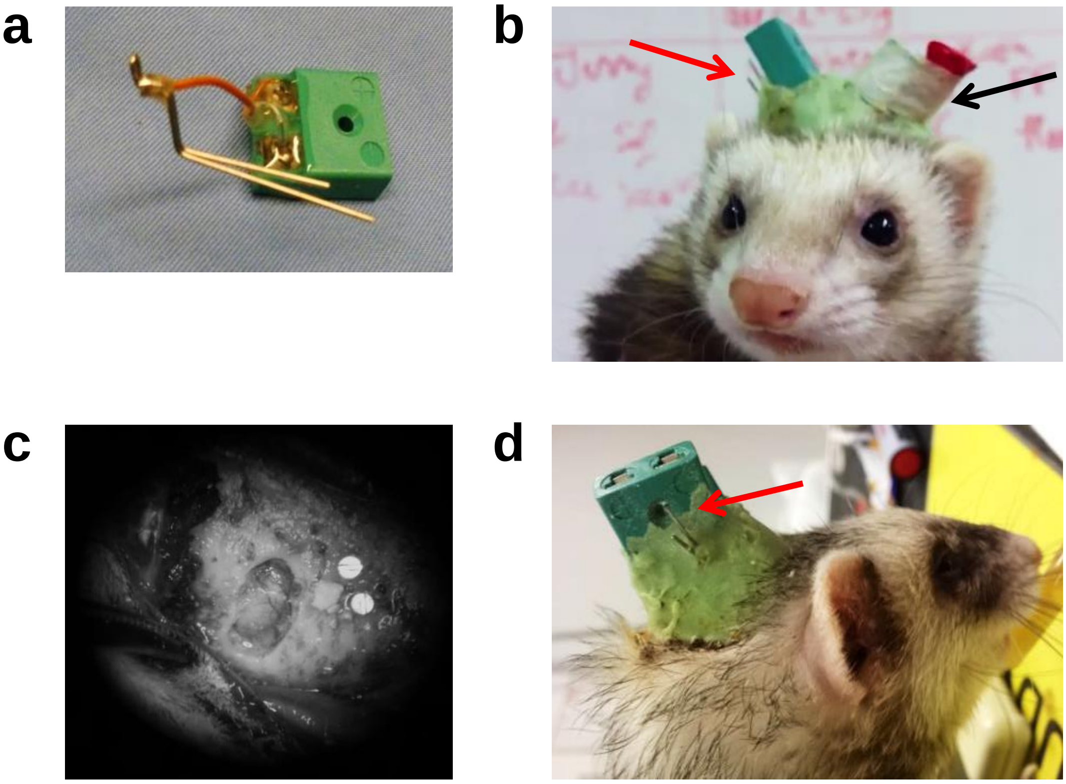


Figure 5.2 – Cryoloop implant. [a] shows the cooling loop implant, with cooling loop and temperature sensor attached to a green female thermocouple connector. A micro-thermocouple is attached to the base of the loop by solder and epoxy resin glue. [b and d] Red arrows indicate the ends of the cryoloops and the black arrow shows the protective cap covering the cryoloop. [c] shows an image of the craniotomy over auditory cortex.

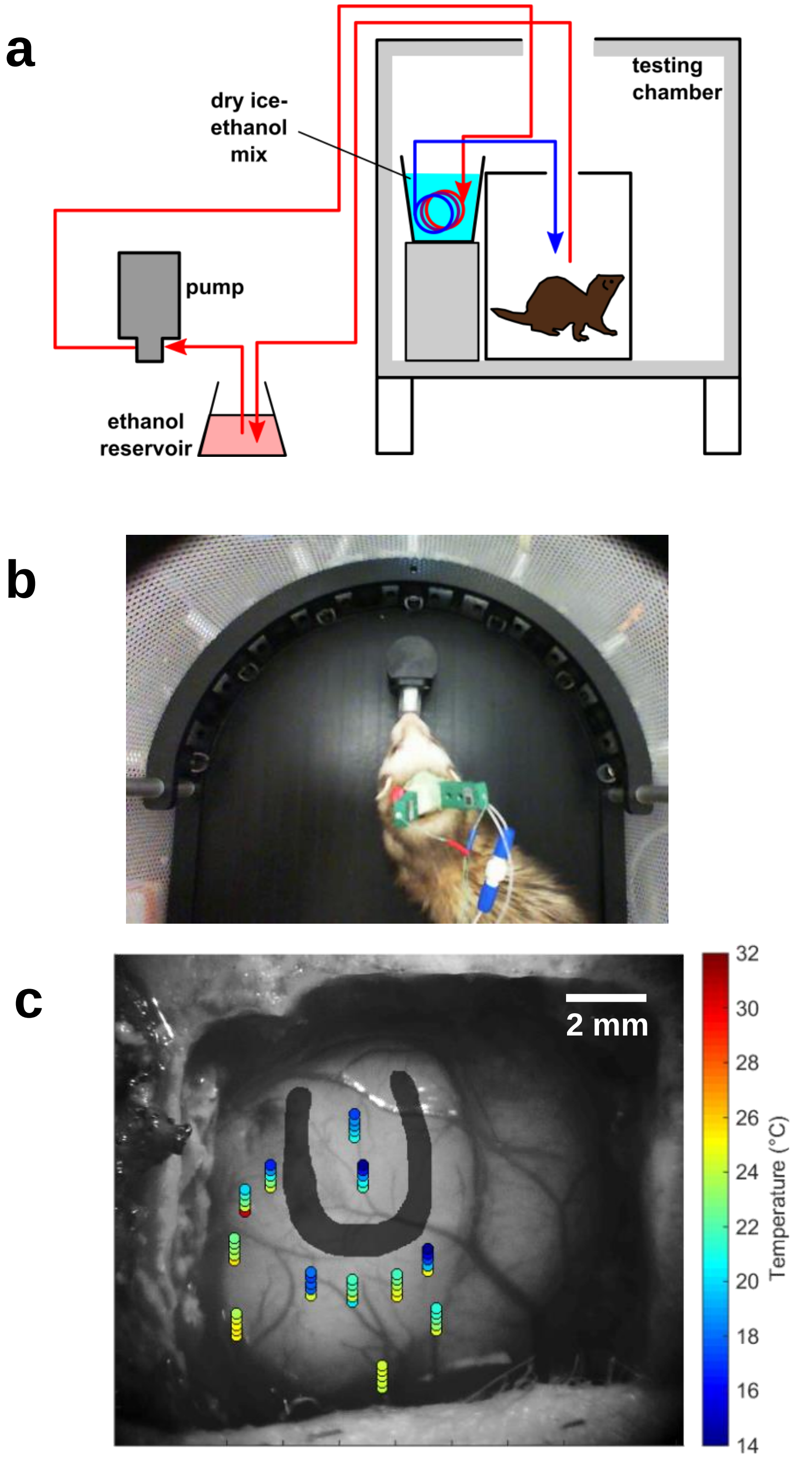


Figure 5.3 – Cooling set-up. [a] shows the set-up of the cooling. Ethanol is pumped around a tubing system, represented by blue and red lines, with arrows representing the direction of ethanol flow. Warm ethanol from the ethanol reservoir is pumped into the chamber where the tubing is coiled in an insulated container of dry ice and ethanol (-70°C). This cools the ethanol inside the tubing before proceeding to the loops on the ferrets' head. The ethanol is returned to the ethanol reservoir, in a closed system. [b] shows an image of a ferret performing the task while the right hemisphere is cooled and the temperature sensors connected bilaterally. [c] shows temperature measurements at different depths (500-2500 µm in 500 µm steps, coloured circles) from auditory cortex with a cooling loop (black shading) held at 10 °C.

outside of the cryoloop and fed ethanol flow through the cryoloop. A short piece of 0.5 mm i.d. PTFE tubing (approximately 20 cm) was pushed over the other end of the cryoloop and was connected to 0.8 mm i.d. PTFE tubing via a two-way connector which fed the ethanol back to the reservoir, thus completing the cooling circuit. The ends of the tubing that connected to the cryoloop were trimmed after each cooling session so that a fast seal could be obtained around the cryoloop. The tubing connected to an implanted cryoloop for unilateral cooling can be seen in Figure 5.3 [b].

5.2.6 Cooling during behavioural testing

Cooling sessions were performed in one of the testing sessions of days 2-5 of a 5-day long testing run. For a cooling session, the apparatus was set up as detailed above (see Figure 5.3 [a]) and the system was pre-cooled with spare cooling loops prior to testing a ferret. When the spare loops reached temperatures below zero, the system was thought of as ‘pre-cooled’. The ‘in’ and ‘out’ ends of the tubing were then attached to the cryoloop on the ferret’s implant. Male thermocouple connectors were attached to the female thermocouple connectors on the ferret’s implant and the temperature was monitored on a PC using a wireless transfer system (UWTC-1, Omega Engineering Ltd., Manchester, UK. See Figure 5.3 [b]). The speed of the pumps were adjusted so that the temperature of each loop was taken down to 7-10 °C, this temperature was maintained by manually adjusting the speed of the pumps during testing of the ferret. After initial coupling to the cooling system, the ferret was held for approximately 5 minutes while the temperature was reduced as necessary before being placed in the testing chamber. Testing proceeded as a normal training session. The other session of the same day was used as the warm control for that testing session, during this session, the cooling apparatus was attached to the ferret but no cooling was performed.

5.2.7 Statistical Analysis

For comparisons where there were two animals in the testing, paired Student’s *t*-test were used to see if there were any significant changes in performance between the warm and cooled conditions. Where multiple comparisons were made, Bonferroni correction was applied. When more than one type of comparison was important and there was an n value greater than two, RM ANOVAs were used to investigate if there were any significant effects on performance. *Post-hoc* testing was used to investigate significant effects (Bonferroni's multiple comparison test, $p < 0.05$).

5.2.8 Anaesthetised preparation

All animal procedures were approved by the local ethical review committee and performed under license from the UK Home Office in accordance with the Animal (Scientific Procedures) Act 1986. Two adult, female, pigmented ferrets (Mustela putorius) were used in this study. All animals received regular otoscopic examinations before the experiment, to ensure that both ears were clean and disease free. Anaesthesia was induced by a single dose of a mixture of medetomidine (Domitor; 0.022 mg/kg/h; Pfizer) and ketamine (Ketaset; 5 mg/kg/h; Fort Dodge Animal Health). The left radial vein was cannulated and anaesthesia was maintained by a continuous infusion of medetomidine (0.022 mg/kg/h), ketamine (5 mg/kg/h), atropine sulphate to reduce bronchial secretions (0.06 mg/kg/h, C-Vet veterinary products) and dexamethasone to reduce cerebral edema (0.5 mg/kg/hr, Dexadreson, Intervet UK) in Hartmann's solution, supplemented with 5% glucose, throughout the experiment. The ferret was intubated, placed on a ventilator (683 small animal ventilator; Harvard Apparatus) and ventilated with oxygen. Body temperature, end-tidal CO_2, and the electrocardiogram were monitored throughout the experiment. Experiments typically lasted between 36 and 60 h. The animal was placed in a stereotaxic frame and the temporal muscles on both sides were retracted to expose the dorsal and lateral parts of the skull. A metal bar was cemented and screwed into the right side of the skull, holding the head without further need of a stereotaxic frame. On the left side, the temporal muscle was largely removed, and the suprasylvian and pseudosylvian sulci were exposed by a craniotomy, exposing auditory cortex (Kelly et al., 1986). The dura was removed and the cortex covered with 3%

agar. The animal was then transferred to a small table in a soundproofed chamber.

5.2.9 Temperature measurement in auditory cortex and effects on neural activity

The temperature in auditory cortex during cooling was mapped in an anaesthetised ferret with a cooling loop positioned over primary auditory cortex and cooled to 10°C. Temperatures were measured at 5 depths in each location (500, 1000, 1500, 2000 and 2500 μm from the surface of the brain) using a hypodermic needle temperature probe (Omega, Stamford, USA) controlled by a micromanipulator (Harvard Apparatus, USA). Position of the cryoloop and temperatures can be seen in Figure 5.3 [c]. The temperature decrease caused by the cryoloop is rapidly attenuated as distance from the loop increases. Only at locations in the immediate vicinity of the loop does the temperature reach less than 20 °C which is required for cessation of neural firing (Lomber and Payne, 1999). At 2500 μm (auditory cortex in ferrets is typically 2000 μm thick), none of the locations tested were below the 20 °C threshold. The temperatures achieved in the chronically implanted ferret may differ from those achieved in this anaesthetised preparation for a number of different reasons; the brain is not exposed as in this acute preparation, the ferret is awake and thus blood flow to the area will probably be stronger and the surface of the loop is enclosed under the silicone and dental acrylic protection. Figure 5.4 [a] shows the temperatures in another anaesthetised preparation with the cooling loop positioned over a visual cortical area, SSY. Temperatures were measured at a depth of 1000 μm, it is clear that the decreased temperatures caused by cooling of the loop (in this case to 7.5 °C) have not spread across to different brain area (auditory cortex). It is evident from Figure 5.4 [b] that the evoked activity of units around the cooling loop has been dramatically decreased compared with before cooling. Figures 5.4 [c & d] illustrate the time course of two individual units during cooling, one unit from SSY where the evoked firing rate decreases as the temperature decreases and one unit from auditory cortex which is not affected by cooling. The analysis for this figure was done

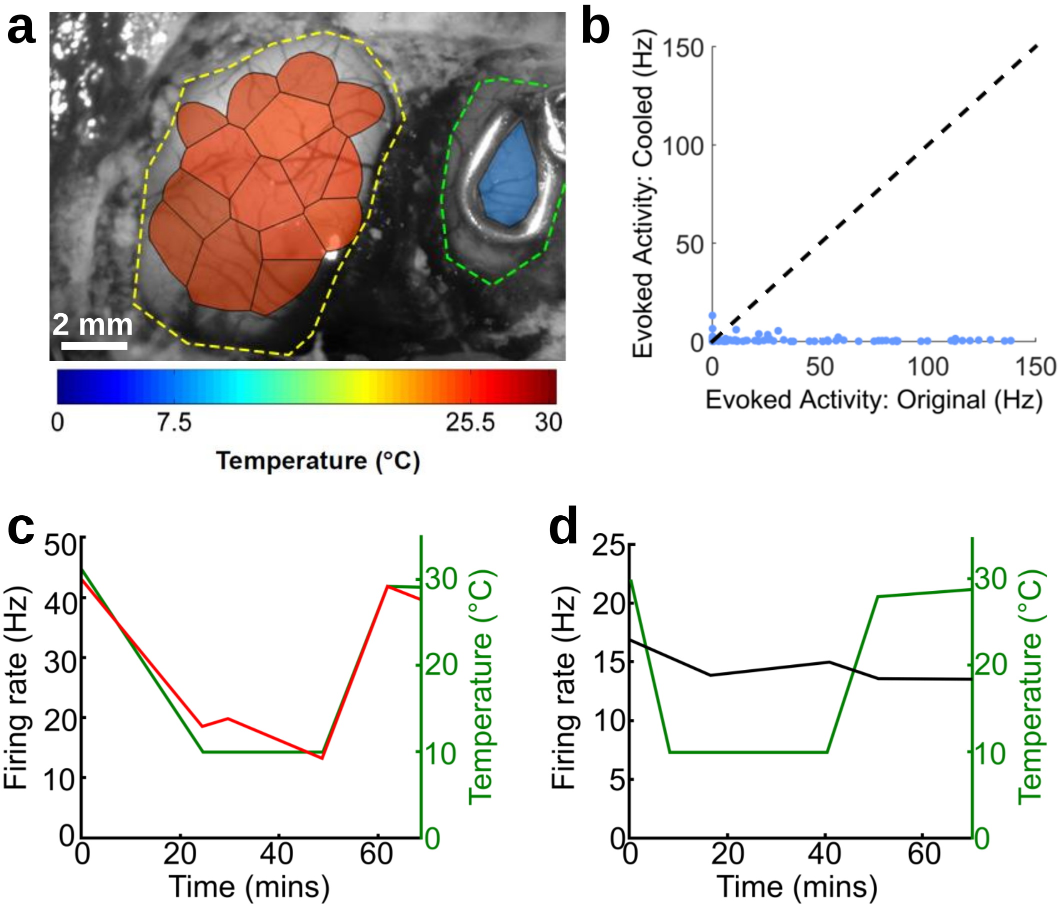


Figure 5.4 – Effect of cooling on neuronal activity. [a] shows temperature measurements from auditory cortex (yellow dashed outline) and SSY (green dashed outline) during cooling of SSY with the cryoloop held at 7.5 °C. Temperatures of underlying cortex are indicated by the tessellated coloured blocks (measured at 1000 µm depth). [b] shows evoked activity of units recorded from SSY in response to visual stimuli before and during cooling. The dashed black line indicates the line of equality. [c] Activity of a single MU in response to a white LED flash recorded from SSY during cooling (red line), the temperature is indicated in green. [d] shows evoked firing rate to a BBN burst of an individual MU recorded from auditory cortex during the course of cooling (black line). The green line indicates the temperature of the cryoloop.

by Dr. Stephen Town; the data were collected by Dr Town and me in collaboration (Town et al., 2013).

5.3 Results

Ferrets were trained to localise noise bursts of 100, 250 and 500 ms duration in the azimuthal plane, they were then tested on this localisation task during unilateral and bilateral inactivation by cooling of primary auditory cortex. As a control experiment, two ferrets were trained to localise visual stimuli (LEDs), this experiment controlled for any motor or motivational effects of cooling. A further experiment involved cooling in a pulse-rate discrimination task to investigate the effects of cooling primary auditory cortex in a non-spatial task, this also acted as a control for motivation and motor deficits that may have been caused by the cooling.

5.3.1 Effect of inactivation of primary auditory cortex on azimuthal sound localisation

Figure 5.5 illustrates the effect that cooling of A1 has on sound localisation performance. Cooling causes a modest decrease in behavioural performance contralateral to the cooled hemisphere (dotted lines show control performance, solid lines performance during cooling, Figure 5.5 [a & b]), or in both hemispheres in the case of bilateral cooling (Figure 5.5 [c]). To investigate the effects of cooling further, in an approach similar to that used by Malhotra and Lomber (2007), data were divided according to the side of space and hemisphere of inactivation.

Figure 5.6 shows the performance of each ferret in each side of space during unilateral cooling of the left or right auditory cortex and more clearly illustrates the contralateral performance deficit elicited by unilateral inactivation. A two-way RM ANOVA (independent variables: speaker side and cooling condition. Dependent variable: % correct) within each duration revealed a main effect of cooling condition for 100 ms sounds ($F_{(2,4)} = 15.72$, $p = 0.013$, n = 3), *post-hoc* pairwise comparisons (Bonferroni's multiple comparison test, $p < 0.05$) showed that the warm condition was different from left cooled. There was no effect of speaker side ($F_{(1,2)} = 0.001$, $p = 0.98$) and

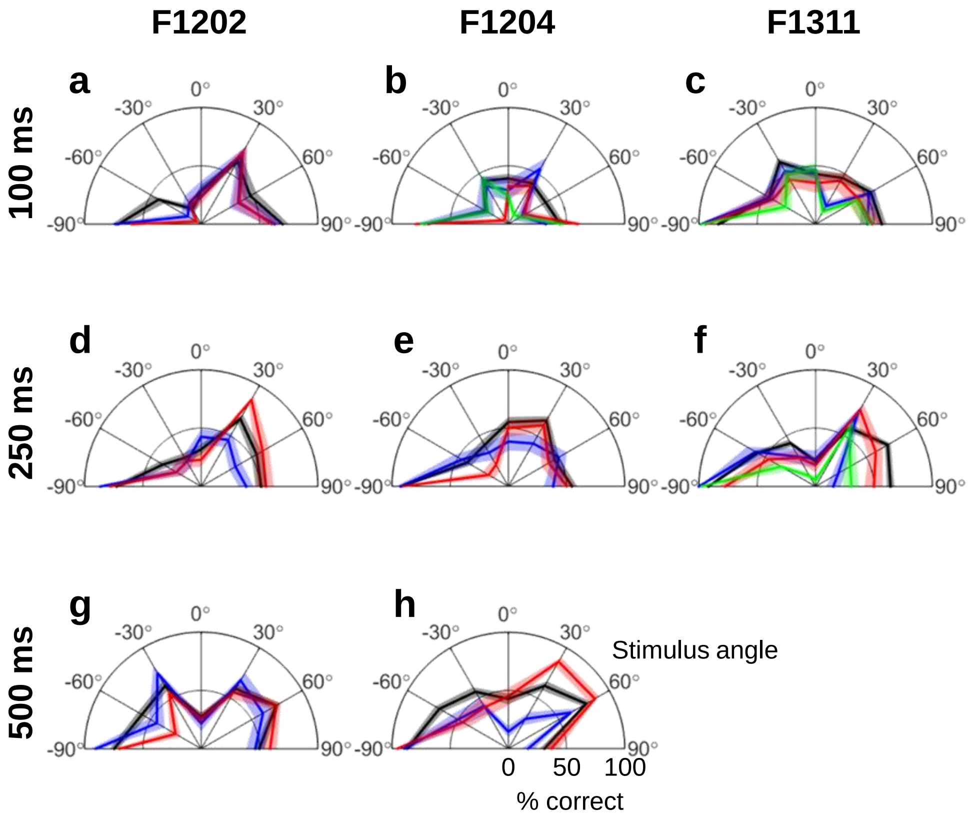


Figure 5.5 – Mean localisation performance at each location for each ferret. Performance varied between ferrets. The mean performance (± standard error of the mean) of each ferret (columns) is indicated for each sound duration (rows) at each location in the warm condition (black line), during cooling of the left primary auditory cortex (blue), right auditory cortex (red) and bilateral cooling (green). Each data point represents a mean of at least 23 trials in each condition. Ferrets identification is indicated above the top row. Negative angles indicate the left side of the testing arena.

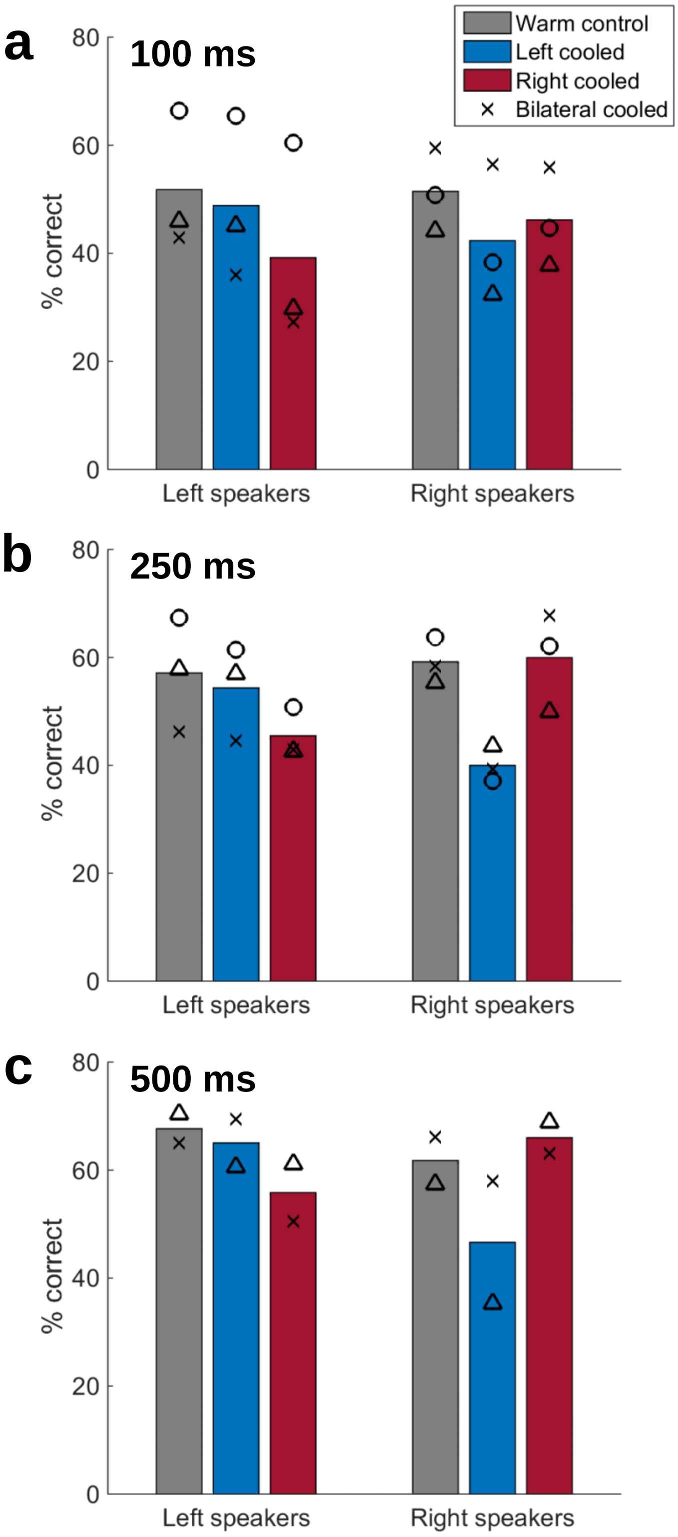


Figure 5.6 – Mean localisation performance split by the side of sound presentation and hemisphere cooled. Performance was averaged across speaker locations in left or right space (the central location was not included) [a] shows the mean performance in each hemisphere for the warm control (grey), when the left hemisphere was cooled (blue) and when the right hemisphere was cooled (red) for 100 ms duration sounds. The symbols indicate individual ferrets' performance. The same is shown for 250 ms [b] and 500 ms [c] duration sounds.

no interaction. An RM ANOVA in the 250 ms duration revealed no effects of speaker side ($F_{(1,2)} = 0.07$, $p = 0.81$) or cooling condition ($F_{(2,4)} = 3.22$, $p = 0.15$) however there was an interaction between cooling condition and speaker side ($F_{(2,4)} = 32.75$, $p = 0.003$) suggesting that the performance in each hemifield of space depends on which side A1 is cooled. In the 500 ms condition, none of the cooled conditions were different from the warm controls (paired Student's *t*-test, $p > 0.0125$, Bonferroni correction for multiple comparisons).

To investigate this interaction further, the data were combined according to whether the speaker locations were ipsilateral or contralateral to cooling (Figure 5.7). These data show that for sound durations of 100 ms and 250 ms, performance was significantly different during cooling (RM ANOVA with independent variable: warm, ipsilateral or contralateral cooling. Dependent variable: % correct. 100 ms: $F_{(2,10)} = 13.06$, $p = 0.0002$. 250 ms: $F_{(2,10)} = 12.13$, $p = 0.0002$) but not with 500 ms duration stimuli ($p = 0.148$, paired Student's *t*-test), possibly owing to the fact that there is only data from two ferrets at this duration. *Post-hoc* analysis (Bonferroni's multiple comparison test, $p < 0.05$) revealed that the side contralateral to cooling was significantly different to the warm condition for 100 ms and 250 ms stimuli.

Previous work has revealed that the midline location is not affected by unilateral inactivation of primary auditory cortex (Kavanagh and Kelly, 1987; Malhotra et al., 2008). Consistent with this, performance at the midline location in all durations was not affected by unilateral cooling (Figure 5.8, paired Student's *t*-tests, 100 ms: $p = 0.143$, 250 ms: $p = 0.329$, 500 ms: $p = 0.366$).

In order to assess further the changes in behaviour of the ferrets during unilateral cooling, the error magnitudes were calculated during unilateral cooling and compared with the error magnitude in warm control sessions (Figure 5.9). On incorrect trials, the ferrets tended to respond more towards the side being cooled.

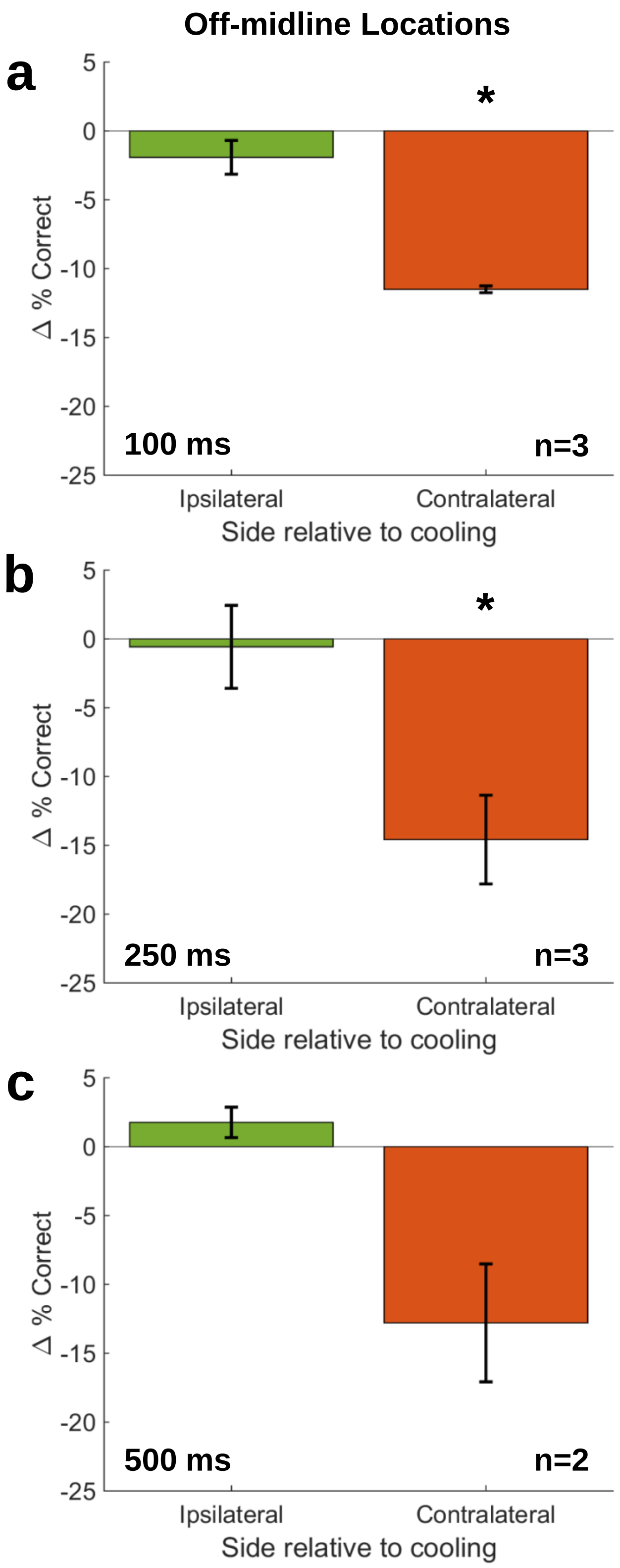


Figure 5.7 – Mean change in localisation performance by side relative to cooling. [a] shows the mean change in performance in the ipsilateral hemisphere (green) and the contralateral hemisphere (orange) relative to cooling for 100 ms sound durations. Mean performance change is shown for 250 ms [b] and 500 ms [c] sound durations. Error bars indicate the standard error of the mean. Black stars indicate that performance is significantly different to the warm control data across hemisphere (2-way repeated measures ANOVA, *post-hoc,* Bonferroni's multiple comparisons test $p < 0.05$. 500 ms: paired Student's T-test, p=0.1478).

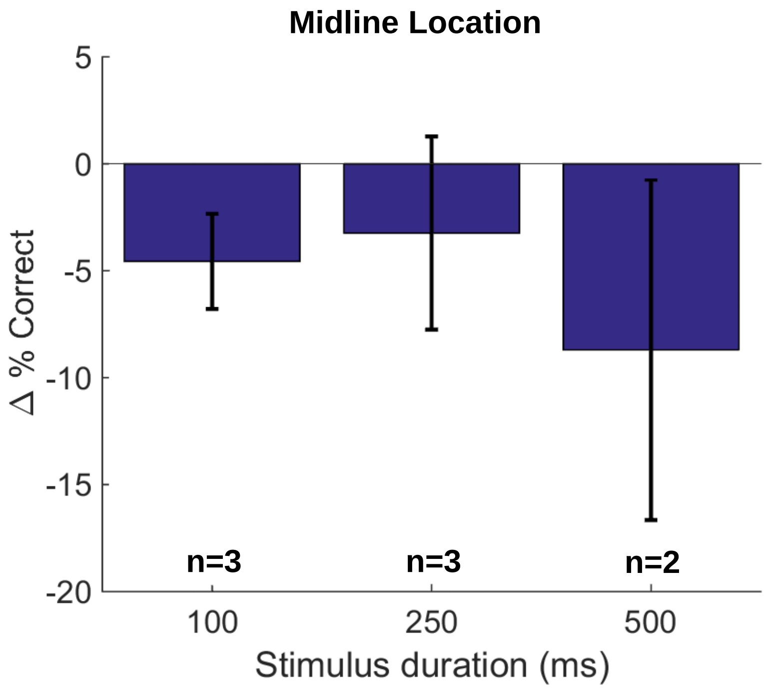


Figure 5.8 – Mean change in localisation performance at the midline location during unilateral cooling. Showing the mean change in performance at the midline during ipsilateral cooling, compared to the warm control data, for 100, 250 and 500 ms sound durations. Error bars indicate the standard error of the mean. Student's *t*-tests revealed no significant differences from the warm control in any of the stimulus durations ($p > 0.05$).

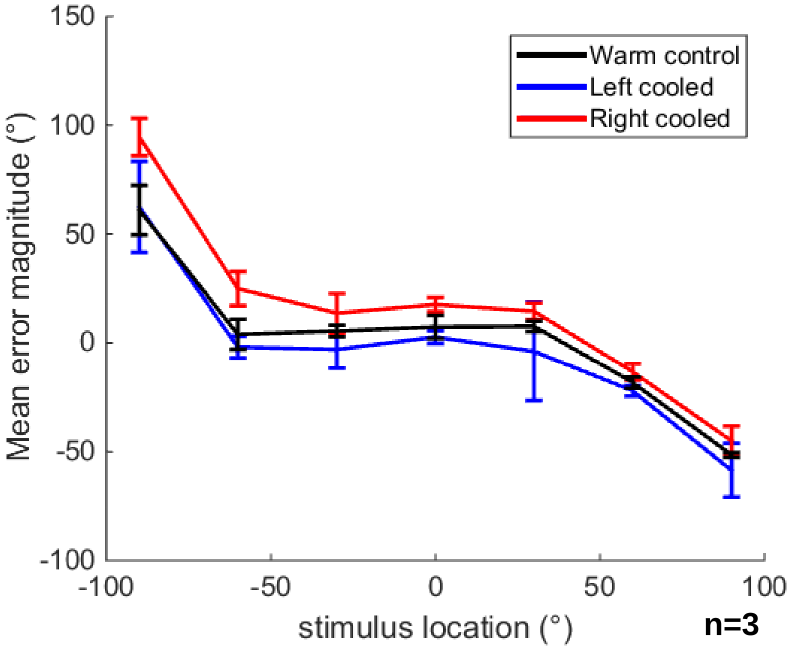


Figure 5.9 – Mean error magnitude by stimulus location during unilateral cooling. Errors made by the ferrets tended to be further toward side ipsilateral to cooling at all locations. Black line indicates the error magnitude in the warm control, the red line during unilateral cooling of the right and the blue line during unilateral cooling of the left. Error bars indicate the standard error of the mean. Mean across stimulus durations of 100 and 250 ms, where a significant ipsilateral vs. contralateral performance difference was observed. n=3.

The effect of bilateral cooling of primary auditory cortex has been shown to reduce performance in an azimuthal localisation task across all of frontal space (Malhotra et al., 2008). Consistent with this, performance overall was significantly reduced compared to warm controls (Figure 5.10) for sound durations of 100 ms (two animals, paired Student’s *t*-test, $p = 0.019$) and was reduced for one animal at 250 ms.

5.3.2 Effect of inactivation of primary auditory cortex on azimuthal light localisation

This experiment served as a control to indicate whether cooling is localised to A1 and that inactivation of A1 is not affecting the ability of the animal to approach a target but rather its ability to localise sounds, as it was reasoned that inactivation of primary auditory cortex should not affect the azimuthal localisation of visual stimuli (Lomber et al., 2010). Figure 5.11 [a] plots the change in performance for one ferret (F1311) tested with light and sound trials interleaved within the same testing sessions, where the duration of the light was adjusted such that the performance in light and sound localisation was matched (sound duration: 250 ms, light duration: 750 ms). Performance in the sound localisation task was greatly affected in contralateral space during unilateral cooling and across all space during bilateral cooling for one animal, while performance ipsilateral to cooling and at the midline during unilateral cooling were not greatly affected compared with the warm control. In contrast, performance in the visual localisation task contralateral to cooling and during bilateral cooling was barely affected. Performance at the midline and ipsilateral to unilateral cooling improved slightly.

Figure 5.11 [b] shows the change in performance for a different ferret (F1202) tested with sound and light localisation in separate testing sessions. The sound duration was selected to be matched in difficulty to the visual localisation (both 500 ms). During visual localisation, the ferret also heard a sound from above simultaneously presented with the light, which offered no localisation cue to the ferret. Performance in the auditory localisation was greatly reduced in contralateral space during unilateral cooling whereas ipsilateral to cooling and the midline location, performance was reduced only

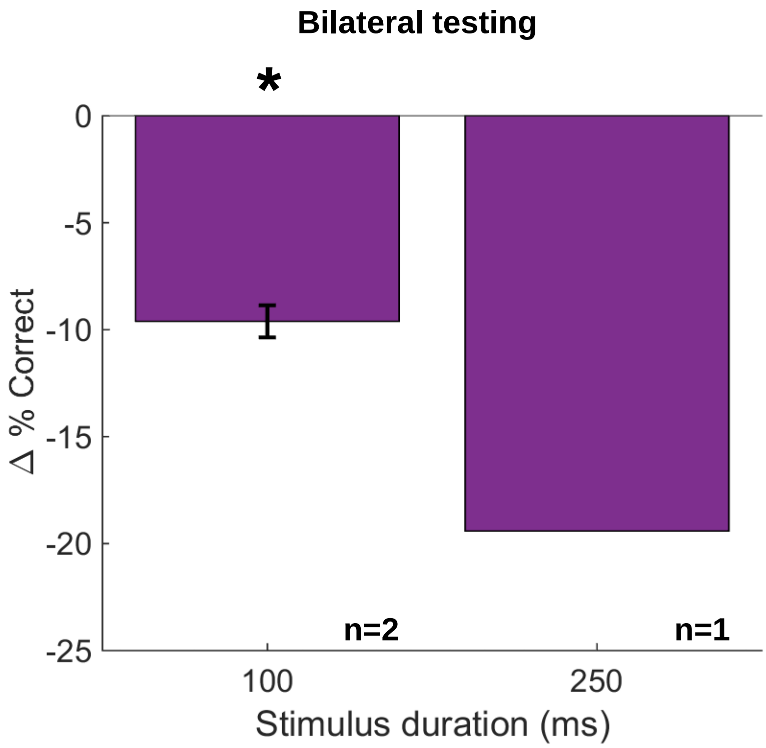
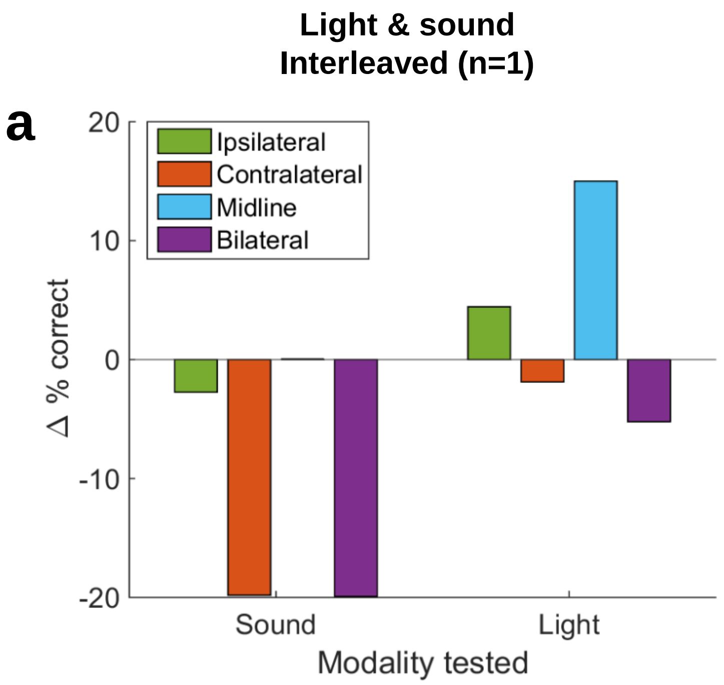


Figure 5.10 – Mean change in localisation performance during bilateral cooling. The mean change in performance during bilateral cooling from the warm control data, for different sound durations. Error bars indicate the standard error of the mean. Black star indicates that performance is significantly different to the warm control performance ($p < 0.05$, 100 ms: paired Student's t-test)



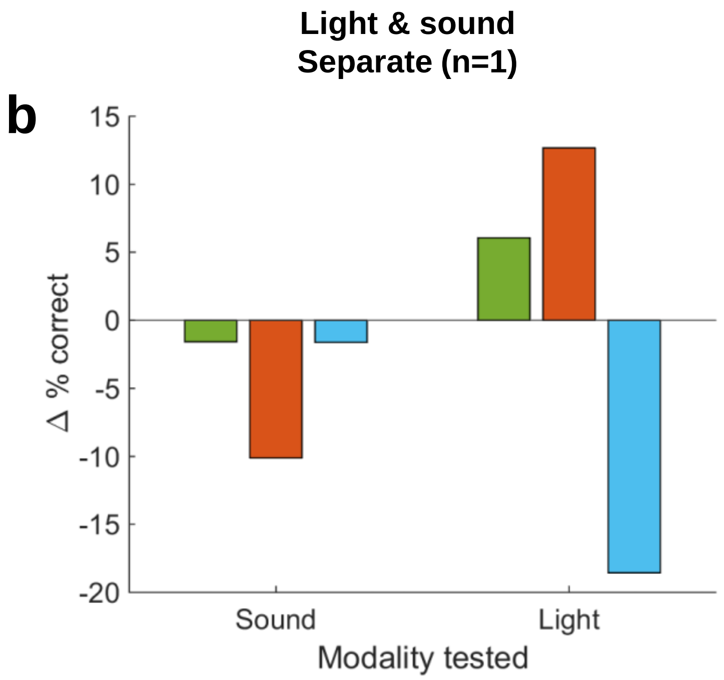


Figure 5.11 – Mean change in localisation performance for control light stimuli with matched performance sound stimuli. [a] shows the mean change in performance from the warm control during ipsilateral cooling (green), contralateral cooling (orange), at the midline location during unilateral cooling (light blue) and bilateral cooling (purple) for sound and light stimuli. Sound and light stimuli for this ferret (F1311) were interleaved. Sound stimuli were 250 ms and light stimuli were 750 ms in duration. Black stars indicate that performance is significantly different to the warm control data. [b] shows the mean change in performance from the warm control during ipsilateral cooling (green), contralateral cooling (orange) and at the midline location during unilateral cooling (light blue) for sound and light stimuli for a different ferret (F1202). Sound and light stimuli for this ferret were presented in separate testing sessions. Sound and light stimuli were 500 ms.

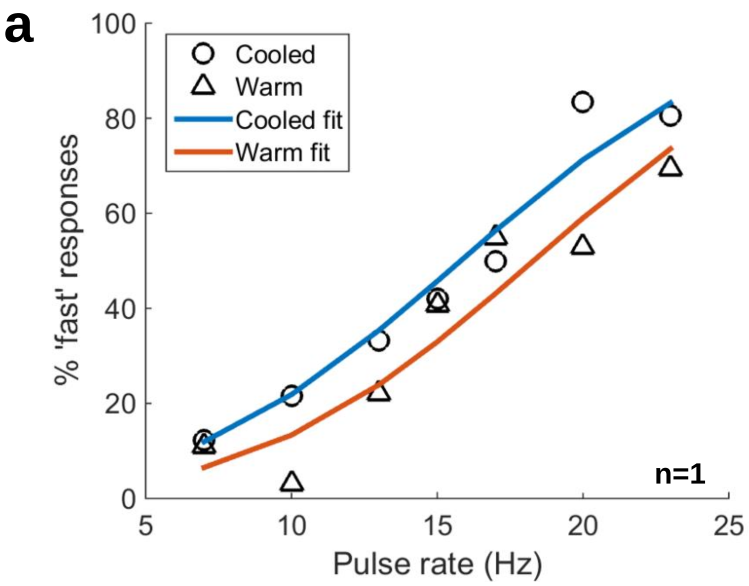
slightly. In contrast, performance in the visual localisation was improved in the ipsilateral and contralateral locations and decreased at the midline.

5.3.3 Effect of inactivation of primary auditory cortex on pulse-rate discrimination

The effects of inactivation of primary auditory cortex have been investigated in a non-localisation task involving discrimination of pulse-rate. It was unknown whether cooling A1 would have an effect during the pulse-rate discrimination task, which would require some form of temporal integration to perform. Previous work has shown that inactivation of a secondary area, AAF, in cats caused a deficit in an auditory temporal pattern discrimination task, where temporal integration is required to perform the task, whereas it did not cause a deficit in an auditory azimuthal localisation task (Lomber and Malhotra, 2008), the effects of inactivating A1 however are unknown. Figure 5.12 [a] shows data from one ferret (F1202) performing the pulse-rate discrimination task at variable pulse rates in the warm control condition and during unilateral cooling of A1 (right hemisphere). The data are fit by binomial logistic regression and the fit of the data was significantly improved by the inclusion of a predictor term indicating cooling or not cooling (analysis of deviance, X^2 distribution, $p = 0.0075$). This ferret was trained with the contingency of responding to the right when the pulse-rate was fast and left when it was slow. Further analysis of the data in the cooled and warm conditions showed that the maximum gradients in the fits were very similar (cooled: 5.25 % pulse-rate^{-1}, warm: 5.21 % pulse-rate^{-1}), however the midpoint of responses (i.e. 50% point) decreased by 2.5 Hz (from 18.3 Hz to 15.8 Hz), indicating that sensitivity to the pulse-rate was not affected but the ferret became more inclined to respond to the right, in this case the side that was cooled, this had the effect of making the ferret less biased overall since she already had a left bias (as indicated by a relatively high midpoint of 18.3 Hz).

Figure 5.12 [b] shows data from a different ferret (F1303) performing the pulse-rate discrimination task at variable pulse rates in the warm control and during bilateral cooling of A1. The data are fit by binomial logistic regression

Unilateral cooling



Bilateral cooling

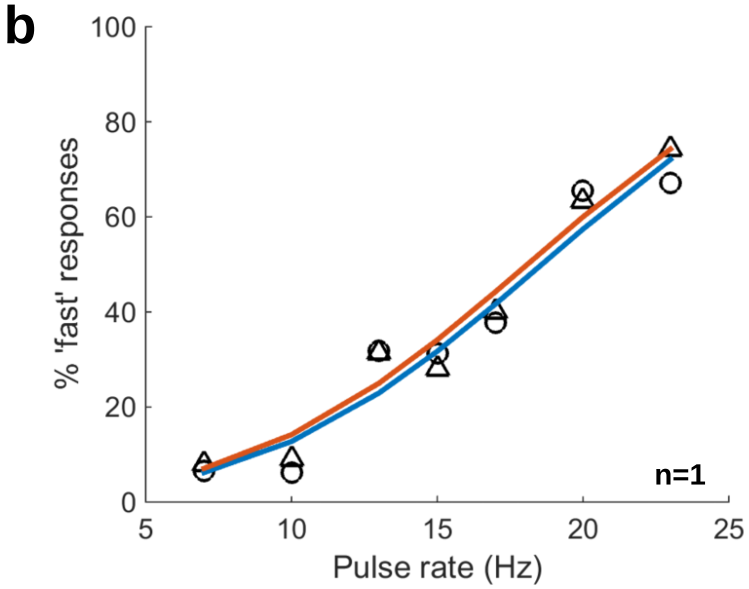


Figure 5.12 – Pulse-rate discrimination performance during cooling. [a] shows the percentage of responses that were ‘fast’ during unilateral cooling (circles) and in the warm control (triangles). The data are fit with a binomial logistic regression (blue – cooled data, red – warm data). Ferret was F1202, right cooled, contingency was to respond right for fast stimuli. The fit of the data was significantly improved by the inclusion of a term taking account of whether there was cooling or not (*p* = 0.0075, analysis of deviance, X^2 distribution) [b] shows the percentage of responses that were ‘fast’ during bilateral cooling. Ferret was F1303, contingency was to respond right for fast stimuli. The fit of the data were not significantly improved by the additional predictor term of cooling or not cooling (*p* = 0.5329, analysis of deviance, X^2 distribution).

and the fit of the data was not improved by the inclusion of a predictor term of cooling or not cooling (analysis of deviance, X^2 distribution, $p = 0.5329$), indicating cooling did not significantly affect performance. Again, this ferret was trained with the contingency of responding to the right when the pulse-rate was fast and left when it was slow.

5.4 Discussion

The data in this chapter demonstrate that cooling is a viable technique for reversibly inactivating auditory cortex in the behaving ferret. Expected performance deficits in sound localisation were observed and data from two control experiments measuring effect of cooling on performance in a visual localisation task and a non-spatial pulse-rate discrimination task indicated no effects caused by motivation or motor deficits. Together with experiments determining the spread of cooling and the consequences of cooling on neuronal activity, these experiments demonstrate that cortical cooling is a robust method of reversibly silencing neuronal activity in the ferret during behaviour over long timescales (>1 year).

Cooling of auditory cortex in a sound localisation task demonstrated that performance of the ferrets is impaired in the hemifield contralateral to cooling for stimulus durations of 100 and 250 ms, when compared with warm controls. While there was a trend at 500 ms for a deficit in contralateral space, results were not significant, possibly because only two animals took part at this sound duration. This is consistent with previous work in cats which also show contralateral localisation deficit during cooling of A1 (Malhotra et al., 2008). The deficit strongly supports successful application of cooling for cortical inactivation in the chronically implanted ferrets. The deficit caused by inactivation of primary auditory cortex is consistent with other forms of inactivation in the ferret; unilateral ablation of primary auditory cortex of the ferret caused a profound contralateral localisation deficit (Kavanagh and Kelly, 1987), and bilateral ablation and bilateral inactivation of primary auditory cortex by pharmacological means causes a deficit in localisation ability across space (Kavanagh and Kelly, 1987; Smith et al., 2004; Nodal et al., 2010, 2012). Unlike in cats where it has been shown that certain parts of primary and secondary auditory cortex contribute to sound localisation ability, namely A1, PAF, AES and DZ (see Figure 1.5 for anatomy) (Malhotra et al., 2004, 2008), both the major secondary areas of auditory cortex in the ferret appear to contribute to sound localisation ability (Nodal et al., 2012).

The deficits caused by cooling in the present study appear relatively modest when compared directly with those seen in A1 inactivation in the cat (Malhotra and Lomber, 2007; Malhotra et al., 2008). This may be in part owing to the design of the behavioural experiments. In the cat localisation studies, the cats were given an alternative option to localising the stimuli during testing; they could receive a 'lower value' reward for approaching the 0° position when the acoustic stimulus could not be localized, in the present study, ferrets were forced to guess the location of the stimulus even if they were not sure, of course in some trials they will guess correctly and they also respond to some locations more frequently than others. Deficits in other ferret inactivation, by lesion or tonic application of muscimol, studies of sound localisation are also relatively modest (Smith et al., 2004; Nodal et al., 2012) compared with the deficits observed in cooling of cat A1 (Malhotra and Lomber, 2007; Malhotra et al., 2008).

Although the change in the error magnitude was small, the direction of the change between the unilateral cooling and the warm control was consistent, the ferrets tended to respond more towards the side ipsilateral to cooling, i.e. towards the side where performance is maintained during unilateral cooling. One ferret was also tested unilaterally in the pulse-rate discrimination task and it was found that the bias of the ferret to respond to the left was reduced during cooling of the right A1, indicating that the ferret responded more toward the side that was cooled. Thus, these changes in error magnitude and direction could be an effect of the cooling rather than any perceptual effect related to the encoding of space in A1.

As discussed in the introduction to this chapter, cooling has many advantages over other methods of inactivation: Unlike ablation, it is reversible. Unlike pharmacological methods (e.g. implantation of musicmolloaded Elvax; Smith et al., 2004), the implant is long term, cryoloops were viable for longer than a year and the majority were still working at the time of culling. Because cooling and thus inactivation is acute there is no time for the ferret to adapt to the inactivation. With pharmacological methods, where the brain area is inactive continuously for up to 4 weeks or until implant removal, ferrets showed adaptation to the inactivation and performance in a localisation task improved over time (Smith et al., 2004).

The cooling loops were cooled to between 7 and 10 °C during testing, according to previous work in anaesthetised guinea pigs, cooling the surface of the cortex to 2 °C was sufficient to reduce the temperature at 2 mm cortical depth to below 20 °C when the temperature probe was placed in the centre of the cryoloop. In an anaesthetised ferret (Figure 5.3 [c]), when the temperature of the cryoloop was held at 10 °C, the temperature achieved at 2 mm depth in the centre of the loop was 21.1 °C. Lomber and Payne (1994; 1999) found that at a temperature of 20 °C in the awake-behaving cat neuronal activity was severely restricted, a finding corroborated by work on the anaesthetised guinea pig (Coomber et al., 2011). However in slices of rat brain temperatures below 10 °C were needed for neuronal inactivation, although even strong stimulation could still evoke spiking at this temperature (Volgushev et al., 2000). Thus there appears to be a difference between the temperatures needed to inactivate neural activity *in vivo* and *in vitro*, possibly because, *in vitro*, neurons may be more excitable in general if they have lost inhibitory inputs from other brain regions. More generally, the environment of the neurons in a brain slice will not be the same as *in vivo* and this can change the excitability of neurons in the slice. The temperature achieved in a chronically implanted behaving animal is likely to be somewhat different to that achieved in an anaesthetised animal with an open craniotomy. In the present study, it is likely that the activity in the majority of the layers of cortex was significantly attenuated. Selecting a temperature at which to hold the loop is necessarily a balance between successfully inactivating the whole cortical depth (1.5-2mm in ferrets) versus restricting the spread of cooling to under and within the area covered by the cooling loop. Figures 5.3 [c] and 5.4 [a] demonstrate that the spread of cooling in the anaesthetised ferret is minimal, and neuronal activity in units recorded from the middle of the cryoloop was attenuated whereas activity of those in another brain area was not.

Localisation deficits during inactivation of A1 are smaller than when larger areas of auditory cortex are inactivated (Nodal et al. 2010) and inactivation of only secondary areas causes a smaller deficit in sound localisation than inactivation of A1 alone (Nodal et al. 2012), suggesting that secondary areas can compensate for the loss of primary auditory cortex to a certain extent. The extent of the cooling in the present study has not been quantified in this thesis. However, an attempt has been made to measure the extent of the inactivation through c-Fos protein staining. Prior to culling, two of the animals (F1202 and F1204) were sedated and placed in a sound-proof booth, here noise bursts varying in virtual spatial location (Mrsic-Flogel et al., 2005) and intensity throughout frontal 180° of space were presented to the ferrets for 40 minutes while one of the cryoloops was cooled to 10 °C. After culling and fixing of the brain, 40 µm sections were stained for c-Fos protein, the product of the gene *c-fos*. Neurons that are activated by an external stimulus have been shown to modulate the expression of 'immediate early genes', of which *c-fos* is one and can be used as a marker for metabolic activity provided the stimulus is of 'interest' (Dragunow and Faull, 1989). Further work is required to ascertain whether c-Fos expression is lower in the cooled cortex than the warm cortex and whether this difference is sufficient to determine the area inactivated. Sections from these two animals and one other (F1311) were also stained for Nissl and SMI-32 immuno-reactivity, which facilitate delineation of primary and secondary areas, in order to locate the exact positions of the cryoloops; again this work is on-going. Images of the brains of all 4 animals in this study were obtained after removal from the animal. In most of the animals, an imprint of the location of the loop was visible on the brain (Figure 5.13) indicating the gross location of the loops. The histology will be required to determine exact locations of primary and secondary cortical areas. It was not possible to perform any c-Fos immuno-reactivity on animal F1303 as she had to be prematurely euthanased due to a sudden illness unrelated to the experiments in this thesis.

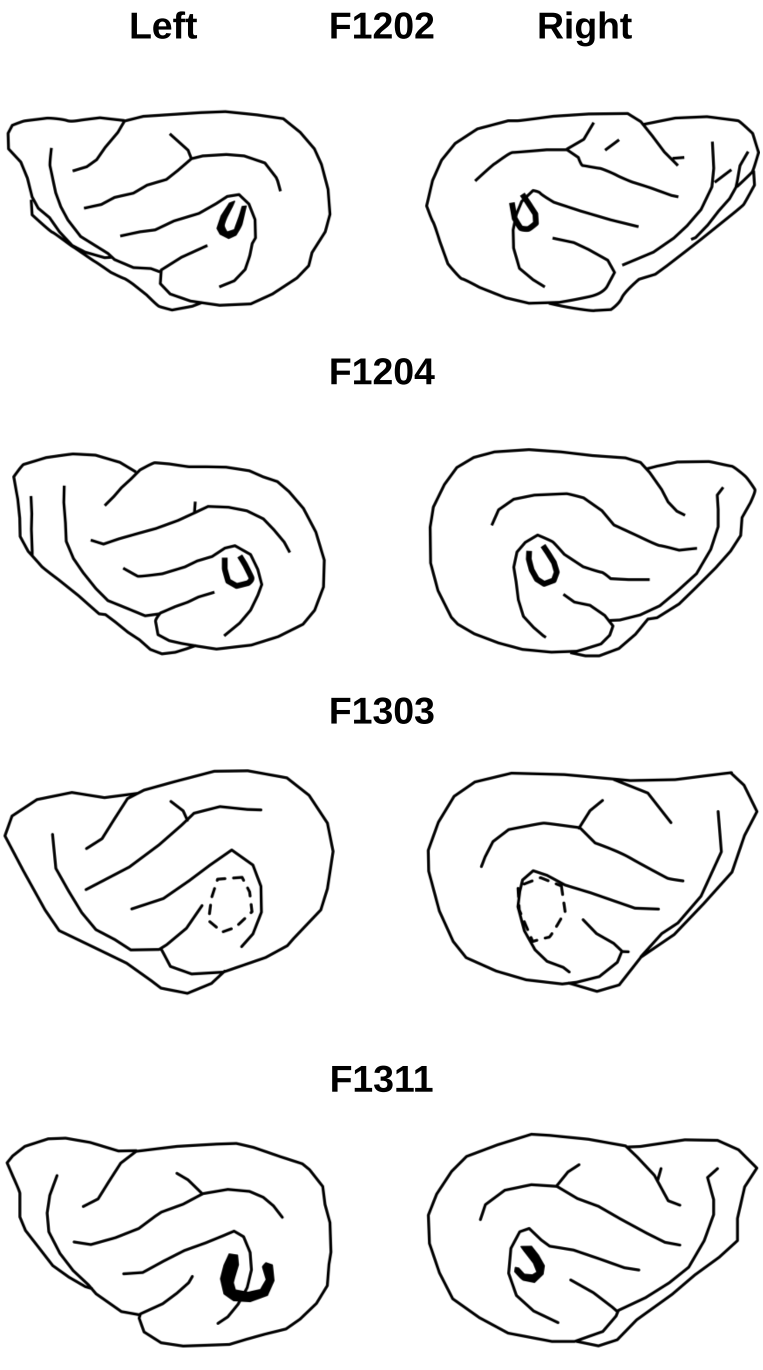


Figure 5.13 – Putative cooling loop locations. Putative cooling loop locations for each ferret. The left column shows the left hemisphere and the right column the right hemisphere. Location of the loop's contact with the brain is indicated by thick black lines on the outline of the brain. Where no imprint of the loop was visible, a dotted line was drawn around the putative area of contact. Location of the loops will be confirmed later with histology.

As well as localising sounds, two ferrets were trained to localise light sources at the same azimuthal locations as the speakers as a control for possible motivation or motor deficits caused by cooling. One ferret (F1202) was trained and tested on light and sound separately and showed no significant performance deficit with unilateral cooling on the right. Unfortunately it was not possible to test her performance bilaterally or unilaterally cooling the left side due to a permanent blockage in the left cryoloop that occurred ~14 months after implantation. The second ferret (F1311) who was trained and tested with sound and light trials interleaved showed no significant changes in performance in the visual localisation. Despite limited testing in the visual localisation task, data from two ferrets with unilateral inactivation of primary auditory cortex showed no deficit in contralateral space and one ferret with bilateral inactivation showed no deficit in visual localisation indicating that the deficit in sound localisation was not simply caused by a decrease in motivation or some form of motor deficit.

Unilateral inactivation of primary auditory cortex during a pulse-rate discrimination task did not change performance but shifted the psychometric function because of a small increase in right bias in a pulse-rate discrimination task in one ferret (F1202); this ferret also took part in the sound localisation and light localisation studies. She was trained to respond to the right response spout when she heard a fast pulse-rate of 23 Hz and to respond to the left spout when she heard a slow pulse-rate of 7 Hz. During testing, varying pulse-rates between these two extremes were presented in order to test the sensitivity of the ferret to the pulse-rates (rates < 15 Hz were rewarded for left responses, >15 Hz were rewarded for right response and rates = 15 Hz were rewarded for responses on either side). Only the right side of auditory cortex was inactivated since at this point the animal had only one functioning cryoloop. The inactivation served to shift the bias of the ferret towards responding right, since the ferret was slightly biased to the left already, this served to make the ferret less biased overall. Another ferret (F1303) tested on the same stimuli with the same contingency showed no change in performance during bilateral inactivation of primary auditory cortex.

Work in the cat has shown that in a 2AFC pattern discrimination task, requiring temporal integration of stimuli much like the pulse-rate discrimination task, in which cats had to report whether a pattern of morse-code like noise bursts was the same or different to preceding presentations of the stimulus, bilateral inactivation of AAF, a core cortical area, reduced performance in this task to chance levels (Lomber and Malhotra, 2008). Whilst it is unknown whether inactivation of A1 would cause a deficit in this same task, the present study suggests not. Although not pattern discrimination, the pulse-rate discrimination task would require some form of temporal integration in order to judge the rate of the pulses presented much like in the pattern task. One way to test this would be to inactivate anterior parts of secondary auditory cortex (e.g. AEG or AAF) in the ferret during the pulse-rate discrimination task.

The same study mentioned above (Lomber and Malhotra, 2008) also inactivated a posterior secondary area in the cat (PAF), bilateral inactivation of this area caused a deficit in a sound localisation task but not in the pattern discrimination task, whereas inactivation of an anterior secondary area (AAF) caused a deficit in pattern discrimination but not localisation. In contrast, inactivation by pharmacological methods (application of $GABA_A$ agonist muscimol) of the anterior secondary auditory cortex (AEG) or the posterior secondary areas (PEG) in the ferret both caused deficits in sound localisation. It has been shown in ferrets that the greatest sensitivity to azimuthal location are found in A1 and ADF (Bizley and King, 2008; Bizley et al., 2009; Walker et al., 2011), whereas in the cat, posterior areas and area DZ have been shown to have the greater spatial sensitivity (Stecker et al., 2003, 2005a; Harrington et al., 2008). Thus the organisation of ferret auditory cortex and/or the hierarchical processing may be different to that in cats.

One of the major shortcomings of the results in the pulse-rate discrimination task is the limited testing that was completed. I decided to present the data here since it provides some evidence that cooling of primary auditory cortex may not necessarily affect all aspects of auditory discrimination in ferrets. The data collected were limited by a set of uncontrollable circumstances whereby one ferret taking part in the study (F1303) became ill during the study and had to be culled before she could complete the testing in the pulse-rate discrimination task and the localisation task. Another ferret (F1202), by the time she was preforming the pulse-rate discrimination task, the localisation study completed, only had one functioning cryoloop. Another ferret (F1204) was unable to learn the localisation of the visual stimuli after training on auditory localisation and had to be culled (due to time limits imposed under our Home Office Licence) before data was able to be collected in the pulse-rate discrimination task. The final ferret (F1311) learned the localisation task with visual stimuli interleaved but was previously trained in a vowel-discrimination task and had to be culled before pulse-rate discrimination could be trained.

During the process of developing the cooling technique for use in the behaving ferret, several improvements were made to the initial methods adopted: In one ferret (not involved in this project) the thermocouple wire had broken, possibly during implantation, and thus we found that epoxying the thermocouple wire both at the connection with the loop and at the thermocouple connector strengthened the connections solving this potential problem. Leaving the cryoloop ends relatively long allows trimming of the ends if blockages occur. The angle of the loops in implantations was adjusted so that the two ends were not too close together or too close to the thermocouple connector, allowing greater access to get the tubing over the ends of the loops. Smaller bits of tubing that connected directly to the implanted cryoloop were used as it was soon discovered that the tubing had to be trimmed after each use in order to maintain a good seal around the cryoloop. This meant that the tubing of the main cooling system remained intact and we could easily replace the tubing that went directly to the implant if it became damaged, blocked or too short. Further refinements that could be introduced are some form of measure to stop the loops being knocked accidentally during surgery – see Figure 5.13, in F1311 half the loop appears to be positioned over a visual cortical area although histology will be required to confirm the positioning of the loops. Neural recordings from auditory cortex could be made prior to implantation to ensure the correct area was targeted, however this approach may cause more problems than it would solve; damage to the target area could make findings ambiguous, could cause greater inflammatory response and more dura regrowth that could limit the effectiveness of the cooling or even spread the cooling further than the target area.

In summary, this chapter has presented work that supports the successful application of inactivation of auditory cortex by cooling. Consistent with previous work in ferrets (Kavanagh and Kelly, 1987; Smith et al., 2004; Nodal et al., 2010, 2012) and other animals, including primates and carnivores (Heffner, 1978; Jenkins and Masterton, 1982; Thompson and Cortez, 1983; Heffner and Heffner, 1990; Malhotra et al., 2004), unilateral inactivation of primary auditory cortex resulted in a contralateral localisation deficit and bilateral inactivation resulted in a deficit across space. The localisation of visual stimuli at the same azimuthal locations as the sounds during cooling acted as a control to demonstrate that motivation and motor coordination were not affected by the cooling. Unilateral inactivation of primary auditory cortex during a pulse-rate discrimination task did not cause a deficit in discrimination whilst it did cause an increase in bias towards the cooled side in one ferret (F1202); suggesting primary auditory cortex in the ferret is not essential for a temporal integration task but plays a major role in approach-to-target localisation. The replication of localisation deficits in a behaving animal by cortical inactivation through cooling is an important finding; it establishes cooling as a viable technique in the ferret thus allowing potential further work using the method for inactivation. Indeed, further cooling experiments in our laboratory have found preliminary evidence for a dissociation of sound localisation and spatial unmasking, a phenomenon also observed in human lesion patients (Thiran and Clarke, 2003; Duffour-Nikolov et al., 2012).

Chapter 6: General discussion

6.1 Context and conclusions

The aim of this thesis was to investigate the coding of auditory space in auditory cortex. Two major models and variations on these have been proposed for encoding of sound location; the hemispheric two-channel model, where the location of a sound in space is given by the relative firing rates of two broadly, contralaterally tuned populations of neurons with peak firing rates at ±90°, with one channel in each hemisphere (McAlpine et al., 2001). A modification of this model, the opponent two-channel model was proposed after observing neurons in the anaesthetised cat auditory cortex that were tuned to contralateral and ipsilateral space (Stecker et al., 2005b), here instead of the two channels represented by each hemisphere, the channels are represented by ipsilateral and contralaterally tuned units distributed across each hemisphere. The labelled-line model is a variation of the topographic model (Jeffress, 1948) where the location of a sound in space is given by maximal firing in a particular group of neurons that represents that location in space (Day and Delgutte, 2013) without organisation of the group of neurons into an isomorphic map of auditory space as predicted by the topographic model. In a modified version of this model, the midline is overrepresented and more units are tuned to the midline area than the periphery.

The models create different predictions about what should be observed in the spatial receptive fields (SRF) of neurons recorded from auditory cortex and the distribution of these SRFs. The hemispheric two-channel model predicts that all the units will be broadly tuned to 90° in contralateral auditory space, while the opponent two-channel model predicts that there will be units within each hemisphere broadly tuned to 90° in ipsilateral and contralateral space. The labelled line model predicts that units that there will be sharply tuned units distributed throughout space, the distribution across hemispheres would be contralateral tuning based on observed contralateral deficits when auditory cortex is lesioned or inactivated (see Chapter 5 and Kavanagh and

Kelly, 1987; Malhotra et al., 2008). The modified version of the labelled line again predicts sharply tuned units but with more tuned to contralateral space.

To investigate these predictions, ferrets were trained in a relative localisation task, where they had to report the relative location of a target sound relative to a reference sound. Once trained, they were implanted with arrays of electrodes in primary auditory cortex and recordings of neural activity were made while the ferret performed the relative localisation task. The task was developed first with psychophysical testing in human subjects in order to characterise results and provide a measure to relate the ferret work back to human performance. Predictions made by the hemispheric two-channel model and the modified labelled-line model could not be distinguished and they both predicted that performance in the task would be best around the midline and worse in the periphery with no difference for inward and outward-moving stimuli. The labelled-line model predicted again no difference for inward or outward-moving stimuli but that performance would be equal across space. Results from testing with broadband stimuli and narrow-band stimuli, where ILDs were the dominant localisation cue, were inconsistent with all the predictions made by the models. However, results with low-pass stimuli, where only ITDs were available, were consistent with the hemispheric two-channel model and the modified labelled-line model. That the models could not be distinguished in the psychophysics served to highlight the need for invasive testing to measure the SRFs in cortex where predictions made by the different models were disambiguated.

Ferrets were trained in the relative localisation task and recordings were made during performance of the task. The performance of ferrets in the task was very similar to humans in that they were good at the task about the midline while their performance decreased towards the periphery. Decoding of location from the firing patterns of individual units indicated that many units showed significant information about the location of the stimuli. SRFs were constructed by measuring the modulation of the firing rate by stimulus location during presentation of the reference sound. The shapes of the SRFs were narrower than would be expected with either of the two-channel models

(Figure 4.3 [d]), providing evidence against the two-channel model. When the SRFs of all units were averaged based on the hemisphere they were recorded from or based on the side of space they were tuned to, a coarse two-channel-like SRF was observed (Figure 4.3 [c]). However, this results from averaging of the narrower SRFs of the individual units and the distribution of the units. The distribution of the SRFs indicates that the vast majority of the units were tuned to contralateral space and more units were tuned to the periphery than the midline locations, this is inconsistent with the modified labelled-line model where more units tuned to the midline should be found. Thus the results from the shapes and distributions of the SRFs support the labelled-line model.

In order to explore the models further, the firing rates of populations of neurons were tested with three decoding strategies based on the labelled-line, the hemispheric two-channel and the opponent two-channel models. Success of the models in localising the reference or target stimuli was used to assess the performance of each of the models. The decoded location was determined by comparing single trial population responses to the joint distributions of spike rates in each hemisphere (hemispheric two-channel model), in two populations of units tuned to left or right space (opponent two-channel model) or in individual cells (labelled-line model). Compared with performance of ferrets performing an absolute localisation task in the same testing chamber (Figure 5.5), all the models performed as well as the ferrets could. However, performance of the labelled-line decoder was much better than either of the two-channel models providing further support for the labelled-line model. Since both codes were capable of replicating behaviour, it is possible that the brain could use both codes. However, results from the SRF investigation provide stronger evidence for the labelled-line model in auditory cortex, consistent with results observed in the anaesthetised gerbil (Belliveau et al., 2014).

Evidence in favour of the labelled line model over the two channel model has not only been found in this thesis but also in awake rabbit IC (Day and Delgutte, 2013) and in anaesthetised gerbil IC, results were more equal for the two-channel model and the labelled-line but the labelled line still performed better than the two-channel model (Belliveau et al., 2014). These results appear at odds with representation of space at lower levels of the brain (e.g. McAlpine et al., 2001; Stange et al., 2013) where the peak responses of units lie outside of the physiological range of the animal. However, recently, it has been shown that spatial tuning with peaks within the physiological range can be observed in the gerbil (van der Heijden et al., 2013; Franken et al., 2015), this type of coding is more consistent with a labelled-line code.

Finally, the method of cooling was developed for acute inactivation of brain areas in the behaving ferret. In order to provide evidence for the efficacy for the method in the behaving ferret, a different task was chosen, an absolute localisation task, which provides clear and testable hypotheses for performance deficits during cortical inactivation: Namely, that there will be a contralateral deficit in localisation performance during unilateral inactivation and a bilateral deficit during bilateral inactivation based on previous inactivation and lesion studies in ferrets (Kavanagh and Kelly, 1987; Smith et al., 2004; Nodal et al., 2010). Results indicate that there is a contralateral deficit during unilateral inactivation by cooling (Figure 5.7) and a bilateral deficit during bilateral inactivation by cooling (Figure 5.10) consistent with that predicted by previous inactivation studies. Furthermore, inactivation of auditory cortex had little effect on localisation of visual stimuli providing an important control that the effects observed in the auditory localisation are not due to some general effect of cooling but inactivation of a brain area necessary for sound localisation. The evidence supports successful application of cooling for inactivation. Addressing the question of how auditory cortex encodes the location of sounds, given the distribution of SRFs which is mainly contralateral and narrow (Figure 4.3) one would expect a contralateral deficit with unilateral inactivation of A1, as was observed.

In summary, this is the first body of work to analyse the question of the models of coding in auditory cortex of an awake and behaving animal.

Results provide stronger evidence for a labelled-line encoding of auditory space than for either type of two-channel model.

6.2 On-going work

As mentioned in the discussion of chapter 4 (Section 4.4) there are several avenues of work that are still on-going that will add to aspects of the work presented here. Many of the units that were recorded from in the AC have also been recorded from during a passive presentation of sounds of different frequency and level such that it is possible to determine the frequency tuning of the unit. This information will fulfil two goals. Firstly it can be used to help determine the locations of the units in A1, since A1 is tonotopically organised, the tuning of units across the implant can indicate the direction of the tonotopicity and also if there are any reversals in tonotopicity, which would suggest the electrodes are in another brain area (see Figure 1.5 [c] for information about the layout of AC in the ferret). Secondly, the data collected can be analysed by characteristic frequency, one might expect that high frequency units are more likely to respond to the BPN and HPN stimuli than the LPN stimuli and this may provide more information about the task in the BPN/HPN condition than low frequency units. Likewise for low frequency units, one might expect that these units will respond more strongly to the LPN stimuli and may contain more information about the LPN stimuli than the HPN/BPN. Two ferrets are still performing the relative localisation task and so their remaining data will be added to the work presented here.

Recordings have been made from ferrets performing the task with the longer interval between the reference and target stimuli and also in the presence of the background noise. This data is yet to be analysed and compared with the relevant conditions presented here. The local field potential data is also yet to be analysed and may provide an interesting comparison with the electro-encephalography experiments performed on humans (e.g. Magezi and Krumbholz, 2010).

6.3 Potential further investigation

Inactivation of primary auditory cortex appears only to inhibit the localisation ability of ferrets in approach-to-target tasks but not in a head orienting behaviour (Smith et al., 2004; Nodal et al., 2010), whereas more extensive lesions containing part of higher auditory cortex also cause deficits in head orienting behaviour in the ferret (Nodal et al., 2010). Nodal et al. (2008) showed that in normal animals performing an approach-to-target sound localisation task, for incorrect trials, the initial head orienting movement correlated more highly with the location approached by the animal than with the target location suggesting that this initial response is involved in approach-to-target localisation tasks. Furthermore, unilateral inactivation of the superior colliculus by cooling has been shown to produce a contralateral deficit in head orienting behaviour in response to auditory stimuli in cats. This deficit is abolished during bilateral inactivation and head orienting behaviour returns to normal (Lomber et al., 2001) and inactivation of superior colliculus contralateral to primary auditory cortical lesions can restore localisation deficits in auditory space contralateral to the cortical lesion (Lomber et al., 2007a). This suggests that there may be feedback connections from auditory cortex to the superior colliculus involved in transformation of the location information relative to oneself into an absolute location that can be approached by the animal. There are cortical projections from secondary areas of AC to SC (Bajo et al., 2010; Manger et al., 2010; Chabot et al., 2013) and these areas in the ferret are likely to have been included in the larger lesions in the Nodal et al. (2010) study, possibly explaining the deficit observed in head orienting behaviour. However, these connections are mainly ipsilateral so it could be that the initial head orienting response is as a result of some separate ascending pathway and can be modified by descending projections from AC, which receives mainly contralateral information.

With a view to further work, a preliminary experiment to establish the role of the descending connections from secondary auditory cortex to the SC could be specific inactivation of these fibres. This could be by an optogenetic experiment, injecting viral mediated ArchT in secondary areas of auditory cortex and inactivating the terminals in SC by shining the light here. Alternatively, since optogenetic experiments in the ferret are difficult, one could perform a photolysis experiment similar to that performed by Bajo et al. (2009), here, injections would be made in SC with photolysis performed in secondary auditory cortex to selectively destroy neurons projecting to the SC. This has the disadvantage of not being reversible like an optogenetic experiment. Performance in an approach-to-target localisation task could be tested with light (i.e. projections inactivated) and without light or pre-lesion and post lesion of the projecting neurons. One may expect to see a disruption of the head orienting movement or the approach to the target locations or both if these projections are involved in the transformation of head orientation to absolute location. The possible involvement of SC in encoding of auditory direction (as mentioned in Section 6.4) could also make the inactivation of auditory cortex or these secondary areas of auditory cortex in animals performing the relative localisation task an informative experiment.

It is also possible that information about location is transferred from the contralateral auditory cortex through callosal connections between the two cortices. To test whether information from the contralateral auditory cortex is required to coordinate approach-to-target localisation, one could also perform an optogenetic experiment, injecting virally mediated ArchT in one AC and inactivating the AC contralateral to the injection. One may expect to see a deficit in performance in space contralateral to the light-inactivated AC.

I suggest that a transformation of the encoding of auditory space occurs across the ascending auditory pathway from a two-channel like encoding towards an object-oriented encoding as auditory cortex is approached. There is much evidence to suggest a two-channel encoding of space in brain structures prior to auditory cortex (reviewed in Grothe and Pecka, 2014) and a two-channel model is advantageous for adaptive encoding of auditory space. The location of the cross-over point of the two channels can be shifted according to recent stimulation history for both ILDs and ITDs (Magnusson et al., 2008; Dahmen et al., 2010; Stange et al., 2013) and this adaptation is shown to have psychophysical effects in spatial perception

(Phillips and Hall, 2005; Vigneault-MacLean et al., 2007). Dahmen et al. (2010) showed that changes in IC encoding of ILDs (in anaesthetised ferrets) could account for shifts in spatial perception in human listeners. Changes in the location of the cross-over point appear to serve to improve the relative segregation of the adapting sound source and subsequent sound sources (Getzmann, 2004). Consistent with this, Maddox et al. (2014) show that a visual stimulus that carries information about the location of proceeding auditory stimulus can improve discrimination of stimuli presented in the same region, they suggest this is as a result of a change in spatial receptive fields comparable with a shift in the cross-over point of a two-channel model.

Evidence for two-channel encoding of auditory space in the cortex of human listeners has also been observed in *passively* listening subjects (Salminen et al., 2009, 2010b; Magezi and Krumbholz, 2010). However, when two identical sound sources are presented simultaneously, the perception of their location is fused and it is this fused location that is represented in auditory cortex of human listeners (Salminen et al., 2015a) suggesting that cortex is representing the perceived location of sounds rather than their actual location. Carlile et al. (Carlile et al., 2014) also found evidence for a more topographic-like encoding of space when they presented two simultaneous stimuli and asked listeners to judge the azimuthal distance between them. Furthermore, a recent study in the cat (Middlebrooks and Bremen, 2013) has demonstrated that when two streams of sounds are presented from different locations with different rhythms, neurons in auditory cortex that were previously broadly tuned (two-channel-like) became dramatically sharper and responded to sounds presented from one location or the other – a form of spatial segregation. When only single sounds are presented, there is no requirement for formation of objects in space relative to one another and thus what is observed in auditory cortex is a feed through of encoding of space observed in earlier brain areas, i.e. two-channel encoding. When there are multiple sound sources, I propose that auditory cortex is in fact encoding the auditory objects in relative space rather than the location explicitly, a representation that can easily be confused with a labelled-line encoding of space explicitly.

It is possible that the formation of auditory objects occurs prior to auditory cortex in the IC or thalamus. In the IC, there is conflicting evidence as to whether a two-channel code is present or a topographic-like encoding. Recent studies in awake rabbits (Day and Delgutte, 2013) and with data from anaesthetised guinea pig IC in simulated complex listening environments (Goodman et al., 2013) suggest that a labelled-line encoding of space in the firing patterns of IC neurons is more compatible with behaviour and unilateral ablation studies. These findings are also consistent with the idea that auditory objects are formed at the level of the IC. However, a large body of work from the IC supports a two-channel encoding of space (e.g. Dahmen et al., 2010; Lesica et al., 2010), often from anaesthetised animals, so it is unclear where the formation of objects may start in the ascending auditory pathway. The formation of auditory objects occurring somewhere between the level of the IC and AC is compatible with inactivation studies where unilateral inactivation or ablation of AC results in a contralateral deficit in approach-to-target sound localisation tasks.

During formation of auditory objects, sounds can be grouped or fused according to spatial location but often the spatial location of sounds can be overridden by stronger grouping cues such as fundamental frequency (Shackleton and Meddis, 1992). There are experiments required to resolve the question of whether in auditory cortex what we see is a topographic representation of auditory space or the formation of auditory objects segregated by space. I would suggest the following experiments in animals with simultaneous recording of neural activity in auditory cortex: Present to an animal trained to localise single sounds two sounds from different locations that will be fused together to form a percept located between the two stimuli and allow the animal to localise this sound. I would predict that the animal will localise the stimulus to the fused location and if auditory cortex were representing this perceived location rather than the two stimuli separately, that one would observe neural firing patterns consistent with presentation of a single sound source at this location. This would provide basic evidence of auditory object formation in AC and repeat an experiment performed in humans (Salminen et al., 2015a).

It has been shown in humans that harmonic complexes of tones can ‘capture’ a harmonic tone with a different ITD to the complex such that its location is indistinguishable from the complex. However, if the tone is not harmonic with the complex then its ITD can be discriminated (Buell and Hafter, 1991). This type of capture has been attributed to the formation of auditory objects (reviewed in Best et al., 2007). In a more complex task, one could train an animal to localise tones, and present the animal with probe trials where a tone is presented simultaneously with a harmonic or inharmonic complex of differing locations to the tone – one would predict that the location of tone would be captured by a harmonic complex and the ferret would localise the complex however with an inharmonic complex one would predict localisation of the tone. Since one would need control over the phase of the stimuli, this experiment may be better performed over headphones and the task reduced to a lateralisation (i.e. 2AFC) experiment, sound could be presented in virtual acoustic space such that it appeared ‘external’ to the animal – ferrets have been trained to perform tasks wearing headphones (Keating et al., 2014). Recording of units from auditory cortex while the animal performed these experiments should resolve the problem of whether auditory cortex is encoding spatial locations or perceived spatial locations/auditory objects.

Chapter 7: Appendices

7.1 Author contributions

The present author (KCW) performed experimental design, surgical implantation, data collection, data analysis and interpretation. Ferret training sessions were run collaboratively according to a rota within the lab in which KCW participated. Analysis of the data presented in Figure 5.4 was performed by Dr Stephen Town, KCW collected the data in collaboration with Dr Town.

7.2 Publications arising from this thesis

Work presented in chapter 2 was published in The Journal of the Acoustical Society of America. Volume 138, Issue 2, pages 674 – 686. Relative sound localisation abilities in human listeners. Wood, K. C. & Bizley, J. K. (2015).

7.3 MI decoding statistics

7.3.1 Decoding of reference location

7.3.1.1 Effect of stimulus condition and bin size on % of units with significant MI

Binomial logistic regression compares each predictor (bin size and stimulus condition) to a constant alone, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. If both significant, individual predictors are tested against a regression containing both predictors, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. Post-hoc comparisons were conducted within any significant predictors using a two-proportion *Z*-test with level of significance Bonferroni correction for multiple comparisons, bin size: $p < 0.0167$ and stimulus condition: $p < 0.0083$.

Binomial logistic regression, each predictor *vs.*:
Constant model: bin size: $p =$ **0.0079**, stimulus condition $p =$ **0.0020**
Full model: bin size: $p =$ **0.0020**, stimulus condition $p =$ **0.0078**
***Post-hoc* comparisons:**
Bin size: 50 ms > 15 ms & 150 ms, 150 ms > 15 ms

Stimulus condition: LPN < BBN & BPN, HPN < BBN

7.3.1.2 Effect of stimulus condition and bin size on % maximum MI of significant units

2-way ANOVA with dependent variable % maximum MI and independent variables of bin size and stimulus condition, $p < 0.05$. *Post-hoc* comparisons, $p < 0.05$ Tukey-Kramer.

	Degrees of freedom	F	p
Bin size	2	5.34	**0.001**
Stimulus condition	3	6.34	**<0.001**
Bin size * Stimulus condition	6	0.29	0.940
Error	1019		
Total	1030		

***Post-hoc* comparisons:**

Bin size: 15 ms > 150 ms

Stimulus condition: BBN > HPN, BPN > LPN & HPN

7.3.2 Decoding of target location

7.3.2.1 Effect of stimulus condition and bin size on % of units with significant MI

Binomial logistic regression compares each predictor (bin size and stimulus condition) to a constant alone, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. If both significant, individual predictors are tested against a regression containing both predictors, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. Post-hoc comparisons were conducted within any significant predictors using a two-proportion Z-test with level of significance Bonferroni correction for multiple comparisons, bin size: $p < 0.0167$ and stimulus condition: $p < 0.0083$.

Binomial logistic regression, each predictor *vs.*:

Constant model: bin size: p **= 0.0086**, stimulus condition p = **0.0018**

Full model: bin size: p = **0.0018**, stimulus condition p = **0.0084**

***Post-hoc* comparisons:**

Bin size: 50 ms > 15 ms & 150 ms, 150 ms > 15 ms

Stimulus condition: LPN < BBN & BPN, HPN < BBN

7.3.2.2 Effect of stimulus condition and bin size on % maximum MI of significant units

2-way ANOVA with dependent variable % maximum MI and independent variables of bin size and stimulus condition, $p < 0.05$. *Post-hoc* comparisons, $p < 0.05$ Tukey-Kramer.

	degrees of freedom	*F*	*p*
Bin size	2	6.83	**0.001**
Stimulus Condition	3	7.52	**<0.001**
Bin size * Stimulus condition	6	0.28	0.945
Error	950		
Total	961		

***Post-hoc* comparisons:**

Bin size: 15 ms > 150 ms

Stimulus condition: BBN > HPN, BPN > LPN & HPN

7.3.3 Rate decoding of reference location in a moving 50 ms time window

7.3.3.1 Effect of stimulus condition and bin size on % of units with significant MI

Binomial logistic regression compares each predictor (bin size and stimulus condition) to a constant alone, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. If both significant, individual predictors are tested against a regression containing both predictors, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. Post-hoc comparisons were conducted within any significant predictors using a two-proportion *Z*-test with level of significance Bonferroni correction for multiple comparisons, bin size: $p < 0.0167$ and stimulus condition: $p < 0.0083$.

Binomial logistic regression, each predictor *vs.*:

Constant model: bin size: $p = 0.8251$, stimulus condition $p = 0.0258$

7.3.3.2 Effect of stimulus condition and bin size on % maximum MI of significant units

2-way ANOVA with dependent variable % maximum MI and independent variables of bin size and stimulus condition, $p < 0.05$. *Post-hoc* comparisons, $p < 0.05$ Tukey-Kramer.

	degrees of freedom	*F*	*p*
Bin size	2	0.2	0.8181
Stimulus Condition	3	0.88	0.4488
Bin size * Stimulus condition	6	1.02	0.4141
Error	692		
Total	703		

7.3.4 Rate decoding of target location in a moving 50 ms time window

7.3.4.1 Effect of stimulus condition and bin size on % of units with significant MI

Binomial logistic regression compares each predictor (bin size and stimulus condition) to a constant alone, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. If both significant, individual predictors are tested against a regression containing both predictors, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. Post-hoc comparisons were conducted within any significant predictors using a two-proportion *Z*-test with level of significance Bonferroni correction for multiple comparisons, bin size: $p < 0.0167$ and stimulus condition: $p < 0.0083$.

Binomial logistic regression, each predictor *vs.*:

Constant model: bin size: *p* < **0.0001**, stimulus condition *p* = 0.9047

***Post-hoc* comparisons:**

Bin size: 100–150 ms < 0-50 & 50-100

7.3.4.2 Effect of stimulus condition and bin size on % maximum MI of significant units

2-way ANOVA with dependent variable % maximum MI and independent variables of bin size and stimulus condition, $p < 0.05$. *Post-hoc* comparisons, $p < 0.05$ Tukey-Kramer.

	degrees of freedom	*F*	*p*
Bin size	2	4.94	**0.0074**
Stimulus Condition	3	0.98	0.3994
Bin size * Stimulus condition	6	1.33	0.2408
Error	619		
Total	630		

***Post-hoc* comparisons:**

Bin size: 100-150 ms < 0-50 ms

7.3.5 Rate decoding of reference location over an increasing duration time window

7.3.5.1 Effect of stimulus condition and bin size on % of units with significant MI

Binomial logistic regression compares each predictor (bin size and stimulus condition) to a constant alone, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. If both significant, individual predictors are tested against a regression containing both predictors, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. Post-hoc comparisons were conducted within any significant predictors using a two-proportion *Z*-test with level of significance Bonferroni correction for multiple comparisons, bin size: $p < 0.0167$ and stimulus condition: $p < 0.0083$.

Binomial logistic regression, each predictor *vs.*:

Constant model: bin size: *p* < **0.0001**, stimulus condition *p* = **0.0190**

Full model: bin size: *p* = **0.0178**, stimulus condition *p* < **0.0001**

***Post-hoc* comparisons:**

Bin size: 50 ms < 100 & 150 ms

Stimulus condition: HPN < BBN & BPN, LPN < BBN

7.3.5.2 Effect of stimulus condition and bin size on % maximum MI of significant units

2-way ANOVA with dependent variable % maximum MI and independent variables of bin size and stimulus condition, $p < 0.05$. *Post-hoc* comparisons, $p < 0.05$ Tukey-Kramer.

	degrees of freedom	*F*	*p*
Bin size	2	10.73	**<0.0001**
Stimulus Condition	3	1.01	0.3888
Bin size * Stimulus condition	6	1.16	0.3266
Error	904		
Total	915		

***Post-hoc* comparisons:**

Bin size: 50 ms < 100 & 150 ms

7.3.6 Rate decoding of target location over an increasing duration time window

7.3.6.1 Effect of stimulus condition and bin size on % of units with significant MI

Binomial logistic regression compares each predictor (bin size and stimulus condition) to a constant alone, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. If both significant, individual predictors are tested against a regression containing both predictors, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. Post-hoc comparisons were conducted within any significant predictors using a two-proportion *Z*-test with level of significance Bonferroni correction for multiple comparisons, bin size: $p < 0.0167$ and stimulus condition: $p < 0.0083$.

Binomial logistic regression, each predictor *vs.*:

Constant model: bin size: p = **0.0017**, stimulus condition p = **0.0065**

Full model: bin size: p = **0.0064**, stimulus condition p = **0.0016**

***Post-hoc* comparisons:**

Bin size: 50 ms < 100 & 150 ms

Stimulus condition: BBN > LPN & HPN

7.3.6.2 Effect of stimulus condition and bin size on % maximum MI of significant units

2-way ANOVA with dependent variable % maximum MI and independent variables of bin size and stimulus condition, $p < 0.05$. *Post-hoc* comparisons, $p < 0.05$ Tukey-Kramer.

	degrees of freedom	*F*	*p*
Bin size	2	5.28	**0.0053**
Stimulus Condition	3	5.72	**0.0007**
Bin size * Stimulus condition	6	0.86	0.5246
Error	863		
Total	874		

***Post-hoc* comparisons:**

Bin size: 50 ms < 100 & 150 ms

Stimulus condition: LPN < BBN & BPN

7.3.7 Decoding of target location in the context of direction of the stimulus

7.3.7.1 Effect of stimulus condition and bin size on % of units with significant MI

Binomial logistic regression compares each predictor (bin size and stimulus condition) to a constant alone, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. If both significant, individual predictors are tested against a regression containing both predictors, analysis of deviance performed on the results, X^2 distribution, $p < 0.025$. Post-hoc comparisons were conducted within any significant predictors using a two-proportion *Z*-test with level of significance Bonferroni correction for multiple comparisons, bin size: $p < 0.0167$ and stimulus condition: $p < 0.0083$.

Binomial logistic regression, each predictor *vs.*:

Constant model: bin size: *p* < **0.0001**, stimulus condition *p* = **0.0149**

Full model: bin size: *p* = **0.0143**, stimulus condition *p* < **0.0001**

***Post-hoc* comparisons:**

Bin size: 150 ms < 15 & 50 ms

Stimulus condition: HPN < BBN & LPN & BPN

7.3.7.2 Effect of stimulus condition and bin size on % maximum MI of significant units

2-way ANOVA with dependent variable % maximum MI and independent variables of bin size and stimulus condition, $p < 0.05$. *Post-hoc* comparisons, $p < 0.05$ Tukey-Kramer.

	degrees of freedom	*F*	*p*
Bin size	2	14.94	**<0.0001**
Stimulus Condition	3	8.3	**<0.0001**
Bin size * Stimulus condition	6	0.56	0.7597
Error	847		
Total	858		

***Post-hoc* comparisons:**

Bin size: 150 ms < 15 & 50 ms

Stimulus condition: BPN > BBN & LPN & HPN

7.3.8 Comparing the target and direction, target alone and direction alone decoders

7.3.8.1 Effect of stimulus condition, bin size and decoder type on % of units with significant MI

Binomial logistic regression compares each predictor (bin size, stimulus condition and decoder type) to a constant alone, analysis of deviance performed on the results, X^2 distribution, $p < 0.0167$. If more than one was significant, these individual predictors were tested against a regression containing the significant predictors, analysis of deviance performed on the results, X^2 distribution, $p < 0.05$/number of comparisons. *Post-hoc* comparisons were conducted within any significant predictors using a two-proportion Z-test with level of significance Bonferroni corrected for multiple comparisons, bin size: $p < 0.0167$, stimulus condition: $p < 0.0083$ and decoder type: $p < 0.0167$

Binomial logistic regression, each predictor *vs.*:

Constant model: bin size: $p <$ 0.4485, stimulus condition: $p <$ **0.0001**, decoder type: $p <$ **0.0001**

Full model: stimulus condition: $p <$ **0.0001**, decoder type: $p <$ **0.0001**

***Post-hoc* comparisons:**

Stimulus condition: BBN > LPN & HPN, BPN > HPN

Decoder type: Direction & Target > direction alone & target alone, target alone > direction alone

7.3.8.2 Effect of stimulus condition, bin size and decoder type on % maximum MI of significant units

3-way ANOVA with dependent variable % maximum MI and independent variables of bin size, stimulus condition and decoder type; $p < 0.05$. *Post-hoc* comparisons, $p < 0.05$ Tukey-Kramer.

	degrees of freedom	*F*	*p*
Bin size	2	16.9	**<0.0001**
Stimulus Condition	3	0	1
Decoder type	2	1158.5	**<0.0001**
Bin size * Stimulus condition	6	0	1
Bin size * decoder type	4	7.04	**<0.0001**
Stimulus condition * decoder type	6	0	1
Error	3728		
Total	3751		

***Post-hoc* comparisons:**

Bin size: 150 ms < 15 & 50 ms

Decoder type: Direction & Target > direction alone & target alone, target alone > direction alone

Chapter 8: References

Aaronson, N. L., and Hartmann, W. M. (**2014**). “Testing, correcting, and extending the Woodworth model for interaural time difference,” J. Acoust. Soc. Am., **135**, 817–23.

Alain, C., Arnott, S. R., Hevenor, S., Graham, S., and Grady, C. L. (**2001**). “‘What’ and ‘where’ in the human auditory system,” Proc. Natl. Acad. Sci. U. S. A., **98**, 12301–12306.

Arnott, S. R., Binns, M. A., Grady, C. L., and Alain, C. (**2004**). “Assessing the auditory dual-pathway model in humans,” Neuroimage, **22**, 401–408.

Atiani, S., Elhilali, M., David, S. V, Fritz, J. B., and Shamma, S. A. (**2009**). “Task difficulty and performance induce diverse adaptive patterns in gain and shape of primary auditory cortical receptive fields,” Neuron, **61**, 467–80.

Bajo, V., Korn, C., Reynolds, K., Boyden, E., Nodal, F., and King, A. J. (**2013**). “Behavioral and neural effects of silencing the auditory cortex in adult ferrets using optogenetics,” Tucker Davis Technol. Symp. Adv. Perspect. Audit. Neurophysiol., 8.

Bajo, V. M., Nodal, F. R., Moore, D. R., and King, A. J. (**2009**). “The descending corticocollicular pathway mediates learning-induced auditory plasticity,” Nat. Neurosci., **13**, 253–260.

Bajo, V., Nodal, F., Bizley, J., and King, A. (**2010**). “The non-lemniscal auditory cortex in ferrets: convergence of corticotectal inputs in the superior colliculus,” Front. Neuroanat., **4**, 18.

Bajo, V. V., Nodal, F. F., Bizley, J. J., Moore, D., and King, A. (**2007**). “The ferret auditory cortex: descending projections to the inferior colliculus,” Cereb. Cortex, **17**, 475–566.

Beitel, R. E., and Kaas, J. H. (**1993**). “Effects of bilateral and unilateral ablation of auditory cortex in cats on the unconditioned head orienting response to acoustic stimuli,” J Neurophysiol, **70**, 351–369.

Belliveau, L. A. C., Lyamzin, D. R., and Lesica, N. A. (**2014**). “The Neural Representation of Interaural Time Differences in Gerbils Is Transformed from Midbrain to Cortex,” J. Neurosci., **34**, 16796–16808.

Benson, D. A., Hienz, R. D., and Goldstein, M. H. (**1981**). “Single-unit activity in the auditory cortex of monkeys actively localizing sound sources: spatial tuning and behavioral dependency,” Brain Res., **219**, 249–316.

Bernstein, L. R. (**1985**). “Lateralization of low-frequency, complex waveforms: The use of envelope-based temporal disparities,” J. Acoust. Soc. Am., **77**, 1868.

Bernstein, L. R., and Trahiotis, C. (**1994**). “Detection of interaural delay in high-frequency sinusoidally amplitude-modulated tones, two-tone complexes, and bands of noise,” J. Acoust. Soc. Am., **95**, 3561.

Best, V., Gallun, F. J., Carlile, S., and Shinn-Cunningham, B. G. (**2007**). “Binaural interference and auditory grouping,” J. Acoust. Soc. Am., **121**, 1070–1076.

Bizley, J., Atilgan, H., Jones, G., Wood, K. C., and Town, S. M. (**2015**). “Inviting Ferrets to the Cocktail Party: A Behavioral Model for Complex Sound Recognition and Selective Attention,” Assoc. Res. Otolaryngol. 38th Annu. MidWinter Meet., 47.

Bizley, J. K., Elliott, N., Wood, K. C., and Vickers, D. (**2016**). “Simultaneous assessment of speech identification and sound localisation: a potential testing approach for bilateral cochlear implant users?,” Trends Hear.,.

Bizley, J. K., and King, A. J. (**2008**). “Visual-auditory spatial processing in auditory cortical neurons,” Brain Res., **1242**, 24–60.

Bizley, J. K., Nodal, F. R., Parsons, C. H., and King, A. J. (**2007**). “Role of auditory cortex in sound localization in the midsagittal plane,” J Neurophysiol, **98**, 1763–1774.

Bizley, J. K., Walker, K. M., King, A. J., and Schnupp, J. W. (**2013**). “Spectral timbre perception in ferrets: discrimination of artificial vowels under different listening conditions,” J Acoust Soc Am, **133**, 365–376.

Bizley, J. K., Walker, K. M. M., Nodal, F. R., King, A. J., and Schnupp, J. W. H. (**2013**). “Auditory cortex represents both pitch judgments and the corresponding acoustic cues,” Curr Biol, **23**, 620–625.

Bizley, J. K., Walker, K. M., Silverman, B. W., King, A. J., and Schnupp, J. W. (**2009**). “Interdependent encoding of pitch, timbre, and spatial location in auditory cortex,” J. Neurosci., **29**, 2064–2075.

Bizley, J., Nodal, F., Nelken, I., and King, A. (**2005**). “Functional organization of ferret auditory cortex,” Cereb. Cortex, **15**, 1637–1690.

Blauert, J. (**1997**). *Spatial Hearing: The Psychophysics of Human Sound Localization*, MIT Press, Cambridge, MA, 494 pages.

Brainard, D. H. (**1997**). “The Psychophysics Toolbox,” Spat Vis, **10**, 433–436.

Brand, A., Behrend, O., Marquardt, T., McAlpine, D., and Grothe, B. (**2002**). “Precise inhibition is essential for microsecond interaural time difference coding,” Nature, **417**, 543–550.

Briley, P. M., Kitterick, P. T., and Summerfield, A. Q. (**2013**). “Evidence for opponent process analysis of sound source location in humans,” J. Assoc. Res. Otolaryngol., **14**, 83–101.

Brugge, J. F., Reale, R. a, and Hind, J. E. (**1998**). "Spatial receptive fields of primary auditory cortical neurons in quiet and in the presence of continuous background noise," J. Neurophysiol., **80**, 2417–2432.

Brugge, J., Reale, R., and Hind, J. (**1996**). "The structure of spatial receptive fields of neurons in primary auditory cortex of the cat," J. Neurosci., **16**, 4420–4457.

Brugge, J., Reale, R., Jenison, R., and Schnupp, J. (**2001**). "Auditory cortical spatial receptive fields," Audiol. Neurootol., **6**, 173–180.

Buell, T. N., and Hafter, E. R. (**1991**). "Combination of binaural information across frequency bands," J. Acoust. Soc. Am., **90**, 1894–1900.

Campbell, R. A., Schnupp, J., Shial, A., and King, A. (**2006**). "Binaural-level functions in ferret auditory cortex: evidence for a continuous distribution of response properties," J. Neurophysiol., **95**, 3742–3797.

Carlile, S. (**2014**). "The plastic ear and perceptual relearning in auditory spatial perception," Front. Neurosci., **8**, 237.

Carlile, S., Delaney, S., and Corderoy, A. (**1999**). "The localisation of spectrally restricted sounds by human listeners," Hear. Res., **128**, 175–264.

Carlile, S., Fox, A., Leung, J., Orchard-Mills, E., and David, A. (**2014**). "Azimuthal Distance Judgements Produce a 'Dipper' Sensitivity Function," Assoc. Res. Otolaryngol. 38th Annu. MidWinter Meet., San Diego, 249–250.

Carlile, S., and King, a J. (**1994**). "Monaural and binaural spectrum level cues in the ferret: acoustics and the neural representation of auditory space," J. Neurophysiol., **71**, 785–801.

Carr, C., and Konishi, M. (**1990**). "A circuit for detection of interaural time differences in the brain stem of the barn owl," J. Neurosci., **10**, 3227–3246.

Cavanaugh, J., Monosov, I. E., McAlonan, K., Berman, R., Smith, M. K., Cao, V., Wang, K. H., et al. (**2012**). "Optogenetic inactivation modifies monkey visuomotor behavior," Neuron, **76**, 901–7.

Chabot, N., Mellott, J. G., Hall, A. J., Tichenoff, E. L., and Lomber, S. G. (**2013**). "Cerebral origins of the auditory projection to the superior colliculus of the cat," Hear. Res., **300**, 33–45.

Chase, S. M., and Young, E. D. (**2008**). "Cues for sound localization are encoded in multiple aspects of spike trains in the inferior colliculus," J. Neurophysiol., **99**, 1672–82.

Clarke, S., Bellmann, A., Meuli, R., Assal, G., and Steck, A. (**2000**). "Auditory agnosia and auditory spatial deficits following left hemispheric lesions:

evidence for distinct processing pathways," Neuropsychologia, **38**, 797–1604.

Coleman, P. D. (**1963**). "An analysis of cues to auditory depth perception in free space," Psychol. Bull., **60**, 302–315.

Coomber, B., Edwards, D., Jones, S. J., Shackleton, T. M., Goldschmidt, J., Wallace, M. N., and Palmer, A. R. (**2011**). "Cortical inactivation by cooling in small animals," Front. Syst. Neurosci., **5**, 53.

Cotillon-Williams, N., Huetz, C., Hennevin, E., and Edeline, J.-M. (**2008**). "Tonotopic control of auditory thalamus frequency tuning by reticular thalamic neurons," J. Neurophysiol., **99**, 1137–51.

Dahmen, J. C., Keating, P., Nodal, F. R., Schulz, A. L., and King, A. J. (**2010**). "Adaptation to Stimulus Statistics in the Perception and Neural Representation of Auditory Space," Neuron, **66**, 937–948.

Day, M. L., and Delgutte, B. (**2013**). "Decoding sound source location and separation using neural population activity patterns," J. Neurosci., **33**, 15837–47.

Diester, I., Kaufman, M. T., Mogri, M., Pashaie, R., Goo, W., Yizhar, O., Ramakrishnan, C., et al. (**2011**). "An optogenetic toolbox designed for primates," Nat. Neurosci., **14**, 387–97.

Dingle, R. N., Hall, S. E., and Phillips, D. P. (**2010**). "A midline azimuthal channel in human spatial hearing," Hear. Res., **268**, 67–74.

Dingle, R. N., Hall, S. E., and Phillips, D. P. (**2012**). "The three-channel model of sound localization mechanisms: Interaural level differences," J. Acoust. Soc. Am., **131**, 4023.

Dingle, R. N., Hall, S. E., and Phillips, D. P. (**2013**). "The three-channel model of sound localization mechanisms: interaural time differences," J. Acoust. Soc. Am., **133**, 417–24.

Dragunow, M., and Faull, R. (**1989**). "The use of c-fos as a metabolic marker in neuronal pathway tracing," J. Neurosci. Methods, **29**, 261–265.

Duffour-Nikolov, C., Tardif, E., Maeder, P., Thiran, A. B., Bloch, J., Frischknecht, R., and Clarke, S. (**2012**). "Auditory spatial deficits following hemispheric lesions: Dissociation of explicit and implicit processing," Neuropsychol. Rehabil., **22**, 674–696.

Eliades, S. J., and Wang, X. (**2008**). "Chronic multi-electrode neural recording in free-roaming monkeys," J. Neurosci. Methods, **172**, 201–14.

Fitzpatrick, D. C., Kuwada, S., Kim, D. O., Parham, K., and Batra, R. (**1999**). "Responses of neurons to click-pairs as simulated echoes: auditory nerve to auditory cortex," J. Acoust. Soc. Am., **106**, 3460–3472.

Franken, T. P., Roberts, M. T., Wei, L., Golding, N. L., and Joris, P. X. (**2015**). *In vivo coincidence detection in mammalian sound localization generates phase delays.*, Nat. Neurosci., Available: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=25664914&retmode=ref&cmd=prlinks\npapers3://publication/doi/10.1038/nn.3948, (date last viewed: 11-Nov-15).

Freigang, C., Schmiedchen, K., Nitsche, I., and Rübsamen, R. (**2014**). “Free-field study on auditory localization and discrimination performance in older adults,” Exp. brain Res., **232**, 1157–72.

Fritz, J., Elhilali, M., and Shamma, S. (**2005**). “Differential dynamic plasticity of A1 receptive fields during multiple spectral tasks,” J. Neurosci., **25**, 7623–7658.

Fritz, J., Elhilali, M., and Shamma, S. (**2005**). “Active listening: task-dependent plasticity of spectrotemporal receptive fields in primary auditory cortex,” Hear. Res., **206**, 159–76.

Fritz, J., Shamma, S., Elhilali, M., and Klein, D. (**2003**). “Rapid task-related plasticity of spectrotemporal receptive fields in primary auditory cortex,” Nat. Neurosci., **6**, 1216–1239.

Furukawa, S., and Middlebrooks, J. C. (**2001**). “Sensitivity of auditory cortical neurons to locations of signals and competing noise sources,” J. Neurophysiol., **86**, 226–266.

Furukawa, S., and Middlebrooks, J. C. (**2002**). “Cortical representation of auditory space: information-bearing features of spike patterns,” J. Neurophysiol., **87**, 1749–62.

Georgopoulos, A. P., Schwartz, A. B., and Kettner, R. E. (**1986**). *Neuronal Population Coding of Movement Direction*, Science (80-.)., Available: http://www.sciencemag.org/content/233/4771/1416.long, (date last viewed: 18-Nov-15).

Getzmann, S. (**2004**). “Spatial discrimination of sound sources in the horizontal plane following an adapter sound,” Hear. Res., **191**, 14–20.

Glendenning, K. K., Baker, B. N., Hutson, K. A., and Masterton, R. B. (**1992**). “Acoustic chiasm V: inhibition and excitation in the ipsilateral and contralateral projections of LSO,” J. Comp. Neurol., **319**, 100–22.

Gold, J. R., Nodal, F. R., Peters, F., King, A. J., and Bajo, V. M. (**2015**). “Auditory Gap-in-Noise Detection Behavior in Ferrets and Humans,” Behav. Neurosci.,.

Good, M., and Gilkey, R. (**1996**). “Sound localization in noise: the effect of signal-to-noise ratio,” J. Acoust. Soc. Am., **99**, 1108–1125.

Goodman, D. F. M., Benichoux, V., and Brette, R. (**2013**). “Decoding neural responses to temporal cues for sound localization,” Elife, **2013**, e01312.

Green, D. M., and Swets, J. A. (**1974**). *Signal detection theory and psychophysics*, R. E. Krieger Pub. Co., Huntington, N.Y., xiii, 479 p. pages.

Grothe, B., and Pecka, M. (**2014**). “The natural history of sound localization in mammals--a story of neuronal inhibition,” Front. Neural Circuits, **8**, 116.

Grothe, B., Pecka, M., and McAlpine, D. (**2010**). “Mechanisms of sound localization in mammals,” Physiol. Rev., **90**, 983–1995.

Hackett, T. a (**2011**). “Information flow in the auditory cortical network,” Hear. Res., **271**, 133–146.

Harper, N., and McAlpine, D. (**2004**). “Optimal neural population coding of an auditory spatial cue,” Nature, **430**, 682–688.

Harper, N. S., Scott, B. H., Semple, M. N., and McAlpine, D. (**2014**). “The neural code for auditory space depends on sound frequency and head size in an optimal manner,” PLoS One, **9**, e108154.

Harrington, I. a., Stecker, G. C., Macpherson, E. a., and Middlebrooks, J. C. (**2008**). “Spatial sensitivity of neurons in the anterior, posterior, and primary fields of cat auditory cortex,” Hear. Res., **240**, 22–41.

Hartmann, W., and Rakerd, B. (**1989**). “On the minimum audible angle--a decision theory approach,” J. Acoust. Soc. Am., **85**, 2031–2072.

Hebrank, J., and Wright, D. (**1974**). “Spectral cues used in the localization of sound sources on the median plane,” J. Acoust. Soc. Am., **56**, 1829–1834.

Heffner, H. (**1978**). “Effect of auditory cortex ablation on localization and discrimination of brief sounds,” J Neurophysiol, **41**, 963–976.

Heffner, H. E. (**1997**). “The Role of Macaque Auditory Cortex in Sound Localization,” Acta Otolaryngol. Suppl., **532**, 22–7.

Heffner, H. E., and Heffner, R. S. (**1990**). “Effect of bilateral auditory cortex lesions on sound localization in Japanese macaques,” J. Neurophysiol., **64**, 915–946.

van der Heijden, M., Lorteije, J. a M., Plauška, A., Roberts, M. T., Golding, N. L., and Borst, J. G. G. (**2013**). *Directional hearing by linear summation of binaural inputs at the medial superior olive.*, Neuron, Available: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3741096&tool=pmcentrez&rendertype=abstract, (date last viewed: 11-Nov-15).

Ingham, N. J., Hart, H. C., and McAlpine, D. (**2001**). “Spatial receptive fields of inferior colliculus neurons to auditory apparent motion in free field,” J. Neurophysiol., **85**, 23–33.

Jeffress, L. (**1948**). “A place theory of sound localization,” J. Comp. Physiol. Psychol., **41**, 35–44.

Jenkins, W., and Masterton, R. (**1982**). “Sound localization: effects of unilateral lesions in central auditory system,” J. Neurophysiol., **47**, 987–2003.

Jenkins, W., and Merzenich, M. (**1984**). “Role of cat primary auditory cortex for sound-localization behavior,” J Neurophysiol, **52**, 819–847.

Kacelnik, O., Nodal, F. R., Parsons, C. H., and King, A. J. (**2006**). “Training-induced plasticity of auditory localization in adult mammals,” PLoS Biol., **4**, e71.

Kavanagh, G. L., and Kelly, J. B. (**1987**). “Contribution of auditory cortex to sound localization by the ferret (Mustela putorius),” J Neurophysiol, **57**, 1746–1766.

Keating, P., Dahmen, J. C., and King, A. J. (**2013**). “Context-specific reweighting of auditory spatial cues following altered experience during development,” Curr. Biol., **23**, 1291–9.

Keating, P., Dahmen, J. C., and King, A. J. (**2015**). “Complementary adaptive processes contribute to the developmental plasticity of spatial hearing,” Nat. Neurosci., **18**, 185–7.

Keating, P., Nodal, F. R., Gananandan, K., Schulz, A. L., and King, A. J. (**2013**). “Behavioral Sensitivity to Broadband Binaural Localization Cues in the Ferret,” J. Assoc. Res. Otolaryngol., **14**, 561–572.

Keating, P., Nodal, F. R., and King, A. J. (**2014**). “Behavioural sensitivity to binaural spatial cues in ferrets: evidence for plasticity in the duplex theory of sound localization,” Eur. J. Neurosci., **39**, 197–206.

Kelly, J. B., Rooney, B. J., and Phillips, D. P. (**1996**). “Effects of bilateral auditory cortical lesions on gap-detection thresholds in the ferret (Mustela putorius),” Behav. Neurosci., **110**, 542–50.

King, A. J., Bajo, V. M., Bizley, J. K., Campbell, R. A., Nodal, F. R., Schulz, A. L., and Schnupp, J. W. (**2007**). “Physiological and behavioral studies of spatial coding in the auditory cortex,” Hear Res, **229**, 106–115.

King, A. J., and Hutchings, M. E. (**1987**). “Spatial response properties of acoustically responsive neurons in the superior colliculus of the ferret: a map of auditory space,” J Neurophysiol, **57**, 596–624.

Kleiner, M., Brainard, D., and Pelli, D. (**2007**). “What’s new in Psychtoolbox-3?,” Perception, **36**, 14.

Knudsen, E. I., Knudsen, P. F., and Masino, T. (**1993**). “Parallel pathways mediating both sound localization and gaze control in the forebrain and midbrain of the barn owl,” J. Neurosci., **13**, 2837–52.

Knudsen, E., and Konishi, M. (**1978**). “A neural map of auditory space in the owl,” Science (80-.).,.

Konishi, M. (**2003**). “Coding of auditory space,” Annu. Rev. Neurosci., **26**, 31–86.

Koppl, C. (**1997**). “Phase Locking to High Frequencies in the Auditory Nerve and Cochlear Nucleus Magnocellularis of the Barn Owl, Tyto alba,” J. Neurosci., **17**, 3312–3321.

Kuhn, G. F. (**1977**). “Model for the interaural time differences in the azimuthal plane,” J. Acoust. Soc. Am., **62**, 157.

Lee, C. C., and Middlebrooks, J. C. (**2013**). “Specialization for sound localization in fields A1, DZ, and PAF of cat auditory cortex,” JARO - J. Assoc. Res. Otolaryngol., **14**, 61–82.

Lee, C.-C., and Middlebrooks, J. C. (**2011**). “Auditory cortex spatial sensitivity sharpens during task performance,” Nat. Neurosci., **14**, 108–114.

Lesica, N., Lingner, A., and Grothe, B. (**2010**). “Population coding of interaural time differences in gerbils and barn owls,” J. Neurosci., **30**, 11696–12398.

Lewald, J., and Ehrenstein, W. H. (**1996**). “The effect of eye position on auditory lateralization,” Exp. brain Res., **108**, 473–85.

Lingner, A., Wiegrebe, L., and Grothe, B. (**2012**). “Sound Localization in Noise by Gerbils and Humans,” J. Assoc. Res. Otolaryngol.,.

Litovsky, R. Y., Colburn, H. S., Yost, W. a., and Guzman, S. J. (**1999**). “The precedence effect,” J. Acoust. Soc. Am., **106**, 1633.

Lomber, S. G., Cornwell, P., Sun, J. S., MacNeil, M. A., and Payne, B. R. (**1994**). “Reversible inactivation of visual processing operations in middle suprasylvian cortex of the behaving cat,” Proc. Natl. Acad. Sci., **91**, 2999–3003.

Lomber, S. G., Malhotra, S., and Sprague, J. M. (**2007**). “Restoration of acoustic orienting into a cortically deaf hemifield by reversible deactivation of the contralesional superior colliculus: the acoustic ‘Sprague Effect,’” J. Neurophysiol., **97**, 979–93.

Lomber, S. G., Meredith, M. A., and Kral, A. (**2010**). “Cross-modal plasticity in specific auditory cortices underlies visual compensations in the deaf,” Nat. Neurosci., **13**, 1421–7.

Lomber, S. G., Payne, B. R., and Cornwell, P. (**2001**). “Role of the superior colliculus in analyses of space: superficial and intermediate layer contributions to visual orienting, auditory orienting, and visuospatial discriminations during unilateral and bilateral deactivations,” J. Comp. Neurol., **441**, 44–57.

Lomber, S., and Malhotra, S. (**2008**). “Double dissociation of ‘what’ and ‘where’ processing in auditory cortex,” Nat. Neurosci., **11**, 609–625.

Lomber, S., Malhotra, S., and Hall, A. (**2007**). “Functional specialization in non-primary auditory cortex of the cat: areal and laminar contributions to sound localization,” Hear. Res., **229**, 31–76.

Lomber, S., and Payne, B. (**1999**). “The cryoloop: an adaptable reversible cooling deactivation method for behavioral or electrophysiological assessment of neural function,” J. Neurosci. Methods,.

Lorenzi, C., Gatehouse, S., and Lever, C. (**1999**). “Sound localization in noise in normal-hearing listeners,” J. Acoust. Soc. Am., **105**, 1810–1830.

Macmillan, N. A., and Creelman, C. D. (**2004**). *Detection Theory: A User’s Guide*, Psychology Press, 24-45 pages.

Macpherson, E., and Middlebrooks, J. (**2000**). “Localization of brief sounds: effects of level and background noise,” J. Acoust. Soc. Am., **108**, 1834–1883.

Macpherson, E., and Middlebrooks, J. (**2002**). “Listener weighting of cues for lateral angle: the duplex theory of sound localization revisited,” J. Acoust. Soc. Am., **111**, 2219–2236.

Maddox, R. K., Pospisil, D. a, Stecker, G. C., and Lee, A. K. C. (**2014**). “Directing eye gaze enhances auditory spatial cue discrimination,” Curr. Biol., **24**, 748–52.

Magezi, D., and Krumbholz, K. (**2010**). “Evidence for opponent-channel coding of interaural time differences in human auditory cortex,” J. Neurophysiol., **104**, 1997–4004.

Magnusson, A. K., Park, T. J., Pecka, M., Grothe, B., and Koch, U. (**2008**). “Retrograde GABA signaling adjusts sound localization by balancing excitation and inhibition in the brainstem,” Neuron, **59**, 125–37.

Maier, J. K., Hehrmann, P., Harper, N. S., Klump, G. M., Pressnitzer, D., and McAlpine, D. (**2012**). “Adaptive coding is constrained to midline locations in a spatial listening task,” J. Neurophysiol.,.

Makous, J., and Middlebrooks, J. (**1990**). “Two-dimensional sound localization by human listeners,” J. Acoust. Soc. Am., **87**, 2188–2388.

Malhotra, S., Hall, A., and Lomber, S. (**2004**). “Cortical control of sound localization in the cat: unilateral cooling deactivation of 19 cerebral areas,” J. Neurophysiol., **92**, 1625–1668.

Malhotra, S., and Lomber, S. G. (**2007**). “Sound localization during homotopic and heterotopic bilateral cooling deactivation of primary and nonprimary auditory cortical areas in the cat,” J. Neurophysiol., **97**, 26–43.

Malhotra, S., Stecker, G., Middlebrooks, J., and Lomber, S. (**2008**). “Sound localization deficits during reversible deactivation of primary auditory cortex and/or the dorsal zone,” J. Neurophysiol., **99**, 1628–1670.

Malone, B. J., Scott, B. H., and Semple, M. N. (**2002**). “Context-dependent adaptive coding of interaural phase disparity in the auditory cortex of awake macaques,” J. Neurosci., **22**, 4625–38.

Malone, B. J., and Semple, M. N. (**2001**). “Effects of auditory stimulus context on the representation of frequency in the gerbil inferior colliculus,” J. Neurophysiol., **86**, 1113–30.

Manger, P. R., Restrepo, C. E., and Innocenti, G. M. (**2010**). “The superior colliculus of the ferret: Cortical afferents and efferent connections to dorsal thalamus,” Brain Res., **1353**, 74–85.

May, B. (**2000**). “Role of the dorsal cochlear nucleus in the sound localization behavior of cats,” Hear. Res., **148**, 74–161.

McAlpine, D. (**2005**). “Creating a sense of auditory space,” J. Physiol., **566**, 21–29.

McAlpine, D., Jiang, D., and Palmer, A. (**2001**). “A neural code for low-frequency sound localization in mammals,” Nat. Neurosci., **4**, 396–797.

McAlpine, D., Jiang, D., Shackleton, T. M., and Palmer, a R. (**2000**). “Responses of neurons in the inferior colliculus to dynamic interaural phase cues: evidence for a mechanism of binaural adaptation,” J. Neurophysiol., **83**, 1356–65.

Michalka, S. W., Kong, L., Rosen, M. L., Shinn-Cunningham, B. G., and Somers, D. C. (**2015**). “Short-Term Memory for Space and Time Flexibly Recruit Complementary Sensory-Biased Frontal Lobe Attention Networks,” Neuron, **87**, 882–892.

Mickey, B. J., and Middlebrooks, J. C. (**2003**). “Representation of auditory space by cortical neurons in awake cats,” J Neurosci, **23**, 8649–8663.

Mickey, B. J., and Middlebrooks, J. C. (**2005**). “Sensitivity of auditory cortical neurons to the locations of leading and lagging sounds,” J. Neurophysiol., **94**, 979–989.

Middlebrooks, J. C. (**2015**). “Sound localization,” Handb. Clin. Neurol., **129**, 99–116.

Middlebrooks, J. C., and Bremen, P. (**2013**). “Spatial stream segregation by auditory cortical neurons,” J. Neurosci., **33**, 10986–1001.

Middlebrooks, J. C., and Green, D. M. (**1991**). “Sound localization by human listeners,” Annu. Rev. Psychol., **42**, 135–159.

Middlebrooks, J. C., and Knudsen, E. I. (**1984**). “A neural code for auditory space in the cat’s superior colliculus,” J. Neurosci., **4**, 2621–2634.

Middlebrooks, J. C., and Onsan, Z. A. (**2012**). “Stream segregation with high spatial acuity,” J. Acoust. Soc. Am., **132**, 3896–911.

Middlebrooks, J., Clock, A., Xu, L., and Green, D. (**1994**). “A panoramic code for sound location by cortical neurons,” Science, **264**, 842–846.

Middlebrooks, J., and Pettigrew, J. (**1981**). “Functional classes of neurons in primary auditory cortex of the cat distinguished by sensitivity to sound location,” J Neurosci, **1**, 107–120.

Middlebrooks, J., Xu, L., Eddins, A., and Green, D. (**1998**). “Codes for sound-source location in nontonotopic auditory cortex,” J. Neurophysiol., **80**, 863–944.

Miller, L., and Recanzone, G. (**2009**). “Populations of auditory cortical neurons can accurately encode acoustic space across stimulus intensity,” Proc. Natl. Acad. Sci. U. S. A., **106**, 5931–5936.

Mills, A. (**1960**). “Lateralization of High-Frequency Tones,” J. Acoust. Soc. Am., **30**, 237–246.

Mills, A. W. (**1958**). “On the Minimum Audible Angle,” J. Acoust. Soc. Am., **45**, 237–246.

Moore, J., Tollin, D., and Yin, T. (**2008**). “Can measures of sound localization acuity be related to the precision of absolute location estimates?,” Hear. Res., **238**, 94–203.

Moshitch, D., and Nelken, I. (**2014**). “The Representation of Interaural Time Differences in High-Frequency Auditory Cortex,” Cereb. Cortex,.

Mrsic-Flogel, T., King, A., and Schnupp, J. (**2005**). “Encoding of virtual acoustic space stimuli by neurons in ferret primary auditory cortex,” J. Neurophysiol., **93**, 3489–3992.

Musial, P. ., Baker, S. ., Gerstein, G. ., King, E. ., and Keating, J. . (**2002**). “Signal-to-noise ratio improvement in multiple electrode recording,” J. Neurosci. Methods, **115**, 29–43.

Musicant, A. D. (**1990**). “Direction-dependent spectral properties of cat external ear: New data and cross-species comparisons,” J. Acoust. Soc. Am., **87**, 757.

Musicant, A. D., and Butler, R. A. (**1984**). “The influence of pinnae-based spectral cues on sound localization,” J. Acoust. Soc. Am., **75**, 1195.

Musicant, A. D., and Butler, R. A. (**1985**). “Influence of monaural spectral cues on binaural localization,” J. Acoust. Soc. Am., **77**, 202–8.

Nakamoto, K. T., Jones, S. J., and Palmer, A. R. (**2008**). “Descending projections from auditory cortex modulate sensitivity in the midbrain to cues for spatial position," J. Neurophysiol., **99**, 2347–2403.

Nakamoto, K. T., Shackleton, T. M., and Palmer, A. R. (**2010**). "Responses in the Inferior Colliculus of the Guinea Pig to Concurrent Harmonic Series and the Effect of Inactivation of Descending Controls," J. Neurophysiol., **103**, 2050–2061.

Neff, W. D., Fisher, J. F., Diamond, I. T., and Yela, M. (**1956**). "Role of auditory cortex in discrimination requiring localization of sound in space," J. Neurophysiol., **19**, 500–512.

Nelken, I., Chechik, G., Mrsic-Flogel, T. D., King, A. J., and Schnupp, J. W. H. (**2005**). "Encoding stimulus information by spike numbers and mean response time in primary auditory cortex," J. Comput. Neurosci., **19**, 199–221.

Nelken, I., and Young, E. (**1994**). "Two separate inhibitory mechanisms shape the responses of dorsal cochlear nucleus type IV units to narrowband and wideband stimuli," J. Neurophysiol., **71**, 2446–2462.

Niwa, M., Johnson, J. S., O'Connor, K. N., and Sutter, M. L. (**2013**). "Differences between primary auditory cortex and auditory belt related to encoding and choice for AM sounds," J. Neurosci., **33**, 8378–95.

Nodal, F. R., Bajo, V. M., and King, A. J. (**2012**). "Plasticity of spatial hearing: behavioural effects of cortical inactivation," J. Physiol., **590**, 3965–3986.

Nodal, F. R., Bajo, V. M., Parsons, C. H., Schnupp, J. W., and King, A. J. (**2008**). "Sound localization behavior in ferrets: comparison of acoustic orientation and approach-to-target responses," Neuroscience, **154**, 397–805.

Nodal, F. R., Kacelnik, O., Bajo, V. M., Bizley, J. K., Moore, D. R., and King, A. J. (**2010**). "Lesions of the auditory cortex impair azimuthal sound localization and its recalibration in ferrets," J. Neurophysiol., **103**, 1209–25.

Packer, A. M., Roska, B., and Häusser, M. (**2013**). "Targeting neurons and photons for optogenetics," Nat. Neurosci., **16**, 805–15.

Palmer, A. R., and King, A. J. (**1982**). "The representation of auditory space in the mammalian superior colliculus," Nature, **299**, 248–249.

Parker, A., and Newsome, W. (**1998**). "Sense and the single neuron: probing the physiology of perception," Annu. Rev. Neurosci., **21**, 227–277.

Parsons, C. H., Lanyon, R. G., Schnupp, J. W., and King, a J. (**1999**). "Effects of altering spectral cues in infancy on horizontal and vertical sound localization by adult ferrets," J. Neurophysiol., **82**, 2294–2309.

Pecka, M., Brand, A., Behrend, O., and Grothe, B. (**2008**). "Interaural time difference processing in the mammalian medial superior olive: the role of glycinergic inhibition," J. Neurosci., **28**, 6914–25.

Phillips, D. P., and Hall, S. E. (**2005**). "Psychophysical evidence for adaptation of central auditory processors for interaural differences in time and level," Hear. Res., **202**, 188–99.

Plakke, B., Hwang, J., and Romanski, L. M. (**2015**). "Inactivation of Primate Prefrontal Cortex Impairs Auditory and Audiovisual Working Memory," J. Neurosci., **35**, 9666–9675.

Quiroga, R. Q., Nadasdy, Z., and Ben-Shaul, Y. (**2004**). "Unsupervised spike detection and sorting with wavelets and superparamagnetic clustering," Neural Comput., **16**, 1661–1687.

Raposo, D., Sheppard, J. P., Schrater, P. R., and Churchland, a. K. (**2012**). "Multisensory Decision-Making in Rats and Humans," J. Neurosci., **32**, 3726–3735.

Rauschecker, J., and Tian, B. (**2000**). "Mechanisms and streams for processing of 'what' and 'where' in auditory cortex," Proc. Natl. Acad. Sci. U. S. A., **97**, 11800–11806.

Rayleigh, Lord (**1907**). "On our perception of sound direction," Philos. Mag. Ser. 6, **13**, 214–232.

Razavi, B., O'Neill, W. E., and Paige, G. D. (**2007**). "Auditory spatial perception dynamically realigns with changing eye position," J. Neurosci., **27**, 10249–58.

Reale, R. A., Jenison, R. L., and Brugge, J. F. (**2003**). "Directional sensitivity of neurons in the primary auditory (AI) cortex: effects of sound-source intensity level," J. Neurophysiol., **89**, 1024–38.

Recanzone, G. H., and Cohen, Y. E. (**2010**). "Serial and parallel processing in the primate auditory cortex revisited," Behav. Brain Res., **206**, 1–8.

Recanzone, G. H., Guard, D. C., Phan, M. L., and Su, T. K. (**2000**). "Correlation between the activity of single auditory cortical neurons and sound-localization behavior in the macaque monkey," J. Neurophysiol., **83**, 2723–2739.

Recanzone, G., Makhamra, S., and Guard, D. (**1998**). "Comparison of relative and absolute sound localization ability in humans," J. Acoust. Soc. Am., **103**, 1085–1182.

Rice, J., May, B., Spirou, G., and Young, E. (**1992**). "Pinna-based spectral cues for sound localization in cat," Hear. Res., **58**, 132–184.

Romanski, L., Tian, B., Fritz, J., Mishkin, M., Goldman-Rakic, P., and Rauschecker, J. (**1999**). "Dual streams of auditory afferents target multiple domains in the primate prefrontal cortex," Nat. Neurosci., **2**, 1131–1137.

Salminen, N. H., May, P. J. C., Alku, P., and Tiitinen, H. (**2009**). “A population rate code of auditory space in the human cortex,” PLoS One, **4**, e7600.

Salminen, N. H., Takanen, M., Santala, O., Alku, P., and Pulkki, V. (**2015**). “Neural realignment of spatially separated sound components,” J. Acoust. Soc. Am., **137**, 3356–3365.

Salminen, N. H., Takanen, M., Santala, O., Lamminsalo, J., Altoè, A., and Pulkki, V. (**2015**). “Integrated processing of spatial cues in human auditory cortex,” Hear. Res., **327**, 143–152.

Salminen, N. H., Tiitinen, H., Miettinen, I., Alku, P., and May, P. J. C. (**2010**). “Asymmetrical representation of auditory space in human cortex,” Brain Res., **1306**, 93–99.

Salminen, N. H., Tiitinen, H., Yrttiaho, S., and May, P. J. C. (**2010**). “The neural code for interaural time difference in human auditory cortex,” J. Acoust. Soc. Am., **127**, EL60–L65.

Schnupp, J., and Carr, C. (**2009**). “On hearing with more than one ear: lessons from evolution,” Nat. Neurosci., **12**, 692–699.

Schnupp, J., Nelken, I., and King, A. (**2011**). *Audtiory Neuroscience : Making Sense of Sound*,.

Scott, B. H., Malone, B. J., and Semple, M. N. (**2009**). “Representation of dynamic interaural phase difference in auditory cortex of awake rhesus macaques,” J. Neurophysiol., **101**, 1781–1799.

Shackleton, T. M., and Meddis, R. (**1992**). “The role of interaural time difference and fundamental frequency difference in the identification of concurrent vowel pairs,” J. Acoust. Soc. Am., **91**, 3579.

Shaw, E. A., and Vaillancourt, M. M. (**1985**). “Transformation of sound-pressure level from the free field to the eardrum presented in numerical form,” J. Acoust. Soc. Am., **78**, 1120–1123.

Slee, S. J., and Young, E. D. (**2011**). “Information conveyed by inferior colliculus neurons about stimuli with aligned and misaligned sound localization cues,” J Neurophysiol, **106**, 974–985.

Smith, A. L., Parsons, C. H., Lanyon, R. G., Bizley, J. K., Akerman, C. J., Baker, G. E., Dempster, A. C., et al. (**2004**). “An investigation of the role of auditory cortex in sound localization using muscimol-releasing Elvax,” Eur. J. Neurosci., **19**, 3059–3072.

Spitzer, M. W., and Semple, M. N. (**1993**). “Responses of inferior colliculus neurons to time-varying interaural phase disparity: effects of shifting the locus of virtual motion,” J. Neurophysiol., **69**, 1245–63.

Spitzer, M. W., and Semple, M. N. (**1998**). “Transformation of binaural response properties in the ascending auditory pathway: influence of time-varying interaural phase disparity,” J. Neurophysiol., **80**, 3062–3076.

Stange, A., Myoga, M. H., Lingner, A., Ford, M. C., Alexandrova, O., Felmy, F., Pecka, M., et al. (**2013**). “Adaptation in sound localization: from GABA(B) receptor-mediated synaptic modulation to perception,” Nat. Neurosci., **16**, 1840–7.

Stanislaw, H., and Todorov, N. (**1999**). “Calculation of signal detection theory measures,” Behav. Res. Methods, Instruments, Comput., **31**, 137–149.

Stecker, G. C., Harrington, I. a, Macpherson, E. a, and Middlebrooks, J. C. (**2005**). “Spatial sensitivity in the dorsal zone (area DZ) of cat auditory cortex,” J. Neurophysiol., **94**, 1267–1347.

Stecker, G. C., Mickey, B. J., Macpherson, E. a, and Middlebrooks, J. C. (**2003**). “Spatial sensitivity in field PAF of cat auditory cortex,” J. Neurophysiol., **89**, 2889–903.

Stecker, G. C., and Middlebrooks, J. C. (**2003**). “Distributed coding of sound locations in the auditory cortex,” Biol. Cybern., **89**, 341–349.

Stecker, G., Harrington, I., and Middlebrooks, J. (**2005**). “Location coding by opponent neural populations in the auditory cortex,” PLoS Biol., **3**, e78.

Steinmetz, M. A., Motter, B. C., Duffy, C. J., and Mountcastle, V. B. (**1987**). “Functional properties of parietal visual neurons: radial organization of directionalities within the visual field,” J. Neurosci., **7**, 177–91.

Stepien, I., Stepien, L., and Lubinska, E. (**1990**). “Function of dog’s auditory cortex in tests involving auditory location cues and directional instrumental response,” Acta Neurobiol. Exp. (Wars)., **50**, 1–12.

Stern, R., and Shear, G. (**1996**). “Lateralization and detection of low-frequency binaural stimuli: Effects of distribution of internal delay,” J. Acoust. Soc. Am., **100**, 2278–2288.

Stevens, S., and Newman, E. B. (**1936**). “The localization of actual sources of sound,” Am. J. Psychol., **48**, 297–306.

Sumner, C. J., and Palmer, A. R. (**2012**). “Auditory nerve fibre responses in the ferret,” Eur. J. Neurosci., **36**, 2428–39.

Thiran, A. B., and Clarke, S. (**2003**). “Preserved use of spatial cues for sound segregation in a case of spatial deafness,” Neuropsychologia, **41**, 1254–1261.

Thompson, G. C., and Cortez, A. M. (**1983**). “The inability of squirrel monkeys to localize sound after unilateral ablation of auditory cortex,” Behav. Brain Res., **8**, 211–216.

Tian, B., Reser, D., Durham, a, Kustov, a, and Rauschecker, J. P. (**2001**). “Functional specialization in rhesus monkey auditory cortex,” Science, **292**, 290–293.

Tollin, D. J., Populin, L. C., and Yin, T. C. T. (**2004**). “Neural correlates of the precedence effect in the inferior colliculus of behaving cats,” J. Neurophysiol., **92**, 3286–3297.

Tolnai, S., Litovsky, R. Y., and King, A. J. (**2014**). “The precedence effect and its buildup and breakdown in ferrets and humans,” J. Acoust. Soc. Am., **135**, 1406.

Town, S. M., Atilgan, H., Wood, K. C., and Bizley, J. K. (**2015**). “The role of spectral cues in timbre discrimination by ferrets and humans,” J. Acoust. Soc. Am., **137**, 2870.

Town, S. M., Wood, K. C., and Bizley, J. K. (**2013**). “Multisensory Integration in Ferret Auditory Cortex: Effects of Inactivating Visual Cortex,” Br. Soc. Audiol. Annu. Conf.,.

Tsunada, J., Lee, J. H., and Cohen, Y. E. (**2011**). “Representation of speech categories in the primate auditory cortex,” J. Neurophysiol., **105**, 2634–2646.

Vigneault-MacLean, B. K., Hall, S. E., and Phillips, D. P. (**2007**). “The effects of lateralized adaptors on lateral position judgements of tones within and across frequency channels,” Hear Res, **224**, 93–100.

Volgushev, M., Vidyasagar, T. R., Chistiakova, M., Yousef, T., and Eysel, U. T. (**2000**). “Membrane properties and spike generation in rat visual cortical cells during reversible cooling,” J. Physiol., **522**, 59–76.

Walker, K. M., Bizley, J. K., King, A. J., and Schnupp, J. W. (**2011**). “Multiplexed and robust representations of sound features in auditory cortex,” J. Neurosci., **31**, 14565–14641.

Walker, K. M., Schnupp, J. W., Hart-Schnupp, S. M., King, A. J., and Bizley, J. K. (**2009**). “Pitch discrimination by ferrets for simple and complex sounds,” J Acoust Soc Am, **126**, 1321–1335.

Wang, X. (**2007**). “Neural coding strategies in auditory cortex,” Hear. Res., **229**, 81–93.

Wang, X., Lu, T., Snider, R. K., and Liang, L. (**2005**). “Sustained firing in auditory cortex evoked by preferred stimuli,” Nature, **435**, 341–346.

Wang, Y., Gutfreund, Y., and Peña, J. L. (**2014**). “Coding space-time stimulus dynamics in auditory brain maps,” Front. Physiol., **5 APR**, 135.

Wang, Y., and Peña, J. L. (**2013**). “Direction selectivity mediated by adaptation in the owl’s inferior colliculus,” J. Neurosci., **33**, 19167–75.

Van Wanrooij, M. M., and Van Opstal, A. J. (**2007**). “Sound localization under perturbed binaural hearing,” J. Neurophysiol., **97**, 715–26.

Wightman, F. L., and Kistler, D. J. (**1999**). “Resolution of front-back ambiguity in spatial hearing by listener and source movement,” J Acoust Soc Am, **105**, 2841–2853.

Witte, R. S., and Kipke, D. R. (**2005**). “Enhanced contrast sensitivity in auditory cortex as cats learn to discriminate sound frequencies,” Brain Res. Cogn. Brain Res., **23**, 171–84.

Wood, K. C., and Bizley, J. K. (**2015**). “Relative sound localisation abilities in human listeners,” J. Acoust. Soc. Am., **138**, 674–686.

Woods, T. M., Lopez, S. E., Long, J. H., Rahman, J. E., and Recanzone, G. H. (**2006**). “Effects of Stimulus Azimuth and Intensity on the Single-Neuron Activity in the Auditory Cortex of the Alert Macaque Monkey,” J. Neurophysiol., **96**, 3323–3337.

Woodworth, R., and Schlosberg, H. (**1962**). *Experimental Psychology*, Holt, 348-361 pages.

Yin, P., Fritz, J. B., and Shamma, S. A. (**2010**). “Do ferrets perceive relative pitch?,” J. Acoust. Soc. Am., **127**, 1673–1753.

Yin, T. C., and Chan, J. C. (**1990**). “Interaural time sensitivity in medial superior olive of cat,” J. Neurophysiol., **64**, 465–488.

Yost, W. A., and Zhong, X. (**2014**). “Sound source localization identification accuracy: bandwidth dependencies,” J. Acoust. Soc. Am., **136**, 2737–46.

Zalocusky, K., and Deisseroth, K. (**2013**). “Optogenetics in the behaving rat: integration of diverse new technologies in a vital animal model,” Optogenetics, **1**, 1–17.

Zatorre, R., and Penhune, V. (**2001**). “Spatial localization after excision of human auditory cortex,” J. Neurosci., **21**, 6321–6329.

Zhou, B. (**1992**). “Characterization of external ear impulse responses using Golay codes,” J. Acoust. Soc. Am., **92**, 1169.

Zikopoulos, B., and Barbas, H. (**2006**). “Prefrontal projections to the thalamic reticular nucleus form a unique circuit for attentional mechanisms,” J. Neurosci., **26**, 7348–61.

Zurek, P. M. (**1992**). “Binaural advantages and directional effects in speech intelligibility,” In G. A. Studebaker and I. Hochberg (Eds.), Acoust. Factors Affect. Hear. Aid Performance, 2nd Ed., Allyn & Bacon, Boston, MA, 2nd ed., pp. 255–276.


LEVEL
SPIKE
TRAINED
CONSISTENT
PRESENT
USED
SPEAKER
ITD
DATA
FIRING
TIME
STUDIES
TASK
STIMULI
INFORMATION
DIFFERENT
MIDLINE
SPATIAL
USING
MODEL
FERRET
SOUND
KHZ
SPOUT
BPN
MS
MAY
PERFORM
TOPOGRAPHIC
PRESENTED
REFERENCE
FOUND
CHANGES
CONTRALATERAL
DIRECTION
RESPONSE
STUDY
NEURONS
EFFECT
RELATIVE
DISCRIMINATION
CONDITION
PERFORMANCE
CHANGE
LOCATIONS
ILD
ITDS
TWO-CHANNEL
NOISE
BBN
UNITS
PERFORMED
SHOWN
SHOWED
CUES
CORTEX
ACROSS
TESTED
BRAIN
ANALYSIS
LEFT
EXPERIMENT
STIMULUS
LOCALISATION
RIGHT
SIGNIFICANT
INACTIVATION
MODELS
SPACE
DB
ANIMAL
SPEAKERS
AREAS
MEAN
CONDITIONS
TESTING
FREQUENCY
AVAILABLE
SPECTRAL
LPN
TARGET
RATE
HEAD
PATTERN
AUDITORY
NEURAL
A1
TUNING
COOLING
SIDE
MADE
ENCODING
WORK