PET investigations in focal epilepsy

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Abstract

Epilepsy is the most common serious disease of the brain. Positron Emission Tomography (PET) provides information on neurotransmitter systems and the pathophysiology of the epilepsies. Evaluation of PET together with anatomical information from high quality MRI correlates structure and function.

Aims:
- To develop methods to anatomically label neuroimaging datasets
- To investigate and quantify GABA<sub>A</sub> receptor abnormalities in temporal lobe epilepsy (TLE) due to hippocampal sclerosis (HS), malformations of cortical development (MCD) and patients with localisation-related epilepsy and normal MRI
- To investigate and quantify abnormalities of opioid receptors in MCD

Methods: Atlases of normal neuroanatomy were created from 21 high resolution MRI datasets. [<sup>11</sup>C] flumazenil PET scans of 48 controls, 15 preoperative patients with HS, 10 patients with MCD, 18 patients with TLE and normal MRI, and 44 patients with neocortical localisation-related epilepsy and normal MRI, as well as [<sup>11</sup>C] diprenorphine PET scans of 20 controls and 15 patients with MCD, were analysed with statistical parametric mapping (SPM), a volume-of-interest (VOI) approach with partial volume effect correction (PVC), or both.

Findings: A method for automatical anatomical labelling and a probabilistic anatomical atlas were developed.

8/15 patients with HS had abnormalities of [<sup>11</sup>C] flumazenil binding outside the hippocampus. White matter (WM) binding correlated with neuron content.

MCD patients showed abnormalities of [<sup>11</sup>C] flumazenil binding within lesions and in the adjacent/overlying cortex.

Patients with TLE and normal MRI had increased [<sup>11</sup>C] flumazenil binding in the temporal lobe WM, indicating microdysgenesis, in addition to ipsilateral>contralateral hippocampal decreases. 33/44 patients with neocortical localisation-related epilepsy and normal MRI had abnormalities, often WM increases suggestive of migration disturbances. In some, the PET findings were surgically useful.

More than three quarters of MCD patients had abnormalities of [<sup>11</sup>C] diprenorphine binding, implying abnormalities of opioid neurotransmission which could be a novel target for pharmacological intervention.
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<td>ACPC</td>
<td>anterior-posterior commissure</td>
</tr>
<tr>
<td>ADNFLE</td>
<td>autosomal-dominant nocturnal frontal lobe epilepsy</td>
</tr>
<tr>
<td>AED(s)</td>
<td>antiepileptic drug(s)</td>
</tr>
<tr>
<td>AMPA</td>
<td>alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate</td>
</tr>
<tr>
<td>AMT</td>
<td>alpha-methyl-L-tryptophan</td>
</tr>
<tr>
<td>Arg</td>
<td>arginine</td>
</tr>
<tr>
<td>ARSAC</td>
<td>Administration of Radioactive Substances Advisory Committee</td>
</tr>
<tr>
<td>BGO</td>
<td>bismuth germanate</td>
</tr>
<tr>
<td>BH</td>
<td>band heterotopia</td>
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<tr>
<td>B\textsubscript{max}</td>
<td>available receptor density</td>
</tr>
<tr>
<td>CA</td>
<td>cornu ammonis</td>
</tr>
<tr>
<td>CBF</td>
<td>cerebral blood flow</td>
</tr>
<tr>
<td>cBZR</td>
<td>central benzodiazepine receptor</td>
</tr>
<tr>
<td>Cl\textsuperscript{-}</td>
<td>chloride ion</td>
</tr>
<tr>
<td>cm</td>
<td>centimeters</td>
</tr>
<tr>
<td>CMV</td>
<td>cytomegalovirus</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>CPS</td>
<td>complex partial seizure(s)</td>
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<tr>
<td>CPU</td>
<td>central processing unit</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>CT</td>
<td>(X-ray) computed tomography</td>
</tr>
<tr>
<td>CV</td>
<td>coefficient of variation</td>
</tr>
<tr>
<td>DAMGO</td>
<td>[D-Ala\textsuperscript{2},N-Me-Phe\textsuperscript{4},Gly-ol\textsuperscript{5}]-enkephalin</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>DNET</td>
<td>dysembryoplastic neuro-epithelial tumour</td>
</tr>
<tr>
<td>DPDPE</td>
<td>[D-Pen2,5]-enkephalin</td>
</tr>
<tr>
<td>DTI</td>
<td>Diffusion Tensor Imaging</td>
</tr>
<tr>
<td>DWI</td>
<td>Diffusion-Weighted Imaging</td>
</tr>
<tr>
<td>ECoG</td>
<td>electrocorticography</td>
</tr>
<tr>
<td>EDE</td>
<td>effective dose equivalent</td>
</tr>
<tr>
<td>EEG</td>
<td>Electroencephalogram</td>
</tr>
<tr>
<td>FC</td>
<td>febrile convulsion(s)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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<tr>
<td>FCD</td>
<td>focal cortical dysplasia</td>
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<tr>
<td>$[^{18}\text{F}]\text{DG}$</td>
<td>$[^{18}\text{F}]$-fluorodeoxyglucose</td>
</tr>
<tr>
<td>FLAIR</td>
<td>fluid attenuation inversion recovery</td>
</tr>
<tr>
<td>fMRI</td>
<td>functional Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>FOV</td>
<td>field of view</td>
</tr>
<tr>
<td>FPEVF</td>
<td>familial partial epilepsy with variable foci</td>
</tr>
<tr>
<td>FWHM</td>
<td>full width at half maximum</td>
</tr>
<tr>
<td>GABA</td>
<td>gamma-aminobutyric acid</td>
</tr>
<tr>
<td>GAD</td>
<td>glutamic acid decarboxylase</td>
</tr>
<tr>
<td>GE</td>
<td>General Electric</td>
</tr>
<tr>
<td>GM</td>
<td>grey matter</td>
</tr>
<tr>
<td>GTCS</td>
<td>generalised tonic-clonic seizure(s)</td>
</tr>
<tr>
<td>HCV</td>
<td>hippocampal volume</td>
</tr>
<tr>
<td>His</td>
<td>histidine</td>
</tr>
<tr>
<td>HMPAO</td>
<td>99m-technetium-d,1-hexamethylpropyleneamine oxime</td>
</tr>
<tr>
<td>HS</td>
<td>hippocampal sclerosis</td>
</tr>
<tr>
<td>5-HT</td>
<td>serotonin</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>ICV</td>
<td>intracranial volume</td>
</tr>
<tr>
<td>IDEX</td>
<td>iododexetimide</td>
</tr>
<tr>
<td>ILAE</td>
<td>International League Against Epilepsy</td>
</tr>
<tr>
<td>IQ</td>
<td>intelligence quotient</td>
</tr>
<tr>
<td>IRF</td>
<td>Impulse Response Function</td>
</tr>
<tr>
<td>JME</td>
<td>Juvenile Myoclonic Epilepsy</td>
</tr>
<tr>
<td>$K_\text{d}$</td>
<td>receptor affinity</td>
</tr>
<tr>
<td>MAM</td>
<td>methylazoxymethanol</td>
</tr>
<tr>
<td>MAO-B</td>
<td>monoamine oxidase type B</td>
</tr>
<tr>
<td>MCD</td>
<td>malformation(s) of cortical development</td>
</tr>
<tr>
<td>mm</td>
<td>millimeter(s)</td>
</tr>
<tr>
<td>MNI</td>
<td>Montreal Neurological Institute</td>
</tr>
<tr>
<td>MRA</td>
<td>magnetic resonance angiography</td>
</tr>
<tr>
<td>MRC</td>
<td>Medical Research Council</td>
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<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
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<tr>
<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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</table>
mSv milli-Sievert
NEMA National Electrical Manufacturers Association
NEX number of excitations
NMDA N-methyl-D-aspartate
NMPB N-methyl-4-piperidyl benzylate
ns nanoseconds
NSE National Society for Epilepsy
PD proton density
SD standard deviation
SNH subependymal nodular heterotopion/heterotopia
SPECT Single Photon Emission Computed Tomography
SPM statistical parametric mapping
SPS simple partial seizure(s)
p. page
PET Positron Emission Tomography
PNH periventricular nodular heterotopion/heterotopia
PSF point spread function
rCBF regional cerebral blood flow
RNA ribonucleic acid
ROI region of interest
SPECT Single Photon Emission Computed Tomography
SPM statistical parametric mapping, statistical parametric map
TE time of echo
TR time of repetition
UK United Kingdom
VBM voxel-based morphometry
V_d volume-of-distribution
VOI volume of interest
WM white matter
1 INTRODUCTION AND BACKGROUND

1.1 The epilepsies

1.1.1 Classification of epileptic seizures and syndromes

Epilepsy is generally defined as a condition characterised by the occurrence of recurrent epileptic seizures of primary cerebral origin. Epileptic seizures consist of a paroxysmal dysfunction of cerebral neurophysiological function involving synchronisation of nerve cell firing and, in general, have a correlate on the electroencephalogram (EEG).

Classification of epilepsy is fraught with difficulty. There is an enormous variety of clinical manifestations of seizures; unless video telemetry data is obtained when the clinical events are frequent enough, the physician has to rely on the necessarily incomplete account of the seizures by the patients themselves or by eye witnesses whose accounts may be biased and inaccurate (Rugg-Gunn et al., 2001b). Aetiologies are equally varied and may not be known; some EEG patterns (Jasper and Kershman, 1941) are specific enough so they can aid in the classification; some signs and symptoms cluster together in an age-related fashion, forming age-specific syndromes; and there is some evidence that other characteristics of a person's epilepsy like seizure frequency at onset might be usefully included in classifications as well (Kwan and Brodie, 2000).

The International League Against Epilepsy (ILAE) first introduced a classification of seizure type in 1969 of which the revised version is currently in use (Commission on Classification and Terminology of the International League against Epilepsy, 1981). This classification distinguishes three groups of seizures: generalised, partial and unclassifiable, due to lack of data. Generalised seizures are further divided into absence (typical and atypical), myoclonic, clonic, tonic, tonic-clonic (GTCS) and atonic. Partial seizures, i.e. seizures beginning focally or locally, are divided into simple partial (SPS) without impairment of consciousness and complex partial (CPS) with alteration of consciousness. If seizures begin as partial seizures and then spread to become generalised, they are termed secondarily generalised.
This classification scheme has certainly been useful in providing a common language for the summarising description of a seizure's semiology. EEG data was taken into account whereas age was not. Furthermore, the absence of readily available neuroimaging methods at the time of redaction of the classification is reflected in the absence of aetiology and anatomical location.

The ILAE proposed a new scheme in 1989 which takes into account seizure type, EEG, prognostic, pathophysiological and aetiological data, the classification of the epilepsies and epilepsy syndromes and related seizure disorders (Commission on Classification and Terminology of the International League against Epilepsy, 1989). An epilepsy syndrome is a disorder characterised by a cluster of signs and symptoms which tend to occur together. The main subdivision is into focal or localisation-related epilepsies and epilepsy syndromes on the one hand and generalised epilepsies and epilepsy syndromes on the other. Two further categories are recognised in this classification: one of epilepsies and epilepsy syndromes where a categorisation into localisation-related or generalised is not possible, due to either the occurrence of both generalised and focal seizures or due to the absence of clearly distinguishing features; and another of special syndromes, e.g. situation-related seizures.

Each main classification (localisation-related and generalised) is further subdivided into idiopathic and symptomatic forms. Cryptogenic epilepsies and epilepsy syndromes are those in which a symptomatic aetiology is suspected but the aetiology is not known. More than a decade has passed since this classification. The outstanding advances in neuroimaging have since allowed identification of an increasing number of underlying aetiologies and thus markedly decrease the proportion of epilepsies and epilepsy syndromes which are considered cryptogenic. The aetiology determines in part the prognosis (Semah et al., 1998), and it is to be expected that advances in neuroimaging (Commission on Neuroimaging of the International League Against Epilepsy, 1997; Duncan, 1997a) - and therefore in the correlation between semiology and anatomy which would not have been possible previously - will be incorporated in future classifications.

All conditions investigated in this thesis are localisation-related epilepsies, either symptomatic (temporal lobe epilepsy (TLE) due to hippocampal sclerosis (HS),

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localisation-related epileptic syndromes due to malformations of cortical development) or cryptogenic (localisation-related epileptic syndromes with normal magnetic resonance imaging (MRI)).
1.2 TLE and hippocampal sclerosis

1.2.1 Introduction

Temporal lobe epilepsy (TLE) is the most common form of localisation-related epilepsy, characterised by recurrent simple partial seizures and complex partial seizures (Commission on Classification and Terminology of the International League against Epilepsy, 1989). Mesial temporal lobe epilepsy (mTLE) is a specific chronic epilepsy which is associated with hippocampal sclerosis (HS) and regarded as a discrete syndrome (Engel, 1992; Wieser, 1980). These patients typically have medically refractory seizures (Stephen et al., 2001) but a predictably good outcome following limited temporal lobe resections, with some 70% becoming seizure free after surgery (Berkovic et al., 1995). While cases of neocortical TLE occur, mTLE is by far more frequent, and HS is the underlying aetiology in about 60% of cases referred for epilepsy surgery (Babb et al., 1984).

1.2.2 Clinical features of mTLE

Clinically (Wieser et al., 1993), patients with HS have an increased incidence of epilepsy in their family history. There is an increased incidence of febrile convulsions (FC), particularly prolonged FC during the first year of life. This may be due to some factor predisposing the child to both FC and later HS (Baulac et al., 1998; Fernandez et al., 1998), or FC may indeed be the cause for HS (Holthausen, 1994; Wiesmann et al., 1997). The majority of children who suffer from FC, however, will not develop epilepsy (Annegers et al., 1987; Nelson and Ellenberg, 1978; Verity and Golding, 1991). Onset of non-febrile seizures is typically in the second half of the first decade of life, often initially with secondarily generalised seizures, and there may be a seizure-free period of several years with antiepileptic drugs.

1.2.3 Seizure types in mTLE

Seizure types (Delgado-Escueta et al., 1982; Duncan and Sagar, 1987; Wieser and Hauser, 1987) are auras (SPS, no loss of consciousness) which may occur in isolation, CPS, often evolving from SPS, and sometimes secondarily generalised seizures. Seizures are generally longer than frontal lobe seizures, typically lasting longer than 2 minutes, have a more gradual onset and offset and a slower evolution.
Auras typically comprise visceral, cephalic, gustatory, dysmnestic, affective, perceptual or autonomic symptoms. Ascending epigastric sensations are particularly common. Autonomic symptom, if present, include changes in skin colour, blood pressure, heart rate, and pupil size. There is usually no or only severely reduced speech, but occasionally repetitive vocalisation with formed words may occur if the seizure originates in the non-dominant temporal lobe. Of affective symptoms, fear is the commonest and can be very intense; its occurrence is thought to indicate amygdala involvement. Other affective symptoms occurring in TLE include depression, anger and irritability and, more rarely, elation, erotic thoughts, serenity or exhilaration.

Absence (altered consciousness) with motor arrest, the 'motionless stare' (Williams et al., 1987), is a prominent feature of the progression of the aura (SPS) to a CPS. When the aura is short, unreported or absent, this is often the first manifestation of a seizure noticed by eyewitnesses.

Automatisms are defined as 'a more or less coordinated involuntary motor activity occurring during the state of clouding of consciousness either in the course of, or after, an epileptic seizure, usually followed by amnesia of the event' (Delgado-Escueta et al., 1982). Automatisms in TLE are usually oro-alimentary or gestural. The former include lip-smacking, chewing and swallowing and the latter include fumbling, fidgeting, repetitive motor actions, undressing, walking, and sexually-directed actions. They can sometimes be prolonged or semi-purposeful, e.g. rearranging items on a desk. Limb automatisms are usually ipsilateral to the focus, with contralateral dystonic posturing. Vocalisation of identifiable words suggests a non-dominant seizure focus. For both the absence and the automatisms, amnesia is the rule even though a patient may not recognize that consciousness was fully lost.

After the seizure itself, post-ictal confusion and headache are common. Dysphasia, if present, is a useful lateralising sign indicating seizure origin in the dominant temporal lobe. Another useful lateralising sign in the post-ictal phase is nose-rubbing which is ipsilateral to the focus in 90% of cases (Geyer et al., 1999a). Peri-ictal headache is common, occurring in approximately half of patients, often has migraine-like characteristics and is ipsilateral to the focus in 90% of cases (Bernasconi et al., 2001a).
Secondary generalisation is much less common than in extratemporal lobe epilepsy (particularly frontal lobe epilepsy). Psychiatric or behavioural disturbances are more commonly part of the epileptic syndrome than in extratemporal epilepsy.

1.2.4 EEG features in mTLE

The EEG in mTLE typically shows anterior or mid-temporal spikes. These are best shown on superficial sphenoidal or zygomatic electrodes. Intermittent or persistent slow activity may be present over the temporal lobes. The EEG abnormalities can be unilateral or bilateral, but are usually more marked ipsilateral to the focus.

1.2.5 Imaging features of mTLE

MRI imaging will frequently reveal the cause of the mTLE. By far the most frequently encountered lesion is hippocampal sclerosis which is demonstrated by unilateral decrease in hippocampal volume and increase in signal on T2-weighted MRI scans (Jackson et al., 1990; Van Paesschen et al., 1997). Awareness of both normal anatomy (Cook et al., 1992; Duvernoy, 1998; Klingler, 1948; Niemann et al., 2000a) and relevant imaging abnormalities, advances in MRI instrumentation, acquisition sequences and post-acquisition processing of imaging data all contribute to the ability of MRI to identify HS (Jackson et al., 1990). The hippocampus is a curved structure with its concave surface facing the brainstem and with its longitudinal axis at approximately 35° to the traditionally used axial imaging plane for both X-ray CT and MRI, the orbito-meatal line (Duvernoy, 1998). The sensitivity to find abnormalities can be increased when coronal images are acquired perpendicular to the long axis of the hippocampus (Press et al., 1989). This is achieved by determining the imaging planes on a sagittal scout image: the axial plane of the hippocampus is in the line joining the base of the splenium of the corpus callosum with the inferoposterior border of the frontal lobe. The ensuing coronal plane is perpendicular to this, parallel to the anterior border of the brainstem. The sensitivity can be further increased if volume and T2 relaxation times are quantified with suitable methods (Cook et al., 1992; Duncan et al., 1996b; Free et al., 1995).

1.2.6 Pathology and pathophysiology in mTLE/HS

HS is typically unilateral and diffuse (Quigg et al., 1997) but more localised damage may occur (Woermann et al., 1998). Even if hippocampal damage is bilateral the atrophy is often asymmetrical, with greater cell loss on the epileptogenic side. The
classical pattern of neuronal loss and gliosis affects the different hippocampal subfields differently. Expressed in the nomenclature introduced by Lorente de Nó (Lorente de Nó, 1934), neuronal loss and gliosis is most severe in the CA (cornu ammonis) sector CA1 (Sommer, 1880). There is often an abrupt transition to the preserved neurones of the adjacent (pro)subiculum. Loss of pyramidal cells is less severe in CA3 and CA4, followed by hilar and granule cells. CA2 pyramidal cells are typically resistant to damage (Spielmeyer, 1925). A special form of HS is end folium sclerosis in which hippocampal damage is centered on the hilus and dentate gyrus. It is important as it is usually undetectable on MRI, associated with a later onset of onset and with a poorer outcome after epilepsy surgery (Engel, 1996). There is little doubt that HS may be acquired, and some direct evidence for developing HS following status epilepticus (Nohria et al., 1994; Wieshmann et al., 1997). It is less clear, however, if there is commonly progression once HS is established (Van Paesschen et al., 1998).

While the typical picture of HS is unequivocal and striking, several uncertainties remain, regarding the natural history/aetiology and pathogenesis of HS. Falconer et al. first suggested that prolonged febrile convulsions in infancy or early childhood may lead to the development of TLE with intractable CPS (Falconer et al., 1964) in susceptible individuals, and a strong association between febrile convulsions and HS has subsequently been confirmed (Kuks et al., 1993). Thus, there seems to be a period of vulnerability during which seizures can cause unilateral HS in later life, and the same seems to be true for the relationship between bilateral HS and febrile convulsions or other insults (Barr et al., 1997; Free et al., 1996; Van Paesschen et al., 1997). How HS develops, however, is far from clear. The original hypothesis of a selective vulnerability of CA1, CA3 and CA4 to ischemia/hypoxia due to a particular vascular supply (Pfeifer, 1930) has been abandoned in favour of the excitotoxic hypothesis which states that inundation with excitatory amino acid neurotransmitters (glutamate, aspartate) leads to calcium-mediated neuronal death (Sloviter and Dempster, 1985). CA2 might be relatively resistant due to a higher abundance of calcium binding proteins in its pyramidal cells (Sloviter et al., 1991). Still, it is unclear why only certain children with appropriate insult develop HS. One explanation may be the existence of underlying malformations of the hippocampus which may predispose to HS or potentially to both febrile convulsions and HS (Baulac et al., 1998; Fernandez et al., 1998). While malformations including the
hippocampus can certainly only explain a small proportion of the HS cases seen in adult epileptology, more subtle disorganisation of the granule cells of the dentate gyrus have been described in approximately a third of HS specimens (Harding et al., 1998). As there is proliferation of granule cell precursors in the dentate gyrus of adult primates (Gould et al., 1998) and humans (Eriksson et al., 1998a), and as postnatal migration and differentiation of primitive granule cells have been demonstrated in humans up to 15 months of age (Mathern et al., 1994), such disorganisations could be due to disturbances in the normal migration of granule cells and then in turn lead to aberrant connections. There is also the intriguing possibility of neurogenesis in the adult dentate gyrus, possibly even in response to seizures (Sankar et al., 2000) which could then lead to aberrant connections and thus be part of the pathogenetic process (Scharfman et al., 2000). Animal models still give equivocal answers but as inhibition of neurogenesis fails to prevent epileptogenesis it seems established that neurogenesis cannot be the sole explanation (Parent et al., 1999).

A role for genetic factors is suggested by the existence of familiar malformations with HS (see above) as well as the existence of families with several members with HS, some of whom may have a very mild phenotype with single seizures or febrile seizures only (Kobayashi et al., 2001).

A well-established finding in both the kindling and kainic acid model of mTLE in rats and in human specimens, however, is mossy fibre sprouting. Mossy fibres are the axons of the glutamatergic, excitatory granule cells in the dentate gyrus (Ramón y Cajal, 1903). They terminate at the dendrites of the pyramidal neurons of the neighbouring fields CA3 and CA4. Sprouting of the mossy fibres results in additional aberrant innervation of other granule cells in the dentate gyrus and of pyramidal neurons in CA1 and CA2, resulting in feedback and feedforward excitation (Babb et al., 1992; Mathern et al., 1995). The resulting recurrent excitation is generally considered to be sufficient to maintain hyperexcitability in the hippocampus, leading to the development of recurrent seizures. Mossy fiber sprouting may either be initiated following the loss of hilar cells which would normally act as a target or may be independent of hilar cell loss and be induced by neurotrophic growth factors (Adams et al., 1997). Neurotrophins that may play a role, as their mRNAs have been found to be elevated in human dentate gyrus granule cells from HS specimens and
correlate with mossy fibre sprouting, are BDNF, NGF and NT-3 (Mathern et al., 1997b).

Subsets of *inhibitory interneurons* are labelled by various calcium-binding proteins: calbindin D-28k, parvalbumin and calretinin (Andressen et al., 1993). As mentioned above, CA2 pyramidal neurons contain calbindin D-28k which may confer them their relative resistance to damage in epilepsy (Leranth and Ribak, 1991). Similarly, calretinin immunoreactive interneurons have been shown to be preserved or even increased in HS in a quantitative study (Blumcke et al., 1996a). In sclerotic tissue, however, absolute determination of neuronal numbers and densities is always difficult due to the considerable reduction of the reference volume (Van Paesschen and Revesz, 1998), and a number of calretinin immunoreactive interneurons have been shown to have the morphology of Cajal-Retzius cells (Blumcke et al., 1999) (see chapter on Malformations of Cortical Development) which may indicate a disturbed formation of the archicortex rather than persistence of a subclass of inhibitory interneurons. Sloviter *et al.* have proposed the "dormant basket (inhibitory interneuron) cell" hypothesis, stating that loss of excitatory input to GABAergic interneurons results in a net disinhibition of granule cells (Sloviter, 1991). There is indeed experimental evidence that direct stimulation of basket cells in epileptogenic tissue in rats leads to normal inhibitory postsynaptic potentials in the absence of excitatory neurotransmission, whereas inhibitory postsynaptic potentials evoked indirectly by stimulation of excitatory inputs to basket cells were reduced compared to controls (Bekenstein and Lothman, 1993).

Finally, alterations of *neurotransmitter systems* have been investigated in HS. Immunohistochemical studies have shown a reduction of N-methyl-D-aspartate (NMDA) receptor 1 and AMPA (GluR1/2) (Blumcke et al., 1996b) as well as GABA_\text{A} receptor (Wolf *et al.*, 1994) which paralleled neuronal loss in all hippocampal subfields studied. An autoradiographic study, however, demonstrated that loss of the GABA_\text{A} receptor in the CA1 subfield is over and above the level of neuronal loss (Hand *et al.*, 1997). A further autoradiographic study using $^{125}$I-iomazenil and cell counts in resected human HS specimens showed a reduction of central benzodiazepine receptor (cBZR) binding greater than the loss of neurons but while this was present as a tendency in all subfields it was only significant over and above loss of neurons in the CA1 and CA3 subfields (Johnson *et al.*, 1992). A study using immunocytochemical staining in the limbic status model in rats has shown
increased staining for both excitatory AMPA and NMDA receptors and inhibitory GABA_A receptors in the fascia dentata molecular layer (Mathern et al., 1997c). In situ hybridisation techniques have suggested an increase in hippocampal pyramidal and granule cell AMPA (GluR1/2) and NMDA (NMDAR1/2) receptor mRNA in human specimens (Mathern et al., 1997a). In a similar vein, quantitative autoradiographic analyses of glutamate receptor subtypes have shown an increase in receptor density in CA1, CA4 and the molecular layer in human specimens (Brines et al., 1997). GABA_B receptor mRNA and autoradiographically measured GABA_B receptor binding were both increased per remaining neuron in human hippocampal sclerosis specimens, compared to postmortem controls (Billinton et al., 2001a; Billinton et al., 2001b). Neuroreceptor transporter systems may also show changes in human HS (Mathern et al., 1999). Finally, within receptor classes, there may be subunit reorganisations which may have functional significance and may predate the development of epilepsy in rats (Brooks-Kayal et al., 1998).

Taken together, these results suggest a shift of the balance between excitation and inhibition towards excitation, due to cell population alterations, "rewiring" of remaining cells, and changes in neurotransmitter/receptor systems.

### 1.2.7 Clinical features of neocortical TLE

There is considerable overlap between the clinical and EEG features of mesiobasal and lateral temporal lobe epilepsy (Wieser and Muller, 1987; Williams et al., 1987). Some authors have advocated further subclassifications of partial seizures arising from the temporal lobes (opercular, temporal polar, temporo-neocortical posterior and basal or limbic) (Wieser, 1983) but the semiological overlap and the frequent spread of discharges from one area to another make it debatable whether such a detailed classification scheme is clinically useful.

In neocortical TLE, there is usually a detectable structural pathology, for example glioma, angioma, hamartoma, dysembryoplastic neuroepithelial tumour, other benign tumours, other malformations of cortical development or post-traumatic change. In contrast to mTLE, there is no association with a history of febrile convulsions. Clinically, consciousness may be preserved for longer than in a typical mesial temporal seizure. The typical aura includes hallucinations which are often structured and of visual, auditory, gustatory or olfactory forms, or illusions of size, shape, weight, distance or sound. Discharges arising in the superior temporal gyrus may lead to simple auditory phenomena such as humming, buzzing or hissing. Seizures
arising from the Sylvian region can start with olfactory sensations; these are usually unpleasant and difficult to describe. More complex hallucinatory or illusory states occur with seizure arising from association areas and may include structured visual hallucinations, complex visual patterns, musical sounds and speech. While illusions of size (i.e. macropsia or micropsia) and shape, distance, weight, distance or sound usually occur with parieto-occipital discharges, they may be seen in neocortical TLE as well, particularly with discharges arising in the posterior temporal regions. Affective, visceral or psychic auras may occur but are less common than in mTLE. The automatisms can be unilateral and have more prominent motor manifestations than in mTLE. Post-ictal phenomena, amnesia for the attack and psychiatric comorbidity are probably equally common in neocortical temporal epilepsy as in mTLE.

1.2.8 EEG features of neocortical TLE

The interictal EEG in neocortical TLE often shows spikes over the temporal region, maximal over the lateral convexity rather than inferomesial electrodes. Hippocampal volumes and T2 measurements on MRI are usually normal, in contradistinction to mTLE. MRI will reliably demonstrate extrahippocampal structural lesions responsible for the epilepsy even though imaging studies may be normal in some patients.

1.2.9 Imaging features of neocortical TLE

CT may sometimes show an underlying structural lesion in mTLE, particularly if the lesion is calcified. As for mTLE, however, MRI is much more sensitive and the imaging method of choice (Kuzniecky et al., 1993b; Sperling et al., 1986; Theodore et al., 1986a).

1.2.10 Other neocortical epilepsies

The other forms of neocortical epilepsies and their underlying causes are discussed in chapters 1.4 (p. 69) and 1.6.1.3 (p. 99).
1.3 Malformations of cortical development (MCD)

1.3.1 Introduction

Malformations of cortical development (MCD) are due to disorders of neuronal and glial proliferation, abnormal neuronal migration, or abnormal postmigrational cortical organisation, which in turn are the consequence of a variety of inherited and acquired insults. Since the availability of MRI, MCD are increasingly recognised as a cause for medically refractory epilepsies. MRI-visible MCD are very rare in healthy controls, e.g. 1/46 in a National Hospital series (Raymond et al., 1995) and in 0/170 in the NSE MRI study of structural brain abnormalities in adult epilepsy patients and healthy controls (Everitt et al., 1998), and occur only in 3% of patients who have a first seizure (Everitt et al., 1998). They are important in epileptology, however, as they are present in 15-20% of adults with intractable partial seizures, some of whom are candidates for epilepsy surgery (Kuzniecky and Jackson, 1997). Surgical resection of areas of MCD in patients with drug resistant epilepsy, however, results in at best about 40% of patients becoming seizure free (Cascino et al., 1993a; Sisodiya, 2000), compared with 70% in patients with hippocampal sclerosis (Berkovic et al., 1995). A possible explanation is that the area of functional cortical abnormality may be greater than the structural abnormality shown by conventional magnetic resonance imaging (MRI) techniques (Richardson et al., 1997a; Richardson et al., 1996; Sisodiya et al., 1995).

Animal models have shown functional abnormalities in cortex adjacent to MCD, possibly due to formation of aberrant thalamocortical connections subsequent to the presence of MCD in the original projection area (Jacobs et al., 1999a; Jacobs et al., 1999b). Abnormal adjacent cortex might in part explain the low surgical success rate in MCD and the observation that lesionectomies tend to have a less good outcome than more extended resections (Raymond et al., 1995).

1.3.2 Normal macroscopic brain development

Many aspects of the normal macroscopic brain development in man have been studied in detail (see, for example, (Norman et al., 1995a; Sarnat, 1992; Sidman and Rakic, 1973; Sidman and Rakic, 1982)). Neuroectoderm, forming the neural plate, appears on the 16th postconceptional day. The neural plate then folds into a tube; this process is complete by about the 26th day. A zonal division is already present, exemplified in the arrangements in the future spinal cord: The most dorsal portion of
the neural tube is destined to become the somato-afferent part, and the viscero-
afferent part is located slightly more ventrally, followed by the sulcus limitans which
separates the afferent parts (alar plate) from the efferent parts (basal plate) of the
neural tube. Within the ventral portion, the viscero-efferent part lies more dorsally
than the somato-efferent part.

The rostral portion of the neural tube then grows extensively and differentiates into
the brain. Around day 28, three primary brain vesicles appear: prosencephalon,
mesencephalon and rhombencephalon. By about day 35, the first and third of these
vesicles have both grown into two vesicles. The resulting five secondary brain
vesicles are

1. telencephalon (to become the cortex, striatum and olfactory system)
2. diencephalon (to become thalamus, hypothalamus and subthalamus)
3. mesencephalon (to become the midbrain)
4. metencephalon (to become pons and cerebellum) and
5. myelencephalon (to become the medulla oblongata).

1.3.3 Normal microscopic brain development

By far the majority of neurons and glial cells are derived by proliferation of
neuroepithelial cells in the cortical ventricular or subventricular zone (also called
germinall matrix). The highest rate of cell division occurs in the second trimester of
gestation. The number of cells created is far larger than the final number of neurons
in the adult brain. Many cells undergo programmed (apoptotic or histogenetic) cell
death, particularly if appropriate synaptic contacts have not been formed. This is the
case for substantial proportions of cells - there is, for example, a 35% decline in
human fetal motoneurons between 11 and 25 weeks of gestation (Forger and
Breedlove, 1987).

Four anatomical migratory pathways have classically been described: via the corpus
pontobulbare to the pontine and medullary nuclei; via the corpus gangliothalamicum
to the basal ganglia and thalamus; to the cerebellum; and centrifugal migration from
the cortical ventricular zone to the cerebral cortex. The known malformations of
cortical development discussed in this thesis involve errors in this fourth migration
path.

Neurons that are generated in the ventricular zone may have to migrate several
hundred cell-body distances to arrive at their final destination within the six-layered
mammalian cerebral cortex, even though the distances are far shorter in the early
development (reviewed in (Gleeson and Walsh, 2000)). Since the late nineteenth century it has been known that cortical neurons originate in the ventricular zone and then migrate towards the pial surface (reviewed in (Sarnat, 1992)) where they accumulate below the marginal zone to ultimately form the cortical plate. More recently, it was firmly established that this migration occurs along radial glial fibers which span the entire width from the ventricle to the marginal zone (e.g. (Rakic, 1972)). Radial glia play a central role in cortical organisation even though not all neurons migrate along these pathways ((Walsh and Cepko, 1992) and see below).

The earliest neurons arriving at what is to become cerebral cortex form the so-called preplate at around embryonic week 8-9, and the subsequent cortical plate is formed within this preplate between weeks 10-18. This process divides the preplate into an outer zone (the marginal zone), directly proximal of the pial surface and basal lamina, and a deeper layer called the subplate. The layer proximal of the preplate and then later proximal of the subplate is termed intermediate zone. The technique of "birthdating" neurons has shown that somewhat surprisingly the neurons forming the basic six-layered organisation within the cortical plate are arranged in an 'inside-out' order. Neurons newly arriving in the cortical plate penetrate both the subplate and the previously deposited cortical plate neurons so that the newest neurons always add to the cortical plate distally from present neurons and just proximally from the marginal zone (Angevine and Sidman, 1961). This leads to a radial organisation of newly formed neocortex, but tangential or orthogonal migration (Walsh and Cepko, 1992) as well as the establishment of connections leading to an increase in neuropil mean the original radial organisation develops into the laminar organisation of the adult cortex.

Recently, it has been established that the processes outlined above apply to pyramidal neurons as well as neuroglia. The mammalian cerebral cortex, however, contains not only excitatory pyramidal cells that project to cortical and subcortical targets but also inhibitory, GABAergic, nonpyramidal cells, the cortical interneurones. The vast majority of the latter seem to be generated in a distinct proliferative zone, not in the cortical ventricular zone but in the ganglionic eminence of the ventral telencephalon (reviewed by (Parnavelas, 2000)). The lateral ganglionic eminence gives rise to the striatum and olfactory bulb in rodents as well as amygdala and claustrum (Norman et al., 1995b), while the medial ganglionic eminence is the primordium for some parts of pallidum and striatum (Parnavelas, 2000) as well as
nucleus accumbens septi and the bed nucleus of the stria terminalis (Sidman and Rakic, 1982) and provides nonpyramidal cells for the neocortex (Parnavelas, 2000). In contrast to the radial migration of pyramidal cells, these nonpyramidal cells use tangential migratory paths to reach their positions in the cortex, possibly along axonal bundles of the developing corticofugal fibre system. They first appear in the marginal zone, some in the form of Cajal-Retzius cells. These are unique neurons in the cell-sparse marginal zone which provide signals - e.g. Reelin (see below) - that are important in the migration of (mainly pyramidal) neurons to the cortical plate (Frotscher, 1997). Somewhat later, nonpyramidal cells appear in the lower intermediate zone and in the cortical plate itself. Thus, cells belonging to the excitatory and inhibitory system seem to use two rather different migration systems, but their development may be spatially and temporally linked.

1.3.4 Normal microscopic development of the archicortex

As is the case for the neocortex, neuroblasts for the hippocampus are generated in the ventricular zone and migrate to the the cortical plate which in this area is designated "ammonic plate" [(Stanfield and Cowan, 1988), reviewed by (Raymond et al., 1995)]. In contrast to the normal neocortical development, however, the migrating neurons dissociate themselves earlier from the radial glial fibers and accumulate in the deeper aspects of the ammonic plate. During a subsequent phase, these young neurons are reorganised to more superficial positions so that the formation of ammon's horn follows an inside-out gradient similar to that of the neocortex, but the result is the more simple histological organisation of the archicortex. The development of the dentate gyrus is different in many ways. Neurogenesis occurs initially in the ventricular zone, but a second, subpial germinal centre develops at a later stage and appears to become the dominant germinal centre (Schlessinger et al., 1975). Another difference is the assembly of the laminar architecture which seems to follow an outside-in pattern, with neurons arriving later being added to the deeper layers. Tangential migration seems to be more important than radial migration, and migration is nearer the pial surface. During maturation of the archicortex, the dentate granule cell layers and the hippocampal pyramidal cell layers which are originally in continuity become separated, probably in the course of the complex folding of the hippocampus (Duvernoy, 1998; Klingler, 1948; Stanfield and Cowan, 1988). A possibly unique feature of the development of the dentate gyrus is its timing. Granule cell generation continues well into postnatal life, with the peak of neurogenesis in the
early postnatal period (Schlessinger et al., 1975), and it is now known that this is a lifelong process (Eriksson et al., 1998b) and has functional relevance at least in rodents (Shors et al., 2001), contradicting the long-held belief that neurogenesis does not occur in adults (Rakic, 1985).

1.3.5 Factors influencing migration

Various migration abnormalities have known causes (reviewed in (Norman et al., 1995c)). There are chromosomal errors, many syndromes due to single gene mutations, teratogens like isoretinoin, chemicals like methylmercury and radiation. Polymicrogyria is often associated with destruction or necrosis which may or may not have the same etiology.

While radial glia is certainly a fundamental mechanical prerequisite to corticogenesis, numerous other factors are known or suspected to be involved in normal neuronal migration. All of the following may be disturbed in human disease or animal models.

1.3.5.1 Proliferation and differentiation

Proliferation and cell-fate determining factors, studied in the fly Drosophila, include Notch1 which is inherited variably during neuroblast division depending on the orientation of the line of cell cleavage (Chenn and McConnell, 1995).

1.3.5.2 Initiation of migration

Filamin 1 is a large actin-binding/crosslinking protein whose gene is located on chromosome Xq28. It plays an important role in migration onset, and defects generally lead to periventricular nodular heterotopia (PNH), i.e. neurons differentiate but do not initiate migration (reviewed in (Gleeson and Walsh, 2000)). Some of its defects are due to sporadic mutations, but some severe mutations are inherited in an X-linked fashion and generally lead to PNH and epilepsy in females, while affected males generally do not survive to term. Mutations leading to less severe loss of function may be seen in males with PNH, and interestingly, there is no evidence for mosaicism in these patients (Sheen et al., 2001). Again, other factors such as integrins, Ra1A and presenilin 1 may be involved in the initiation of migration (reviewed by (Gleeson and Walsh, 2000)).

As for the nonpyramidal cells originating from the ganglionic eminence, the mechanisms that initiate their tangential migration are not well known. The slit
protein is produced in regions within the ganglionic eminence of the embryonic mouse telencephalon, and it appears to provide diffusible repulsive activity that drives GABAergic nonpyramidal neurons to migrate away from the ganglionic eminence to the neocortex.

1.3.5.3 Ongoing migration

Insight into the mechanisms of migration between the ventricular zone and the cortical plate comes from two disorders where migration is arrested partway between source and target, lissencephaly type I and a form of laminar heterotopia (double cortex; band heterotopia, BH).

Lissencephaly ("smooth brain") type I is characterized by a roughly four-layered cortex the layers of which bear no clear relationship to the normal six-layered structure of neocortex. Lissencephaly can be caused by mutations in at least two genes.

The LIS1 gene is located on chromosome 17p13.3 (Cardoso et al., 2002; Dobyns et al., 1991). Its autosomal mode of inheritance causes haploinsufficiency in affected individuals and the lissencephaly phenotype (severe mental retardation and intractable epilepsy), suggesting that two wildtype copies of the gene are necessary for normal cortical development. LIS1 co-localises with microtubules and likely exercises its role in migration through interaction with the microtubule cytoskeleton (Morris et al., 1998).

In males, a second lissencephaly locus on the X chromosome, termed DCX gene, leads to a phenotype that is similar to the one caused by the LIS1 mutation. Affected females, however, retain a working copy of the gene and have laminar heterotopia - they show a normal six-layered outer cortex but an additional collection of neurons located in the subcortical white matter, together with a milder phenotype. They usually show only mild to moderate mental retardation and less severe epilepsy. The pathological manifestation may be due to random inactivation of the X chromosome. The gene product, DCX, has an unknown function in migration but, like LIS1, it is a microtubule-associated protein (Gleeson et al., 1999). It should be noted that laminar heterotopia may be due to other factors, evidenced through the fact that it may occur sporadically and in males.

As for the nonpyramidal cells, the nature of the cellular elements used for migration is still under study, but it is likely that axons provide a substrate for non-radial
migration and that a variety of chemical cues plays a role (reviewed by (Parnavelas, 2000)).

1.3.5.4 Penetration of migrating neurons through the subplate

To achieve correct placement in the developing cortical plate in the 'inside-out' order, migrating neurons need to penetrate both the subplate and any previously deposited layers of neurons. This process is disturbed in the reeler mouse, a naturally occurring and well studied mouse mutant (Caviness and Sidman, 1973). Newly arrived neurons pile up sequentially underneath the preplate, reversing the normal inside-out pattern and creating a superplate through obliteration of the marginal zone which merges with the preplate. The malformation may be due to defective penetration of the preplate, or possibly due to defective dissociation of the neurons from the radial glia (reviewed in (Gleeson and Walsh, 2000)). Cerebellar development is severely disturbed as well, which probably accounts for the phenotype with severe unsteadiness of gait. The gene product, reelin, is a large extracellular protein which is secreted from the horizontally oriented Cajal-Retzius cells of the preplate and marginal zone, suggesting that signaling from the marginal zone to incoming neurons is a crucial step in cortical plate formation.

Studies in several other mutations in which the phenotype is similar to the reeler mutant have recently provided evidence that both the ApoE receptor 2 (alias Lrp8) and the very low density lipoprotein receptor are probably the reelin receptors, with some regional specificity but also redundancy. The reelin signal seems to be transduced to the mouse homolog of *Drosophila disabled* (Dab1) which encodes an intracellular phosphoprotein and whose mutation leads to the scrambler (Sweet et al., 1996) or yotari (Yoneshima et al., 1997) phenotypes which replicate the reeler phenotype. Mutations in the genes encoding cyclin-dependent kinase 5 (alias Cdk5) and its regulator, p35 (alias Cdk5r2), also produce mutants with inverted cortical plates. They do not, however, affect the marginal plate, and lamination is less disrupted than in reeler. The cyclin-dependent kinase 5 mutation leaves the subplate in the middle of the cortical plate, indicating that early cortical-plate neurons can penetrate the subplate but neurons arriving later are unable to do so. In contrast, the p35 or cyclin-dependent kinase 5 regulator mutation leaves the subplate proximal to the cortical plate. The main abnormality, therefore, is the inversion of the normal layering, indicating that only the penetration of newly arriving neurons through existing cortical plate neuronal layers is disturbed. Although the mechanisms by
which these two proteins affect migration are not entirely clear, they are again likely to be mediated through the cytoskeleton (review in (Gleeson and Walsh, 2000)).

1.3.5.5 Defective pial limiting membrane

Lissencephaly type II (cobblestone lissencephaly) in humans, the related Fukuyama disease (Kobayashi et al., 1998) and Walker-Warburg syndrome (Kanoff et al., 1998) all produce disruptions of the architecture of the developing cerebral wall and severe disorders of neuronal migration. The pial limiting membrane is defective, and neurons migrate out of the CNS and into the meninges [reviews by (Gleeson and Walsh, 2000; Golden, 2001)]. The pial-glial barrier seems, therefore, to play an important, possibly mechanical role in the final positioning of neurons. Similar extrusions of neurons out of the CNS have been described in much more subtle disorders of cortical development, namely microdysgenesis, and it is possible that environmental factors play a role as well as the genetic factors outlined above.

1.3.5.6 Disorders of migration of nonpyramidal neurons

In mice, mutations the homeobox-containing genes Dlx1 and Dlx2 which are expressed in the developing forebrain, lead to an accumulation of partially differentiated cells in the lateral ganglionic eminence, and the neocortex contains only a quarter of the normal number of GABAergic interneurons (Bulfone et al., 1993). Similarly, mice that have a mutation of the Nkx2.1 homeobox gene lack a functional medial ganglionic eminence, and show an approximately 50% reduction in cortical interneurons (see (Parnavelas, 2000)).

Many of the mouse mutants do not yet have clear corresponding syndromes in humans, but the general mechanisms are similar. It is likely that in the future, more than the few discrete stages described above will be identified and that interactions between these mechanisms will be found. As will become evident later in the thesis, subdividing the different forms of MCD by their mechanisms enhances understanding of the receptor changes found by ligand PET.

Some specific syndromes of MCD are relevant to this study and warrant more detailed description.
1.3.6 Abnormal neuronal and glial proliferation: Focal cortical dysplasia, tuberous sclerosis

1.3.6.1 Focal cortical dysplasia

1.3.6.1.1 Definition and epidemiology

Focal cortical dysplasia (FCD) describes a specific localised MCD with a characteristic histological appearance (Taylor et al., 1971). FCD may involve any lobe but is more commonly found in the temporal or frontal lobes or around the central sulcus. It is usually unilateral and in many cases has a sharp demarcation from normal appearing cortex. Epilepsy with early onset of partial seizures seems to be a constant feature of FCD (Barkovich and Kjos, 1992b).

The true incidence and prevalence are not known. In the National Hospital series, out of 100 patients with MCD (Raymond et al., 1995), histologically confirmed FCD was seen in four patients, but in some of the seven patients with minor gyral abnormalities and in some of the 16 patients with focal macrogyria, FCD may well have been the underlying pathology, and has indeed been shown in one patient with focal macrogyria who underwent surgery (Raymond et al., 1995). Kuzniecky and Barkovich estimate FCD to be "probably the most common form of focal developmental disorder diagnosed in patients with intractable focal epilepsy", based on their experience (Kuzniecky and Barkovich, 2001), and Sisodiya found FCD to be the most common form of MCD in surgical series (Sisodiya, 2000). This does, however, probably reflect selection bias. In the NSE MRI study, 1 out of 110 patients with newly diagnosed epilepsy and 1 of 174 patients with chronic epilepsy had FCD on MRI [Dr Alex Everitt, personal communication]. FCD seems to be exceedingly rare in persons without epilepsy and was not seen in 500 controls in one series (Wolf et al., 1995) and not seen in 170 controls in the NSE MRI study (Everitt et al., 1998).

1.3.6.1.2 Neuropathology

Macroscopically, smooth cortex with lack of sulci, coarse gyri of blurring of the grey-white matter junction have been described (Thom, 2001).

Histology (Norman et al., 1995d; Prayson and Estes, 1995; Taylor et al., 1971) shows, beneath a molecular layer that is usually normal, cortical laminar architectural disorganisation with hypercellular cortex, possibly with persistent columnar alignment. Clusters of atypical neurons and glia can occur within the cortex, and
heterotopic nerve cells may also be present in increased numbers in the underlying juxtacortical white matter.

Cytology (Norman et al., 1995d; Thom, 2001) shows aberrant differentiation of nerve cells which are described as bizarre and cytomegalic and scattered in all cortical layers. They maintain an overall pyramidal shape and are usually greater than 20 microns. The Nissl substance is often prominent and appears 'tigroid' and unevenly distributed. These nerve cells often appear randomly orientated, with loss of the normal vertical polarity of the apical dendrites. Nerve cells may cluster as well. Dendritic processes have been found to be increased, abnormally branched and arborised, but other studies have found a diminution in the number of such processes [reviewed by (Thom, 2001)]. In a large proportion of cases, abnormal and enlarged astrocytic cells of up to 50 microns diameter are present. These are mostly called "balloon cells", but other terms include "balloon cell glia", "grotesque cells", "dysplastic glial cells" or "uncommitted cells". Their appearance is intermediate between nerve cell and astrocyte. Glassy cytoplasm is abundant, nuclei are large and sometimes multiple. They are preferentially located in the deeper regions of the cortex or in the juxtacortical white matter. They resemble similar cells seen in cortical tubers in tuberous sclerosis and subependymal giant cell astrocytoma. A frequently used classification system is based on the absence (FCD type I) or presence (FCD type II) of these balloon cells (Kuzniecky and Barkovich, 2001). Different cases show great variability in appearance (Norman et al., 1995d), and this may in part explain the multiple classification systems.

Immunohistochemical findings in FCD have recently been reviewed by Thom (Thom, 2001). There were various findings regarding the abnormal nerve cells. Firstly, cytoskeletal abnormalities have been found. Intracytoplasmatic fibrillar inclusions are thought to be due to progressive accumulation and phosphorylation of neurofilaments, possibly due to a failure of normal axon transport, or otherwise due to increased production. Independent studies have also shown increased expression of microtubule-associated proteins, thought to reflect increased plasticity and remodelling of dendrites. Secondly, the persistent staining of nerve cells with the embryonal form of N-CAM (despite a reduced amount of total N-CAM in tissue samples), together with expression of nestin and internexin in these nerve cells, suggests some failure of cell maturation in FCD. Balloon cells are interesting in that they show variable intensity of staining with antibodies to glial fibrillary acidic
protein (GFAP) while some cells label with neuronal markers, and some cells even show dual labelling with both neuronal markers (e.g. synaptophysin, neurofilament or protein gene product 9.5) and glial markers. Balloon cells resemble neoplastic gemistocytic astrocytes, but investigation with a proliferation marker showed virtual absence of labelling.

Other lesions may coexist with FCD. Tumours have been reported [e.g. (Daumas-Duport et al., 1988; Prayson and Estes, 1995; Prayson et al., 1993)], most commonly slow-growing mixed glial-neuronal hamartomatous tumours such as ganglioglioma and dysembryoplastic neuroepithelial tumour, and there is the possibility of a common origin of both. Other tumours that have been reported to occur adjacent to FCD are pilocytic astrocytomas, fibrillary astrocytoma and meningioangiomatosis. Associations with degenerative, destructive or inflammatory pathologies, including Rasmussen's encephalitis, have been described in a smaller proportion of cases [reviewed by (Thom, 2001)]. Hippocampal sclerosis is relatively frequently found, in 9% (Prayson and Estes, 1995) and 12% (Wolf et al., 1995) respectively in two series. Conversely, out of 100 patients with hippocampal sclerosis, 15 had some form of MCD, including one case of FCD (Raymond et al., 1994a). Whether FCD causes hippocampal sclerosis through some sort of kindling mechanism or whether both lesions are due to the same developmental abnormality is unclear at the moment.

1.3.6.1.3 Mechanisms of formation of focal cortical dysplasia

There may be a genetic predisposition in some instances, particularly when FCD is diffuse, as suggested by a case report of two brothers (Kuzniecky, 1994). Similarly, histological resemblances between the cortical tubers of tuberous sclerosis and FCD have been noted, and indeed some believe FCD to be a "forme fruste" of tuberous sclerosis, meaning that the stigmata of tuberous sclerosis were confined to the brain (Palmini et al., 1991b).

The presence of environmental factors, however, is strongly suggested through the usually sporadic presentation of FCD and a case report of monozygotic twins discordant for both epilepsy and FCD (Briellmann et al., 2001).

Pathogenetic factors could conceivably act during proliferation, migration, in post-migrational maturation, differentiation, or interfere with programmed cell death. Alterations of the catecholaminergic neuronal circuitry within neuropathologically confirmed FCD was found by using antisera to tyrosine hydroxylase and dopamine-beta-hydroxylase with the former being increased in the surrounding areas of seizure
propagation as well, suggesting that catecholamines may play a role in seizure propagation and/or limitation (Trottier et al., 1994). The amino acids ethanolamine and glycine have been found to have higher biochemical concentrations in tissue with cortical dysplasia (Hamberger et al., 1993). In a case of FCD containing dysplastic nerve cells, abnormalities in the distribution and morphology of inhibitory interneurons were found using calbindin and parvalbumin immunoreactivity (Ferrer et al., 1992).

A decrease of calcium-binding protein immunopositive (GABAergic) interneurons within FCD was seen in three cases (Spreafico et al., 1998). Abnormal baskets of parvalbumin-positive terminals around excitatory (pyramidal and large, round shaped) neurons were also found. Abnormalities in the distribution of glutamate receptors on pyramidal cells (Najm et al., 2000), and in the distribution and morphology of parvalbumin and calbindin inhibitory neurons, have been demonstrated in FCD (Garbelli et al., 1999) and are thought to be relevant to intrinsic hyperexcitability - for example, GABAergic terminals clustering around giant neurons failed to establish synapses (Garbelli et al., 1999). A study of single neurons microdissected from postmortem temporal cortex, FCD tissue and nondysplastic temporal cortex, both obtained at epilepsy surgery, found increased levels of mRNA coding for several glutamate receptor subunits in dysplastic compared with pyramidal and heterotopic neurons (glutamate receptor 4, N-methyl-D-aspartate (NMDA)-receptor 2B and 2C). Dysplastic neurons also contained decreased mRNA levels for the NR(2A) subunit of the NMDA receptor and for the β1 subunit of the GABA_A receptor. Messenger RNA for the GABA_A receptor subunits α1, α2 (but not α3-6) and β2 was reduced in both dysplastic and heterotopic neurons, as well as mRNA for one subunit of the glutamate receptor (1) (Crino et al., 2001).

Another mechanism which may be relevant is over-expression of drug resistance proteins in FCD and other MCD which may also contribute if not to epileptogenesis then to the development of refractory seizures (Sisodiya et al., 1999b; Sisodiya et al., 2002; Sisodiya et al., 2001b).

1.3.6.1.4 Clinical features and neurophysiology

Barkovich et al. have described the clinical features in 31 patients with focal cortical gyral abnormalities, presumed to correspond to dysplasia (Barkovich and Kjos, 1992b). In patients with bilateral FCD, generalised developmental delay was very frequent. In patients with unilateral FCD, contralateral spastic hemiplegia or
monoplegia was present in the majority, but dysarthria was uncommon regardless of
the location of the FCD. Compared to patients with HS, patients with FCD have a
younger age at seizure onset (Hirabayashi et al., 1993), and a low intelligence
quotient (IQ) is common (Palmini et al., 1991b). The majority of FCDs are located
extra-temporally, and the frontal lobes and pericentral areas appear to be involved
particularly often (Kuzniecky and Barkovich, 2001).

Onset of epilepsy is usually in the first decade, usually after the age of two to three
years but occasionally shortly after birth. Seizures are expectedly mainly partial or
secondarily generalised (Kuzniecky and Barkovich, 2001) and often refractory to
treatment with antiepileptic drugs, even though occasional cases have been described
in whom the epileptic syndrome was mild or in whom remission was achieved
(Gambardella et al., 1997). Epilepsia partialis continua may occur (Desbiens et al.,

EEG features were very variable in a retrospective review of 94 cases in children
(Quirk et al., 1993). EEG could be normal despite widespread cortical dysplasia, and
an EEG with very high amplitude rhythmic activity, while not very sensitive, was
found to be highly specific for severe cortical dysplasia. In another study, EEG
abnormalities were more widespread than the MRI lesion in 17 of 30 cases,
sometimes even contralateral to a unilateral lesion (Palmini et al., 1991b). The same
group found continuous "ictal-like" spiking during electrocorticography (ECoG) in
21/32 patients but in none with cortical tumours (Palmini et al., 1994). As
abnormalities were recorded directly over FCD, this was seen as suggestive of
intrinsic epileptogenicity of FCD (Palmini, 2000) and contrasted with the findings in
tumour patients in whom abnormalities were localised in surrounding cortex.
Rhythmic epileptiform discharges on scalp EEG occurred frequently in focal cortical
dysplasia (15 of 34 patients, 44%) but in none of 40 patients with other structural
lesions, and 12 of the 15 patients with rhythmic epileptiform discharges on scalp
EEG were found to have continuous epileptiform discharges on ECoG (Gambardella
et al., 1996).

Patients are often refractory to treatment with antiepileptic drugs, and therefore
surgery is frequently considered. The case for surgery for FCD has recently been
reviewed in depth by Sisodiya (Sisodiya, 2000). Intrinsic epileptogenicity has been
repeatedly suggested by indirect or direct evidence (see above), and completeness of
resection of the lesion is likely to be important. Notwithstanding, not all cases in
whom resection is complete by histological criteria become seizure free (Palmini et al., 1995), and the overall seizure free rate at at least two years after surgery is only around 38% (Sisodiya, 2000). In an animal model, excitability changes have been shown inside but also outside the area of FCD (Redecker et al., 1998), and similar changes in humans might explain some of the surgical failures. This would suggest that intraoperative monitoring with ECoG is particularly important in FCD, and careful monitoring with ECoG, together with completeness of resection as far as permitted by the extent of eloquent cortex, was the conclusive recommendation for surgery for FCD in Sisodiya's review (Sisodiya, 2000).

1.3.6.1.5 Neuroimaging

CT is negative in the majority of cases with FCD (Raymond et al., 1995), while MRI has long been recognised to allow good delineation of the abnormalities (Barkovich et al., 1987; Raymond et al., 1995). MRI features are blurring of the grey-white matter interface, focal thickening of the cortex, gyral thickening and abnormal T2 signal, particularly in the underlying white matter (Kuzniecky and Barkovich, 2001). MRI was, however, found to be normal in surrounding areas of cortex in which histology was still abnormal in a study in the early 1990s (Palmini et al., 1994). It is unclear whether this would still be true with currently used MRI techniques. The abnormalities can occur anywhere in the neocortex but are found more frequently in the frontal lobes and in the perirolandic areas.

1.3.6.2 Tuberous sclerosis

1.3.6.2.1 Definition and epidemiology

Tuberous sclerosis is part of a group of genetically programmed embryopathies which may be called "neurocutaneous" because of their prominent involvement of the main ectodermal derivatives, nervous system and skin (Sarnat, 1992), even though organs and tissues derived from other germ layers are also affected. The term "ectomesodermal syndromes" (Norman et al., 1995d) emphasises the same aspect of this group of diseases, while the traditional term to denote these cytological dysgeneses is "phakomatosis" (van der Hoeve 1923). The latter, derived from the greek work for lentil or lentil-shaped, is a purely descriptive term referring to the shape of some of the malformations and tumours, and is no longer favoured by many authors. Other examples of clinically important ectomesodermal syndromes are
neurofibromatosis (von Recklinghausen disease), encephalofacial angiomatosis (Sturge-Weber disease) and retinocerebellar angiomatosis (von Hippel-Lindau disease).

Tuberous sclerosis is an autosomal-dominantly inherited condition which is clinically characterised by a triad of skin changes, epilepsy and learning difficulties. It is the commonest of the mendelian-inherited conditions causing epilepsy, present in about 1 in 10 000 live births (Gomez, 1988). Beside autosomal-dominant transmission, new mutations seem to be responsible for a significant proportion of cases, estimated at up to 75% (Guerrini and Carrozzo, 2001). Mutations in at least two genes can cause the condition. TSC1 encodes hamartin and TSC2 encodes tuberin. Both are tumour suppressor genes, and their gene products were unknown prior to the study of tuberous sclerosis. Mutations in either gene lead to the tuberous sclerosis phenotype, and their interaction is a necessary requirement for their function (Hodges et al., 2001). Linkage studies have revealed loci for TSC1 on chromosome 9q34 (Consortium, 1993) and on 16q for TSC2 (van Slegtenhorst et al., 1997).

1.3.6.2.2 Epilepsy

Epilepsy occurs in about 80% of cases in a strongly age-related pattern (Duncan et al., 1996c). Seizures may be present in the neonatal period. The commonest manifestation of tuberous sclerosis between the ages of 4 and 8 months is the syndrome of early infantile epileptic encephalopathy. Between 1 and 7 years of age, tuberous sclerosis is the most common identified pathological cause of "Lennox-Gastaut syndrome", a descriptive term for severe epilepsy and mental deterioration in this age group. Infantile spasms, myoclonic, atonic or tonic seizures are said to be common. Febrile convulsions may be the first manifestation. Later in life, the condition can present with partial or generalized seizures. The EEG has no specific features.

1.3.6.2.3 Learning difficulties and other neurological manifestations

Learning difficulties are said to occur in about 50% of identified cases, but as asymptomatic persons may not be detected, the true proportion of cases with mental handicap might be less. The incidence of mental deficiencies is greater in those who develop seizures early, before the age of 2 years. Other possible neurological deficits include hemiplegia, ataxia, stroke-like episodes, behavioural disturbance and psychosis. Patients need to be monitored for signs and symptoms of raised
intracranial pressure, usually due to a growing astrocytoma or hamartoma close to the foramina of Monro or other ventricular outflow tracts, causing obstructive hydrocephalus. The cerebral lesions may also undergo malignant transformation, causing other progressive neurological signs. Ophthalmic signs are common, with retinal hamartoma (which are considered a diagnostic feature) or phakomata occurring in about 50% of cases. Other ophthalmic signs include retinal pigmentary changes, vascular changes, anomalous discs, megalocornea, pigmentary changes on the iris, cataract, poliosis of the eyebrow, strabismus and third nerve palsy.

1.3.6.2.4 Skin lesions

Skin changes are almost invariably present. Hypomelanotic patches are found in 90% of cases when patients are examined under ultraviolet light. Facial angiofibromata (termed adenoma sebaceum in older publications), classic shagreen patch and periungual fibroma are all considered diagnostic when present. Atypical shagreen patch, hypomelanotic patches, forehead fibrous plaque and poliosis occur.

1.3.6.2.5 Other manifestations

Other organs manifestations are less prominent but common and may be life-threatening, requiring regular monitoring. In the urinary tract, renal angiomyolipomata occur and are considered diagnostic when they are bilateral and multiple. Myolipomas and asymptomatic renal cysts are frequently found, and fibrodysplasia of the renal arteries also occurs. Hamartomatous abnormalities can also be found in the liver, pancreas, spleen and gastrointestinal tract. Angiolipomas of the bones are found on radiographical skeletal surveys in over 50%. Cardiac rhabdomyomata occur in some 40% and can often be asymptomatic. In the lungs, pulmonary cysts and fibrosis occur. Endocrine abnormalities include sexual precocity, ovarian or testicular defects, lesions in the thyroid and adrenals. Dental changes include gingival fibroma and enamel defects.

1.3.6.2.6 Neuropathology

The condition is characterized by both cortical tubers and subependymal nodules. The tubers from which the condition derives its name are palpable in the cortex (Norman et al., 1995d). They are either widened gyri or round flat nodules with a central dimple. The boundary between grey and white matter may be blurred in the myelinated brain. They are typically located at the crown of a gyrus rather than in the
depth of a sulcus (Sarnat, 1992). There is a coarse subpial gliosis, and the normal
cortical lamination has been replaced with a mixture of astrocytes, abnormal glial
cells, neurons and large atypical neuronlike cells, some with two or three nuclei.
Imperfect cortical lamination containing normal cellular elements occurs in
transitional zones adjacent to tubers. The large dysplastic neurons occur in the grey
and underlying white matter. The subependymal nodules are protrusions into the
ventricles which consist of large abnormal astrocytes. They may enlarge as giant cell
astrocytomas and may then obstruct the flow of CSF. Adjacent or contained vessels
may be calcified. Cytological studies in tuberous sclerosis have generally concluded
that the cells are unique, i.e. they do not correspond to normal cells in either the
mature or developing brain, may remain undifferentiated, and if they differentiate,
this may be incomplete along either neuronal or glial lines. Even the cells that do
differentiate along the neuronal lines show a paucity of spines and beaded
varicosities (Huttenlocher and Heydemann, 1984), often no definite axons, and have
abnormally low immunoreactivity to synaptophysin I (Lippa et al., 1993), probably
indicating little connectivity. Glutamate and GABA receptor subunit messenger
ribonucleic acid (mRNA) expression may be selectively altered in dysplastic neurons
and giant cells of the cortical tubers (White et al., 2001).

1.3.6.2.7 Clinical features

As with most autosomal dominant diseases, affected individuals show widely
varying manifestations and the features within a family may also vary, both within
and across generations. In view of the family planning implications, examination of
family members is indicated even if they appear neurologically normal. Screening
includes dermatological examination under ultraviolet light to show hypomelanotic
patches, ophthalmological examination, renal ultrasound and neuroimaging. CT is
particularly sensitive to calcified subependymal lesions but MRI, particularly when
T2 and FLAIR sequences are included, will readily show cortical tubers and/or
subependymal nodules and is therefore preferable. Skeletal radiographs and
echocardiographs can be considered. Genetic counselling can then inform affected
individuals that there is a 50% chance of passing the condition to offspring, and
unaffected individuals that there is no genetic propensity. The prognosis in any
individual is very variable. On one end of the spectrum, there are patients with few
signs, normal intelligence and mild epilepsy. On the other end of the spectrum, there
are individuals with severe epilepsy and mental retardation who often cannot live
independently and whose life expectancy is shortened by the disease. Death may be due to status epilepticus, complications due to the mental retardation, growth of the cerebral tumours with ensuing obstructive hydrocephalus or malignant transformation.

Treatment of the epilepsy is with antiepileptic drugs and follows the usual guidelines (Duncan et al., 1996c). Surgery can be considered and has most chances of success in patients with focal seizures and good imaging-EEG correlation. In one series, seven of 18 patients achieved seizure-free outcomes (Engel class IA and IB) (Guerreiro et al., 1998).

1.3.6.2.8 Structural imaging appearances of tuberous sclerosis

CT was abnormal in four out of five patients in a large series of patients with malformations of cortical development (Raymond et al., 1995). Multiple subependymal calcifications were seen in four and additional cortical or subcortical calcifications in three. MRI showed multiple subependymal and subcortical or cortical nodules. These were hyperintense to brain tissue on T2 and proton density weighted images. Tuberous sclerosis thus has a characteristic MRI appearance, but the abnormalities are most readily seen on T2 weighted or fluid attenuation inversion recovery (FLAIR) images rather than T1 weighted images commonly used to depict abnormality. Identified large tubers commonly show concordance with surface EEG recordings in terms of localisation. In an early study, MRI has not, however, been able to reliably differentiate between the forme fruste of tuberous sclerosis and other malformations of cortical development (pachygyria and focal cortical dysplasia) (Palmini et al., 1991b). Recent MRI techniques have not been systematically applied to this issue.

1.3.7 Abnormal neuronal migration resulting in heterotopic grey matter: Microdysgenesis, subependymal nodular heterotopia, laminar heterotopia, and subcortical heterotopia

The mechanism of normal corticogenesis and its disturbances have been described above. Heterotopic grey matter refers to collections of neurons that lie between the ependyma lining the lateral ventricles and the cortex, and classification is by anatomical location and morphological appearance. By convention, only collections of neurons big enough to be readily seen by the naked eye are included in this category, whereas microdysgenesis refers to collections of neurons which are too
scattered to be detected macroscopically. By extension, it follows that microdysgenesis cannot be seen on MRI which can currently only resolve clusters of neurons greater than 1mm in diameter.

1.3.7.1 Subependymal nodular heterotopia

1.3.7.1.1 Definition and epidemiology
Subependymal nodular heterotopia is a condition characterised by periventricular (subependymal) collections of neurons which form nodules, with patients typically presenting with epilepsy in the second decade of life.

The nodules measure typically 2-10mm in diameter (Eksioglu et al., 1996), but may merge to form an irregular band of multiple confluing nodules (Raymond et al., 1994b). They may be unilateral or bilateral, and in published series comprising seven (Huttenlocher et al., 1994), eight (Barkovich and Kjos, 1992a), 13 (Raymond et al., 1994b), 20 (Raymond et al., 1995) and 33 patients (Dubeau et al., 1995), respectively, they were seen bilaterally in 40-100%. While the whole length of the ventricles may be involved, the trigones and occipital horns are more commonly involved (Raymond et al., 1995). Frontal and temporal horns are less frequently involved, and the third and fourth ventricle are spared. The nodules can also be located lateral to basal ganglia or thalamus (Norman et al., 1995c).

Subependymal nodular heterotopia are among the more common forms of MCD encountered in adult epilepsy patients, accounting for 20/100 patients with MCD in the National Hospital series (Raymond et al., 1995), but the true prevalence is not known. Subependymal nodular heterotopia has occasionally been seen in people undergoing MRI as presumably healthy controls, one 25 year old male out of 46 healthy controls in one series (Raymond et al., 1995), and three patients who were investigated for memory impairment, vertigo and transient ischemic attacks, respectively (Dubeau et al., 1995). One of these had a strong family history of epilepsy with subependymal nodular heterotopia shown in one daughter. The same series contained a three year old female with nodules but without epilepsy who had an affected mother, so seizures later in life cannot be excluded. In the NSE MRI study, 2 out of 110 patients with newly diagnosed epilepsy and 1 of 174 patients with chronic epilepsy, but none of 170 controls had subependymal nodular heterotopia on MRI [Dr Alex Everitt, personal communication]. In a histological survey of 7374
brains of asymptomatic subjects, severe abnormalities of migration were found in only 1.7% (Meencke and Veith, 1992).

There is a female preponderance which may be due to familiar cases with X-linked inheritance of diffuse bilateral subependymal nodular heterotopia. The gene, FLN1 (named after its gene product, filamin), has been linked to Xq28, and affected hemizygous males in typical pedigrees do not appear to survive gestation.

Subependymal nodular heterotopia can be associated with other CNS malformations, for example Chiari II malformations or agenesis of the corpus callosum, and metabolic disorders such as Zellweger's syndrome or neonatal adrenoleukodystrophy [reviewed by (Kuzniecky and Barkovich, 2001)]. In Dubeau's series of 33 patients with subependymal nodular heterotopia, 13 also had subcortical nodular heterotopia (Dubeau et al., 1995), and this combination was present in 2 of 20 patients in Raymond's series as well (Raymond et al., 1995). These cases were mostly unilateral, and abnormalities of the overlying cortex were frequently seen. Hippocampal sclerosis has also been described in association with subependymal nodular heterotopia (Dubeau et al., 1995; Raymond et al., 1994b), and the association of hippocampal sclerosis with subependymal nodular heterotopia or other MCD is commonly referred to as "dual pathology" (Cendes et al., 1995). The hippocampus may also be distorted when subependymal nodular heterotopia involve the temporal horn (Raymond et al., 1994b).

1.3.7.1.2 Neuropathology

The heterotopia are composed of islands of relatively mature nerve cells which resemble cortical neurons rather than the neurons of deep grey nuclei, in keeping with their likely provenance. They contain multiple neuronal types but no dysplastic nerve cells, and calcifications are not seen. Synaptophysin immunohistochemistry has shown dense presynaptic terminals around the heterotopic cells (Eksioglu et al., 1996) but the origin of these afferents is not established. A study of nodular heterotopia in four children using dye tracing methods has shown limited connectivity of neurons between nodules (Hannan et al., 1999), for which there is indirect evidence through functional imaging studies that show task-induced activation in heterotopic gray matter (Pinard et al., 2000; Richardson et al., 1998a; Spreer et al., 2001). The detailed autopsy study in children (Hannan et al., 1999) showed abnormal calretinin-positive neurons in the nodules and far less arborisation of dendritic trees; interneurons in the nodules appeared generally immature.
Mechanisms of formation of SNH

The best studied syndrome that includes subependymal nodular heterotopia is the X-linked mutation in FLN1 ([Fox et al., 1998] and see above). The gene product, filamin, is an actin binding/cross-linking protein, suggesting that the role of filamin in neuronal migration might be mediated through modulation of the actin cytoskeleton (Gleeson and Walsh, 2000). The prevailing view has been that random X inactivation leads to a mosaic phenotype with cells expressing the wildtype gene migrating properly and cells expressing the gene form the mutant X chromosome failing to migrate (Gleeson and Walsh, 2000). This view was also consistent with the observation of male death in utero, presumably as a consequence of the absence of any wildtype gene and possibly due to a coagulation defect (Fox et al., 1998). This view has recently been challenged, however. Firstly, a pedigree with X-linked female-to-male transmission has been described, showing that at least some neurons carrying a mild filamin germline mutation are able to migrate [reviewed by (Sheen et al., 2001)]. Secondly, in a study using single-strand confirmation polymorphism analysis of the entire FLN1 gene, while mutations were detected in 83% of pedigrees with subependymal nodular heterotopia and typical MRI features, there was a female with a single amino acid substitution, presumably causing a partial loss of function, who had five alive male offspring of whom only two had mental retardation (Sheen et al., 2001). Thirdly, in the same study, two males out of 24 had sporadic mutations of FLN1, and both had either little or no wildtype bands on single-strand confirmation polymorphism analysis of peripheral lymphocytes, which makes cerebral mosaicism unlikely (Sheen et al., 2001). Taken together, these data indicate that the developmental mechanism underlying the formation of subependymal nodular heterotopia need not solely be mosaicism of the FLN1 allele or X inactivation. Rather, interactions of the mutated filamin protein with cell surface or extracellular signals which may change with time and location may prevent a subset of neurons from migrating.

These results also show that formation of subependymal nodular heterotopia is by no means a monocausal event - a case of monozygotic twins discordant for both bilateral subependymal heterotopia and epilepsy is a further example (Briellmann et al., 2001). Exogenous factors such as focal subependymal haemorrhages or ischaemia have also been implicated as possible praenatal or perinatal events, and there is a relatively high incidence of nonspecific antenatal or perinatal problems,
e.g. 8/20 in one series (Raymond et al., 1995). Dubeau et al. even reported abnormal pregnancies in 17/33 patients, and 12/33 had congenital malformations (Dubeau et al., 1995). In one series, the vast majority of females with bilateral confluent symmetrical nodules and a positive family history had mutations of FLN1, while no mutation was seen in seven females with sporadic disease and atypical MRI appearances. It is likely that with the description of more cases a more detailed aetiological classification will be possible, probably taking the imaging appearances into account.

1.3.7.1.4 Clinical features and neurophysiology

Epilepsy is common even though not universal (Raymond et al., 1995). As the onset of seizures is typically in the second decade of life, relatively later than in other forms of MCD, the prevalence of epilepsy may be underestimated in series including young patients. Focal, multifocal and generalized seizures can occur; at the severe end of the spectrum, infantile spasms and Lennox-Gastaut syndrome are seen (Golden, 2001). Epileptic phenomena are frequently discordant or contralateral to the side or location of the lesion; in one series, this was true of 10/20 patients (Raymond et al., 1995) while this was far less prominent in another including both periventricular and subcortical nodular heterotopia (Dubeau et al., 1995).

Mental retardation is relatively uncommon and occurred in only 1/20 patients in the National Hospital series (Raymond et al., 1995). Bilateral and more extensive subependymal nodular heterotopia, however, are associated with more severe disorders of cognition (Kuzniecky and Barkovich, 2001). If the subependymal nodular heterotopia is part of a syndrome, neurodevelopmental delay or other organ dysgeneses may be prominent.

The alpha rhythm is mostly preserved on interictal EEGs (Raymond et al., 1995), and the interictal EEG can occasionally be entirely normal. Localised slow activity and focal or lateralized epileptiform discharges can be seen. Importantly, epileptiform EEG changes can be bilateral even when subependymal nodular heterotopia only appear unilateral on MRI. Not infrequently, patients present with an electroclinical syndrome mimicking idiopathic generalized epilepsy with ≥3Hz spike or sharp and slow wave discharges [e.g. (Dubeau et al., 1995)]. Patients with an electroclinical diagnosis of typical absences have been described (Fish, 1995). There is direct evidence for epileptogenicity of heterotopic neuronal clusters (Francione et al., 1994; Kothare et al., 1998; Mattia et al., 1995; Palmini et al., 1995; Sisodiya et al., 1999a).
Treatment of the epilepsy is with antiepileptic drugs following the usual principles (Duncan et al., 1996c). Temporal lobectomy for temporal-lobe onset seizures on the basis of HS in the presence of subependymal nodular heterotopia was uniformly unsuccessful in nine patients (Li et al., 1997), with the best result in a patient in whom the majority of the subependymal lesion was removed as well. In another series, four of six patients with subependymal nodular heterotopia undergoing temporal lobectomy and followed up for at least six months failed to improve. One of these four had additional subcortical heterotopia, as did the remaining two patients of whom one became seizure free and one improved significantly (Dubeau et al., 1995). With these caveat in mind, surgery might be considered only in exceptional cases when removal of the entire lesion seems possible, all data seems concordant and, rather than a genetic cause, an exogenous cause as suggested by the presence of additional subcortical heterotopia (see below) seems likely. One would need to keep the possibility in mind that heterotopic grey matter may be functionally important (Pinard et al., 2000; Richardson et al., 1998a; Spreer et al., 2001).

1.3.7.1.5 Neuroimaging

Nodules do not calcify and are not commonly seen on CT (Raymond et al., 1995) unless they are large or clearly protrude into the ventricle. In contrast to the subependymal hamartomas of tuberous sclerosis, they do not enhance after injection of contrast medium (Barkovich and Kjos, 1992a). On MRI, the isolated or confluing nodules are seen as isointense to normotopic grey matter on all standard MR sequences but are most readily seen on T1 weighted images (Raymond et al., 1995). Coexisting hippocampal sclerosis (dual pathology) is relatively frequent (Cendes et al., 1995; Raymond et al., 1995).

1.3.7.2 Subcortical heterotopia

1.3.7.2.1 Definition and epidemiology

Subcortical heterotopia is a condition characterised by collections of neurons in the hemispheric white matter. These are separated from both the ventricles and the cortex through white matter, in contrast to subependymal nodular heterotopia. Patients typically present with epilepsy in the first or second decade of life (Kuzniecky and Barkovich, 2001). Subcortical heterotopia are much rarer than subependymal heterotopia, accounting for only 3/100 malformations in the National
Hospital series (Raymond et al., 1995). Subcortical heterotopia are very often associated with other malformations, e.g. with subependymal nodular heterotopia (Raymond et al., 1995), often in the same area (personal observation), or complex malformations (Raymond et al., 1995). In distinction to subependymal nodular heterotopia, they do seem more likely to interfere with normal function. Motor and intellectual disturbances are seen, their severity paralleling the extent of the malformation to some degree (Kuzniecky and Barkovich, 2001).

1.3.7.2.2 Neuropathology

The nodules are of widely varying size, from less than 5mm to 20 or more mm in diameter. They may frequently be associated with subependymal nodular heterotopia, particularly when the latter is unilateral. In one study, 13 of 19 cases with unilateral subependymal nodular heterotopia showed subcortical heterotopia as well, but none of the 14 cases with bilateral subependymal nodular heterotopia (Dubeau et al., 1995); in another study, of three patients with subcortical heterotopia two had associated subependymal nodular heterotopia and one had focal macrogyria/polymicrogyria associated with a cleft. Abnormalities of the overlying cortex may be present [e.g. (Battaglia et al., 1996; Guerrini et al., 1996)]. Histology shows unlayersed neurons which may look normal on inspection (Battaglia et al., 1996), but closer study suggests an imbalance between excitation and inhibition within the heterotopia (Hannan et al., 1999).

1.3.7.2.3 Mechanisms of formation of subcortical heterotopia

Subcortical heterotopia are often sporadic, indicating that somatic rather than germline mutations may be involved (Kuzniecky and Barkovich, 2001). There is also evidence pointing towards focal nongenetic causes - exclusive association with unilateral rather than bilateral subependymal nodular heterotopia (Dubeau et al., 1995), discordant occurrence in monozygotic twins (Kuzniecky et al., 1995), better outcome after surgery than for the commonly genetically caused subependymal nodular heterotopia (Dubeau et al., 1995). In one single case report, intractable epilepsy due to occipital subcortical nodular heterotopia was associated with an ipsilateral hypoplastic posterior cerebral artery, implicating a prenatal ischemic aetiology (Reutens et al., 1993) or a process causing both subcortical nodular heterotopia and hypoplastic artery.
1.3.7.2.4 Clinical features and neurophysiology

Epilepsy is the rule rather than the exception and usually develops during the first or second decade (Kuzniecky and Barkovich, 2001). Patients with small unilateral subcortical heterotopia may be neurologically normal (Barkovich and Kjos, 1992a), whereas patients with extensive unilateral heterotopia are likely to present with hemiplegia. At the severe end of the spectrum, patients with bilateral, large or spatially extended lesions may present with moderate to severe developmental delay and neurological deficits (Kuzniecky and Barkovich, 2001).

EEG studies usually show regional rather than focal abnormalities. Heterotopic grey matter (Francione et al., 1994; Kothare et al., 1998; Sisodiya et al., 1999a) and neocortex in migration disorders (Mattia et al., 1995) may be intrinsically epileptogenic. As expected, however, acute recordings do not always show epileptiform activity (Preul et al., 1997).

Treatment of the epilepsy is with antiepileptic drugs. There are few case reports on epilepsy surgery. As expected with intrinsically epileptogenic lesions, incomplete resection does not lead to seizure freedom (Dubeau et al., 1995; Preul et al., 1997) whereas complete resection had a good outcome in a report of one case (Francione et al., 1994). Surgery for subcortical heterotopia associated with ipsilateral subependymal nodular heterotopia seems to carry a better prognosis than surgery for subependymal nodular heterotopia alone. In one series, two of three patients with both pathologies became seizure free, whereas none of three patients with subependymal nodular heterotopia alone improved significantly after surgery (Dubeau et al., 1995). This may reflect the different aetiologies of the two conditions.

1.3.7.2.5 Neuroimaging

Nodules do not calcify and are not commonly seen on CT (Raymond et al., 1995). On MRI, the subcortical nodules are seen as isointense to normotopic grey matter (Raymond et al., 1995). They may extend in a curvilinear course from the ventricular surface to the cortex (Kuzniecky and Barkovich, 2001). The overlying cortex may look normal but may also be thin with small gyri and shallow sulci. The volume of the affected hemisphere may be reduced overall, but nodules may also exert a "mass effect", displacing white matter into the ventricles. Coexisting abnormalities are frequent, Kuzniecky and Barkovich claim that an agenetic or hypogenetic corpus callosum is present in approximately 70% of affected brains (Kuzniecky and
Barkovich, 2001), and other malformations, particularly subependymal nodular heterotopia, are frequently seen (Cendes et al., 1995; Raymond et al., 1995).

1.3.7.3 Laminar heterotopia

1.3.7.3.1 Definition and epidemiology

Laminar heterotopia (also called band heterotopia, or, misleadingly, "double cortex syndrome") is one of the generalised disorders of migration. It is a condition characterised by laminar collections of heterotopic neurons ("bands") in the subcortical white matter, usually affecting the fronto-central and/or parieto-occipital areas and sparing of the temporal, inferior frontal and cingulate/medial frontal cortices. Patients usually develop epilepsy during the first two decades of life.

Laminar heterotopia is not a very common form of MCD found in adult epilepsy patients, accounting for 8/100 patients with MCD in the National Hospital series (Raymond et al., 1995), and the true prevalence is unknown. Notwithstanding, it is the migration disorder studied in greatest detail and the one usually featuring most prominently in reviews (Golden, 2001; Guerrini and Carrozzo, 2001; Kuzniecky and Barkovich, 2001; Ross and Walsh, 2001), owing to its occurrence as an X-linked condition leading to laminar heterotopia in affected females and lissencephaly in males (see below).

1.3.7.3.2 Neuropathology

The laminae are composed of differentiated, randomly orientated and focally clustered neurons of all types, accompanied by glial cells. In most cases, the overlying cortex shows the normal six-layered architecture, but layers V and VI may be poorly delineated and merge with the subcortical white matter and the lateral parts of the laminae. It is in these cases that macrogyric or pachygyric appearances may be seen. When the laminae are thick, they are more likely to be associated with thickening of the overlying cortex (Barkovich and Kjos, 1992a; Palmini et al., 1991a).

1.3.7.3.3 Mechanisms of formation of laminar heterotopia and the relationship to lissencephaly

A developmental link between lissencephaly (agyria) and laminar heterotopia was reported in a study of two families. Females had laminar heterotopia and hemizygous
male offspring had lissencephaly (Pinard et al., 1994). Subsequently, mutations of the gene, named DCX after its gene product, doublecortin, or XLIS after the mode of transmission, have been identified on the X chromosome (Des Portes et al., 1998). Mutations have also been seen in some sporadic cases where DCX mutations account for some 85% of cases, whereas mutations have been found in 11 of 11 pedigrees with X-linked transmission of laminar heterotopia [reviewed by (Ross and Walsh, 2001)]. The different expression in males and females is thought to be due to males only possessing one defective copy of the gene, while females inactivate one copy early in embryogenesis through random X inactivation. Skewing of this process could then lead to the lissencephaly phenotype in females carrying the DCX/XLIS mutation (Ross et al., 1997). Doublecortin has only been detected in neurons of both the central and peripheral nervous system. It is a microtubule-associated protein expressed by migrating neurons (Gleeson et al., 1999). While its precise function is unknown, it is thought to play a role in regulation of the microtubule cytoskeleton (Gleeson and Walsh, 2000).

A linked phenotype is seen in classical lissencephaly (Type I lissencephaly, "smooth brain" with four-layered cortex), which includes Miller-Dieker syndrome. The latter is caused by a deletion of 17p13.3 in over 90% of cases, the deletion including the LIS1 gene but probably also affecting contiguous genes which are responsible for the facial appearance of patients with Miller-Dieker syndrome. LIS1 mutations are often found alone (i.e. not affecting contiguous genes) in isolated lissencephaly sequence, but up to a quarter of isolated lissencephaly sequence cases could involve another gene (Ross and Walsh, 2001). The LIS1 gene product (Lis1 protein) is a noncatalytic subunit of platelet activating factor-acetylhydrolase (Pafah1b1) and is known to regulate platelet activating factor (Ross and Walsh, 2001), a lipid first messenger involved in many intracellular processes.

Interestingly, patients with classic (Type I) lissencephaly can show relative sparing of the frontal lobes which distinguishes this type from the X-linked form of both lissencephaly and laminar heterotopia where the frontal lobes are usually more severely affected. Moreover, mutational heterogeneity can lead to milder phenotypes [for reviews, see (Golden, 2001; Ross and Walsh, 2001)].

Comparison with two animal models shows some of the difficulties in elucidating mechanisms of migration disorders. It was previously believed that formation of the X-linked laminar heterotopia in females and lissencephaly in males was due to
random X inactivation and arrest of the neurons in which the mutation is expressed beneath the subplate, whereas the neurons expressing the wildtype gene penetrated the subplate to form normal cortex, in analogy to the reeler mouse model. Engineered LIS1 mutants, however, have shown poor layer specificity of migrating neurons rather than arrest beneath the subplate as in reeler [reviewed by (Gleeson and Walsh, 2000)]. On the other hand, the spontaneous, autosomal-recessively transmitted mutation present in the tish rat leads to a phenotype similar to human laminar heterotopia, with a fronto-parietal band. It has been shown, however, that the heterotopia arise from heterotopic cell proliferation rather than disturbed migration, and tish thus represents an example of a similar phenotype arising from a different mechanism [reviewed by (Ross and Walsh, 2001)].

1.3.7.3.4 Clinical features and neurophysiology
While in earlier series most patients were mildly to moderately mentally retarded (Palmini et al., 1991a), this is by no means the rule (Raymond et al., 1995). Thicker laminae and more disorganised overlying cortex are more likely to be associated with mental retardation (Barkovich et al., 1994; Palmini et al., 1991a; Raymond et al., 1995). Most if not all patients develop epilepsy, usually within the first two decades. A variety of seizure types is seen, including infantile spasms, Lennox-Gastaut syndrome, or other forms of secondary generalized, focal or multifocal epilepsy. Partial seizures are, however, most frequent (Barkovich et al., 1994). EEG may be normal, show widespread theta and focal spikes (Barkovich et al., 1994) or sometimes generalised spike-wave discharges.

The response to medical treatment is variable. One study reported a high incidence (60%) of drop attacks which could be considerably reduced by callosotomy (Palmini et al., 1991a). In another series of eight patients with laminar heterotopia and regional or focal seizure onset despite mostly multilobar EEG changes, a variety of surgical procedures was undertaken. Three patients were improved, one of whom significantly, but the authors concluded that their data did not support surgical approaches in bilateral laminar heterotopia (Bernasconi et al., 2001c).

1.3.7.3.5 Neuroimaging
Laminar heterotopia was not visible on CT in 8 cases (Raymond et al., 1995). On MRI, the laminar heterotopia are usually bilateral and approximately symmetrical. As with the other heterotopia, they are isointense to normotopic grey matter on all
sequences. They predominate in the superolateral centrum semiovale of the frontal through to the occipital lobes, but may be seen in the temporal lobes only. The bands may be less than 5 mm but also some 20 mm thick (Raymond et al., 1995). When they are thin, they may appear (or be) discontinuous. Typically, the medial border of the lamina is smooth, whereas the lateral border follows the white matter into the crowns of the gyri. The cortex overlying the laminae may appear macrogyric. In patients with the DCX/XLIS mutation, both patients with laminar heterotopia and relatives with lissencephaly show an anterior>posterior gradient of severity.

1.3.8 Abnormal postmigrational cortical organisation and unclassified MCDs: Schizencephaly, polymicrogyria, dysembryoplastic neuroepithelial tumours

1.3.8.1 Polymicrogyria

1.3.8.1.1 Definition and epidemiology
Polymicrogyria arises when neurons move out to the cortical surface but organise abnormally to produce multiple small gyri. It may be focal (but often bilateral), occurring predominantly in perisylvian, frontal, parieto-occipital or mesial occipital areas, and diffuse. In diffuse forms, the medial occipital region seems less often affected (Kuzniecky and Barkovich, 2001).

The true incidence and prevalence are not known. In the National Hospital series, out of 100 patients with MCD (Raymond et al., 1995), only one was diagnosed as having polymicrogyria on MRI with certainty, but polymicrogyria could not be excluded in the 16 patients classified as focal macrogyria, and another seven patients out of 11 in the subcategory "focal macrogyria/polymicrogyria associated with a cleft" had polymicrogyric cortex lining the cleft (see schizencephaly below). In patients with diffusely thickened cortex seen on MRI, Kuzniecky et al. estimated that polymicrogyria is more common than lissencephaly, based on personal experience (Kuzniecky and Barkovich, 2001).

1.3.8.1.2 Neuropathology
Polymicrogyria is characterised by many small microgyri separated by shallow sulci, abnormally thin cortex which appears thickened through the juxtaposition of the tightly packed microgyri, neuronal heterotopia and often enlarged ventricles (Kuzniecky and Barkovich, 2001). Two patterns are recognised, four-layered
polymicrogyria, in which there is a molecular layer, an organised outer layer, a cell sparse layer and a disorganised inner layer, and an unlayered (disorganised) form. These two forms likely reflect the timing of the causative insult during corticogenesis (Norman et al., 1995c). Associated MCD may be present, including neural and glial leptomeningeal ectopia, areas resembling FCD, clefts, and calcification. There is an excess of reelin immunopositive Cajal-Retzius cells in the region of the malformation which may be part of the malformation or a may represent a reaction to an area of cortical injury (Eriksson et al., 2001b). Bilateral perisylvian polymicrogyria is relatively frequent and considered a discrete congenital syndrome by some (Kuzniecky et al., 1993a) but not others (Raymond et al., 1995).

1.3.8.1.3 Mechanisms of formation of polymicrogyria

Polymicrogyria is generally considered to be due to environmental insults rather than genetic causes, and diffuse polymicrogyria has classically been associated with intrauterine cytomegaly virus (CMV) infection (Barkovich, 1995), while perfusion failure due to CMV, toxoplasma, syphilis, or maternal hypoxia has been suggested as a mechanism for diffuse polymicrogyria (McBride and Kemper, 1992). The environmental hypothesis is further strengthened by the rat freeze model where a focal freezing injury leads to the formation of a microgyrus (Dvorák and Feit, 1977; Jacobs et al., 1999a) and epilepsy through hyperexcitability caused by reorganisation of neural networks in perilesional areas (Jacobs et al., 1999b; Jacobs et al., 1999c). Lesion experiments suggest mechanisms that interfere with radial glial function and ensuing secondary disturbances, but this need not be the mechanism in all cases (Ross and Walsh, 2001). The presence of several mechanisms is suggested through the observation that polymicrogyria usually occurs at the margins of disruptions (e.g. porencephaly) [reviewed by (Friede, 1989)] but also in the setting of primary malformations such as Zellweger’s syndrome and triploidy (Golden, 2001). Focal patterns and transmission within families (Ferrie et al., 1995) are recognised and suggest several implicated genes (Barkovich et al., 1999). An autosomal recessive inheritance is possible in purely frontal polymicrogyria: Thirteen cases appeared sporadic, but there was parental consanguinity in two of the families (Guerrini et al., 2000). Genetic heterogeneity has also been indicated in kindreds with perisylvian polymicrogyria, with both X-linked (Cardoso et al., 2000; Guerreiro et al., 2000; Leventer et al., 2000; Yoshimura et al., 1998) and autosomal (dominant with incomplete penetrance, or recessive) patterns (Borgatti et al., 1999; Leventer et
al., 2000). The genetic locus for bilateral perisylvian polymicrogyria has recently been mapped to Xq28 by linkage analysis in five families (Villard et al., 2002). No inheritance pattern was discernible in nine individuals with parieto-occipital polymicrogyria (Guerrini et al., 1997).

1.3.8.1.4 Clinical features and neurophysiology

Epilepsy and mental retardation are common. Neurological signs are more likely to occur when the MCD is located in or near eloquent cortex (Raymond et al., 1995). The best-studied form is the bilateral perisylvian form. Clinical features include pseudobulbar palsy with often prominent tongue movement disturbance and dysarthria, and variable cognitive deficits. A unique seizure type consists of peri-oral seizures with bilateral facial involvement (Kuzniecky and Barkovich, 2001). EEG shows generalised slow spike-wave in 80% and (multi)focal abnormalities in 20% (Kuzniecky et al., 1994). A small minority do not have epilepsy (Guerrini et al., 1992).

1.3.8.1.5 Neuroimaging

CT is often normal in focal and even diffuse forms (Raymond et al., 1995). The polymicrogyria may be visible on high resolution MRI or be indistinguishable from macrogyria/pachygyria (Raymond et al., 1995).

1.3.8.2 Schizencephaly

1.3.8.2.1 Definition and epidemiology

Schizencephaly ("split brain") is a term coined by Yakovlev and Wadsworth (Yakovlev and Wadsworth, 1946a; Yakovlev and Wadsworth, 1946b) to describe full-thickness clefts of the cerebral hemisphere lined by grey matter which may appear normal or be polymicrogyric. Schizencephaly may be "closed-lipped" (type I) when the edges of the cleft are in apposition to each other or "open-lipped" (type II) when the appearance is that of a wide cleft. It may be unilateral or bilateral. In one series of 20 patients, approximately one third of patients had bilateral schizencephaly and one third had the open-lipped form (Barkovich and Kjos, 1992c). Clefts which do not span the entire thickness of the cerebral hemispheres are occasionally described as schizencephalies as well even though they do not meet the criteria set by Yakovlev and Wadsworth.
The true incidence and prevalence are not known, but schizencephaly is thought to be among the rarer forms of MCD (Sisodiya, 2000). Only two patients out of 100 with MCD in the National Hospital series fulfilled the Yakovlev/Wadsworth criteria for true bilateral schizencephaly, whereas a further nine had focal macrogyria/polymicrogyria associated with clefts which appeared to be normal fissures or sulci which were widened or deepened but did not span the entire thickness of the hemispheres (Raymond et al., 1995).

1.3.8.2.2 Neuropathology
Apart from the cleft, the neuropathological appearance corresponds to polymicrogyria and indeed schizencephaly has been thought of as an extreme form of polymicrogyria (Barkovich and Kjos, 1992c).

1.3.8.2.3 Mechanisms of formation of schizencephaly
A vascular lesion during development has been suggested (Landrieu and Lacroix, 1994), and in view of the similarities with polymicrogyria, a similar variety of aetiological factors seems likely. More than half (6/11) of the patients with focal macrogyria/polymicrogyria associated with a cleft in the National Hospital series had antenatal or perinatal problems including exposure to thalidomide and rubella (Raymond et al., 1995). Other areas of the brain, outside the schizencephaly, may be histologically abnormal (Packard et al., 1997).

While most cases are sporadic, there is a minority of cases of open-lipped (type II) severe schizencephaly which is due to germline mutations in EMX2 (Brunelli et al., 1996), a homeobox gene involved in cell fate determination [reviewed by (Ross and Walsh, 2001)]. A monozygotic twin pair has been described in whom one twin had epilepsy and a closed-lipped schizencephaly in the anterior temporal lobe and the other twin who did not have epilepsy had an arachnoid cyst in the same location, suggesting a combination of genetic and environmental factors (Briellmann et al., 2001).

In analogy to polymicrogyria, epileptogenic tissue is likely to be located adjacent to the lesion.

1.3.8.2.4 Clinical features and neurophysiology
Neurological signs are common, may be accompanied by mental retardation and are usually related to the type of defect, with open-lipped (type II) defects and bilateral
clefts leading to more severe clinical pictures (Barkovich and Kjos, 1992c; Kuzniecky and Barkovich, 2001). Epilepsy seems to be the rule and is often intractable.

The EEG tends to show regional rather than focal epileptiform discharges (Kuzniecky and Barkovich, 2001).

Surgical treatment can be considered. In the published cases reviewed by Sisodiya (Sisodiya, 2000), in most the cleft itself was not completely excised, and extraoperative intracranial EEG studies or electrocorticography (ECoG) guided the resection to adjacent areas, in analogy to the situation in polymicrogyria (see above).

1.3.8.2.5 Neuroimaging

The cleft may be seen on CT, and MRI may be able to clearly show the polymicrogyric nature of the thickened grey matter lining the clefts (Raymond et al., 1995).

1.3.8.3 Dysembryoplastic neuroepithelial tumour

1.3.8.3.1 Definition and epidemiology

Dysembryoplastic neuroepithelial tumour (DNET) is a predominantly intracortical developmental lesion associated with early onset of epilepsy and cortical dysgenesis which carries a good prognosis after surgical resection (Daumas-Duport, 1993; Daumas-Duport et al., 1988; Raymond et al., 1994c). The location is temporal in the vast majority of cases, 24/39 (62%) in the original series (Daumas-Duport et al., 1988), 59/74 (80%) in a recent large study (Honovar et al., 1999) and 20/21 (95%) in the National Hospital series (Raymond et al., 1995). Frontal lobe including cingulate gyrus is the next likely location, but rarely DNETs have been seen in deep grey nuclei (Cervera-Pierot et al., 1997), midline structures and cerebellum [reviewed by (Thom, 2001)].

The true incidence and prevalence are not known, but DNETs are certainly among the more frequent MCD causing refractory epilepsy, accounting for 21% of cases in the National Hospital series (Raymond et al., 1995). In the NSE MRI study, none out of 110 patients with newly diagnosed epilepsy but 2 of 174 patients with chronic epilepsy had DNETs [Dr Alex Everitt, personal communication].
1.3.8.3.2 Neuropathology

DNETs have a complex nodular or multinodular architecture. The cellular composition is usually heterogenous with nerve cells, astrocytes and oligodendroglial-like cells, all of normal or abnormal morphology [neuropathology reviewed by (Thom, 2001)]. Nodules of oligodendroglial-like cells which may have immunohistochemical features of neurons or glia cells, and astrocytic areas are common findings. Occasional mitoses may be seen. Adjacent malformations have been noted in about a quarter (Honovar et al., 1999) to 90% (Prayson et al., 1996) of cases; the variation may be due to availability of marginal tissue after resection. In general, therefore, DNETs are remarkable through their histopathological variation (Daumas-Duport, 1993; Thom, 2001).

1.3.8.3.3 Mechanisms of formation of DNETs

The occurrence of occasional mitoses, together with deformities of the overlying calvarium, and serial MRI studies, supports the classification of DNETs as benign neoplasms. Early descriptions considered these unusual lesions to be hamartomatous in nature, however (Cavanagh, 1958), and the participation of many distinct neuroepithelial cell lines, as well as the common association with malformative lesions in the adjacent cortex indicate that DNET arise on a background of cortical malformation.

Neoplasms of the brain other than DNET have also been found in association with cortical dysgenesis, indicating that they, too, may have a similar dysgenetic origin (Prayson et al., 1993).

1.3.8.3.4 Clinical features and neurophysiology

Epilepsy is the rule; only very occasionally patients with DNET who do not have epilepsy have been reported (Raymond et al., 1995). Intellect is normally preserved although mild memory impairment can occur with temporal lesions. There is usually no family history of epilepsy, and developmental milestones and neurological examination are normal.

EEG frequently shows localised slow activity or focal epileptiform discharges, superimposed on normal background rhythms (Raymond et al., 1995; Raymond et al., 1994c).

Seizures tend to be refractory. Surgical resection can lead to seizure freedom in 53-90% of cases after mean follow-up times of 3 up to more than 5 years (Aronica et al.,
2001; Daumas-Duport et al., 1988; Fomekong et al., 1999; Lee et al., 2000). One report claimed that outcome was significantly better after a temporal lobectomy rather than a lesionectomy (Raymond et al., 1995). This should be viewed with caution, however, as three patients in the "lesionectomy" group only had stereotactic biopsies which would not be expected to be curative, and indeed the difference between lobectomies and lesionectomies regarding postoperative seizure freedom is no longer significant when these three cases are not considered. Further, data on completeness of resection was not given in all cases and the mesial temporal structures were involved by the DNET in five cases. It therefore seems premature to base a recommendation of extended temporal lobectomy for DNET on this series.

1.3.8.3.5 Neuroimaging

DNETs may be visible on CT, especially when calcification is present (in about a quarter of cases) (Raymond et al., 1994c). MRI is clearly superior in providing detailed anatomical information and a presumptive histological diagnosis. Typical features of DNETs include intracortical location (although white matter involvement may be seen), temporal lobe location, often close to mesial temporal lobe structures, circumscribed hyperintensity on T2 weighted and hypointensity on T1 weighted images, and cyst formation (Raymond et al., 1994c). If the DNET is in superficial cortex, erosion of the cranial vault may occur. Approximately one third of DNETs enhance after contrast medium has been given, and associated cortical dysplasia is seen relatively frequently (Duncan et al., 1996c).
1.4 Cryptogenic ("MRI-negative") localisation-related epilepsy

1.4.1 Introduction

Cryptogenic epilepsies and epilepsy syndromes are those in which a symptomatic aetiology is suspected but the aetiology is not known (Commission on Classification and Terminology of the International League against Epilepsy, 1989). In practical terms, cryptogenic localisation-related epilepsies are therefore epilepsies in which clinical semiology and EEG features point towards a partial onset of seizures and in which contemporary neuroimaging, i.e. optimal MRI, does not detect a definitive abnormality.

While advances in MRI have allowed the identification of structural abnormalities that are presumed to be the seizure focus in 70-80% of localisation-related epilepsies (Duncan, 1997a), this means that there remain 20-30% in whom no lesion is identified by current optimal MRI. Surgery has a less favorable outcome when the presumed epileptogenic region is removed in the absence of identifiable pathology on imaging or the resected specimen (Berkovic et al., 1995; Jack et al., 1992).

MRI may be normal even when histopathological examination of resected specimens detects a variety of pathologies, for example focal cortical dysplasia or hippocampal sclerosis (Chugani et al., 1990; Desbiens et al., 1993; Kuzniecky et al., 1991; Van Paesschen et al., 1997), and these have been discussed in previous chapters.

One particular form of underlying pathology is microdysgenesis. By definition, microdysgenesis is neither visible on neuroimaging studies nor on naked eye examination of the cortical tissue (Raymond et al., 1995; Thom, 2001), and is therefore a prototype of pathology underlying cryptogenic localisation-related epilepsies. The concept of microscopic abnormalities underlying epileptogenesis was formulated in the mid-1980s (Meencke and Janz, 1984), and microdysgenesis is now increasingly recognised as an underlying cause of epilepsy.

1.4.2 Microdysgenesis

1.4.2.1 Microdysgenesis: Definition and epidemiology

Microdysgenesis describes a variety of neuropathological abnormalities (see below). Common to all of them is their relatively subtle nature, leading to indetectability on neuroimaging and macroscopic examination. Many of the hallmarks of
microdysgenesis also point towards an excess of nerve cells rather than a deficit (Thom, 2001).

The true incidence and prevalence are not known, and mild microdysgenetic changes can occur in neurologically normal subjects (Kaufmann and Galaburda, 1989). Lack of standardised criteria for the diagnosis leads to widely varying estimates, and it has been claimed that microdysgenesis accounts for up to 37% of the malformative lesions found in the brains of patients with epilepsy (Meencke and Veith, 1992). If the definition is restricted to white matter neuronal densities, findings are more comparable between laboratories. There is general agreement that there is a large range of normal variation [e.g. (Rojiani et al., 1996; Thom et al., 2001)], a difference between patients with temporal lobe epilepsy (Emery et al., 1997; Hardiman et al., 1988; Kasper et al., 1999; Mitchell et al., 1999; Thom et al., 2001) and controls, a difference between patients with idiopathic generalised epilepsy (Meencke and Janz, 1984) and controls, and considerable overlap between patients and controls. Some researchers have found a "cut-off" for an upper limit of normal (Hardiman et al., 1988).

1.4.2.2 Microdysgenesis: Neuropathology

The original description of microdysgenesis was based on the detailed study of eight autopsy specimens of patients with idiopathic generalised epilepsy (Meencke and Janz, 1984). Seven of eight showed a variety of 'disturbances of the brain architecture', including an excess of nerve cells in the subpial region, increased numbers of single neurons or clusters of neurons in the molecular layer of the cortex, an indistinct boundary between cortical layers I and II, protrusions of nervous tissue into the pia mater, persistent columnar alignment of cortical nerve cells, increased numbers of heterotopic neurons in the white matter, heterotopic nerve cells in the hippocampus and dystopic Purkinje cells in the cerebellar cortex (Meencke and Janz, 1984). White matter neuronal heterotopia is easier to quantify than many of the other features and has received more attention recently. Another abnormality recently reported in the context of microdysgenesis in epilepsy is the presence of abnormal myelinated fibres in the superficial cortex (Thom et al., 2000).

1.4.2.3 Mechanisms of formation of microdysgenesis and of epileptogenesis

Microdysgenesis is thought to be due to a disturbance of cortical development occurring after the seventh month of gestation (Meencke and Veith, 1992). The
heterotopic nerve cells in the molecular layer may represent either Cajal-Retzius cells or additional or misplaced pyramidal cells from adjacent layers (Meencke, 1985). The heterotopic neurons within the white matter are thought to represent neurons which have failed to migrate fully to the cortex or which have failed to undergo programmed cell death (Rojiani et al., 1996). A possible role in epileptogenesis is also suggested by findings of neurotransmitters in remnant/migrating cells [reviewed by (Rojiani et al., 1996)]. In order to avoid programmed cell death, such neurons should be part of signalling networks, and recently there has been direct evidence for involvement of heterotopic neurons in methylazoxymethanol-(MAM-) treated rats in both hippocampal and neocortical circuitry (Chevassus-au-Louis et al., 1999; Chevassus-au-Louis et al., 1998) and evidence for aberrant connections of heterotopic layer I neurons in New Zealand black mice (Jenner et al., 2000).

There is evidence for both genetic influences, with a malformation similar to microdysgenesis in a proportion of New Zealand black mice (Sherman et al., 1985), and environmental causes, with lesions similar to microdysgenesis in a rat freeze model (Humphreys et al., 1991) and the rat MAM model (Chevassus-au-Louis et al., 1999; Chevassus-au-Louis et al., 1998).

1.4.2.4 Microdysgenesis: Clinical features and neurophysiology

The clinical features of microdysgenesis are not well defined as the diagnosis requires histopathological examination of the brain and cannot be made during the life of the individual.

Microdysgenesis has been described in cases of idiopathic generalised epilepsy (Meencke, 1985; Meencke and Janz, 1984), West syndrome (Meencke and Gerhard, 1985), in conjunction with hippocampal sclerosis (Emery et al., 1997; Kasper et al., 1999; Meencke and Veith, 1992; Thom et al., 2001) and normal MRI appearances (Mitchell et al., 1999) and others. Microdysgenesis in the temporal lobe has been associated with an older age of onset of epilepsy than in patients with hippocampal sclerosis, a clinical feature that has repeatedly been noted in patients with normal MRI. No specific EEG pattern has been described.

One of the difficulties with the concept of microdysgenesis is the mostly nonspecific character of the abnormalities (Lyon and Gastaut, 1985; Meencke and Janz, 1985). This may be the reason why microdysgenetic changes have also been described in conditions that are not associated with epilepsy, for example dyslexia (Humphreys et al., 1990). Whether microdysgenetic changes are truly nonspecific or whether such
differences in minimal disorganisation of cerebral cortex are specific on a subcellular level, with some forms leading to epilepsy and other forms leading to other conditions, remains to be seen.

1.4.2.5 Microdysgenesis: Neuroimaging

By definition, microdysgenesis is not visible on conventional MRI scans (Raymond et al., 1995). Anterior temporal lobe abnormalities have been described in isolation (O'Brien et al., 2000) or in combination with hippocampal sclerosis (Mitchell et al., 1999), but the link to microdysgenesis is not clear. Diffusion Tensor Imaging (DTI) shows abnormalities in temporal lobe white matter in TLE patients which would be compatible with increased nerve cell content (Rugg-Gunn et al., 2001a).

1.4.3 Other "MRI-negative" localisation-related epilepsies

As outlined in the previous chapters, the brain in "MRI-negative" localisation-related epilepsies is not necessarily structurally normal. There are, however, several forms of such epilepsies (Picard et al., 2000) where the underlying abnormality is microstructural and therefore not visible on standard structural MRI sequences.

One form which has attracted much interest in recent years is autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE) (Scheffer et al., 1994), in which the α4 (Phillips et al., 1995; Steinlein et al., 1995) or β2 (De Fusco et al., 2000; Phillips et al., 2001) subunits of the nicotinergic acetylcholine receptor are affected by mutations. The clinical spectrum is wide, and manifestations like enuresis or sleep-related violent behaviour can be confounded with parasomnias (Oldani et al., 1998). In one study of 40 patients, sudden awakenings with dystonic posturing or dyskinetic movements were seen in 42% of patients on video-polysomnigraphic recordings. The EEG showed ictal epileptiform activity, predominating over frontal areas, in 32% of patients, and anterior ictal rhythmic slow in another 47% (Oldani et al., 1998). Less than 20% of patients had received a correct diagnosis of epilepsy. Recognition of the epileptic syndrome is important as the clinical manifestations are readily controlled with antiepileptic drugs in about three quarters of patients. ADNFLE is highly sensitive to carbamazepine, and mutant alpha4 subunits reconstituted in Xenopus oocytes as α4β2 nicotinic acetylcholine receptors have been found to have threefold higher sensitivity to carbamazepine than wildtype receptors (Picard et al., 1999).
Other forms of autosomal dominant partial epilepsies with no gross structural brain abnormality include familial temporal lobe epilepsy (Berkovic et al., 1994; Depondt et al., 2002), a subsyndrome of which is autosomal dominant partial epilepsy with auditory features (Ottman et al., 1995); familial partial epilepsy with variable foci (FPEVF) (Scheffer et al., 1998); and benign epilepsy of childhood with centrotemporal spikes [reviewed by (Ottman, 2001)]. Similar syndromes with relatively mild mutations affecting ion channels or neurotransmitter receptors are likely to underly some cases of "MRI-negative" localisation-related epilepsies. Such mutations may, however, be important during brain development, too, and with the further development of sophisticated imaging analysis techniques, abnormalities beyond the cellular level may eventually be found.
1.5 Neurotransmission

1.5.1 GABAergic transmission

1.5.1.1 Introduction

The amino acid \( \gamma \)-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the brain. Between 17% and 50% of all synapses in the brain have been estimated to be GABAergic (Bloom and Iversen, 1971; Mody et al., 1994; Young and Chu, 1990). GABA exerts its effect through GABA<sub>A</sub>, GABA<sub>B</sub> and GABA<sub>C</sub> receptors (see below). The clinical importance of the GABAergic system is reflected in the wide range of drugs modifying GABAergic transmission (mainly GABA<sub>A</sub> receptors), including anticonvulsants (benzodiazepines and barbiturates), steroids, anaesthetics, and anxiolytics. The paramount importance of GABA<sub>A</sub> receptors is reflected in their multiple roles. They are involved in controlling the excitability of the brain, in the modulation of anxiety, of feeding and drinking behaviour, circadian rhythms, cognition, vigilance, memory and learning [reviewed by (Sieghart et al., 1999)].

1.5.1.2 Metabolism

The metabolism of GABA is closely related to glutamate, an excitatory amino acid. Glutamate is the predominant precursor of GABA. Glutamine is converted to glutamic acid by glutaminase, releasing ammonia. Glutamic acid is converted to GABA through decarboxylation, catalysed through the enzyme glutamic acid decarboxylase (GAD). GAD is present in nerve terminals and acts as a marker for GABAergic neurons.

After release from the nerve terminal into the synaptic cleft, extracellular GABA is removed by sodium-dependent reuptake into neurons and uptake into glia. GABA is then metabolised into succinic semialdehyde, catalysed through GABA transaminase; in the process, \( \alpha \)-ketoglutarate is transformed into glutamate. Within glia cells, glutamic acid is metabolised into glutamine by fixating ammonia, catalysed through glutamine synthetase. This is an important elimination step for neurotoxic ammonia, and the necessary glutamate/glutamic acid can be provided via \( \alpha \)-ketoglutarate which is an intermediate product of the citric acid cycle. Glutamine is then passed on from glia back to neurons.
1.5.1.3 GABA receptors

The actions of GABA are mediated through GABA\textsubscript{A}, GABA\textsubscript{B} and GABA\textsubscript{C} receptors. GABA\textsubscript{A} and GABA\textsubscript{B} receptors are distinguished by their respective sensitivity and insensitivity to the antagonist bicuculline (Hill and Bowery, 1981), while GABA\textsubscript{C} receptors are stimulated by cis-4-aminocrotonic acid but not the ligands of GABA\textsubscript{A} and GABA\textsubscript{B} receptors. In the rat brain, GABA\textsubscript{A} receptors predominate in most areas; GABA\textsubscript{B} receptors are more numerous only in the molecular layer of the cerebellum and the mesencephalic interpeduncular nucleus (Bowery, 1989), while in the thalamus numbers are approximately equal (Crunelli and Leresche, 1991). GABA\textsubscript{C} receptors are mainly located on subpopulations of retinal neurons, but their \(\rho2\) subunit has also been found in the spinal cord, optic tectum, cerebellum and hippocampus (see below).

1.5.1.3.1 GABA\textsubscript{A} receptors

The GABA\textsubscript{A} receptor belongs to a superfamily of ligand-gated ion channels that includes the nicotinic acetylcholine receptor, the 5-hydroxytryptamine type 3 receptor, and the glycine receptor (Bertrand and Changeux, 1995). They are multisubunit, heteromeric ion channels directly activated by their transmitter, GABA. They seem to be regularly composed of five subunits, although a minority may have four subunits only [reviewed by (Sieghart et al., 1999)]. All GABA\textsubscript{A} receptor subunits are transmembrane proteins and consist of a large N-terminal extracellular domain, four transmembrane domains and a large intracellular loop between the transmembrane domains 3 and 4 (Schofield et al., 1987). At least six \(\alpha\)-, three \(\beta\)-, three \(\gamma\)-, one \(\delta\)-, one \(\epsilon\)-, one \(\pi\)-, one \(\theta\)- and three \(\rho\)-subunits have been cloned from the mammalian nervous system (Barnard et al., 1998). Depending on their subunit composition, receptors exhibit distinct physiological and pharmacological properties (Barnard et al., 1998). If all subunits could be combined randomly, there would be over 150,000 different receptor subtypes. Few subunits, however, can form recombinant homo-oligomeric receptors, and not all GABA\textsubscript{A} receptor subunit combinations can form recombinant hetero-oligomeric receptors [reviewed by (Sieghart et al., 1999)]. \(\alpha\), \(\beta\), and \(\gamma\) subunits have to combine to to produce GABA\textsubscript{A} receptors with a pharmacology resembling that of native receptors. Stoichiometric studies have converged onto a most likely composition of 2\(\alpha\), 2\(\beta\) and 1\(\gamma\) subunit in the form of four alternating \(\alpha\) and \(\beta\) subunits, connected by a \(\gamma\) subunit (Figure 1.1, p. 75).
In a part of receptors, two different α and/or β subunits may be present. The γ subunit may be replaced by δ, ε or π subunits, while a β subunit may be replaced by a θ subunit [reviewed by (Sieghart et al., 1999)]. The number of different receptors actually present in the brain has thus been estimated at about 500 (Sieghart, 2000). Mapping of subunits has revealed several patterns. The commonest type of receptor seems to consist of α1, β2 and γ2 subunits, but some subunits are expressed in specific regions. For example, in a study of 13 subunits in the adult rat brain, α6 subunits were only found in granule cells of the cerebellum and cochlear nucleus (Pirker et al., 2000). Furthermore, within a given region, there may be distinct patterns of subunits on the somata of neurons on the one side and on the dendrites on the other side. In the example of the rat hippocampus and using immunocytochemistry, somata of neurons showed staining for α1 and α3 subunits but not α5 or α2, while diffuse staining, i.e. staining of the processes, was seen for subunits α1, α2 and α5 but not for α3 (Pirker et al., 2000).

GABA binding to the β subunit causes the chloride channel to open (Figure 1.1). Due to the large difference in extra-and intracellular chloride concentrations, the membrane becomes hyperpolarised and therefore less excitable (DeLorey and Olsen, 1992).

The α subunit determines benzodiazepine affinity, and the γ subunit is required for benzodiazepine binding to modulate GABA-chloride coupling (Pritchett et al., 1989). The benzodiazepine binding site is situated at the interface of α- and γ-subunits (see Figure 1.1) (Sigel and Buhr, 1997). The different α subunits have different affinities for various benzodiazepines. For example, most GABA_A receptors containing α1, α2, α3, and α5 subunits have high affinities for the "classical benzodiazepine agonists" diazepam, flunitrazepam, clonazepam, while GABA_A receptors containing α4 or α6 subunits are insensitive to classical benzodiazepine agonists (Barnard et al., 1998). Such differences are probably underlying the differences in receptor binding noted historically which had led to the concept of benzodiazepine receptor subtypes 1 and 2 (BZ1 and BZ2), a distinction which is now obsolete (Barnard et al., 1998).
Flumazenil (Ro 15-1788) is a competitive antagonist, and Ro 15-4513 is a partial agonist, both with high affinity, at GABA$_A$ receptors containing $\alpha_1$, $\alpha_2$, $\alpha_3$ and $\alpha_5$ subunits (except for the combination $\alpha_1$$\beta_x$$\gamma_1$ where they have low affinity (Barnard et al., 1998)). They have low affinity for $\alpha_4$ and $\alpha_6$ containing GABA$_A$ receptors and binding is not influenced by GABA (Sivilotti and Nistri, 1991; Wisden et al., 1992).

Endogenous ligands for the benzodiazepine binding site (endozepines) have been isolated and shown to displace $[^3]$H flunitrazepam. Some modulate the action of GABA on chloride conductance positively. They were present at potentially physiologically active concentrations and implicated in idiopathic recurrent stupor
(Rothstein et al., 1992a; Rothstein et al., 1992b). Their action at the mitochondrial benzodiazepine receptor, however, where it stimulates endogenous steroid production, seems to be more important biologically (Costa and Guidotti, 1991). It has long been known that receptors have distinct pharmacological and electrophysiological properties depending on their subunit composition [for a review, see, for example, (Barnard et al., 1998)]. Unravelling the behavioural function of GABA_A receptor subtypes containing certain subunits has not been possible pharmacologically due to a lack of sufficiently specific substances (Sieghart, 2000). Genetic engineering can produce so-called "knockout" mice in which genes encoding certain subunits are inactivated. A "knockout" of the γ2 subunit is lethal (Günther et al., 1995). Inactivation of the α6 subunit does not result in an overt phenotype (Jones et al., 1997). Inactivation of the β3 subunit results in mice with epilepsy and some behavioural characteristics of the Angelman syndrome in humans (DeLorey et al., 1998).

More recently, an even more specific approach to study the effects of lack of certain subunits on the whole organism has been developed, based on the introduction of point mutations into the genes encoding single subunits of the GABA_A receptor. It can be shown that such point mutations can be targeted in a way that renders the altered subunit insensitive to modulation by classical benzodiazepines without altering their sensitivity to GABA. In mice with such a selective mutation in the gene encoding the α1 subunit (Histidin in position 101 to Arginin, His101Arg), diazepam failed to show the sedative, amnesic and (partly) the anticonvulsant actions it shows in wildtype mice. The "anxiolytic", myorelaxant and ethanol-potentiating effects, however, were preserved, suggesting that those are mediated via GABA_A receptors containing α2, α3 or α5 subunits (McKernan et al., 2000; Rudolph et al., 1999).

Similarly, the α2 and α3 subunits in mice were rendered insensitive to diazepam by point mutations in the gene for the α2 subunit (Histidine in position 101 to Arginine, His101Arg) and for the α3 subunit (Histidine in position 126 to Arginine, His126Arg). In summary, these studies showed that the anxiolytic effect of diazepam is mediated by GABA_A receptors containing the α2 subunit, which is expressed particularly in the limbic system, but not by GABA_A receptors containing the α3 subunit, which predominates in the reticular activating system (Löw et al., 2000). The α5 subunit may play a role in learning and memory (Sieghart, 2000).
1.5.1.3.2 GABA<sub>B</sub> receptors

GABA<sub>B</sub> receptors are less numerous than GABA<sub>A</sub> receptors but are still widely distributed in the brain. In contrast to GABA<sub>A</sub> receptors, they are metabotropic; their effects on membrane conductance occur via a calcium-dependent secondary messenger system involving G-protein (Sivilotti and Nistri, 1991). Furthermore, they are insensitive to benzodiazepines. Many are thought to have presynaptic locations where they impair release of both excitatory and inhibitory neurotransmitter from vesicles. Such presynaptic GABA<sub>B</sub> receptors have been found on GABAergic neurons, and their activation reduces GABA release, while their inhibition facilitates GABA release (Hill and Bowery, 1981). At postsynaptic sites, GABA<sub>B</sub> receptor activation results in an outward flux of potassium ions. The resulting membrane hyperpolarisation is of slower onset, weaker and longer than the one mediated through GABA<sub>A</sub> receptor activation.

GABA<sub>B</sub> receptors are thought to be responsible for some forms of epilepsy, particularly epilepsy with absence seizures (Bernasconi et al., 1992; Liu et al., 1992). GABA<sub>B</sub> receptors are distributed differently from GABA<sub>A</sub> receptors both in terms of anatomical regions and in terms of cellular localisation. For example, within the hippocampal pyramidal cell layers, GABA<sub>B</sub> receptor mediated feed-forward inhibition predominates in dendritic regions, while feedback inhibition is mediated through GABA<sub>A</sub> receptors and is most prevalent in perisomatic regions (Lacaille and Schwartzkroin, 1988).

1.5.1.3.3 GABA<sub>C</sub> receptors

GABA<sub>C</sub> receptors are ionotropic and composed of ρ subunits (Cutting et al., 1991). These subunits can form homo- or hetero-oligomeric channels with other ρ subunits but do not seem to combine with GABA<sub>A</sub> receptor subunits. Since ρ subunits are structurally part of the family of GABA<sub>A</sub> receptor subunits, the classification of ρ containing receptors as a specialised set of GABA<sub>A</sub> receptors has recently been recommended by the International Union of Pharmacology (Barnard et al., 1998).

1.5.1.3.4 GABA receptors and epilepsy

The importance of GABA receptors for epilepsy is described in the sections describing the different forms of epilepsy studied in this thesis (chapters 1.2-1.4).
1.5.2 Opioid transmission

1.5.2.1 Introduction and history

Various preparations of the opium poppy papaver somniferum have been used for pain relief for centuries. Morphine was first isolated in the early 19th century (Chaturvedi et al., 2000) and later shown to be almost entirely responsible for the analgesic action of opium. It emerged that structure and stereochemistry were essential for the analgesic actions of morphine and other opiates, and this led to the hypothesis of the existence of specific receptors. Receptors were identified simultaneously by three laboratories in 1973 (Pert and Snyder, 1973; Simon et al., 1973; Terenius, 1973). The different pharmacological activity of different agonists provided evidence for the existence of multiple receptors (Martin et al., 1976). In the early 1980s, there was evidence for the existence of at least three types of opiate receptors: μ, κ and δ (Kosterlitz et al., 1981; Lord et al., 1977). Endogenous opioids (originally opposed to exogenous opiates, but the distinction got lost) have been identified (Pleuvry, 1991). Endogenous opioids were first thought to be proconvulsive following the demonstration that direct central injection of enkephalins and β-endorphin led to epileptiform EEG activity [reviewed by (Tortella, 1988a)]. A growing body of pharmacological and pathophysiological evidence has subsequently demonstrated that these and other opioid neuropeptides are mainly anticonvulsant and that an endogenous antiepileptic substance, opioid in nature, is released at the time of seizures [reviewed by (Tortella, 1988a)].

1.5.2.2 Derivation, release, peptide action and metabolism

All opioid peptides are derived from three different gene products, proopiomelanocortin, proenkephalin, and prodynorphin. Pro-nociceptin is the precursor for orphanin FQ/nociceptin, the endogenous ligand for a fourth type of receptor, the "orphan" receptor (see below), but orphanin FQ/nociceptin does not interact directly with the other opioid receptors. Proopiomelanocortin gives rise to β-endorphin and nonopioid peptides; proenkephalin contains four copies of different enkephalins; and prodynorphin gives rise to several dynorphin peptides and neoendorphin (see Table 1.1, below) (Alexander et al., 1999).
Precursor protein | pro-opio-melanocortin | pro-enkephalin | pro-dynorphin | pro-nociceptin/orphanin FQ
---|---|---|---|---
**Derived ligands** | β-endorphin $(\mu, \delta)$ | [Met]enkephalin [Leu]enkephalin (both $\delta > \mu$) metorphamide $(\mu)$ | dynorphin A dynorphin A (1-8) dynorphin B α-neoendorphin β-neoendorphin (all $\kappa > \mu, \delta$) | nocistatin orphanin FQ/nociceptin

**Table 1.1** Endogenous opioid peptides, their protein precursors, their affinities to the various receptor subtypes and derived ligands. For endomorphins (1, 2), no precursor has so far been identified. Endomorphins bind to $\mu$ receptors.

Similarly to other neuropeptides, the opioids are stored in large vesicles called dense-core vesicles because their high protein content confers them an electron-dense appearance (Simmons and Chavkin, 1996). They are distinguishable from the small clear vesicles containing the fast-acting transmitters such as GABA and glutamate. Exocytosis of the dense-core vesicles is calcium-dependent (Bayon et al., 1978) but not limited to restricted zones of the plasma membrane, unlike small clear vesicles. Exocytosis requires high frequency stimulation of the opioid-containing neurons (Neumaier and Chavkin, 1989; Wagner et al., 1991). A possible reason for this could be a higher level of calcium influx required to raise cytoplasmatic calcium levels, in contrast to the small clear vesicles which are located near the active zones where the local calcium level may be controlled by the high local density of calcium channels [e.g. (Verhage et al., 1994); reviewed by (Simmons and Chavkin, 1996)].

In contrast to amino acid transmitters, opioid peptides can affect the excitability of neurons at a relatively large distance (50-100 micrometers) (Drake et al., 1994) from the site of release due to their diffusion through the extracellular space and their very high affinities for their receptors, with nanomolar concentrations of the peptides being effective (Corbett et al., 1982).

After release from nerve terminals, opioid peptides are rapidly degraded by a variety of carboxypeptidases and aminopeptidases (Pleuvry, 1991). Discrete localisation of
one degradative enzyme thought to be involved in the temporal or spatial restriction of opioid action has been described and may add to regionally different actions (Pollard et al., 1989).

1.5.2.3 Receptors and ligands

There are three main types of opioid receptors, \( \mu \), \( \delta \) and \( \kappa \). For all, the existence of subtypes has been proposed (Alexander et al., 1999), and one subtype each has been cloned in several species including man. The receptor proteins consist of about 370 amino acids (Chaturvedi et al., 2000). They belong to the G-protein coupled receptor family and have their characteristic structure with seven hydrophobic transmembrane domains, connected by relatively short intracellular and extracellular loops [reviewed by (Chaturvedi et al., 2000)] (Figure 1.2, p. 83). The amino acid sequences are about 60% identical between opioid receptor types and about 90% identical between the same receptor types cloned from different species [reviewed by (Knapp et al., 1995)]. There are marked differences in the degree of sequence conservation between domains. The transmembrane segments (except the fourth) and the three intracellular loops are highly conserved [reviewed by (Chaturvedi et al., 2000)], the latter probably because they mediate the G-protein coupling. Extracellular loops are more variable and are responsible for receptor selectivity and affinity. The determinants of the opioid receptor binding pocket, however, differ from ligand to ligand for a given receptor, although a subset of the determinants are also conserved among ligands [reviewed by (Chaturvedi et al., 2000)]. This means that there is not a single receptor binding site, as with ionotropic receptors where the engagement of a ligand must lead to micromechanical conformation changes, but that opioid receptors are capable of considerable plasticity regarding engagement of ligands and subsequent events leading to receptor activation (Chaturvedi et al., 2000).

There are well-known species differences. In general, there is relatively less \( \delta \) binding in the human compared to the rat brain, and relatively more \( \kappa \) binding (Pfeiffer et al., 1982). The latter finding has been corroborated by quantitative autoradiography (Hiller and Fan, 1996; Mathieu-Kia et al., 2001) and by the detection of a more widespread \( \kappa \) opioid receptor messenger RNA expression in humans compared with rodents (Peckys and Landwehrmeyer, 1999). Other examples include \( \kappa \) opioid receptors found in deep cortical layers in human but not in rat brain, and the lack, in humans, of the typical patchy distribution of \( \mu \) receptors seen in rodents [reviewed by (Pilapil et al., 1987)]. As a more regionally specific example,
endogenous \( \kappa \) opioids, released through high frequency electrical stimulation, competed with the specific \( \kappa \) agonist \[^{3}H\] U69,593 at \( \kappa \) binding sites which were restricted to the molecular layer of the dentate gyrus of the guinea pig (Wagner et al., 1991), while there are either no (Hiller and Fan, 1996) or very few (Mathieu-Kia et al., 2001) \( \kappa \) opioid receptors in the human dentate gyrus. Because of these and other examples, it is prudent to be careful in extrapolating results from rodent studies to humans.

Importantly for PET studies, there is evidence of \( \mu \) receptor binding and, at a lower level, \( \kappa \) receptor binding in the human cerebellum (Schadrack et al., 1999) indicating that it can not be used as a reference region.

Figure 1.2 Structure and membrane topography of opioid receptors. ECL = extracellular loop, TM = transmembrane domain, ICL = intracellular loop (after (Minami and Satoh, 1995)).

The receptor subtypes are unevenly distributed between regions. In an early study using ligands with suboptimal specificity, total opiate binding was determined with \[^{3}H\] diprenorphine (Pfeiffer et al., 1982). This binding consisted of some 40% \( \kappa \) binding, slightly below 40% \( \mu \) binding and slightly more than 20% \( \delta \) binding in the human neocortex (excluding temporal neocortex). The thalamus showed 67% \( \mu \) binding, 24% \( \kappa \) binding and only 9% \( \delta \) binding. In mesial temporal structures
(hippocampus and amygdala subregions), some 60% of binding were attributed to \kappa{} receptors, \mu{} binding represented less than 30% and \delta{} binding around 15%. Using the more selective ligands DAGO, DSLET and bremazocine (plus blockers) for \mu{}, \delta{} and \kappa{} receptors, respectively, the prevalence of \mu{} and \kappa{} binding over \delta{} binding in the human brain was confirmed in four controls aged 52 to 71 (Pilapil et al., 1987). The latter study indicated less \kappa{} binding in the basal temporal neocortex compared to the remainder of the neocortex. Similarly, a more recent quantitative autoradiographic study using DAGO, DSLET and bremazocine (plus blockers) (Hiller and Fan, 1996) found 72-74 fmol receptors / mg protein in the lateral and medial occipitotemporal gyri, compared with around 120-145 fmol receptors / mg protein in the remainder of the neocortex. \kappa{} binding was predominant at around 40-50% in most areas, \delta{} binding accounted for some 30-40% in most areas, and \mu{} binding for 15-25%, again confirming the predominance of \kappa{} binding in humans compared to rats. Selective agonists and antagonists are now available for most subtypes (Table 1.2, below). "Selectivity" is never complete, but the affinity for the subtype or subtypes indicated is usually more than an order of magnitude higher than for the other subtypes. Furthermore, at least for the \mu{} receptor, an influence of age and sex on binding has been found (Zubieta et al., 1999). This study used PET and the \mu{}-selective ligand \[^{11}\text{C}]\text{carfentanil in a total of 36 men and 30 women scanned in two different scanners.}

In the following sections, a very brief description of the anatomical localisation of the receptor subtypes is given. Messenger RNA (mRNA) expression, opioid receptor binding and, where possible, functional coupling of receptors to G proteins are considered separately for neocortex, hippocampus and amygdala. The situation in subcortical nuclei and the spinal cord, albeit relatively well studied, is not relevant for the present thesis and will therefore not be discussed.
<table>
<thead>
<tr>
<th>Receptor</th>
<th>µ (MOR)</th>
<th>δ (DOR)</th>
<th>κ (KOR)</th>
<th>orphan (ORL1, NOP1)</th>
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<td>Endogenous ligand potency</td>
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<td>dynorphin A &gt;&gt; β-endorphin &gt; leu-enkephalin &gt; met-enkephalin</td>
<td>orphanin FQ/ noiceptin</td>
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<td>dynorphin A</td>
<td>&gt;&gt; dynorphin A</td>
<td>&gt;&gt; leu-enkephalin, met-enkephalin</td>
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<th>DPDPE</th>
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<tr>
<td></td>
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<td>DSLET</td>
<td>U69,593</td>
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<td></td>
<td>Dihydromorphine</td>
<td>DADL (δ, μ)</td>
<td>nor-BNI</td>
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<td></td>
<td>CTAP</td>
<td>naltrindole</td>
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<th>methylnaltrindole (MeNTI) (antagonist)</th>
<th>cyclo-FOXY (CFX) (κ, μ) (antagonist)</th>
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<td>diprenorphine (DPN) (partial agonist) (µ, δ, κ)</td>
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Table 1.2 Opioid and opioid-like receptors and their ligands (after (Alexander et al., 1999; Koepp and Duncan, 2000; Pfeiffer et al., 1982; Pilapil et al., 1987; Schadrack et al., 1999)). Abbreviations: See list of abbreviations at beginning of thesis.

1.5.2.3.1 µ receptors

µ receptors are found abundantly in the human thalamus, amygdala, striatum and neocortex, as well as some midbrain and deeper nuclei (Kuhar et al., 1973). In the human neocortex, µ receptor messenger RNA expression is absent in layers I and II and moderate to dense in layers III to VI, with the exception of the occipital cortex (area 17) where few lightly labeled cells are found in layers II to IV (Peckys and Landwehrmeyer, 1999). µ opioid receptor binding is present in all layers (Hiller and Fan, 1996), more dense in layer IIIa and less dense in lower cortical layers (Hiller and Fan, 1996; Peckys and Landwehrmeyer, 1999) [or most dense in layers I and IV according to other authors (Pilapil et al., 1987)], suggesting a somatodendritic
expression. [D-Ala²,N-Me-Phe⁴,Gly-ol⁵]-enkephalin (DAMGO)-stimulated \[^{35}\text{S}]\text{GTP}_{\gamma}\text{S}-binding, indicating functional coupling of \(\mu\) receptors to intracellular signal transduction mechanisms, was around 200% above basal levels and evenly distributed throughout human frontal grey matter (Platzer et al., 2000; Rodríguez-Puertas et al., 2000).

In the human hippocampus, \(\mu\) receptor mRNA expression is weak to moderate in the dentate gyrus, and weak to dense in CA1-CA4. The distribution of \(\mu\) receptor mRNA containing cells was similar to the distribution of GABAergic interneurons, suggesting that \(\mu\) receptor action might inhibit these. The weak labeling of pyramidal cells in CA subregions would support this (Peckys and Landwehrmeyer, 1999). \(\mu\) opioid receptor binding is very light in all areas (Hiller and Fan, 1996; Pfeiffer et al., 1982). DAMGO-stimulated \[^{35}\text{S}]\text{GTP}_{\gamma}\text{S}-binding was moderate (around 50% above basal levels) throughout hippocampal subfields and in the entorhinal cortex with no increase above basal levels in the subiculum (Rodríguez-Puertas et al., 2000).

The human amygdala was not included in the study of mRNA expression by Peckys et al. (Peckys and Landwehrmeyer, 1999). Total opioid receptor binding as estimated by \[^{3}\text{H}]\text{diprenorphine binding was highest in the amygdala of all regions studied, at up to 12.2pmol/g tissue (Pfeiffer et al., 1982), and \(\mu\) opioid receptor binding contributed some 30%. Another study found high \(\mu\) opioid receptor binding in the amygdala as well (Pilapil et al., 1987). A more recent study did not quantify \(\mu\) opioid receptor binding in the amygdala but from the figures it can be deduced that it is much higher than \(\delta\) or \(\kappa\) binding.

Pharmacologically, naloxonazine-sensitive \(\mu_1\) and naloxonazine-insensitive \(\mu_2\) sites can be distinguished, and there are at least seven \(\mu_1\) splice variants with different amino acid sequences in the intracellular (C-terminal) tip (Pasternak, 2001). Whether these subtle differences play a role beyond providing an explanation for incomplete cross-tolerance between \(\mu\)-agonist mediated analgesics remains to be seen.

1.5.2.3.2 \(\delta\) receptors

\(\delta\) receptors are generally less prominent in the human brain than in rodent brains [see above and (Peckys and Landwehrmeyer, 1999) for discussion]. They are most numerous in the striatum and throughout the neocortex, and occur notably in occipital cortex (area striata) (Pfeiffer et al., 1982; Pilapil et al., 1987).

In the human neocortex, \(\delta\) receptor mRNA expression is absent in layer I and weak to dense in layers II to VI. A similar pattern is seen in the occipital cortex (area 17)
δ opioid receptor binding is present in all layers (Hiller and Fan, 1996) and shows peak densities in layers I to IIIa (Hiller and Fan, 1996; Pilapil et al., 1987). DPDPE-stimulated [35S]GTPγS-binding, indicating functional coupling of δ receptors to intracellular signal transduction mechanisms, was evenly distributed throughout human frontal grey matter (Platzer et al., 2000). In the human hippocampus, δ receptor mRNA expression is moderate to dense in the dentate gyrus, and weak to moderate in CA1-CA4. The distribution of δ receptor mRNA containing cells included granular cells in the dentate gyrus and scattered neurons of moderate size in the CA subfields (Peckys and Landwehrmeyer, 1999). δ opioid receptor binding has been reported to be even lower than μ receptor binding in one earlier study (Pfeiffer et al., 1982) but to be moderate in pyramidal cell layers and dense in the dentate gyrus in another (Hiller and Fan, 1996). The latter would be consistent with a somatodendritic local expression.

The human amygdala was not included in the study of mRNA expression by Peckys et al. (Peckys and Landwehrmeyer, 1999). δ opioid receptor binding accounted for only about 10% of the total opioid receptor binding in the amygdala, but the absolute amount of binding would be not far below other areas as total binding in the amygdala was very high (Pfeiffer et al., 1982). Another study found relatively low δ opioid receptor binding in the amygdala as well (Pilapil et al., 1987).

1.5.2.3.3 κ receptors

κ receptors are generally more or much more prominent in the human brain than in rodent brains [see above and (Peckys and Landwehrmeyer, 1999) for discussion]. They are most numerous in neocortex, amygdala, hippocampus and notably sparse in the striatum (Pilapil et al., 1987).

In the human neocortex, κ receptor mRNA expression is absent in layer I and IV and weak to dense in layers II, III, V and VI, with denser binding in layers V and VI. A similar pattern, but with generally weak expression, is seen in the occipital cortex (area 17) (Peckys and Landwehrmeyer, 1999). κ opioid receptor binding is present in all layers (Hiller and Fan, 1996) but is concentrated in layers V and VI (Hiller and Fan, 1996; Pilapil et al., 1987), matching the distribution of mRNA expression. One study estimated κ opioid receptor binding in laminae V and VI in any cortical region to be approximately 50% higher than the highest concentration of δ opioid receptor binding in the superficial layers (I, II and parts of III) and at least two or three times higher than the greatest concentration of μ opioid receptor binding in laminae II and
IV (Hiller and Fan, 1996). Enalolone-stimulated $[^{35}\text{S}]\text{GTP}_\gamma\text{S}$-binding, indicating functional coupling of $\kappa$ receptors to intracellular signal transduction mechanisms, was present throughout human frontal grey matter but twice as strong in lamina V-VI compared to lamina I-IV (Platzer et al., 2000).

In the human hippocampus, $\kappa$ receptor mRNA expression is moderate to dense in the dentate gyrus, and weak to moderate in CA1-CA4. The dentate gyrus signal was intense over the granular cell layer, and low signal was seen over the pyramidal cell layer in subfields CA1-CA4 (Peckys and Landwehrmeyer, 1999). $\kappa$ opioid receptor binding in the human hippocampus was higher than $\mu$ or $\delta$ receptor binding in one study (Pfeiffer et al., 1982). It was restricted to the pyramidal cell layer, where it was very dense, and did not appear in the dentate gyrus in a more detailed study (Hiller and Fan, 1996). In another study, $[^{3}H]$ U69,593 binding was high in CA1, particularly in the stratum lacunosum moleculare, moderate in CA2 but low in the hilus, dentate molecular layer and CA3 (de Lanerolle et al., 1997). The absence of binding sites despite presence of moderate to dense mRNA signal suggests the expression of $\kappa$ opioid receptors on presynaptic sites on mossy fibres, while their presence on pyramidal neurons suggests a postsynaptic function as well [reviewed by (Peckys and Landwehrmeyer, 1999)].

There is strong $\kappa$ receptor mRNA expression in the human amygdala (Simonin et al., 1995). $\kappa$ opioid receptor binding accounted for about 60% of the total, very high, opioid receptor binding in the amygdala (Pfeiffer et al., 1982). Another study confirmed high $\kappa$ opioid receptor binding in the human amygdala (Pilapil et al., 1987), albeit the figures in another study suggest it may be slightly less than $\mu$ binding (Hiller and Fan, 1996).

1.5.2.3.4 "Orphan" receptor (ORL1, NOP1)

The fourth type, the "orphan" receptor (ORL1 or NOP1), displays a high degree of structural homology with the conventional opioid receptors, and was identified through homology with the $\delta$ receptor (Darland et al., 1998). The endogenous ligand, orphanin FQ/nociceptin, does not interact directly with classical opioid receptors (Mogil and Pasternak, 2001). It has, however, been implied in pain mechanisms, and recent data indicate a possible role in epilepsy, with inhibitory and antiepileptogenic properties (Gutierrez et al., 2001; Tallent et al., 2001). It is not labeled by the tracer used in this thesis and will therefore not be discussed further.
1.5.2.3.5 Desensitisation and downregulation

As is the case for most G-protein coupled receptors, short term exposure of opioid receptors to agonists leads to receptor desensitisation, and long term exposure to agonists leads to receptor downregulation [reviewed by (Chaturvedi et al., 2000)]. Desensitisation is achieved through phosphorylation of agonist-activated receptors and subsequent receptor endocytosis via clathrin-coated pits. In the case of δ opioid receptors and in contrast to other G-protein coupled receptors, this process may be irreversible, with recovery of receptor levels over eight hours through de novo protein synthesis. The ubiquitin/proteasome pathway seems to be important in agonist-induced downregulation as well as basal turnover of opioid receptors (Chaturvedi et al., 2000): Ubiquitination is required for endocytosis. While monoubiquinated receptors are simply internalised, ubiquitin chains of more than three units target the receptor to the proteasome. Further downstream, trafficking to lysosomes may play a role.

1.5.2.4 Opioids and epilepsy

The imaging studies in humans are discussed below in the chapters on opioid receptor imaging (chapters 1.6.2.3.1.2 and 1.6.2.3.2.2). This chapter will discuss the evidence for involvement of opioids and their receptors in epilepsy. Most of the experimental work has been carried out in either rats or guinea pigs, and the caveats about species differences outlined above (chapter 1.5.2.3) apply.

Evidence for an implication of the opioid system in the pathophysiology of seizure disorders was found in the early years after the discovery of opioid receptors. Endogenous opioids were thought to act as convulsants based on the emergence of epileptiform EEG activity after central (usually intracerebroventricular) injection of enkephalins and β-endorphin in rats. It may be of importance that usually no convulsive motor activity was observed [reviewed by (Tortella, 1988a)]. This proconvulsant effect is somewhat unexpected in view of the preponderant inhibitory effect of opioid peptides on unit activity. In the hippocampus, however, opioid peptides induce single-unit excitations in a naloxone-reversible manner. This is due to presynaptic disinhibition of pyramidal cells through inhibition of interneurons (Ziegglänsberger et al., 1979), mediated through μ receptors [reviewed by (Tortella, 1988a)] and δ receptors (Haffmans and Dzoljic, 1987).
Application of the nonselective opioid antagonist naloxone has led to inconclusive results. Only very high doses seem to lower the seizure threshold in rats or directly induce convulsive seizures, but this may be an artefact of GABA antagonist properties of naloxone (Snead and Bearden, 1980). Similarly, clinical studies in humans have not shown clearcut anticonvulsant or proconvulsant effects (Tortella, 1988a). Naloxone does not prevent seizures in humans produced by electroconvulsive therapy (Sperling et al., 1989), and it has been proposed that endogenous opioid systems may not be critically important to the initiation and propagation of seizures (Tortella, 1988a).

The anticonvulsant properties of endogenous opioids, however, have been shown in chemical, electrical and genetic models of epilepsy in mice, rats, gerbils, rabbits and baboons. Their ability to suppress seizures is dose-related and sensitive to opioid antagonists in nearly all experiments [e.g. (Tortella, 1988b; Tortella and Long, 1988); reviewed in (Tortella, 1988a)]. Seizure protection can be mediated by all three major receptor subtypes (Tortella, 1988a) but more recent investigations point to different roles of the subsystems (see below). Importantly, the endogenous opioid systems do not seem to be tonically active. Rather, opioid release is triggered by higher firing rates [e.g. (Neumaier and Chavkin, 1989; Wagner et al., 1991); reviewed by (Simmons and Chavkin, 1996; Tortella, 1988a)], and there is ample evidence for opioid release triggered by seizures and a role of opioids in postseizure inhibition [see reviews by (Simmons and Chavkin, 1996; Tortella, 1988a)].

The hippocampus offers unique opportunities for the study of neurotransmission due to its well known anatomical arrangement (Lorente de Nó, 1934; Ramón y Cajal, 1903) which can be preserved for a certain time in vitro using slice preparations. Many aspects of neural transmission including opioid transmission have been studied in much greater detail in the hippocampus than in other brain regions. The hippocampus is also clearly involved in the pathogenesis of partial seizures (see earlier chapters). For these reasons, it is useful to consider it in greater detail, even though most of the data is derived from laboratory animals, and the caveats about species differences outlined above (chapter 1.5.2.3) apply.

In laboratory animals, enkephalins, binding to μ and δ receptors, are present in interneurons in dentate gyrus and CA1-3; in the lateral perifornat path and in mossy fiber collaterals in the dentate gyrus; in mossy fibers (the efferents of the dentate
gyrus granule cells to the dendrites of the pyramidal cells in ammon's horn in the neighbouring CA3 and CA4) and the lateral temporoammonic tract in CA3; and in the lateral temporoammonic tract in CA1/2. Dynorphins, binding to κ receptors, are present in mossy fibers in CA3 and mossy fiber collaterals in the dentate gyrus as well as in granule cell dentrites in the dentate gyrus [reviewed by (Simmons and Chavkin, 1996)].

Receptor distribution in humans has been discussed in the preceding chapters. This section will focus on the comparison between humans and some standard laboratory animals.

In the guinea pig and in humans, the hippocampus has diffuse and low μ receptor binding (Hiller and Fan, 1996; McLean et al., 1987), but it is said to be dense in the stratum lucidum where mossy fibers end (McLean et al., 1987) in guinea pigs as well as squirrels and hamsters. In contrast, in rats μ receptor binding is dense in the pyramidal cell layer and granule cell layer but there were no binding sites for [³H] DAMGO in the rat stratum lucidum, in contrast to the other three species (McLean et al., 1987).

δ receptor binding in humans is likely moderate in pyramidal cell layers and the dentate gyrus (Hiller and Fan, 1996), similar to rats (McLean et al., 1987). In contrast, in the squirrel, guinea pig and hamster, most areas of the hippocampus are rich in δ binding sites (in contrast to rats) but receptor-sparse areas are found both in the stratum lucidum (as in rats) and the dentate gyrus (again in contrast to rats).

κ receptor binding in humans is generally much more prominent in humans than in rodents (see chapters above). In the hippocampus, it seems to be restricted to the pyramidal cell layer and does not appear in the dentate gyrus (Hiller and Fan, 1996) and was very low in the dentate molecular layer, the hilus and area CA3 in another study (de Lanerolle et al., 1997). In all four animal species studied, κ receptor binding was similar to the μ binding pattern in that species, i.e. present in the pyramidal and granule cells layers in rats (the latter in contrast to humans), and dense in the stratum lucidum in squirrels, guinea pigs and hamsters, in contrast to rats (McLean et al., 1987) and humans (de Lanerolle et al., 1997). Interestingly, a more recent study has shown that the above applies to Sprague-Dawley rats but not Long-Evans rats, indicating that there may even be intraspecies differences that need to be taken into account in the interpretation of experiments (Salin et al., 1995). The paucity of κ receptor binding in the stratum lucidum in the Sprague-Dawley rat and
humans is surprising as this is the site of termination of mossy fibers from the granule cells, and granule cells are the only cells in the hippocampus containing dynorphins.

In contrast to other areas of the CNS in which opioids have inhibitory effects, μ and δ receptor agonists increase the excitatory responses from pyramidal cells: The amplitude of population spikes increases, after-depolarisations appear, and the spontaneous firing rate increases in CA1, CA3 and dentate gyrus [reviewed by (Simmons and Chavkin, 1996)]. As mentioned above, this has been shown to be an indirect effect of inhibition of inhibitory GABAergic interneurons (Zieglgansberger et al., 1979). Among other data, evidence for this mechanism includes the lack of effect of opioids on pyramidal cell responses to exogenous GABA (Masukawa and Prince, 1982; Nicoll et al., 1980), and there is direct evidence for interneuron hyperpolarisation by enkephalin (Madison and Nicoll, 1988).

In contrast to the situation of μ and δ mediated opioid actions, κ agonists can decrease the amplitude of excitatory responses in the dentate gyrus, probably mediated through inhibition of excitatory amino acids, notably glutamate, from presynaptic terminals [reviewed by (Simmons and Chavkin, 1996)]. One has to be extremely careful, however, when generalising these results. Unfortunately, the Simmons and Chavkin review is typical for others in stressing many of the species differences outlined above in its introduction, only to proceed to describe results obtained in guinea pigs for their greater reproducibility, generalising findings without taking into account that the κ receptor situation in the guinea pig is actually very different from humans, in whom results from animal experiments should ultimately destined to be useful. There are, however, numerous reports of anticonvulsant effects of κ receptor agonists in epilepsy models in rats and mice, and the selectivity of the effects could be confirmed by reversing it with the selective antagonist norbinaltorphimine [reviewed by (Simonato and Romualdi, 1996)]. Simonato and Romualdi make the interesting observation that κ receptor agonists seem to be maximally effective in models of complex partial seizures, i.e. models in which the hippocampus is more heavily involved (Simonato and Romualdi, 1996).

In the epileptogenic hippocampus, CA1 and CA3 pyramidal cells and dentate gyrus neurons including excitatory granule cells (which give rise to mossy fibers) are reduced in number. As the mossy fiber cells are thought to excite GABAergic interneurons of the hilus, this could explain a reduction of GABA-mediated
inhibition even though many GABAergic interneurons survive, i.e. the dormant basket cell hypothesis [(Sloviter, 1991) and see chapter 1.2]. The mossy fiber sprouting, described in chapter 1.2, contributes to increased excitability through formation of recurrent excitatory synapses (Okazaki et al., 1995; Okazaki et al., 1999). Mossy fiber sprouting could, however, also increase dynorphin levels and thereby lead to increased inhibition. An additional band of dynorphin immunoreactivity has repeatedly been seen in the inner molecular layer in specimens from human TLE patients [e.g. (de Lanerolle et al., 1989; Houser et al., 1990)] and has recently been shown to correspond to mossy fiber terminals (Zhang and Houser, 1999). Mossy fiber sprouting was associated with restoration of inhibition in the kainic acid model in the rat (Sloviter, 1992) but lead to loss of dynorphin A mediated inhibition of calcium channels in human specimens (Jeub et al., 1999), and some aspects of hyperexcitability were more prominent in patients with mossy fiber sprouting than in patients without (de Lanerolle et al., 1997). The quantitative importance of these processes, however, is not clear, and it is unclear what the net effect of these changes is, if there is any (Pitkanen et al., 2000).

During or following seizures in laboratory animals, enkephalin protein levels decrease for several hours but subsequently rise above control levels, and this increase has been reported to last as long as a week [reviewed by (Simmons and Chavkin, 1996)]. In contrast, mRNA levels generally increase for about 3-4 days. Taken together, these data are consistent with a massive release of enkephalins at the time of seizures, leading to depletion and compensatory upregulation of mRNA transcription.

The situation for dynorphins is broadly similar in that the peptide level decreases immediately after seizures and mRNA levels are elevated at some stage. The time course of these changes in the rat, however, is quite different depending on the model used [reviewed by (Simonato and Romualdi, 1996)]. In the electroconvulsive shock model, peptide levels decrease to about 50% after 2-3 hours and return to baseline after about 6 hours. mRNA levels decrease about 50% immediately after the seizure, increase to 150% after 6 hours and return gradually to baseline after 18 hours. In the kainic acid model, decreases of the peptide to 40% of baseline levels have been found by several investigators. These have a much slower time course than in the electroconvulsive shock model, reaching the trough at 6-12 hours and not returning to baseline until after 24-36 hours. It is not clear whether this is followed by an
increase above baseline levels. Prodynorphin mRNA levels increase dramatically immediately after seizures, to 300-1400% of baseline levels after 6-12 hours, returning to baseline after 36-48 hours. The magnitude of changes in various kindling models, still in the rat, is intermediate between the two other models, with a different time course. Peptide levels decrease to about 50% in the first hour after kindled-induced seizures; there is little consistent data for later timepoints but both normal and decreased levels have been reported after 24 hours. mRNA levels increase shortly after kindled-induced seizures but are approximately 60% below baseline after 24 hours. In summary, therefore, for dynorphins, there is less evidence for a rebound of peptide levels than for the enkephalins, but the data are equally consistent with a seizure-triggered massive release of the peptide.

Similarly, in humans, cerebrospinal fluid levels of leu-enkephalin (Cheng and Xie, 1990; Laorden et al., 1985) and beta-endorphin (Pitkanen et al., 1987) have been found to be increased after seizures. Dynorphin immunoreactivity was reduced in both normal and aberrant mossy fibers in one patient who had experienced 2-3 complex partial seizures within the 48 hours preceding surgery (Houser et al., 1990), suggesting that dynorphin is released in humans during seizures as well.

Few studies have examined opioid receptor level changes following seizures. In kindled rats, μ receptor binding, measured with $^{125}$I-FK-33824, was decreased in CA1 and CA2 at day one, and normalised by day 7 (Crain et al., 1987). δ receptor binding, measured with $^{125}$I-[D-Ala$_2$,D-Leu$_5$]enkephalin in the presence of the morphiceptin analog PL-032, was decreased in the dentate gyrus, both at one and 7 days (Crain et al., 1987). Another study found increased hippocampal μ receptor binding in rats 48 hours after the administration of kainic acid (Perry and Grimes, 1989). Following electroconvulsive shock induced seizures as a model for generalised seizures, single seizures did not affect μ or δ receptor binding, but repeated seizures led to a decrease in δ receptor binding lasting between 7 days and 2-3 weeks (Nakata et al., 1985). Some discrepancies between studies may be due to the selectivity of ligands used (Simonato and Romualdi, 1996). In genetic models, increases of opioid binding have been observed in audiogenic seizure-prone rats, El mice and mongolian gerbils [reviewed by (Simonato and Romualdi, 1996)].

Thus, in the hippocampus, opioid peptides are involved in the regulation of signal transmission and excitability in numerous ways. Recurrent seizures lead to short-term and longer-term changes in the expression and amount of opioid peptides and
receptors. In the epileptogenic hippocampus, enkephalins, acting via \( \delta \) (and \( \mu \)) receptors, have proconvulsant effects, while dynorphins, acting via \( \kappa \) opioid receptors, may function as endogenous anticonvulsants.
1.6 Imaging

Imaging is one of the fastest evolving areas of research into the epilepsies. Much progress has been made, particularly during the last decade, with the advent of high resolution imaging devices in PET and acquisition techniques particularly sensitive to the pathology suspected in MRI. The combination of structural and functional imaging further enhances the information obtained from either technique. In this chapter, MRI is considered first, followed by PET studies of glucose metabolism and specific ligands.

1.6.1 Structural Magnetic Resonance Imaging (MRI)

1.6.1.1 Introduction and method

MRI depends on the behaviour of atomic nuclei when placed in orthogonal magnetic and radiofrequency fields. Protons in the nucleus of hydrogen atoms are the most abundant and therefore contribute most to the signal. MRI uses the mobility and magnetic properties of the spatially distributed protons. The technique comprises essentially of three steps: First, a strong homogenous magnetic field is applied to achieve a stable orientation of protons. Secondly, this stable orientation is altered through high frequency electromagnetic energy. Finally, this energy input is terminated and the nuclear magnetic resonance signals measured using suitable detection coils. These raw signals in spatial frequency (k) space can be mathematically reconstructed using fast Fourier transformations to yield tomographic images of the organ of interest.

Image parameters of importance include repetition time, echo time, field of view, slice thickness, and plane, among others. Selection to some degree determines the sensitivity of the study to detect abnormalities in epilepsy patients. MRI is clearly superior to computed tomography (CT) in the detection of the pathology underlying localisation-related epilepsies in both adults and children (Kuzniecky et al., 1993b; Sperling et al., 1986; Theodore et al., 1986a). Its principal clinical applications are to identify patients suitable for epilepsy surgery and to elucidate the basis of (mainly localisation-related) epilepsies. The most commonly identified abnormalities are hippocampal sclerosis, malformations of cortical development, vascular malformations, tumours, and acquired cortical damage (cortical scars). While
hippocampal sclerosis is generally not detectable at all on CT, other pathologies may be visible, and CT may be preferred in the investigation of acutely unwell patients as the patient is more accessible during scanning. Intracranial haematomas and skull fractures are easily identified on CT, and in some instances, CT may be used as a complementary investigation to demonstrate or exclude intracranial calcifications which are not readily seen on MRI. CT may also be used if MRI is contra-indicated. Due to the rapid advances in MRI techniques, abnormalities are not infrequently identified in patients who were previously regarded as being 'MRI-negative', and it is important to describe MRI sequences, MRI field strengths and the criteria by which patients are considered 'MRI-negative' (see Result chapters).

There are various recommendations regarding indications and strategies for neuroimaging of patients with epilepsy (Commission on Neuroimaging of the International League Against Epilepsy, 1997; Commission on Neuroimaging of the International League Against Epilepsy, 1998). Imaging should include T1- and T2-weighted sequences to cover the whole brain in at least two orthogonal planes with thin slices (minimum slice thickness of the scanner used). If possible, sequences should include a volume acquisition with a partition size of 1.5mm or less to allow for the possibility of reformatting the data set in any orientation and three-dimensional reconstruction. The use of the MRI contrast medium, gadolinium, is not generally indicated but may be useful to clarify findings in selected patients (Cascino et al., 1989). In children under the age of two years, incomplete myelination may result in poor grey-white matter contrast, and repetition of the investigation after 1-2 years may be necessary to identify cortical abnormalities.

MRI examination should ideally be part of the investigations in all patients with epilepsy except those with a definite diagnosis of idiopathic generalised epilepsy or benign (rolandic) epilepsy of childhood with centrotegmental spikes. It is particularly important in patients who develop partial seizures at any age; in patients developing generalised or unclassified seizures either in the first year of life or in adulthood; patients with a fixed neurological or neuropsychological deficit; patients refractory to treatment with first-line antiepileptic drugs or patients in whom previously controlled seizures recur or who experience a change in the pattern or type of seizures. When access to MRI is limited, MRI is essential in patients with seizures not controlled by medication and patients who develop progressive neurological or neuropsychological deficits [summarised in (Duncan et al., 1996c)].

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Surgical candidates merit sophisticated imaging. This will usually include sequences acquired orthogonal to the long axis of the hippocampus to allow measurement of hippocampal volumes and T2 relaxation times. They may also benefit from functional imaging with PET and/or SPECT.

1.6.1.2 Recent developments in MRI

Surgical candidates may also benefit from recent developments in MRI. The fluid attenuation inversion recovery (FLAIR) sequence (Bergin et al., 1995) which has high signal in parenchymal lesions but low CSF signal, in contrast to T2 weighted images; diffusion-weighted imaging (DWI) and its sophistication, diffusion tensor imaging (DTI) which are particularly sensitive to water content of tissue and swelling of cells and may identify abnormalities in patients with normal conventional imaging (Eriksson et al., 2001a; Rugg-Gunn et al., 2001a). Voxel-based morphometry (VBM) is a new statistical approach for identifying lesions which seems to be relatively insensitive for the study of individual patients but may yield very interesting results in group studies, as for example the demonstration of increased grey matter in the frontal lobes in patients with juvenile myoclonic epilepsy (Woermann et al., 1999). Magnetic resonance spectroscopy (MRS) can measure total metabolite or neurotransmitter content in circumscribed areas of the brain and has been able to show abnormalities of interest for research and sometimes clinical purposes [reviewed by (Duncan, 1997b)]. Functional MRI (fMRI) is sensitive to changes of approximately 10% of the actual cerebral blood flow through dynamic measurement of concentration changes in oxygenated haemoglobin (Henry, 2000a) and has been used to identify changes in regional blood flow at seizure onset (Jackson et al., 1994). A particularly promising approach is the combination of fMRI with simultaneous EEG to define areas of interictal spike generation (Ives et al., 1993). The pulse artefact can now be corrected (Allen et al., 1998), as well as the acquisition artefact (Lemieux et al., 2001), allowing for good EEG quality and continuous acquisition. Normally, averaged responses are recorded, but approximately one third of single spikes yield a detectable signal (Krakow et al., 2001). fMRI is also beginning to be used in the presurgical evaluation of cortical areas involved in movement, speech and memory [reviewed in (Henry et al., 2000)].
1.6.1.3 Structural abnormalities underlying epilepsy identified with MRI

1.6.1.3.1 Hippocampal sclerosis

Hippocampal sclerosis is the single most common abnormality underlying refractory partial seizure disorders. Its identification is particularly important as it is amenable to surgical treatment; anterior temporal lobe resection renders two thirds of patients seizure free (Berkovic et al., 1995), and ipsilateral hippocampal atrophy is a good prognostic feature for seizure control following surgery (Garcia et al., 1994). MRI imaging is often able to reveal hippocampal sclerosis as the cause of mTLE. Several factors underlie this ability: Awareness of both normal anatomy (Cook et al., 1992; Duvernoy, 1998; Klingler, 1948; Niemann et al., 2000a) and relevant imaging abnormalities (Jackson et al., 1990; Van Paesschen et al., 1997), advances in MRI instrumentation, acquisition sequences and post-acquisition processing of imaging data (Jackson et al., 1990). The hippocampus is a curved structure with its concave surface facing the brainstem. Its longitudinal axis is at approximately 35° to the traditionally used axial imaging plane for both X-ray CT and MRI, the orbito-meatal line (Duvernoy, 1998). The sensitivity to find abnormalities can be increased when two orthogonal planes are used for visualisation with coronal images acquired perpendicular to the long axis of the hippocampus (Press et al., 1989). This is achieved by determining the imaging planes on a sagittal scout image: the axial plane of the hippocampus is in the line joining the base of the splenium of the corpus callosum with the inferoposterior border of the frontal lobe. The ensuing coronal plane is perpendicular to this, parallel to the anterior border of the brainstem.

Hippocampal sclerosis is demonstrated by unilateral decrease in hippocampal volume, demonstrated with coronal T1-weighted images, and increase in hippocampal signal on T2-weighted spin-echo images (Jackson et al., 1990; Van Paesschen et al., 1997). Additional features are decreased T1 signal intensity and disruption of the internal architecture (Jackson et al., 1993a). Possible accompanying features are atrophy of temporal lobe white and grey matter, dilatation of the temporal horn of the lateral ventricle and blurring of the boundary between grey and white matter in the temporal neocortex (Meiners et al., 1994).

The sensitivity can be further increased if volume and T2 relaxation times are quantified with suitable methods (Cascino et al., 1991; Cook et al., 1992; Duncan et al., 1996b; Free et al., 1995). This also permits the localisation of atrophy along the
length of the hippocampus as in some patients one part only, mainly the head, may be affected (Duncan et al., 1996a; Woermann et al., 1998). Morphometry is time-consuming and requires skilled operators and post-processing hardware and software, but so far automated methods are not reliable. While asymmetry of 20% or more is readily detected by experienced neuroimaging specialists on optimally orientated images, morphometry is necessary to detect lesser degrees of asymmetry or bilateral atrophy (Van Paesschen et al., 1995). As for T2 relaxation time quantification, data from healthy controls indicate that the normal range is narrow, indicating that this parameter is a useful absolute measure. While absolute hippocampal volumes have a broad normal range, correction of the hippocampal volume for the total intracranial volume has made the detection of smaller abnormalities possible (Free et al., 1995; Van Paesschen et al., 1997).

Hippocampal atrophy as assessed on MRI correlates with reduction of neuronal density in all hippocampal regions except CA2 (Lencz et al., 1992) as expected (Sommer, 1880; Spielmeyer, 1925). When ictal depth EEG is used as a gold standard, hippocampal atrophy in MRI compared favourably with all other non-invasive means of localisation in TLE (Spencer et al., 1993). Bilateral hippocampal sclerosis on MRI may (Jack et al., 1992) or may not (Jack et al., 1995) be an indicator of a poor prognosis after epilepsy surgery, indicating that other factors than imaging appearance alone need to be taken into account in the presurgical evaluation. The sensitivity of MRI in detecting hippocampal sclerosis is high but not 100%. Subtle pathology, as for example endfolium sclerosis, may remain undetected (Van Paesschen et al., 1995). Another caveat is the possibility of MRI features of hippocampal sclerosis in some patients with extratemporal seizure foci (Cascino et al., 1993b). Conversely, the detection of hippocampal sclerosis MRI must not end the careful study of the remainder of the brain; the coincidence of hippocampal atrophy and other pathology is about 15% (Cendes et al., 1995).

Assessment of the severity of hippocampal sclerosis via quantification of their volumes and the T2 relaxation time on the side of the language-dominant hemisphere is also important as a determinant of the risk of impairment of verbal memory following hippocampal resection. The more severe the atrophy on pre-operative MRI, the less likely it is that there will be a significant decline of verbal memory after surgery (Trenerry et al., 1993). Conversely, the removal of a hippocampus that
appears normal on MRI carries a high risk of noticeable memory impairment (Lencz et al., 1992; Treemery et al., 1993).

1.6.1.3.2 Malformations of cortical development (MCD)

The application of MRI to the investigation of patients with epilepsy (Sostman et al., 1984) has led to the increased recognition of MCDs underlying chronic epilepsy syndromes (Kuzniecky, 1995). The quality of the MRI examination is important: Out of 100 patients with MCDs, 68% of those who had been investigated previously with CT had had normal studies, and previous MRI had been normal in 19/36 patients. The highest sensitivity is obtained using T2 weighted and high resolution T1 weighted volumetric techniques with thin partitions, covering the whole brain and allowing the visualisation of structures in at least two orthogonal planes. During interpretation of the images, attention needs to be paid to the cortical grey matter, the boundary between grey and white matter, and the appearance of the white matter and ventricles (Duncan, 1997a). Subtle abnormalities of sulcal morphology are often difficult to distinguish from normal variation, and three-dimensional rendering of the cerebral surface may help in the analysis (Sisodiya, 2000). In children under the age of two years, the incomplete myelination and subsequently indistinct grey-white matter boundary need to be taken into account.

Even so, MRI may only reveal the 'tip of the iceberg' of abnormal cortical development: Quantitative analysis of the relative volumes of grey and white matter of the normal appearing hemisphere in patients with apparently focal MCDs may show widespread abnormalities (Sisodiya et al., 1995), and this may be part of the explanation why patients with MCDs tend to have a less good outcome after focal cortical resection than patients with other underlying pathologies (Sisodiya, 2000).

The incidence of MCDs in MRI studies is highly dependent on selection bias and the MRI techniques used. The best estimate of the population prevalence comes from community-based studies. In the NSE MRI study, based on a population of approximately 200,000, no MCD were seen on optimal MRI in 170 controls (Everitt et al., 1998), while 2/110 (1.8%) patients with newly diagnosed epilepsy and 4/174 (2.3%) patients with chronic epilepsy were found to have MCDs on MRI [A. Everitt, personal communication]. In a study including patients who were referred for MRI for epileptic seizures, MCD were found in 4.3% of 303 patients with epileptic seizures, 6.7% of patients with established epilepsy, and 13.7% of patients with concomitant mental retardation (Brodtkorb et al., 1992). In a series of 222 patients
with temporal lobe epilepsy, MRI showed MCDs in 7%, including focal cortical
dysplasia, nodular heterotopia, abnormal gyration, limited schizencephaly and
hippocampal malformations. Importantly, clinical and EEG features in these patients
did not differ from those without MCD, indicating the key role of MRI in the
investigation (Lehericy et al., 1995). The prevalence of MCD in patients with
refractory partial epilepsy is as high as 15-20% (Kuzniecky and Jackson, 1997).
The neuroimaging appearance of the various forms of MCD has been described in
detail in a previous chapter in this thesis (chapter 1.3).

1.6.1.3.3 Vascular malformations

Arteriovenous malformations, cavernous haemangiomas, venous angiomas and
telangiectases can all cause symptomatic localisation-related epilepsies (Babb and
Brown, 1987), probably through haemoglobin deposition following haemorrhage
which may be clinically silent. Cavernous haemangiomas and arteriovenous
malformations are the most commonly resected vascular malformations in surgical
series (Cascino, 1997; Dodick et al., 1994). Cavernomas carry up to a 70% chance of
subsequent seizure remission after surgical removal.

Cavernous haemangiomas have a "target" appearance on MRI caused by a region of
T1 and T2 hyperintensity caused by oxidised haemoglobin. There are darker areas on
T1 weighted images, reflecting deoxyhaemoglobin. The central area is surrounded by
an area of T2 hypointensity produced by methaemoglobin deposits in macrophages
following haemorrhages (Dodick et al., 1994; Requena et al., 1991). If there are
calcifications, these appear hypointense on both T1 and T2 weighted images. In
patients with familial cavernous haemangiomas, multiple lesions may occur.
Arteriovenous malformations can show a flow signal on MRI and may be visible on
magnetic resonance angiography (MRA).

1.6.1.3.4 Tumours

Indolent gliomas are clearly identified with MRI. Most commonly, the lesion is ill-
deﬁned, non-cystic, does not enhance after gadolinium injection and appears to arise
from deep white matter. T2 images are most sensitive in revealing foreign tissue
lesions (Bergen et al., 1989), and the sensitivity of MRI approaches 100% for both
tumours and vascular malformations (Cascino, 1997).
1.6.1.3.5 Acquired cortical damage; granulomas

Ischaemic lesions associated with epilepsy are readily seen on MRI (Kilpatrick et al., 1991). They are particularly common in the older age group and partly responsible for the increased incidence of epilepsy in the elderly. Focal and generalised atrophy, scars, cysts and traumatic lesions may all be associated with or underlying epilepsy and are readily demonstrated with MRI.

Granulomas due to tuberculosis or cysticercosis are the most commonly identified causes of epilepsy in developing countries (Garcia-Noval et al., 2001), and epilepsy is the most common manifestation of neurocysticercosis (Aubry et al., 1995). When lesions are calcified, CT will often demonstrate cysticercosis, but MRI is more sensitive especially in the earlier stages (Sanclerette et al., 1991).

1.6.2 Positron Emission Tomography

1.6.2.1 Introduction and background

PET allows tomographic representation of cerebral structures and measurement of local tissue concentrations of injected radiolabeled biologically active compounds (tracers) (Phelps, 1993). This is achieved by combining a mathematical model to describe the observed kinetics of the tracer in vivo and the technique of computed tomography to obtain a three-dimensional image. Subjects can either be scanned in the resting state or before, during or after a variety of experimentally interesting conditions, e.g. performance of a cognitive or motor task, administration of a drug or occurrence of a seizure. Positron emitting isotopes with short half-lives are used in PET, commonly $[^{15}O]$, $[^{11}C]$ and $[^{18}F]$ with half-lives of approximately 2, 20 and 110 minutes, respectively. The availability of PET is hence limited by the need for an on-site cyclotron to produce $[^{15}O]$ and $[^{11}C]$, whereas $[^{18}F]$-labelled substances can be transported off site. $[^{18}F]$ is incorporated into a tracer molecule by substituting for hydrogen or a hydroxyl group and therefore potentially alters the properties of the substituted substance. In the case of $[^{15}O]$ and $[^{11}C]$, the isotopes replace the naturally occurring elements, allowing for the labelling of compounds for use in vivo without altering their biochemical properties. Only minimal or "tracer doses" of neuroreceptor radiotracers need to be injected, usually without clinical effect, to estimate receptor parameters.
Positrons are positively charged electrons. They are emitted from the unstable nuclei of the isotopes as they decay into more stable structures. The positron travels a short distance and is then annihilated following collision with an electron. They release a pair of high energy (511 keV) photons (γ rays) which travel in diametrically opposite directions and can be detected by the PET camera. The PET camera consists of multiple rings of detectors consisting of scintillation crystals composed of bismuth germanate (BGO) (Figure 1.3).

![Schematic representation of a PET camera.](image)

Opposing detectors are linked electronically to accept only those events which are recorded near simultaneously from a source of activity. This coincidence detection establishes the origin of those photons to be somewhere along the line of response, the line linking these two detectors. As the probability of two photons arriving by chance at opposing detectors near simultaneously is very small, PET has a relatively good signal-to-noise ratio compared to techniques relying on single emitted photons (Single Photon Emission Computed Tomography, SPECT).
As an example for the above, the Siemens/CTI ECAT-953B camera consists of two rings of detectors. Each ring consists of 12 buckets; each bucket contains four detectors for a total of $2 \times 12 \times 4 = 96$ detectors. Each detector can detect a maximum of one event per 256 nanoseconds (ns). Each detector is connected in a fan-shape to five buckets per ring on the opposite side, in order to also detect coincidences which do not come from the centre of the field of view. Any event that is recorded within 12 ns in any of the detectors within this fan-shape is recorded as coincident. If, however, a further event is recorded by the detectors within the fan-shape, within 128 ns of the original event, the original event is discarded as background.

The raw data collected by the PET scanner may be mathematically reconstructed to produce tomographic images of tissue radioactivity concentration, normalising for the efficiency of the different detector blocks which changes over time. This is achieved by obtaining weekly scans of uniform phantoms to obtain current detector efficiencies. Another necessary correction concerns the efficiency along the longitudinal ($z$) axis of the field of view which is not uniform and depends on the individual scanner's geometry. Again, this can be measured using uniform phantoms, and the correction applied after reconstruction (Grootoonk, 1995). It is termed "$z$-scaling".

The resolution of the final images is defined as the width of the distribution of counts from a single point source at half the maximum value (full width at half maximum, FWHM), as two point sources can only be distinguished if separated by at least one FWHM. The spatial resolution of PET is limited by the short distance the emitted positron travels before encountering an electron and being annihilated. This distance limits the theoretical best resolution and is different for different isotopes. In the case of $[^1]C$, this theoretical best resolution for human brain studies is approximately 2mm. Other factors like detector size and arrangement influence resolution as well, and in experimental animal scanners, resolutions of approximately 1mm are now possible.

Traditionally, rings of detectors were separated by lead septa, so each ring of detectors collected one transaxial slice of data (two-dimensional or 2D imaging), the lead septa absorbing photons which were deflected from their original trajectory. By
removing the septa, an increase of sensitivity by a factor of 6 can be achieved through an increase in the number of counted events (Bailey, 1992), improving count statistics and reducing the radiation dose for subjects scanned (Hoffman et al., 1981). On the other hand, data collection and reconstruction become more complex, and as deviated photons are no longer absorbed by the septa, the problem of photon deflection, or scatter, increases. With increasing count rates in 3D imaging, the proportion of false coincidences, that is to say of two photons which are not derived from the same annihilation event, is not negligible. To enable accurate quantitation of the radiactivity measures, scatter needs to be taken into account during reconstruction. This can be achieved by either modelling scatter according to the known geometry of the PET camera (the "convolution subtraction" method) (Bailey and Meikle, 1994) or by measuring the scatter simultaneously with the unscattered data (Grootoonk et al., 1996). The latter exploits the possibility to adapt the detector properties so that photons can be measured at two different "energy windows" simultaneously. Scattered photons will have lost some of their energy and will therefore be measured at the lower energy level, whereas unscattered photons will be measured at the higher energy level or window, hence this technique is called "dual-window" scatter correction. The main advantage compared to the convolution subtraction method is the possibility to remove measured rather than modelled scatter, allowing for scatter arising outside of the field of view. Due to the temperature dependence particularly of the lower energy window, the method is, however, more sensitive to small temperature differences in the scanning room (Grootoonk et al., 1996).

Photon energy will be attenuated through the passage through brain and non-brain tissue such as skull, skalp, and muscle. Correction for attenuation can be obtained by obtaining a transmission scan in the same scanning session as the emission scan (Bailey, 1992).

The distribution of the radioligand in the organ of interest will change over time. The scanning period is therefore subdivided into a number of time intervals or "frames". At the beginning of the scan, radioactivity distribution will reflect blood flow and radioligand delivery. This period is best imaged with a series of short (e.g. 15 to 60 seconds) frames. Later scanning times, from about 20 minutes after injection on, will
gradually reflect the pharmacokinetics of the ligand at the receptor rather than blood flow and delivery. As the measurements are affected by the radioactive decay of the isotope, longer frames are required towards the end of the scanning period. The technique of acquiring data subdivided over time is termed "dynamic imaging" and the resulting set of 3D images, with the number of 3D images corresponding to the number of frames, is known as the "dynamic image".

Each frame consists of data from a large number of subvolumes which can be thought of as small image cubes or "voxels". The number of voxels per frame is determined by the number of all imaging levels or planes, (z dimension), multiplied by the number of voxels in the image matrix (x and y dimensions). For each voxel, the radioactivity concentration over time can be determined from the frame sequence and displayed as the time-activity curve for this voxel, typically including a correction for radioactive decay. In addition, the total number of counts over all areas over time can be determined, in the case of brain PET termed "head curve".

The availability of the radiotracer in the organ of interest is influenced by the concentration of the parent radioligand in the blood, its partitioning between plasma and blood cells, its plasma protein binding and its metabolism. Metabolites can be radiolabelled or unlabeled and may be polar or lipophilic, allowing them to penetrate the blood-brain barrier. Therefore, a measure of the availability of the parent radiotracer in the brain is desirable. Arterial blood sampling can be used for this purpose and is preferrable to venous blood sampling where many of the measures will be different. Usually, a continuous measure of the total radioactivity in arterial blood is determined. To assess the availability of the parent radioligand at any time during the scanning period, radiolabelled metabolites in plasma are assayed in intermittent blood samples throughout the scanning period. These data are used to correct the continuous measure of arterial blood radioactivity, in order to produce a time-activity curve for the unmetabolised, available ligand in arterial plasma, termed "input function".

Both the dynamic image set and the arterial input function can be integrated to derive regional parameters of interest, reflecting ligand binding. Examples are the available receptor density ($B_{\text{max}}$), receptor affinity ($K_d$) and volume-of-distribution ($V_d$).
Modelling techniques are used to derive these parameters. A model is a set of internally consistent hypotheses used to explain the behaviour of complex systems, based on available data from that system, which is used to interpret novel observations from that system. These models make the assumption that the time-activity curve at each voxel represents the convolution of the input function and the tissue response function.

1.6.2.2 PET studies of blood flow and metabolism

1.6.2.2.1 Introduction

The spatial resolution of PET data is superior to Single Photon Emission Computed Tomography (SPECT), and the data can be quantified. PET has been used clinically to map (regional) cerebral blood flow ((r)CBF) using $\text{H}_2\text{O}^{15}$O, and regional cerebral glucose metabolism using [18F]-fluorodeoxyglucose ([18F]DG). The development of automated, voxel-by-voxel methods of coregistering MRI and PET data has facilitated the interpretation of clinical and research PET data in conjunction with high resolution structural MRI data (Kiebel et al., 1997; Woods et al., 1993). The application of statistical parametric mapping to the evaluation of [18F]DG PET scans for clinical purposes has been shown to be useful as it allows a rapid and relatively objective evaluation, provided a cohort of normal controls exists (Signorini et al., 1999; Van Bogaert et al., 2000; Wong et al., 1996).

1.6.2.2.2 Localisation-related epilepsies

1.6.2.2.2.1 Introduction

Epileptogenic foci are associated with areas of reduced glucose metabolism as well as reduced bloodflow during the interictal state. Both areas are usually considerably larger than the pathological abnormality (Engel et al., 1982b; Franck et al., 1986; Juhász et al., 2000), likely due to inhibition or deafferentation of neurons around the epileptogenic focus. This interpretation is supported by the finding that some 18% of cavernomas may be associated with an area of hypometabolism around the lesion, independently of the size of the lesion or the presence of seizures but correlated with disruption of connections from limbic structures (Ryvlin et al., 1995). [18F]DG PET provides higher resolution and greater reliability than do rCBF studies using either SPECT or $\text{H}_2\text{O}^{15}$O PET (Leiderman et al., 1992; Theodore et al., 1994). This may in
part be due to uncoupling of regional glucose metabolism, specifically the hexokinase reaction, which is decreased, and rCBF, which may remain unaffected (Fink et al., 1996) or affected to a much lesser degree, leading to a high degree of false lateralisations in both \(H_2^{[15]O}\) PET and 99m-technetium-d,1-hexamethylpropyleneamine oxime (HMPAO) SPECT (Gaillard et al., 1995b).

In contrast to HMPAO-SPECT which can be stored on the ward and reflects rCBF at the time of injection of the tracer, ictal \(H_2^{[15]O}\) PET scans can only be obtained fortuitously because of the two minute half life of \([^{15}O]\). Ictal \([^{18}F]\)DG PET scans are difficult to interpret due to the prolonged and ongoing uptake of \([^{18}F]\)DG. Routine \([^{18}F]\)DG PET is often performed as a single static image rather than a dynamic dataset, and the resulting image will represent an amalgam of ictal and postictal conditions. In general, however, partial seizures are associated with an increase in regional cerebral glucose metabolism and rCBF in the region of the epileptogenic focus, and frequently with a suppression elsewhere (Chugani et al., 1994; Engel et al., 1983). Metabolic abnormalities after a seizure may persist for 24 hours or more (Leiderman et al., 1994). In one patient with fixation-off sensitivity, interictal spikes lead to an increase in rCBF and glucose metabolism (Bittar et al., 1999), whereas there was no effect of interictal spikes on regional glucose metabolism in a study of 11 children with benign childhood epilepsy with centrottemporal spikes and 2 children without seizures but with spikes in a similar location (Van Bogaert et al., 1998b).

PET was available before MRI. Some epilepsy surgery programmes have relied extensively on \([^{18}F]\)DG PET as a tool for localising the epileptogenic focus during presurgical evaluation. The routine use of this technique, with a radiation exposure of approximately 5mSv, needs to be re-evaluated in the light of developments in MRI. The finding of a focal epileptogenic abnormality with MRI, in particular hippocampal sclerosis, may render an \([^{18}F]\)DG PET scan superfluous (Gaillard et al., 1995a; Heinz et al., 1994).

1.6.2.2.2 Temporal lobe epilepsy

\([^{18}F]\)DG PET has been found to be a useful clinical tool in the presurgical evaluation of patients with temporal lobe epilepsy, in particular in patients in whom scalp EEG recordings were non-localising or non-lateralising (Engel et al., 1990; Theodore et al., 1992b). This must be reevaluated in the light of the development, during the 1990s, of MRI techniques that detect hippocampal pathology with high sensitivity
and specificity [e.g. (Cook et al., 1992; Duncan et al., 1996a; Jackson et al., 1990; Jackson et al., 1993b; Van Paesschen et al., 1997)].

Interictal hypometabolism has been shown using [$^{18}$F]DG PET in the ipsilateral temporal lobe in 60-90% of adults and children with temporal lobe epilepsy in multiple studies [e.g. (Abou-Khalil et al., 1987; Engel et al., 1982a; Engel et al., 1982b; Franck et al., 1986; Gaillard et al., 1995c; Kuhl et al., 1980; Ryvlin et al., 1991; Sadzot et al., 1992; Stefan et al., 1987; Theodore et al., 1983)]. The sophistication of equipment and analysis has a major impact on the results obtained. For example, the FWHM spatial resolution of PET systems has been improved by a factor of more than two over the last decade, and quantification of hypometabolism is more accurate than visual assessment (Theodore et al., 1992b). Such methodological considerations are of particular importance when two imaging methods are compared. [$^{18}$F]DG PET is more sensitive than MRI, i.e. may be abnormal in MRI negative temporal lobe epilepsy, but does not add clinically useful information when a definitive MRI abnormality is present (Gaillard et al., 1995a). The same group found [$^{18}$F]DG PET useful when ictal video EEG recordings were non localising (Theodore et al., 1997). Hypometabolism is quite strongly correlated with hippocampal volume, as expected, but partial volume effect correction shows relative hypometabolism per unit grey matter in epileptogenic hippocampi (Knowlton et al., 2001). This is a further indication that [$^{18}$F]DG PET might be useful in MRI negative epilepsies (Lamusuo et al., 2001; O'Brien et al., 2001) (but see notes on outcome below).

Most studies correlating outcome after temporal lobe surgery with hypometabolism on [$^{18}$F]DG PET were performed before the widespread availability of high resolution MRI and suitable quantification methods for the detection of hippocampal sclerosis. Degree and extent of temporal lobe hypometabolism have been strongly correlated with seizure outcome (Delbeke et al., 1996; Radtke et al., 1993b). Asymmetry of lateral but not medial temporal glucose metabolism was greater in patients who became seizure free after surgery compared with those who did not, and patients with an asymmetry of at least 15% were more likely to become seizure free (Theodore et al., 1992b). In another study, [$^{18}$F]DG PET was more sensitive than hippocampal volumetry but did not predict good postoperative outcome in the absence of asymmetrical hippocampal volumes (Knowlton et al., 1997). Multivariate analysis may improve the predictive value (Dupont et al., 2000). While absence of
unilateral temporal hypometabolism does not preclude a good postsurgical outcome (Chee et al., 1993; Knowlton et al., 1997), bilateral temporal hypometabolism indicated bilateral independent seizure onset in 8/15 patients in one series (Koutroumanidis et al., 2000) and is associated with a poor prognosis for seizure remission after surgery (Blum et al., 1998). Hypometabolism is a nonspecific sign of neuronal dysfunction. Accordingly, lateral temporal hypometabolism on $[^{18}\text{F}]$DG PET has been found to correlate with interictal temporal slow waves (Koutroumanidis et al., 1998). Hypometabolic areas are often found beyond the temporal lobe or in subcortical structures (Henry et al., 1993b; Sperling et al., 1990). Therefore, $[^{18}\text{F}]$DG PET is less reliable for precise localisation of the epileptogenic zone than for the question of lateralisation, although some pattern differences exist between mesial and neocortical forms (Hajek et al., 1993). False lateralisations may occur (Sperling et al., 1995) and may be related to interictal epileptiform activity or a postictal state with ensuing hypermetabolism (Chugani et al., 1993), or non-quantitative assessment of $[^{18}\text{F}]$DG PET data, for example with indices of asymmetry which are particularly vulnerable to unexpected hypermetabolism. Furthermore, the demonstration of an area of neuronal dysfunction does not equate with epileptogenicity, which must still be verified electrophysiologically. Consequently, preoperative abnormalities may disappear after temporal lobe surgery (Hajek et al., 1994) although the opposite has been found in the temporal pole after selective amygdala-hippocampectomy (Dupont et al., 2001). A single study of drug-naive patients with cryptogenic temporal lobe epilepsy found hypermetabolism in a bilateral neural network including the temporal lobes, thalami, basal ganglia and cingular cortices; nonsignificant increases were also found in mesial temporal, frontal and cerebellar cortex (Franceschi et al., 1997). Studies correlating hippocampal neuronal loss with degree of hypometabolism have found conflicting results (Henry et al., 1994; Knowlton et al., 2001; O'Brien et al., 1997; Semah et al., 1995). These are most likely due to methodological differences in the degree to which partial volume effects were accounted for, and biological differences in neuronal dysfunction as a response to hippocampal neuronal loss.

1.6.2.2.2.3 Frontal lobe epilepsy

Interictal hypometabolism has been shown using $[^{18}\text{F}]$DG PET in about 60% of patients with frontal lobe epilepsy. In about 90% of those patients who show hypometabolism, however, structural imaging demonstrates a relevant underlying
abnormality. The area of hypometabolism may be much larger than the structural abnormality or the epileptogenic zone, as seen in temporal lobe epilepsy. In contrast to temporal lobe epilepsy, the hypometabolic area may also be restricted to the underlying lesion (Engel et al., 1995; Henry et al., 1991). If there is an area of hypometabolism, the epileptogenic focus is usually contained within it (Henry et al., 1991; Sadzot et al., 1992; Swartz et al., 1989; Theodore et al., 1986b). As with temporal lobe epilepsy, quantitative analysis of $[^{18}F]$DG PET scans was found to be more sensitive and accurate than visual assessment (Swartz et al., 1995).

A study analysing early motor manifestations and their relationship to areas of hypometabolism in 48 patients showed principally contralateral perirolandic hypometabolism and a lesser degree of contralateral frontomesial hypometabolism in those with unilateral clonic seizures. Those with unilateral tonic seizures had unilateral hypometabolism within the frontomesial and perirolandic regions, while patients with versive seizures had no consistent pattern, but areas of hypometabolism were located contralaterally (Schlaug et al., 1997). All groups had bilateral thalamic and cerebellar hypometabolism, and the authors suggested that these may be areas that are involved in symptomatogenesis in addition to epileptogenesis.

Intercital focal neocortical areas of hypermetabolism have been described in early childhood epilepsies (Chugani et al., 1993) but not in adults.

Ictal studies have yielded inconsistent results, including studies of epilepsia partialis continua which been associated with both hypermetabolism and hypometabolism [reviewed by (Henry, 2000b)]. $[^{15}O]$PET studies in epilepsia partialis continua have shown increased blood flow, increased oxygen metabolism and a decreased oxygen extraction fraction contralateral to the focal motor seizure, and the abnormalities were most severe in the frontal lobe (Franck et al., 1986).

In contrast to the situation in temporal lobe epilepsy, absence of an area of hypometabolism on $[^{18}F]$DG PET or presence of a small area of abnormality was associated with better outcome after epilepsy surgery in one study (Swartz et al., 1998).

1.6.2.2.4 Malformations of cortical development

Focal cortical hypometabolism is frequently seen in tuberous sclerosis (Pawlik et al., 1990; Szelies et al., 1983). $[^{18}F]$DG PET was more sensitive than Computed Tomography (CT) (Szelies et al., 1983) and occasionally more sensitive than MRI (Rintahaka and Chugani, 1997). In only two out of 23 children, $[^{18}F]$DG PET was
normal, and the authors suggest this may be due to age under one year and, in one case, a low resolution device.

In migration disorders, [$^{18}$F]DG PET has shown glucose metabolism in laminar heterotopia (De Volder et al., 1994; Miura et al., 1993) and in heterotopic nodules (Bairamian et al., 1985; Falconer et al., 1990). This implies synaptic activity in heterotopic grey matter.

In a study of 17 patients with MCDs, [$^{18}$F]DG PET as well as MRI identified heterotopic grey matter. Hypometabolism concurred with abnormal cortex on MRI but in some cases the area of hypometabolism was more extensive than the MRI abnormality. Both [$^{18}$F]DG PET and MRI were negative in two patients (Lee et al., 1994).

In seven out of eight children with hemimegalencephaly who underwent hemispherectomies, the presence of areas of hypometabolism contralateral to the side of surgery was correlated with a less good prognosis following surgery during limited follow-up (Rintahaka et al., 1993).

In perisylvian dysgenesis, findings were heterogenous in ten patients, five of whom had epilepsy. Normal glucose metabolism within the polymicrogyric cortex was seen in eight patients, patchy hypometabolism in two, and hypometabolism outside the area of MRI abnormalities in three (Van Bogaert et al., 1998a).

1.6.2.2.3 Generalised epilepsies

[$^{18}$F]DG PET has been used in three studies of idiopathic generalised epilepsy (Engel et al., 1985; Ochs et al., 1987; Theodore et al., 1985). Interictal scans were normal (Engel et al., 1985; Ochs et al., 1987; Theodore et al., 1985). Frequent absences provoked by hyperventilation led to diffusely increased glucose metabolism while absence status was associated with a reduction in cerebral glucose metabolism (Theodore et al., 1985). Frequent spike-wave discharges on scalp EEG were associated with a trend towards increased glucose metabolism in two patients with primary generalised epilepsy, but similar changes were not seen in patients with secondary generalised epilepsy or deviations from the typical syndrome of primary generalised epilepsy (Ochs et al., 1987). In three out of four patients in another study, scans performed during frequent absences could be contrasted with scans after medical control of absences. Absences were reported to have led to a 250-350% global increase in cerebral metabolism, without a change of pattern (Engel et al., 1985).
$\text{H}_2^{[15}\text{O}]$ PET has been used to assess rCBF in typical absences, provoked through hyperventilation (Prevett et al., 1995a). Absences lead to a global 15% increase in global CBF, and an additional 4-8% increase in rCBF in the thalamus was seen, with no focal increases in the cortex, and no decreases of rCBF anywhere in the brain. Spike-wave activity is known to oscillate in thalamo-cortical circuits during absence seizures. While this study confirmed the key role of the thalamus in the pathophysiology of absences in man in vivo, the relatively poor temporal resolution of $\text{H}_2^{[15}\text{O}]$ PET (and indeed all methods relying on a haemodynamic response to neuronal activity) means that it could not clarify whether thalamic activation the primary process or results from converging activated cortico-thalamic pathways.

$[^{18}\text{F}]\text{DG PET}$ has been used to study patients with juvenile myoclonic epilepsy (JME) during a visual working memory task. In contrast to controls, patients did not show increased metabolism in the dorsolateral frontal cortex or other frontal areas, while medial temporal structures showed increased metabolism in JME patients but not controls.

1.6.2.2.4 Studies of the effect of antiepileptic drugs

$[^{18}\text{F}]\text{DG PET}$ has been used to study the effect of various antiepileptic drugs on cerebral glucose metabolism. Phenobarbitone was associated with a diffuse 37% reduction (Theodore, 1988), carbamazepine a diffuse 12% reduction (Theodore et al., 1989), phenytoin a non-uniform mean 13% reduction (Theodore, 1988), valproate a diffuse 9% reduction (Gaillard et al., 1996) when given alone to normal volunteers but a diffuse 22% reduction when added to carbamazepine in patients (Leiderman et al., 1991).

Valproate was associated with a reduced blood flow but not a reduced glucose metabolism in the thalamus, and the authors commented that this may be linked to its mode of action against generalised seizures (Gaillard et al., 1996).

1.6.2.2.5 Activation studies

Activation paradigms have been used during $[^{18}\text{F}]\text{DG PET}$ scans with the idea of highlighting an area of abnormal hypometabolism through increased contrast with normal surrounding areas. This may improve the delineation of dysfunctional areas (Bromfield et al., 1991; Pawlik et al., 1994; Pawlik et al., 1990; Swartz et al., 1996). As cerebral uptake of $[^{18}\text{F}]\text{DG}$ continues for at least 15 minutes, however, the time window is difficult to control, and standardisation across subjects would be difficult.
Delineation of eloquent cortical areas, particularly language areas, has been carried out using $\text{H}_2\text{[}^{15}\text{O}]$ PET to help with surgical strategies. Activation studies can also help to demonstrate deficits; for example, impaired naming in patients with left temporal lobe epilepsy has been associated with impaired rCBF in the left fusiform gyrus (Henry et al., 1998b). Due to the absence of radiation and associated better repeatability, as well as more widespread availability, cognitive activation tasks are increasingly carried out using fMRI.

Most vagal nerve stimulator (VNS) implantations, however, make the use of MRI impossible. Repetitive electrical stimulation of the left vagus nerve through an implanted neurostimulator is a palliative procedure for refractory epilepsy. Using $\text{H}_2\text{[}^{15}\text{O}]$ PET, stimulation-associated increases in rCBF have been demonstrated in the medulla, the right post-central gyrus, both hypothalami, thalami and insular cortices, as well as inferior cerebellar hemispheres. Decreases of rCBF were present in both hippocampi, amygdalae and posterior cingulate gyri (Henry et al., 1998a). Thalamic increases were correlated with a therapeutic effect (Henry et al., 1999). Curiously, while the three available $\text{H}_2\text{[}^{15}\text{O}]$ PET studies all find thalamic increases (Henry et al., 1998a; Henry et al., 1999; Ko et al., 1996), the three available SPECT studies all find thalamic decreases (Ring et al., 2000; Van Laere et al., 2000; Vonck et al., 2000). More research is needed to elucidate the mechanism of action of VNS.

1.6.2.2.6 Conclusion

Research using $[^{18}\text{F}]\text{DG PET}$ has defined the major cerebral metabolic associations as well as consequences of epilepsy. $[^{18}\text{F}]\text{DG PET}$ has been shown to be useful in the presurgical evaluation of selected patients. The findings of $[^{18}\text{F}]\text{DG PET}$ are, however, nonspecific with regard to aetiology, and abnormalities are regularly larger than pathological lesions or the epileptogenic zone as defined by other means. The role of $[^{18}\text{F}]\text{DG PET}$ has been reduced by the advances made by MRI over the past decade, in particular the ability of MRI to sensitively and specifically predict hippocampal sclerosis.

Activation studies with $\text{H}_2\text{[}^{15}\text{O}]$ PET can determine the functional anatomy of cerebral processes in both healthy and pathological brains. fMRI is becoming the method of choice for many of these studies. $\text{H}_2\text{[}^{15}\text{O}]$ PET may still be preferred when MRI is contraindicated, i.e. when neurostimulators have been implanted, or when the noise of fMRI would be detrimental, for example in certain studies of speech or hearing.
1.6.2.3 PET studies of specific ligands

1.6.2.3.1 Localisation-related epilepsies

1.6.2.3.1.1 GABA<sub>A</sub> receptors

Flumazenil (FMZ) is a neutral antagonist at the benzodiazepine binding sites of GABA<sub>A</sub> receptors (see above) and is therefore clinically used to reverse the effects of benzodiazepine overdosage. In subjects who have not received benzodiazepines, FMZ can be administered in doses which lead to a significant degree of receptor occupation with negligible effects (Hunkeler et al., 1981). FMZ binds to GABA<sub>A</sub> receptors containing four of the six possible α subunits, namely α 1, 2, 3, and 5. These four are the most prevalent and FMZ, therefore, acts as a good marker for the GABA<sub>A</sub> receptor (Olsen et al., 1990). As most neurons express the GABA<sub>A</sub> receptor, FMZ can also, to some extent, be regarded as a neuronal marker. FMZ is specifically bound exclusively in the brain with high affinity to GABA<sub>A</sub> receptors. Other advantages include easy crossing of the blood-brain barrier, no metabolism in the brain, low nonspecific binding in comparison with labelled benzodiazepine agonists (e.g. <sup>11</sup>C flunitrazepam, <sup>11</sup>C suricone, and <sup>11</sup>C triazolam) (although nonspecific binding still represents 10% of the specific binding which is not negligible), polar metabolites (one of which is radiolabelled) which do not cross the blood-brain barrier, and rapid dissociation from the benzodiazepine binding site (tissue half life estimates vary between 12 and 22 minutes). FMZ reaches peak concentrations in the head by about 10 minutes. It binds principally in neocortex with some binding in hippocampus, basal ganglia, thalamus and cerebellum from which it washes out at a differential rate than from the cortex. As binding to specific receptors is reversible and as there is no cerebral metabolism, the tracer clears slowly from the brain in its unchanged form as the concentration in blood falls towards zero. The rate of clearance of the tracer from a given region is dependent on receptor density and affinity at that site, clearance being most rapid from regions with least receptors. <sup>11</sup>C FMZ volume-of-distribution was not significantly different between two scans in a test-retest study in six volunteers under resting conditions and was not different either between resting scans and visual stimulation scans in another six volunteers, whereas rCBF and K1 showed approproate changes (Holthoff et al., 1991).
GABA is the principal inhibitory neurotransmitter in the brain and exerts its effect through hyperpolarisation of the membrane following the opening of the chloride channel formed by the receptor complex (Meldrum, 1989) (see previous chapters).

The commonest form of localisation-related epilepsy, temporal lobe epilepsy, has been most extensively studied using \[^{11}C\] FMZ PET. The initial report on the use of \[^{11}C\] FMZ PET in localisation-related epilepsy showed an average reduction of \[^{11}C\] FMZ binding of 30% in the epileptogenic focus in all ten patients, compared with homotopic contralateral areas and corresponding areas in five controls (Savic et al., 1988). This finding has been replicated by other groups, and comparative studies with \[^{18}F\] FDG PET have consistently shown the area of reduced \[^{11}C\] FMZ binding to be more restricted than the area of hypometabolism in temporal lobe epilepsy (Debets et al., 1997; Henry et al., 1993a; Savic et al., 1993; Szelies et al., 1996). The first study applying the voxel-by-voxel analysis of statistical parametric mapping (Friston et al., 1995b) to \[^{11}C\] FMZ PET scans of patients with hippocampal sclerosis diagnosed on MRI found decreases of \[^{11}C\] FMZ binding to be restricted to the sclerotic hippocampus, with no abnormalities elsewhere (Koepp et al., 1996).

While such voxel-based analyses have the advantage of increased objectivity compared with hand-drawn volumes of interest and allow the investigation of the entire brain volume, they do not lend themselves to absolute quantification of PET data. This requires an estimation of partial volume effects caused by the limited spatial resolution of PET and therefore comparison with higher-resolution data, i.e. MRI (Labbé et al., 1998; Meltzer et al., 1990; Rousset et al., 1993). If pathological structural changes are present that affect control and patient populations differently, as for example in hippocampal sclerosis, correction for partial volume effects is mandatory. These effects are nonlinear and affect smaller structures more than bigger structures, leading to an artificial apparent decrease of tracer binding in smaller structures (Hoffman et al., 1979).

Correction for partial volume effect increased the sensitivity of \[^{11}C\] FMZ PET in detection of unilateral hippocampal sclerosis from 65% to 100% in 17 patients with MRI-defined hippocampal sclerosis (Koepp et al., 1997c). This means that the sensitivity equalled that of MRI, and it could be argued that in this respect no additional information was gained compared with MRI. \[^{11}C\] FMZ PET was, however, more sensitive than MRI in the detection of contralateral abnormalities which were found in a third of patients with apparent unilateral hippocampal...
sclerosis on MRI (Koepp et al., 1997a). This latter study also showed that loss of GABA_A receptor binding was consistently over and above loss of hippocampal volume, indicating that the loss of binding was not simply due to hippocampal atrophy.

This is in keeping with other in vivo studies which have shown a greater degree of [^{11}C] FMZ binding loss than hippocampal volume loss based on correlational analysis (Henry et al., 1993a) or have demonstrated abnormalities of [^{11}C] FMZ binding even when hippocampal volumes were normal (Koepp et al., 2000; Lamusuo et al., 2000; Szelies et al., 2000). Except for one study (Koepp et al., 2000), these studies suffered from the inclusion of mixed patient populations and/or inadequate modelling of [^{11}C] FMZ binding, but confirm the impression that [^{11}C] FMZ PET may be useful in the investigation of patients with normal MRI and temporal lobe epilepsy.

The underlying pathological basis of reduced [^{11}C] FMZ binding in hippocampal sclerosis has been extensively investigated. Reduced neuron numbers and reduced benzodiazepine site binding, with a further reduction of benzodiazepine site binding per remaining neuron, have been demonstrated in an early histopathological and autoradiographic study using [^{125}I] Ro-160154 (Johnson et al., 1992). Another histopathological and autoradiographic study on 11 specimens from patients operated upon found neuron loss to be most prominent in the CA1 subfield (Burdette et al., 1995), confirming earlier studies (Sommer, 1880). Benzodiazepine binding was highly correlated with neuronal cell loss in the hippocampus, and the authors concluded that this was the predominant source of decreased [^{11}C] FMZ binding observed in vivo. This analysis did not, however, exclude an additional reduction of benzodiazepine binding per remaining neuron. Extrahippocampal abnormalities were present in the form of a diffusely increased lateral temporal neocortical benzodiazepine binding which reached significance in the inferior laminae. Quantitative autoradiographic and neuropathological studies of eight surgically removed hippocampi, compared with six autopsy controls, showed that the number of GABA_A receptors bearing benzodiazepine recognition sites was reduced over and above neuronal loss in the CA1 subregion, while the loss of receptors paralleled the loss of neurons in other subregions. Additionally, increases in affinity were noted in the subiculum, hilus and dentate gyrus (Hand et al., 1997). A direct comparison of quantitative in vivo hippocampal [^{11}C] FMZ binding and quantitative ex vivo [^{3}H]
FMZ autoradiography showed a mean 42% reduction in binding in the hippocampal body in ten patients with hippocampal sclerosis, compared with control material, with both methods, and a good correlation between the in vivo and ex vivo measures in individual patients (Koepp et al., 1998a). This study demonstrated the feasibility and accuracy of measuring the availability of benzodiazepine binding sites per remaining neurons in vivo with $[^{11}\text{C}]$ FMZ PET when partial volume effects are accounted for.

A limited number of studies have investigated patients with extratemporal seizures. In six patients with frontal lobe epilepsy compared with seven controls, $[^{11}\text{C}]$ FMZ PET correctly identified the epileptogenic zone as an area of decreased binding in all, including the five in whom MRI was normal (Savic et al., 1995). In 18 patients with normal high resolution MRI and refractory extratemporal seizures, $[^{11}\text{C}]$ FMZ PET showed focal decreases of $[^{11}\text{C}]$ FMZ volume-of-distribution in six and focal increases in ten (Richardson et al., 1998b). Focal increases of $[^{11}\text{C}]$ FMZ binding had previously only been seen in patients with MCD (Richardson et al., 1996) (see below), and in this more recent study, only decreases were seen in six patients with acquired lesions (Richardson et al., 1996). The implication of these data is that focally increased $[^{11}\text{C}]$ FMZ binding is a marker of MCD and may indicate occult MCD in patients who are MRI negative. An explanation why increases of $[^{11}\text{C}]$ FMZ binding were not seen in earlier studies is that most other studies relied on semiquantitative data and/or asymmetry indices which make the detection of such changes more difficult, and in some studies, visually detected decreases were used for region placement.

The clinical utility of these findings is not entirely clear. In hippocampal sclerosis, $[^{11}\text{C}]$ FMZ PET abnormalities are often more restricted than $[^{18}\text{F}]$ FDG abnormalities, probably reflecting diachisis (Feeney and Baron, 1986). No additional information may be obtained from detecting localised abnormalities of $[^{11}\text{C}]$ FMZ binding in temporal lobe epilepsy with MRI detectable hippocampal sclerosis if standard modified anterior temporal lobe resections are carried out. A clinical series of a total of 100 patients who underwent MRI, $[^{11}\text{C}]$ FMZ PET and $[^{18}\text{F}]$ FDG PET as part of the presurgical evaluation, included 30 with MRI-defined hippocampal sclerosis, and the authors found $[^{11}\text{C}]$ FMZ PET useful in the preoperative delineation of the epileptogenic zone (Ryvlin et al., 1998). $[^{11}\text{C}]$ FMZ
PET also helped to confirm the bilateral origin of seizures in a third of patients with bitemporal epilepsy and identified contralateral abnormalities in a number of cases. The same group later highlighted three patients in whom FMZ decreases were seen contralateral to the epileptogenic temporal lobe and varied in severity on two studies (Ryvlin et al., 1999). Whether these findings were explained through the use of non-modelled 20-40 minute uptake images, the use of a low resolution device (7mm FWHM in-plane, 9mm slices) and 12 controls at least some of whom were scanned on a different machine, or repositioning effects is unclear. Alternative explanations include occupancy by an endogenous ligand or temporary downregulation of receptors. Six of 11 patients with unilateral frontal lobe epilepsy showed focal ipsilateral decreases, either in the frontal or in the temporal lobe, and was judged helpful (Ryvlin et al., 1998).

Malformations of cortical development (MCD) commonly underlie partial seizures and may not be detectable on MRI [e.g. (Desbiens et al., 1993)]. Surgery for seizures is less successful in these patients than in patients with discrete lesions, most likely because of anatomical and functional abnormalities that are more widespread than the visible lesions or the cortex that may be removed. A study using fully quantified $[{\text{11}}C]$ FMZ PET and statistical parametric mapping in 12 patients with MCD and partial seizures found areas of abnormal $[{\text{11}}C]$ FMZ volume-of-distribution in 10. The abnormal regions were frequently more extensive than the abnormality seen with MRI, and were also noted in distant sites which were unremarkable on MRI (Richardson et al., 1996). This study demonstrated, for the first time, focal increases of $[{\text{11}}C]$ FMZ binding in patients with epilepsy which had not been seen in other pathologies. Possible explanations include increased neuronal density, the presence of heterotopic neurons expressing GABA_A receptors, and increased numbers or availability of receptors. Patients with MCD have structurally abnormal brains. A subsequent study used a voxel-based comparison of grey matter and $[{\text{11}}C]$ FMZ binding to address this issue in ten patients. Some regions with abnormal $[{\text{11}}C]$ FMZ binding were indeed accounted for by abnormalities of grey matter volume. In other areas, however, there was disproportionately high or low $[{\text{11}}C]$ FMZ binding compared to the amount of grey matter present, including areas where analysis of the PET data alone had not revealed an abnormality. The latter implies abnormal receptor density per neuron or a change in affinity (Richardson et al., 1997a) and
underlines the importance of interpreting PET data in the light of high resolution structural imaging.

In 17 patients with lesional epilepsy, $^{[11]}$C FMZ PET delineated the spiking cortex well, and the complete resection of this cortex resulted in seizure-free outcome in eight of nine patients, whereas incomplete lesionectomy or lesionectomy without removal of the perilesional cortex was associated with poor seizure control in four cases (Juhász et al., 2000).

While analysis of individual subjects is crucial in clinical practice, the analysis of patients with homogenous epilepsies is of interest when investigating neurobiological hypotheses. One abstract reported that, in a group of ten patients with frontal lobe epilepsy, there was an increase in flumazenil binding in the putamen, particularly ipsilateral to the seizure focus, and in related motor areas (Richardson et al., 1997b). The magnitude of the increase was inversely related to seizure frequency, suggesting the possibility that increased inhibition in the basal ganglia may modulate neocortical seizure threshold. In this study there was no correlation between seizure frequency and and extent of abnormality of neocortical flumazenil binding. In contrast, another study found such a correlation in 19 patients with partial seizures and normal MRI, using manually placed regions of interest (Savic et al., 1996). In patients with daily seizures, reductions were also seen in the primary projection areas of the focus. Similarly, the same group reported decreased binding in some but not all of the primary projection areas in four patients with temporal lobe epilepsy, compared to seven controls. Some of these values but not those of other cortical areas were reported to have increased one year after epilepsy surgery, albeit with a very wide spread (+29 ± 17%). Other groups, however, have failed to replicate a correlation between extent of $^{[11]}$C FMZ binding abnormalities and seizure frequency (Juhász et al., 2000; Koepp et al., 1996).

Studies of drug action have also been performed. While 1.5mg of intravenous unlabelled flumazenil, i.e. 2.5 - 5 times the dose typically used clinically to treat benzodiazepine intoxication, occupied 55% of receptors, 15 mg occupied nearly all receptors (Savic et al., 1991).
1.6.2.3.1.2 Opioid receptors

Diprenorphine (DPN) is a high affinity opiate receptor ligand with similar in vivo affinities for the 3 main receptor subtypes: μ, κ and λ (Pfeiffer et al., 1982). It acts predominantly as an antagonist, but, at higher doses in man, acts as a weak partial agonist and leads to mild sedation. [11C] DPN is established as a useful opioid receptor PET ligand and has been used in the study of normal subjects and patients (Koepp and Duncan, 2000; Mayberg et al., 1991). After intravenous injection it is rapidly taken up into the brain. It is metabolised in the liver and metabolites appear in blood soon after injection, but the metabolites do not cross the blood-brain barrier (Perry et al., 1980). Over time, the tracer washes out slowly from regions with high receptor density and more rapidly from areas with few receptors. 90 minutes after injection of the tracer, 80-90% of the remaining radioactivity represents radioligand specifically bound to receptors. High specific binding is seen in thalamus, caudate, and frontal, temporal and parietal cortex. Binding is lowest in the occipital cortex and white matter (Jones et al., 1988).

There were no significant side-to-side differences in [11C] DPN binding in patients with unilateral temporal lobe epilepsy in two studies (Bartenstein et al., 1994; Mayberg et al., 1991). In contrast, in the same patients and on the side of the epileptogenic focus, higher binding of the μ subtype selective agonist [11C] carfentanil was seen in lateral temporal neocortex, while binding in the amygdala was decreased. The latter finding might be due to partial volume effect which could be corrected for in contemporary studies (Meltzer et al., 1996), but the finding of lateral neocortical increases confirmed an earlier study in 13 patients (Frost et al., 1988). While this earlier study had not shown any difference in amygdala or hippocampus binding, the increases of [11C] carfentanil binding had been noted to be in areas of hypometabolism seen on [18F]DG PET. It has been speculated that an increase in μ receptors in the temporal neocortex may be a manifestation of a tonic antiepileptic system that serves to limit the spread of epileptiform activity from other temporal lobe structures.

Using [18F] cyclofoxy which is a specific antagonist at both μ and κ, but not δ receptor subtypes, increased binding was seen in the ipsilateral temporal lobe in some of the 14 temporal lobe epilepsy patients studied, compared with 14 normal controls, but there was no overall asymmetry in the group (Theodore et al., 1992a). Considering the other available studies, this could be explained through a decrease of
affinity or number of κ receptors and would also be consistent with decreased availability of κ receptors through occupation by an endogenous ligand.

The δ receptor subtype selective antagonist [11C] methylnaltrindole has been used in temporal lobe epilepsy patients who were also investigated with [11C] carfentanyl and [18F]DG PET (Madar et al., 1997). As expected, [18F]DG PET showed ipsilateral widespread decreases, while both opioid tracer showed more localised increases in the ipsilateral temporal cortex. Increases in the δ- and μ-receptor binding showed different regional patterns. Increases in μ receptor binding were confined to the middle aspect of the inferior temporal cortex, whereas binding of delta receptors increased in the mid-inferior temporal cortex and anterior aspect of the middle and superior temporal cortex.

Differences between antagonist ([11C] methylnaltrindole, [18F] cyclofoxy) or partial agonist binding ([11C] DPN) on the one hand and agonist binding ([11C] carfentanyl) on the other may explain some of the differences found (Koepp and Duncan, 2000), as agonist-driven internalisation of receptors may play a role for G-protein coupled receptors (see chapter 1.5.2.3.5, p. 89). Further, quantitative data of macroscopic and microscopic opioid receptor subtype distribution in humans is now becoming available (see chapter 1.5.2.3.1, p. 85, chapter 1.5.2.3.2, p. 86, and chapter 1.5.2.3.3, p. 87), and such data should aid in the interpretation of seemingly disparate results.

Ictal studies have been performed in patients with reading epilepsy with a two-scan (rest-activation) paradigm (Koepp et al., 1998b). Reading epilepsy provides a model for a localisation-related epilepsy and has the advantage that seizures can be easily provoked through reading and do not lead to significant head movement. Comparison of the resting condition in patients with controls revealed no significant differences of [11C] DPN volume-of-distribution, in agreement with the studies cited above. Reading-induced seizures were associated with reduced [11C] DPN binding in the left parieto-temporo-occipital cortex and to a lesser extent in the left middle temporal gyrus and the posterior parieto-occipital junction, implying release of endogenous opioids at the time of seizures. These areas are known to be involved in reading, visual processing and recognition of words.

Further evidence comes for the release of endogenous opioids comes from a fortuitous ictal [18F] cyclofoxy PET scan reported as a personal communication (Koepp and Duncan, 2000). Frequent intermittent right medial temporal discharges started about six minutes after injection, and the time-activity curve for the right
medial temporal lobe remained constantly below that of the contralateral side for the remaining 60 minutes.

1.6.2.3.1.3 N-methyl-D-aspartate receptors

$[^{11}\text{C}] (\text{S})$-[N-methyl]-ketamine ($[^{11}\text{C}]$ ketamine) binds to the excitatory N-methyl-D-aspartate (NMDA) receptor and is thus of great interest in studies of epilepsy. In a pilot study of eight patients with temporal lobe epilepsy, six of whom had unilateral hippocampal sclerosis, an average reduction of binding of 14% was seen on the side of the EEG focus (Kumlien et al., 1999). The difference in radioactivity uptake, however, was established within the first two minutes, with parallel time-activity-curves thereafter. Differences in delivery are possible but rCBF may be altered relatively little in the interictal state in temporal lobe epilepsy (Fink et al., 1996; Gaillard et al., 1995b), and the finding may well be due to partial volume effect in the smaller hippocampus. As sclerotic hippocampi are an average of 30% smaller than their nonsclerotic counterparts (Van Paesschen et al., 1997), and it is even possible that if adequate methodology with partial volume correction had been used, the apparent 14% decrease would have turned out to be an effective increase per unit of grey matter, which would be a less surprising finding for NMDA receptors. Further studies are required.

1.6.2.3.1.4 Serotonergic neurons

Alpha-methyl-L-tryptophan (AMT) is an artificial amino acid and an analogue of tryptophan, the precursor of the neurotransmitter serotonin (5-HT). It has been proposed that it can be used to measure brain 5-HT synthesis when plasma free concentrations are measured (Diksic and Young, 2001), but others have suggested that AMT acts predominantly as a tracer of tryptophan uptake rather than serotonin synthesis (Shoaf et al., 2000). In either case, serotonergic neurons should be labeled. Increased concentrations of serotonin breakdown products and serotonin immunoreactivity have been reported in resected human epileptogenic cortex [reviewed by (Chugani and Chugani, 2000)]. In children with tuberous sclerosis, uptake of $[^{11}\text{C}]$ AMT was increased in some tubers that appeared to be the sites of seizure onset, while other tubers showed decreased uptake. In contrast, $[^{18}\text{F}]$DG PET showed hypometabolism in all tubers (Chugani et al., 1998). This study suggested that $[^{11}\text{C}]$ AMT PET may be useful to detect epileptogenic tubers in patients with tuberous sclerosis. The same group has since used $[^{11}\text{C}]$ AMT PET in 16 children
with refractory localisation-related epilepsy and normal MRI studies. Only preliminary results are currently available (Chugani and Chugani, 2000), but it was suggested that $[^{11}C]$ AMT PET may be useful in this situation, too, particularly when $[^{18}F]$DG PET showed several areas of hypometabolism. A similar conclusion was reached by a different group that studied patients aged 13 to 54 years (mean, 27 years). Seven patients had cortical dysplasia and 11 had MRI-negative and also $[^{18}F]$DG PET negative localisation-related epilepsies (Fedi et al., 2001). $[^{11}C]$ AMT PET showed increased binding in the presumed epileptogenic zone in four of the seven patients with cortical dysplasia and in three of the 11 patients with previously negative imaging studies. This indicates a lower yield of surgically useful results in these patient groups but is still a promising finding, particularly in the difficult-to-treat imaging negative group.

1.6.2.3.1.5 Serotonin receptors

Preliminary data is available for the 5HT(1A) receptor in temporal lobe epilepsy (Toczek et al., 2001). Seven patients with temporal lobe epilepsy (five of whom had hippocampal sclerosis on MRI) were studied with $[^{18}F]$FCWAY, a selective 5HT(1A) antagonist, and compared with 8 controls. The data were partial volume corrected to compensate for activity in the skull. An ROI analysis of temporal regions only revealed decreased $[^{18}F]$FCWAY volume-of-distribution in the inferior medial temporal lobe contralateral to the epileptogenic focus in patients compared with controls. There was no asymmetry in controls, but in patients, the volume-of-distribution in the ipsilateral inferior medial temporal lobe was significantly higher than contralaterally (where it was reduced compared with controls). While promising, these results must be considered preliminary until higher numbers of controls allow an assessment without the use of asymmetry indices.

1.6.2.3.1.6 Histamine receptors

Preliminary investigations on the use of $[^{11}C]$ doxepin, a histamine H(1) receptor antagonist, in patients with epilepsy have shown increased binding in presumed epileptic foci that showed decreased metabolism on $[^{18}F]$DG PET (Linuma et al., 1993; Itoh et al., 1995). This is potentially interesting, but no further studies have been published. One problem with $[^{11}C]$ doxepin is the increased metabolism in patients on enzyme-inducing drugs (Ishiwata et al., 1996); another that it is not a very specific ligand.
1.6.2.3.1.7 Monoamine oxidase type B (MAO-B)

Deprenyl binds with high specificity and affinity to monoamine oxidase type B (MAO-B) which is mainly located in astrocytes. Autoradiographically, higher $[^3]H$-L-deprenyl binding was found in epileptogenic human hippocampi, and binding correlated with neuronal loss (Kumlien et al., 1992). Increased N-[methyl-$^{11}C$]-a,a-di-deutero-L-deprenyl ($[^1]C$deuterium-deprenyl) binding correctly lateralised temporal lobe epilepsy in six out of seven cases (Kumlien et al., 1995), a finding replicated in SPECT studies (Buck et al., 1998). The increased uptake was, however, not limited to the mesial temporal lobe. With simplified methodology, only three of eight patients with temporal lobe epilepsy showed convincing asymmetries (Reutens, 2000). In a more recent study, $[^1]C$deuterium-deprenyl PET was found to be of equivalent use to $[^18]F$DG PET in lateralising temporal lobe epilepsy when volume of distribution images were used (Kumlien et al., 2001). Thirteen of 14 patients were operated upon and found to have hippocampal sclerosis. No MRI data were given, and the usefulness of the technique for MRI-demonstrated hippocampal sclerosis may be questioned.

In the same study, nine patients with seizures of neocortical origin did not show side-to-side differences or differences compared with six healthy controls. $[^1]C$deuterium-deprenyl does not seem to be a useful tracer in neocortical epilepsies.

1.6.2.3.1.8 Peripheral benzodiazepine receptors/activated microglia

$[^1]C$ PK11195 labels macrophages and activated microglia and can be used to image inflammatory responses (Banati et al., 2000) or areas of synaptic reorganisation (Banati et al., 2001) in the brain. In two patients with Rasmussen's encephalitis there was increased binding on the side of the pathology, reflecting the inflammatory nature of the condition. It may be useful in deciding on the site of a biopsy (Banati et al., 1999; Goerres et al., 2001). Binding was similar to that in normal brain in three patients with established hippocampal sclerosis (Banati et al., 1999).

1.6.2.3.1.9 Cholinergic receptors

Muscarinergic acetylcholine receptors have been implied in the pathogenesis of temporal lobe epilepsy where their number or availability may be transiently decreased after seizures but are probably not decreased over and above hippocampal neuronal loss in autoradiographic studies [reviewed by (Pennell, 2000)]. SPECT studies using $[^{123}]I$ iododexetimide (IDEX) have shown decreased binding in the
ipsilateral mesial temporal lobe which disappeared after partial volume effect correction (Pennell, 2000). While earlier PET tracers probing muscarinergic acetylcholine receptors (e.g. $[^{11}C]$ scopolamine or $[^{11}C]$ tropanyl benzilate) had suboptimal in vivo kinetics, $[^{11}C]$ N-methyl-4-piperidyl benzylate (NMPB) has more suitable dissociation rates. $[^{11}C]$ NMPB binding was decreased in the mesial temporal lobe ipsilateral to the epileptogenic hippocampus in one study (Pennell, 2000). Most of the patients had hippocampal sclerosis, and most of the effect seen in vivo is likely due to neuronal loss, as suggested by the earlier autoradiographic studies.

The $\alpha_4$ subunit of the nicotinergic acetylcholine receptor is affected by mutations in the genetic syndrome of autosomal-dominant nocturnal frontal lobe epilepsy (ADNFLE). PET tracers for nicotinergic acetylcholine receptor are under active development but not yet available (Sihver et al., 2000).

1.6.2.3.1.10 Conclusion

$[^{11}C]$ FMZ PET can act as a good marker for neuronal integrity in hippocampus and neocortex and may act as a marker for MCD. It can yield information that is complementary to the information provided by structural imaging. Its future clinical role is likely to be in the presurgical evaluation of patients in whom the usefulness of MRI data is limited, namely those with normal MRI studies and those with MCD. Opioid receptor alterations seem to be involved in temporal lobe epilepsy and a number of other epilepsy syndromes (see below). Some of the earlier findings would benefit from repeating in view of the rapid advances in both MRI and PET neuroimaging over the last decade, and it would be interesting to extend these studies to extratemporal epilepsies and those caused by MCD. As the various tracers seem to be displaceable by endogenous opioids, ictal/interictal comparisons are of great interest.

The NMDA system is of high theoretical interest, but unfortunately only one inadequate study has been published so far.

Of the other systems that can be probed with ligand PET imaging, those who show increased binding in areas of epileptogenesis are less likely to be greatly influenced by partial volume effects through atrophy, and are also less likely to measure changes similar to those seen with established tracers like $[^{11}C]$ FMZ or $[^{18}F]$ FDG. Similarly, tracers that measure nonspecific changes that can be seen using appropriate MRI sequences, for example gliosis, are unlikely to be of clinical benefit.
due to the scarcity and cost of PET compared to MRI. $[^{11}]C$ AMT seems to be one of the more promising tracers, even though the exact interpretation of abnormalities is difficult and recent studies are slightly less enthusiastic than the earlier reports.

1.6.2.3.2 Idiopathic generalized epilepsy

1.6.2.3.2.1 GABA$_A$ receptors

Most studies conducted to date included only small numbers of patients and need to be interpreted with caution. The first report found a slight trend towards a diffuse reduction of cortical $[^{11}]C$ FMZ binding in a heterogenous group of ten patients with primary generalised epilepsy. There were no controls; binding was compared to cortical areas that were thought to be outside the epileptogenic focus in patients with partial seizures (Savic et al., 1990). A follow-up study comparing eight patients with primary generalised epilepsy with eight controls found increased $[^{11}]C$ FMZ binding binding in cerebellar nuclei and decreased binding in the thalami, with unchanged binding elsewhere (Savic et al., 1994). No difference between 16 patients with childhood and juvenile absence epilepsy was found in another study (Prevett et al., 1995b). When the eight patients in this study who were treated with valproate were considered separately, there was a small but significant reduction of global binding of about 9%. When the question of an influence of valproate on FMZ volume-of-distribution was studied specifically, however, in a group of ten patients with idiopathic generalised epilepsy, scanned before and after the addition of valproate and compared with 20 controls, addition of valproate was not associated with any significant change in any brain area. Comparing the initial scans of the IGE patients with the controls, mean FMZ volume-of-distribution was elevated by 11% in the cortex, 14% in the thalamus and 15% in the cerebellum. This finding would be compatible with a larger number of neurons bearing GABA$_A$ receptors, for example through microdysgenesis, which has been described in patients with idiopathic generalised epilepsy (Meencke and Veith, 1992).

One study has investigated possible effects of serial absences provoked by hyperventilation on FMZ binding in five patients scanned twice. There was no difference in any area of neocortex nor in the thalamus, implying no major involvement of GABA$_A$ receptors bearing the benzodiazepine recognition site in the pathophysiology of absence seizures (Prevett et al., 1995c).
1.6.2.3.2 Opioid receptors

Systemic administration of fentanyl, sufentanyl and other μ-receptor agonists increases generalised spike-wave activity in rats (Frey and Voits, 1991) and humans (Kearse et al., 1993), suggesting that opioid transmission may have a role in the pathogenesis of absences. [11C] diprenorphine has similar affinities for the μ, κ and δ receptor subtypes (see above). There was no significant difference in [11C] diprenorphine binding between eight controls and eight patients with childhood and juvenile absence epilepsy. While subtype-specific alterations in opposite directions can not be ruled out, this study suggested there is no overall abnormality of opioid receptors in this condition (Prevett et al., 1994). A dynamic study investigating eight patients with primary generalised epilepsy and eight controls, however, found a faster elimination of [11C] diprenorphine from association cortices but not thalamus, basal ganglia or cerebellum following absences provoked by hyperventilation (Bartenstein et al., 1993). A two compartment model fitted to the single scan data estimated the decrease in specific tracer uptake rate constant (k3) at 15-41%, compared with controls and patients in whom no absences were provoked, compatible with the suggestion that there was a release of endogenous opioids in association cortices at the time of seizures.

1.6.2.3.2.3 Conclusion

[11C] FMZ PET studies have so far yielded inconsistent results. This may be due to small numbers of patients scanned in individual studies, or indeed to syndrome-related or even syndrome-specific changes (Savic, 2000). The finding of diffusely but predominantly frontally increased binding in patients with IGE, mainly JME, benefits from having a pathological correlation in microdysgenesis, and it may be worth extending this study with a homogenous patient sample and PET cameras with fields of view encompassing the entire brain. Probably due to the difference between the benzodiazepine binding site and the GABA binding site on the GABA<sub>A</sub> receptor, [11C] FMZ PET does not seem suited for ictal/interictal comparisons.

No differences in global opioid binding were found between controls and patients with childhood and juvenile epilepsy, but several studies comparing ictal and interictal states were consistent with endogenous opioid release in predicted areas. Such studies should be replicated in other forms of epilepsy and could be extended to
subtype-specific tracers, with the long-term aim of developing antiepileptic drugs acting on the opioid system.
1.7 PET data analysis

1.7.1 Introduction: Modelling

The aim of the PET data analysis is to derive regional quantitative parameters of ligand binding, such as receptor density (B_{max}), receptor affinity (K_d) and volume of distribution (V_d). The data available for derivation of these parameters are the dynamic image set, describing the uptake and subsequent washout of the tracer following intravenous injection, and the metabolite-corrected arterial plasma input function, describing the parent tracer availability. The derivation is performed with a tracer kinetic model which provides the basis for operational equations for estimates of these physiological parameters from the acquired data (Phelps, 1993). The essential underlying assumption is that the observed time course of tissue radioactivity is the convolution of the arterial input and tissue response function.

The various kinetic models can be classified into multivariate models, e.g. Principal Component Analysis, Cluster Analysis or Factor Analysis, and into compartmental models, e.g. Graphical Analysis, Compartmental Modelling or Spectral Analysis. Whereas the former have the advantage of being more data-led, the latter, more model-led analyses are less subject to noise.

In general, a system under study, e.g. tracer pharmacokinetics in brain tissue, can be characterised by means of measuring the input (e.g. metabolite-corrected arterial plasma input function) and the output (e.g. time-activity curve in the tissue). One can estimate physiologically, biochemically or pharmacologically relevant parameters from the transfer function which describes the transfer from the input to the output of the system (Figure 1.4, p. 132).

The parameters can be estimated by finding parameter values such that the function fits the data best. It is possible to measure the quality of the fit by calculating the differences between the actual data points and the fitted function, the so-called residuals, and plotting them over time. As one is interested in the absolute distance of the data points from the fitted function and not in the direction of these distances, the residuals are squared to give the residual sum of squares which is then minimised.
Plasma System Tissue

Figure 1.4: Plasma, system, tissue and their relationship. The system under study is characterised by its input (Plasma) and output (Tissue). The impulse response function describes the transfer from the input to the output of the system.

1.7.1.1 Compartmental models

Compartmental models are the commonest form of a tracer kinetic model used in PET (Cherry and Phelps, 1996). A compartment is a volume within which the tracer rapidly becomes uniformly distributed. Examples in the case of receptor radiotracers include a vascular and a tissue compartment with the tissue compartment subdivided into free, nonspecifically bound and specifically bound (bound to receptor) compartments. Assumptions are that compartments are homogeneous, instant mixing occurs, and the exchange between compartments is constant over time (rate constants). This greatly simplifies the process of tracer kinetic modelling by only allowing a certain number of solutions for the impulse response function.

A model which is too complex results in parameter estimates with large variances. In practice, simplified models and operational equations are developed in which the number of compartments is reduced by grouping them into functional compartments that contain substructures for which no or only partial information is provided. The number, definition and interrelationship of compartments in a compartmental model is developed from knowledge of the biological properties of the radiotracer in question.
1.7.1.1.1 Two compartment model

A two compartment model adequately describes the cerebral kinetics of FMZ (Frey et al., 1991). The compartments consist of a plasma compartment, $C_p$, and a brain compartment incorporating the free, nonspecific and specific compartments, $C_b$. Both are separated by the blood brain barrier, and two rate constants describe the exchange across the blood brain barrier, $K_1$ from plasma to brain and $k_2$ from brain to plasma (Figure 1.5).

![Diagram of two compartment model](image)

**Figure 1.5:** *Example of a two compartment model for FMZ.*

1.7.1.1.2 Three compartment model

The third compartment in this model represents the specifically bound compartment, $C_3$, whereas the remainder of the brain compartment of the two compartment model now represents the free and nonspecifically bound compartment, $C_2$. Two further rate constants, $k_3$ and $k_4$, describe the exchange from $C_2$ to $C_3$ and vice versa (Figure 1.6, p. 134).

A single scan with metabolite corrected arterial plasma input function following a high specific activity injection of tracer can be used to derive $k_3/k_4$ which is equal to $B_{max}/K_d$. This three compartmental approach has been used to measure $B_{max}/K_d$ for $[^{11}C]$ DPN (Cunningham et al., 1991).
For all compartmental models, it must be assumed that all exchanges between compartments occur in steady state. For high specific activity radiotracers, however, only tracer doses are injected, and a steady state of essentially zero receptor occupancy pertains throughout the study (Koepp et al., 1991). For FMZ and DPN, approximately 1 in 1000 molecules injected are labelled; during the PET scan, less than 1% of receptors will be labelled at any one time (Hume et al., 1998).

The volume of distribution of a receptor ligand represents the summed volumes of distribution of free, nonspecifically and specifically bound tracer. As the contributions of free and nonspecifically bound tracer are small, Vd correlates well with the specific binding and is linearly related to $B_{\text{max}}/K_d$.

Non-steady state modelling has been used to obtain measures of $B_{\text{max}}$ and $K_d$ separately; this requires two dynamic PET scans at different levels of receptor occupancy (Sadzot et al., 1991). A bolus injection of a ligand with high specific activity is performed in one study and a bolus injection of the same ligand with low specific activity in the other. During the low activity study, unlabelled ligand will initially occupy a significant number of receptors, but receptor occupancy falls as the unlabelled ligand is cleared from the brain during the dynamic study. The steady state assumption in compartmental analysis does thus not hold.
1.7.1.2 Reference region model

For DPN, but not FMZ studies, an alternative to measuring arterial plasma radioactivity is to use the occipital cortex as a reference tissue as it is virtually devoid of opioid receptors. Specific binding in the occipital lobe can thus be considered to be negligible, and the radioactivity ratio of tissue over plasma in the occipital cortex equals $K_1/k_2$ (cf. Figure 1.6, p. 134). This ratio describes the transport of the tracer between the plasma and tissue compartments. If one assumes this ratio to be identical in occipital cortex and in regions with high specific binding, it can be used to calculate $k_3/k_4$ for regions with specific binding.

The assumption of no specific binding in occipital cortex is not entirely correct, however, and this will introduce an error. In the future, it may be possible to use cluster analysis or other methods to find an ideal reference region. Currently, paired studies in healthy volunteers, one using $[^{11}\text{C}]$ diprenorphine alone and one using $[^{11}\text{C}]$ diprenorphine after blocking specific binding with naloxone, are under way at the Clinical Sciences Centre, Faculty of Medicine, Imperial College, to clarify nonspecific binding and time-activity curves in the white matter [Professor Vincent Cunningham, personal communication]. Attempts are also under way to use the partial volume effect corrected signal from the venous sinuses as an input [Dr Marie-Claude Asselin, personal communication]. As total immobilisation of subjects, achievable in neurosurgery, is impossible in the research setting, using the venous sinuses will require listmode (second-by-second) acquisition of PET data, coupled with real-time movement correction. The latter should be available shortly [Peter Bloomfield, personal communication].

Using a reference region model, $B_{\text{max}}$ and $K_d$ can be derived individually, but again a paired study with different levels of receptor occupancy is necessary (Lammertsma and Hume, 1996).

1.7.1.3 Spectral analysis

In contrast to the compartmental models, spectral analysis requires no assumptions about the number of compartments which are needed to model the data (Cunningham and Jones, 1993). A large but finite number of exponential basis functions which span the expected kinetic behaviour of the tracer in the tissue is defined. In the model
used to calculate both FMZ-$V_d$ and DPN-$V_d$ in these studies, 64 of these exponential basis functions were used. They are convolved with the measured, metabolite-corrected arterial plasma input function to produce a set of convolved basis functions. Time-activity curves are analysed without decay correction which allows convenient limits to be placed on values of the exponents. The slowest possible tissue response then corresponds to irreversible trapping of the radioligand with subsequent isotopic decay and can be defined through the known isotope half-life. The fastest possible tissue response corresponds to rapid transients of radioligand in the vasculature, and an appropriate upper limit of the range of the exponents can be used. The convolved basis functions are weighted and combined by a computer algorithm to obtain a best fit to the measured time-activity curve for each voxel, using a least squares technique. In practice, out of the 64 possible convolved basis functions, only two to four per voxel explain the observed voxel time-activity curve. The equivalent, weighted but unconvolved basis functions are then combined to produce the exponential Impulse Response Function (IRF) for each voxel. Having calculated the IRF for each voxel, a variety of parametric images can be produced. The clearance of tracer from arterial plasma to tissue is shown in the $K_1$ image, with $K_1$ corresponding to the intercept of the IRF and the $y$ axis. As the intercept is poorly defined, an alternative way of obtaining a good approximation of tracer clearance is to produce an image corresponding to the tissue response at 2 minutes. An index of specific retention of the tracer is produced by imaging tissue response at 60 minutes. Finally, images of the volume of distribution of the tracer relative to blood can be obtained from integrating the area under the curve extrapolated to infinity. In more recent implementations of spectral analysis, the fastest component, corresponding to the blood curve, is separated as a blood volume term.

Because all the images are constructed on the basis of the tissue response to the unit impulse activity, the equivalent parametric images are directly comparable between different subjects or between different scanning sessions in the same subject. Within certain limits, they are also independent of the amount of activity injected and of the specific activity of the radiotracer injected.

### 1.7.2 Volume-of-interest based analysis

The general principle in the analysis of most studies in epilepsy using PET has been to define regions of interest (ROIs) on modelled or unmodelled images and
comparing the regional values obtained. A variety of approaches to define these ROIs has been employed (Bartenstein et al., 1991; Debets et al., 1997; Henry et al., 1993a; Juhász et al., 1999a; Juhász et al., 1999b; Lamusuo et al., 2000; Muzik et al., 2000; Prevett et al., 1994; Radtke et al., 1993a; Ryvlin et al., 1998; Savic et al., 1993; Savic et al., 1995; Savic et al., 1990; Szelies et al., 1996; Theodore et al., 1992a).

1.7.2.1 Geometric ROIs versus anatomical ROIs

Most methods for ROI placement are subject to observer bias. Placing ROIs of predetermined shape, for example circles or ellipses, over the structure of interest leads to sampling of both grey and white matter and does not take partial volume effects into account. Partial volume effects will be even greater when anatomically defined ROIs which are surrounded by lower signal are outlined on a coregistered MRI: Only a subset of the PET signal is sampled, as the "spill-out" signal which will appear outside the MRI-defined boundary will not be recovered. Only 50% of the true signal is recovered when using a circular ROI the same size as the FWHM resolution (Hoffman et al., 1979; Mazziotta et al., 1981). An object must be greater than two times the FWHM resolution of the PET system to be recovered with its true activity. Most PET scanners in clinical use have a FWHM resolution of about 6-8 mm, and the best ones of about 4mm. Many structures in the brain including deep nuclei, hippocampi and even the cortical ribbon are smaller than twice this value in at least one dimension. They will therefore appear less intense than their real activity through "spill-out" into adjacent structures with less binding, e.g. CSF, or will appear more intense than their real activity through "spill-in" from adjacent structures with higher activity. It has been observed that the FMZ $V_d$ is significantly more reduced in the affected mesial temporal lobe in TLE than the radiotracer transport ($K_l$) giving indirect evidence that decreases of receptor density are not due to atrophy alone, since $K_l$ and $V_d$ should be equally affected by partial volume effect averaging (Henry et al., 1993a). Our group has previously developed a method to account for partial volume effect in the hippocampus, allowing accurate absolute quantification of the receptor loss in vivo (Koepp et al., 1997a; Labbé et al., 1996). We have now extended this method to enable us to study regions across the brain (Koepp et al., 2000; Labbé et al., 1998).
1.7.2.2 Tissue classes and partial volume effects

A cortical PET signal reflects the average tracer concentration of different compartments: grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF). This is a relatively minor problem when controls subjects are compared with patients with structurally intact brains, as partial volume effects will affect both groups in the same way. When absolute quantification is sought, however, this needs to be taken into account, and partial volume effects are particularly important when conditions are examined in which there is cerebral atrophy. As outlined above, this may lead to both over-or underestimation of the severity of the disease. As a result, it is often impossible to determine the extent to which PET abnormalities represent changes of functional activity in the atrophic structure, purely structural changes, or both together. In mesial temporal lobe epilepsy with hippocampal sclerosis, correction for partial volume effects in the hippocampus alone increases the yield of FMZ-PET to detect the epileptogenic side from 65% to 100% (Koepp et al., 1997c).

1.7.2.3 Correction for partial volume effects

To assess partial volume effects, the size and shape of the structure of interest and their position in the scanner field of view must be known as well as the physical characteristics of the PET scanner used and the presence of neighbouring high or low affinity (Hoffman et al., 1979; Kessler et al., 1984). By incorporating prior knowledge about tissue composition (GM, WM and CSF) from a corresponding MRI scan, true GM activity can be calculated by simply convolving the GM with the PET scanner's measured point spread function (PSF) and dividing the observed GM intensity by this convolved segmented MR image. This three-compartment algorithm, however, depends on two assumptions which are not met in practice, namely that either the observed GM intensity is homogenous across the field of view or that the PET resolution is perfect, with the PSF being equal to a \( \delta \) function. A fourth compartment, a GM VOI, can be incorporated to account for heterogenous GM concentration (Meltzer et al., 1996). By choosing a cuboid box extending twice the FWHM around the VOI to be examined, the error resulting from the assumption of homogenous GM intensity can be contained but not eliminated (Koepp et al., 1997c).
An algebraic method allowing for any number of VOIs can instead be used (Koepp et al., 1997a; Labbé et al., 1998), with the only assumption being that the radioactivity concentration is homogenous in each VOI - an assumption inherent in any VOI analysis.

The observed PET image \( (I_{PET}) \) is the result of the convolution \( (\otimes) \) of the three dimensional PSF with the actual radiotracer concentration in different compartments defined by MRI \( (I_{MRI}) \). \( I_{MRI} \) corresponds to the sum of the actual functional activity in grey matter \( (I_{GM}) \), white matter \( (I_{WM}) \) and cerebrospinal fluid \( (I_{CSF}) \):

\[
I_{PET} = I_{MRI} \otimes \text{PSF} = I_{GM} + I_{WM} + I_{CSF} \tag{1}
\]

This can be expressed for each pixel \((i,j)\) with a set of linear algebraic equations of the form:

\[
PET_{ij} = \text{GM-VOI}_{ij} \cdot C_{GM-VOI} + \text{WM}_{ij} \cdot C_{WM} + \text{VOI}_{ij} \cdot C_{VOI} \tag{2}
\]

where \(PET_{ij}, \text{GM-VOI}_{ij}, \text{WM}_{ij}\) and \(\text{VOI}_{ij}\) are known values corresponding to PET and convolved GM-VOI, WM and VOI images, respectively. \(C_{GM-VOI}, C_{WM}\) and \(C_{VOI}\) are the three unknown values to be solved which are related to as many equations as there are pixels in the image. As there are many more equations than unknowns, the linear set can be solved in high precision.

Equation (2) can be written in matrix form as

\[
A = B \cdot x \tag{3}
\]

where \(A\) represents the matrix of PET values from \(PET_{ij}\) pixels, \(B\) represents the observed convolved GM-VOI, WM and VOI images, and \(x\) is the columnar vector containing the three unknown concentrations to be determined \((C_{GM-VOI}, C_{WM}\) and \(C_{VOI}\)).

The best solution of the matrix equation \(A = B \cdot x\) is the one which comes closest to satisfying all equations simultaneously. If this is defined in the least-squares sense, that is to say that the sum of the squares of the differences between the left-and right-hand sides of the equation be minimised, then the linear set problem reduces to a
solvable linear least-squares problem. The computation of \( x = A \cdot B^{-1} \) was done using singular value decomposition (Press et al., 1988).

The necessary segmentation of MR images into their GM, WM and CSF components was performed as outlined below (Chapter 2).

To avoid intraobserver and interobserver reliability issues and to allow us to rapidly define a large number of VOIs, we used an anatomical region template that can be transformed to match any individual subjects' scan (Chapter 3.1).

1.7.3 Voxel-based analysis

Functional imaging scans can be analysed at the voxel-level. The most widely used method or implementation is Statistical Parametric Mapping (SPM, Wellcome Department of Imaging Neuroscience, London, UK) (Friston, 1997; Friston et al., 1995b). The underlying principle is to transform several functional images into a common reference space so that corresponding voxels can be directly compared using t-tests. This leads to the creation of a statistical parametric map, a spatially extended statistical process. The voxel values are, under the null hypothesis of no regional difference between the functional images used, distributed according to a known probability density function, usually Gaussian. The theory of Gaussian fields can therefore be used to provide p values that are corrected for the brain volume analysed (Friston et al., 1991; Friston et al., 1995b; Worsley et al., 1992). Through the use of appropriate thresholding, corresponding to a correction for multiple comparisons, voxels for which the null hypothesis can be rejected can be found.

Such excursions of the statistical parametric map that are unlikely to be explained by chance are interpreted as regionally specific effects. In activation (blood flow) scans, they can be attributed to the sensorimotor or cognitive process that has been manipulated experimentally and, in the case of ligand PET images, they can be attributed to the underlying (regional) pathological condition.

The experimental design and the model used to test for specific neurophysiological responses or regional PET ligand binding differences are embodied in a mathematical structure called the design matrix. The design matrix is partitioned according to whether the effect is interesting, that is the effect under study, or
uninteresting, for example nuisance effects like global activity. The contribution of each effect to the observed physiological responses is estimated using the general linear model and standard least squares: By examination of the residual error terms, which follow a Student's $t$-distribution, the fit of the data to the model can be examined. The estimated contributions are termed parameter estimates. They can be as simple as the mean activity associated with a particular condition or as complicated as an interaction term in a multifactorial experiment. Regional effects are framed in terms of differences among the parameter estimates and are specified using linear contrasts.

The significance of each contrast is assessed with a statistic whose distribution has Student's $t$ distribution under the null hypothesis. For each contrast, a $t$ statistic is computed for each and every voxel to produce a SPM $\{t\}$. For convenience and easier comparison between studies with different degrees of freedom, the SPM $\{t\}$ is transformed to a Gaussian field or SPM $\{Z\}$. Statistical inferences are then made about local excursions of the SPM $\{Z\}$ above a specified threshold using distributional approximations from the theory of Gaussian fields. As neighbouring voxels are usually spatially correlated for both functional-anatomical and technical reasons (spatial resolution of the camera used and smoothing steps inherent in the method), they are not truly independent. The simple characterisation of the clusters of activation therefore examines both their maximal value (peak height) and their spatial extent, as finding many voxels with peak values below the strictest height threshold but all interconnected is improbable by chance.

The analysis proceeds through three stages, namely spatial normalisation, spatial smoothing, statistical analysis, followed by the statistical inference.

1.7.3.1 Spatial normalisation

To enable voxel-based data from different subjects to be comparable, the data must derive from corresponding parts of the brain. As all brains will be different, functionally homologous parts must therefore be made congruent. This is achieved by applying spatial transformations. Initially, translations, rotations and scaling are applied in three dimensions to perform an affine transformation. In a second step, higher-dimensional spatial deformations are applied using basis functions (Ashburner and Friston, 1999) to warp the images such that their shape approximates
that of a standard or idealised brain, termed "template", which defines a common reference coordinate system. Referring to such a common reference coordinate system also facilitates reporting of results in a convention-based way, even if only single subjects are studied and no intersubject imaging is necessary.

1.7.3.2 Spatial smoothing

After normalisation, the data are convolved with a smoothing kernel. The objectives are threefold: First, while removing high spatial frequency signal, smoothing generally increases signal relative to noise. Second, convolving with a Gaussian kernel conditions the data in the sense that the data conform more closely to a Gaussian field model. Finally, smoothing ensures that changes from subject to subject are assessed on a spatial scale at which homologies in functional anatomy are likely. At the final FWHM of approximately 12-13mm in each direction for our studies, the functional anatomy should not be confounded by microscopic and macroscopic organisational details that are only present in a unique individual.

1.7.3.3 Statistical analysis

In this stage, the data is modelled in order to partition observed binding into components of interest (for example group assignment or covariates), confounds of no interest (for example global binding) and an error term. The general linear model is used at each and every voxel to estimate the contribution of these components as specified in the design matrix. Differences among parameter estimates are specified by a contrast by referring to the error variance, resulting in a t map or SPM \{t\}. This SPM \{t\} is transformed to the unit normal distribution to give a Gaussian field or parametric map of the Z-statistic, SPM \{Z\}.

1.7.3.4 Statistical inference

Inferences can be made at different levels. A P value can be computed for the entire dataset but will not permit inferences about regionally specific effects which one is usually interested in. With an anatomically restricted hypothesis about binding changes in a particular brain region, for example the hippocampus, the Z value in the corresponding region in the SPM \{Z\} can be used to test the hypothesis. This approach was used in the hippocampal sclerosis study.
When there is no regionally specific hypothesis, the null hypothesis is that there is no binding change anywhere in the brain. A correction for multiple nonindependent comparisons must be used, using the theory of Gaussian fields to compute a corrected p value.
2 METHODOLOGY

Many aspects of subject recruitment and data collection and analysis were common to most of the experiments. To avoid repetition, they are described in this section, and referred to in subsequent chapters. Methods specific to each study are included in the relevant chapters.

2.1 Subject recruitment

The patients studied were recruited from the epilepsy clinics of the National Hospital for Neurology and Neurosurgery, Queen Square, London, UK and the National Society for Epilepsy - Chalfont Centre, Chalfont St Peter, UK. Some patients with occipital lobe epilepsy and normal MRI were referred from the epilepsy clinics of St Thomas' Hospital, London, UK. Clinical data and results of any previous imaging were obtained from the hospital notes. Current seizure frequency and time since last seizure were obtained from patients or carers at the time of scanning. Patients who were on regular medication with benzodiazepines or barbiturates within two months of the PET examination were not included in the [11C] Flumazenil studies.

Normal control subjects were all volunteers recruited from friends, colleagues and acquaintances of both patients and investigators as well as from advertisements placed on general noticeboards. All normal controls had no history and no evidence of neurological disorder. They were on no regular medication.

All subjects had not been treated with short course or pro re nata benzodiazepines or barbiturates for at least three weeks before [11C] Flumazenil scans. Consumption of alcohol was not allowed for 48 hours preceding the scan. Patients who were treated with opiate containing drugs on a regular basis were not included in the study of [11C] diprenorphine binding in malformations of cortical development as these drugs could interfere with [11C] diprenorphine binding. All subjects had not taken painkillers containing opioids, e.g. codeine, for at least one week before [11C] diprenorphine scans.
Written informed consent according to the declaration of Helsinki was obtained in all cases and the approvals of local ethics committees and of the UK Administration of Radioactive Substances Advisory Committee (ARSAC) were obtained. In accordance with ARSAC regulations, both male and female patients were studied but pregnancy was excluded in all female patients. No women of childbearing age were included in the normal control groups.
2.2 PET data acquisition

PET scans were performed at the Medical Research Council (MRC) Clinical Sciences Centre, Cyclotron Unit, at Hammersmith Hospital. All scans were acquired in 3D mode on an ECAT 953B PET scanner (Siemens/CTI, Knoxville, Tennessee, USA) which acquires 31 simultaneous slices and has a spatial resolution according to National Electrical Manufacturers Association (NEMA, Rosslyn, VA, USA) specifications of 6.1±0.4 x 6.1±0.4 mm FWHM in scatter 1 cm from the centre of the field of view, and 6.4±0.4 x 6.4±0.4 in conditions comparable to the scanning of subjects [Terry Spinks, personal communication, unpublished data]. The axial resolution is approximately 5.2 mm FWHM in air on the axis and 5.4 mm FWHM in scatter on axis (Bailey, 1992). The resolution deteriorates towards the edges of the field of view, and subjects were therefore positioned in the centre of the field of view.

2.2.1 Continuous Electroencephalogram monitoring

Electroencephalogram (EEG) electrodes were placed on the scalp of each patient using the international 10/20 system of electrode placement. The EEG was monitored continuously during all patient scans with a 20 channel Nihon Kohden instrument. The EEG recordings were used to detect sub-clinical epileptiform activity during the patient scans. This was done in addition to direct patient observation, patient observation via a monitor and asking patients after the scan about any seizure phenomena which might have escaped direct observation.

2.2.2 Arterial plasma parent tracer input function

After the EEG electrode placement and prior to scanning, a 22 gauge cannula was inserted into a radial artery after subcutaneous local anaesthesia with 0.5% bupivacaine. Metabolites appear in plasma after a short delay following the intravenous injection of the parent tracer. Arterial blood sampling was used to determine the availability of the parent radiotracer in the brain. Radioactivity in the arterial blood was measured continuously on-line (Ranicar et al., 1991). Furthermore, intermittent blood samples are assayed in throughout the scanning period. These are separated into cell and plasma components, and radiolabelled metabolites in arterial plasma measured (Luthra et al., 1993). These data are used to correct the continuous measure of arterial blood radioactivity, in order to produce a metabolite-corrected
arterial plasma input function used for subsequent kinetic analyses (Lammertsma et al., 1991; Lammertsma et al., 1993).

2.2.3 Radiotracer

The radioisotopes were produced on site in the Cyclotron Unit and then incorporated into flumazenil (FMZ) and diprenorphine (DPN), respectively, for injection. Each preparation was analysed by HLPC to determine the specific activity of the tracer and to ensure radiochemical purity. Quality control was performed to ensure the preparation matched the specifications and to exclude the presence of pathogens. The tracers were injected intravenously through a 22 gauge cannula inserted into an antecubital fossa vein prior to the emission scan.

2.2.3.1 $[^{11}\text{C}]$ Flumazenil (FMZ)

$[^{11}\text{C}]$ FMZ was prepared using a modification of the technique described by Maziere et al. which is based on methylation of the nor derivative of ethyl 8-fluoro-5,6-dihydro-5-$[^{11}\text{C}]$methyl-6-oxo-4H-imidazo[1,5-a][1,4] benzodiazepine-3-carboxylate (RO 15.1788 1 $[^{11}\text{C}]$) by $^{11}\text{C}$H$_3$ (Maziere et al., 1984). 10 mCi were injected per study, resulting in a total effective dose equivalent (EDE) for each subject of 2.6 milli-Sievert (mSv).

2.2.3.2 $[^{11}\text{C}]$ Diprenorphine (DPN)

$[^{11}\text{C}]$ DPN was prepared by O-methylation of 3-O-trityl, 6-desmethyl-diprenorphine. The use of the base-stable, acid labile trityl protecting group minimises the formation of byproducts and allows reproducible radiosynthesis (S. Luthra, MRC Cyclotron Unit, unpublished data). 10 mCi were injected per study, resulting in a total effective dose equivalent (EDE) for each subject of 2.8 milli-Sievert (mSv).

2.2.4 Head positioning and transmission scans

The axial field of view of the Siemens/CTI 953B PET camera is approximately 10.6cm, preventing imaging of the entire brain simultaneously. The head must therefore be correctly positioned in axial direction. Moreover, the anisotropic voxel size of the 953B camera may lead to slightly different quantitation of radioactivity concentrations small and elongated regions (Mazziotta et al., 1981), and the same tilt must be used for both patients and controls.

Two different scanning positions were used for the studies:
Temporal lobe orientation: For the absolute quantitation study investigating patients with hippocampal sclerosis, all subjects were first positioned with the glabella-inion line parallel to the detector rings. Scans were then performed with the gantry of the scanner tilted forward 20° off the glabella-inion line. Using this orientation, axial images were obtained along the long axis of the hippocampi, and the coronal images were orthogonal to the hippocampus. Partial volume effects are thus minimised (Jackson et al., 1993a; Mazziotta et al., 1981; Press et al., 1989).

Anterior-posterior commissure (ACPC) orientation: All subjects for all other studies were positioned with the glabella-inion line parallel to the detector rings. Using this orientation, axial images were obtained approximately parallel to the intercommissural (ACPC) line, which is the most widely used orientation. Of all subjects scanned in ACPC orientation, most were positioned as to include the entire temporal lobe in the scanner’s field of view, mostly omitting some data at the top of the brain, depending on the size of each subject’s brain. Using DPN, all subjects were scanned in this position. Using FMZ, this concerned 21 control subjects, 18 temporal lobe epilepsy patients with normal MRI and 10 patients with MCD, whereas the patients with extratemporal lobe epilepsy and normal MRI as well as their control subjects were positioned as to include the top slices of the subjects' brains, mostly omitting some data in the inferior temporal lobes.

In order to minimise movement during the scan, subjects' heads were rested in individualised foam head moulds secured with straps. In addition, marks were put on the skin over the forehead and over the maxillary bones, aligned with projected laser lines and observed directly and via a camera throughout the scan. Correct positioning was verified with a 2 minute transmission scan using three rotating $^{68}$Ga/$^{68}$Ge rotatory line sources followed by a 10 minute transmission scan to enable emission scans to be corrected for attenuation (Bailey, 1992).

2.2.5 Scanning protocol

For FMZ, we used a protocol of 20 frames: 30 seconds background (before injection), 4x15 seconds, 4x60 seconds, 2x150 seconds, 2x300 seconds, 7x600 seconds.

The scanning protocol for DPN was originally designed to detect endogenous neurotransmitter release between 30 and 60 minutes after radiotracer injection. It therefore comprises many short time frames covering this period: 30 seconds
background (before injection), 3x300 seconds, 10x120 seconds, 5x300 seconds, 3x600 seconds. All scans were acquired using this protocol to enable the use of the previously acquired control data, reducing the number of control subjects needed.

2.2.6 Image reconstruction, normalisation and scatter correction

After completion of the scan, the raw count data was transferred onto SPARC Ultra workstations (Sun Microsystems Inc., Mountain View, California, USA). Reconstruction into 3D images of radioactivity concentration was performed using CTI software. During reconstruction, images were normalised to account for the current efficiency of the different detector blocks. After reconstruction, all scans were "z-scaled" to correct for the varying efficiency along the longitudinal (z) axis of the field of view (Grootoonk, 1995).

All DPN scans were corrected for scatter using the "dual window" scatter correction, allowing for scatter from outside the field of view (Grootoonk et al., 1996). In contrast, all FMZ scans were corrected for scatter using the "convolution subtraction" technique (Bailey and Meikle, 1994). Whereas this does not allow for the approximately 5% of scatter which may arise from outside the field of view, the technique is insensitive to small changes in ambient temperature. This method was therefore chosen for the FMZ scans as some older control data was used, obtained at a time when such small changes in ambient temperature were not excluded through the use of high-power air conditioning.
2.3 MRI data

MRI scans for coregistration and partial volume effect correction were performed at the Robert Steiner MRI Unit at Hammersmith Hospital, on a 1 Tesla Picker scanner for control subjects. Patients' MRI scans, additionally used for quantification of hippocampal volumes and hippocampal T2 relaxation times, were performed at the MRI Unit of the National Society for Epilepsy, Chalfont St Peter, on a 1.5 Tesla General Electric (GE) Signa Echospeed scanner.

2.3.1 MRI data acquisition

For coregistration with PET, MR images were obtained for each control subject on a 1Tesla Picker scanner using a gradient echo sequence protocol which generated 128 contiguous 1.3 mm thick sagittal images (matrix 256x256 voxels, voxel sizes 1x1x1.3 mm, repetition time (TR) 35 msec; echo time (TE) 6 msec; flip angle 35°). These high resolution volume acquisition MRI scans were coregistered with the parametric images of FMZ binding (Ashburner and Friston, 1997). This approach uses a priori probability maps for each modality which have been previously registered in standard space. They are used to segment the images of each modality separately. Subsequently, a ratio image is calculated and this ratio iteratively minimised through rotation, translation and scaling of the images. This approach works very well for images with characteristics similar to H\textsuperscript{15}O-PET flow images, for example FMZ binding images. For DPN images, which show very little DPN binding in the occipital lobe, this approach does not work satisfactorily as the ratio images are spatially inconsistent. A different approach was therefore used which uses information theory to iteratively maximise mutual information, a measure of cross-correlation between the images (Maes et al., 1997).

Patients were scanned on a 1.5 Tesla GE Signa Echospeed scanner at the National Society for Epilepsy. The standard protocol consisted of:

* Sagittal T1 weighted localiser.* Conventional spin echo, time of echo (TE)/time of repetition (TR)/number of excitations (NEX) 14/640/1, 17 slices of 5mm thickness with 2.5mm gap, field of view (FOV) 24x24cm with a 256x256 matrix, acquisition time 2'47''.

To minimise partial volume effects the hippocampus is best visualised in two planes: along the long axis and perpendicular to the structure. These imaging planes are
readily determined on the sagittal scout image. The axial plane is in the line joining
the base of the splenium of the corpus callosum to the inferior posterior border of the
frontal lobe with the coronal plane perpendicular to this, parallel to the anterior
border of the brainstem.

*Coronal oblique proton density (PD) and T2 weighted.* Conventional spin echo,
TE/TR/NEX 30&120/2000/1, 28 slices of 5mm thickness with 0mm gap, FOV
18x24cm with a 192x256 matrix, acquisition time 10'24".

These slices are orientated perpendicular to the long axis of the hippocampus, to
demonstrate any increase in T2-weighted signal intensity and to obtain hippocampal
T2 relaxation times as described previously (Duncan et al., 1996b).

*Coronal T1 weighted 3D volume.* Inversion recovery prepared fast spoiled gradient
recall (GE), TE/TR/NEX 4.2(fat and water in phase)/15.5/1, time of inversion (TI)
450, flip angle 20°, 124 slices of 1.5mm thickness, FOV 18x24cm with a 192x256
matrix, acquisition time 6'56".

This covers the whole brain with voxel sizes of 0.9375 x 0.9375 x 1.5 mm. The
image data can be resliced using sinc interpolation, to produce cubic voxels, allowing
for reformatting in any orientation, subsequent measurement of hippocampal
morphology and volumes, and for 3D reconstruction and surface rendering. The
inversion recovery prepared spoiled gradient recall sequence used provides good
contrast between grey and white matter.

*Coronal oblique fast fluid attenuation inversion recovery (Fast FLAIR).* Fast FLAIR
sequence, TE/TR/NEX 144/11000/1, TI 2600, 28 slices of 5mm thickness with 0mm
gap, FOV 18x24cm with a 192x256 matrix.

This allows a T2 contrast without interference from the CSF signal.

The coronal T1 weighted volume was used for coregistration with PET, for
hippocampal volume measurements in all temporal lobe epilepsy patients and for the
delineation of individual volumes of interest in the patients with hippocampal
sclerosis, their controls, and patients with malformations of cortical development. It
was further used for the segmentation in grey matter, white matter and CSF prior to
partial volume effect correction.

In the patients with hippocampal sclerosis, hippocampal cross-sectional areas were
outlined manually and measured on consecutive images as previously described
The sum of the hippocampal cross-sectional areas times partition thickness gave total hippocampal volumes (HCV). Intracranial volume (ICV) was measured on the coronal T1 weighted 3D volume dataset. HCV correlates with ICV. Correction for ICV allows detection of bilateral abnormalities in HCV (Free et al., 1995). The correction was performed via a covariance method (Jack et al., 1989). Mean control HCV corrected for ICV was (in mm$^3$ for women/men) 2604±154/2932±233 on the right and 2531±157/2893±218 on the left. Note that the correction method leaves the mean unchanged, only the coefficient of variation (CV, standard deviation (SD)/mean) is reduced to give tighter normal ranges. The lower limits (mean -3SD) was (in mm$^3$ for women/men) 2142/2233 on the right and 2060/2239 on the right. A slightly different, equally validated method for outlining the hippocampi was used in the development of the anatomical region template and its assessment (Niemann et al., 2000a).

Hippocampal volume ratio was determined as the volume of the hippocampus with higher T2 relaxation time divided by the volume of the hippocampus with lower T2 relaxation time. The control hippocampal volume ratio was 0.96 (SD=0.03), with 0.90 (-2 SD) as the lower limit of normal (Free et al., 1995).

Hippocampal T2 relaxation time measurements were obtained for all temporal lobe epilepsy patients on a 1.5 Tesla GE Signa Echospeed scanner, as previously described (Duncan et al., 1996b). 91 milliseconds was taken as the upper limit of normal, which is 2 standard deviations above the mean control hippocampal T2 relaxation time for the hippocampal body on the machine used.

MRI-based partial volume effect correction requires the images to be segmented into grey and white matter, CSF and non-brain tissue. For the hippocampal sclerosis and the malformations of cortical development study using FMZ; this was performed using a clustering algorithm with a modified mixture model (Hartigan, 1975) and a priori information about the likelihoods of each voxel being one of a number of different tissue types, as implemented under Matlab version 4 (The MathWorks, Natick, Massachusetts, USA) in Statistical Parametric Mapping (SPM96, Wellcome Department of Imaging Neuroscience, Institute of Neurology, London). For the malformations of cortical development study using DPN, an improved segmentation
method was available and used. First, the artefact that causes similar tissues to have different intensities in different parts of the image, known as nonuniformity, is removed using software from the Montréal Neurological Institute (MNI), N3 (Sled et al., 1998). This performs a nonuniformity correction of the MR images as a first step, without assumptions about the tissue classes present, and can therefore be used in the presence of abnormal brain structure. Subsequently, the actual tissue segmentation was performed using software developed at the MRI Unit, National Society for Epilepsy, Chalfont St Peter. The algorithm uses automated thresholding and morphological operations and is based on a few assumptions about brain anatomy, e.g. "white matter constitutes a single connected component" and "grey matter intensity is greater than background intensity and lower than white matter intensity" and has been shown to be accurate and reproducible (Lemieux, 2001; Lemieux et al., 1999). The resulting probability images are visually superior to the ones obtained with the previous method, and the simple assumptions make it possible to use the algorithm in the presence of structural pathology.

To avoid intraobserver and interobserver reliability issues and to allow us to rapidly define a large number of VOIs, we used an anatomical region template that can be transformed to match any individual subjects' scan (Chapter 3.1). This defined multiple regions with known accuracy. For the second MCD study using diprenorphine, an improved probabilistic template based on 20 normal MRI data sets was available. Regions of special interest which require millimetric precision - the hippocampus in the hippocampal sclerosis study and the malformations and adjacent/overlying cortex in both MCD studies - were all outlined manually.
2.4 Data analysis

In the implementation of SPM used in the hippocampal sclerosis study, SPM96, the foci of significant differences were characterised in terms of spatial extent \( k \) and peak height \( \mu \). The significance of each regional difference was estimated using distributional approximations from the theory of Gaussian fields. This characterisation was in terms of the probability that a region of the observed number of voxels (or bigger) could have occurred by chance \( P(n_{\text{max}}>k) \), or that the peak height observed (or higher) could have occurred by chance \( P(Z_{\text{max}}>{\mu}) \) over the entire volume analysed, so a corrected p-value for both peak height and extent was obtained (Friston et al., 1995a; Friston et al., 1995b). The threshold chosen for both \( P(n_{\text{max}}>k) \) and \( P(Z_{\text{max}}>{\mu}) \) was <0.05.

In the implementation of SPM used for the subsequent studies, SPM99, the strict correction for spatial extent was abandoned for statistical reasons and following simulation experiments. Instead, the SPM \{t\} is subject to standard procedures developed for statistical parametric mapping using the theory of Gaussian fields. These procedures produce P values that pertain to different levels of inference - either in terms of the number of activated or different regions (number of clusters), number of activated or different voxels comprising a particular region or P values for each voxel within that region.

For the voxel-based analysis of the FMZ-PET study of patients with hippocampal sclerosis, a template in temporal lobe orientation was created on the basis of five individual controls scanned in this orientation. Their FMZ-PET scans were flipped, superimposed with the help of a three-dimensional transformation and then averaged to produce a symmetrical template. Symmetry was necessary as, for the purposes of statistical analysis of the patients as a group, the FMZ-PET scans of patients with left sided hippocampal sclerosis were flipped so that the hippocampal sclerosis appeared on the same, right, side in all patients before the ensuing spatial normalisation process. An asymmetrical template can lead to artificial systematic differences in this situation [own observation, unpublished data].
For the voxel-based analyses of the FMZ-PET studies in patients with partial epilepsy and normal MRI, a FMZ template could be created in common reference space. The FMZ-$V_d$ images of 6 normal volunteers were flipped and coregistered with their respective T1 weighted MRI scans, giving 12 datasets. The T1 weighted MRI scans were then spatially normalised to the average T1 template in common reference space. This average T1 template has been created through linear transformations and averaging of 305 normal control scans at the Montréal Neurological Institute (Evans et al., 1994). The parameters necessary for a near-perfect transformation of the individual flipped and unflipped coregistered MRI scans were determined, stored and then applied to the individual flipped and unflipped FMZ-$V_d$ images. The resulting FMZ-$V_d$ images in common reference space were used to create an average template, weighted per plane for the number of datasets used for this plane. A corresponding procedure was used to create a DPN-$V_d$ template in common reference space for the voxel-based analysis of the DPN-$V_d$ scans of the patients with malformations of cortical development.

For the analysis of individual patients and groups of patients, the aim of the analysis was to identify focal areas of cerebral ligand binding showing significant differences, after global differences had been excluded by comparing global binding. In SPM, global binding was therefore designated in the design matrix as a confounding factor covariate in the sense of an analysis of covariance (Friston et al., 1990).
3 RESULTS OF INVESTIGATIONS

3.1 Implementation and application of a brain template for multiple volumes of interest

3.1.1 Summary

We created a region template and a protocol for transforming that template to define anatomical volumes of interest (VOIs) in the human brain without operator intervention, based on software contained in the SPM99 package (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, London, UK). We used an MRI of a reference brain to create an anatomical template of 41 VOIs, covering the entire brain, that can be spatially transformed to fit individual brain scans. Modified software allows for the reslicing and adaptation of the transformed template to any type of coregistered functional data. Individually defined VOIs can be added. This chapter presents an assessment of the necessary spatial transformations and a comparison of results obtained for scans acquired in two different orientations.

To evaluate the spatial transformations, 11 landmarks distributed throughout the brain were chosen. Euclidean distances between repeat samples at each landmark were averaged across all landmarks to give a mean difference of 1.3±1.0mm. Average Euclidean distances between landmarks (MRI:transformed template) were 8.1±3.7mm in anterior-posterior commissure (ACPC) and 7.6±3.7mm in temporal lobe (TL) orientation.

We use [11C] flumazenil (FMZ) PET as an example for the application of the region template. 34 healthy volunteers were scanned, 21 in standard ACPC orientation, 13 in TL orientation. All had high resolution MRI and FMZ-PET. The average coefficient of variation (CV) of FMZ binding for cortical regions was 0.15, comparable with CVs from manually defined VOIs. FMZ binding was significantly different in 6/19 anatomical areas in the control groups obtained in the different orientations, probably due to anisotropic voxel dimensions.

This new template allows for the reliable and fast definition of multiple VOIs. It can be used for different imaging modalities and in different orientations. It is necessary that imaging data for groups compared are acquired in the same orientation.
3.1.2 Introduction

Brain imaging data can be analyzed in two complementary ways, either with voxel-based methods (e.g. Statistical Parametric Mapping, SPM) or with region-based methods. Whereas the former allows comparisons at the voxel-level, normalisation and smoothing steps are necessary, with interpolation of the original data, and the method does not normally allow for the absolute quantitation of brain imaging data (Strul and Bendriem, 1999). Region-based methods, which do not alter the original brain imaging data, allow for the correction for partial volume effect which is particularly important when dealing with anatomically abnormal and atrophic cerebral structures (Labbé et al., 1998; Müller Gartner et al., 1992; Rousset et al., 1993).

Traditional region-based analyses for the absolute quantification of brain imaging data, however, require neuroanatomically trained observers, are very time-consuming and suffer from observer bias. Furthermore, scans are usually acquired along the plane defined by the anterior and posterior commissure (ACPC), but many studies in epilepsy, psychiatry and neuropsychology focus on the temporal lobe and its substructures and are acquired in temporal lobe (TL) orientation (Ryvlin et al., 1998; Van Paesschen et al., 1997) to minimize partial volume effect in mesial temporal structures (Jackson et al., 1993a; Mazziotta et al., 1981; Press et al., 1989). The different appearance of anatomical structures in different orientations needs to be taken into account when defining protocols for outlining VOIs.

A standard anatomical template makes the definition of multiple VOIs feasible and can improve consistency of outlining (Evans et al., 1988). Several techniques have been described (e.g. (Bajcsy et al., 1983; Bohm et al., 1991; Christensen et al., 1997; Collins et al., 1999; Evans et al., 1988; Evans et al., 1991; Greitz et al., 1991; Kosugi et al., 1993)).

As functional imaging data typically have relatively low spatial resolution, all methods obtain higher spatial frequency information from other sources. Usually, structural data (typically MRI) from the same subject is coregistered with the
functional data. Earlier methods had to use fiducial markers for this step (Evans et al., 1991) or tried to ensure direct comparability by employing head masks (Evans et al., 1988). Automatic voxel-based methods for coregistration have been shown to be superior to manual techniques (Collins et al., 1994). Automatic techniques use a variety of approaches, for example a priori spatial information (Ashburner and Friston, 1997; Friston et al., 1995a), ratio images (Woods et al., 1993) or mutual information (Maes et al., 1997; Studholme et al., 1997). They achieve excellent results (Kiebel et al., 1997) and have generally replaced the earlier methods.

The other step required is some spatial transformation to achieve correspondence between atlas and an individual subject's data. Several methods have been used in the past (van den Elsen et al., 1993). Our method is based on the widely used algorithm as contained in the SPM99 package (Statistical Parametric Mapping, Wellcome Department of Imaging Neuroscience, London, UK, available via http://www.fil.ion.ucl.ac.uk). This algorithm first uses a twelve-parameter affine registration (Ashburner and Friston, 1997) to achieve global correspondence between data sets, followed by nonlinear transformations using basis functions to accommodate interindividual differences on a smaller scale. The sum of squared differences between the image to be normalised and the target image or template is minimized, while the smoothness of the transformation is maximized using a maximum a posteriori approach (Ashburner and Friston, 1999). For the spatial transformation of an individual dataset to a group template, the addition of nonlinear transformations has been shown to be superior to linear matching alone (Ashburner and Friston, 1999). It is a reasonable assumption that the same will be true if the software is used for the inverse process, namely the adaptation of a standard MRI dataset to an individual one.

There is a need for an easy, fast and observer-independent way of defining multiple VOIs on individual, non-warped functional data, making use of state-of-the-art methods of coregistration and spatial warping and the low cost of high-speed computing. Here, we used an MRI scan of a single brain obtained at high resolution at the Montreal Neurological Institute (MNI) (Holmes et al., 1998) as delivered with the SPM99 package and an interactive algorithm to create a brain template consisting of 41 VOIs. A list of the VOIs outlined with their attribution to areas is given in Table 3.1.1, p. 164. This can be spatially transformed to fit any individual brain scan, leaving the individual's imaging data unchanged. It can then be used to identify
structures in the target image and to automatically measure the imaging parameter in those anatomical structures. Individually defined VOIs can be added to the transformed template if desired, leaving the space of the original data unwarped. We present the assessment of the accuracy of the necessary spatial transformations, using manual labeling of landmarks distributed throughout the brain. Furthermore, we compare results obtained for scans acquired in two different orientations. $^{[1]}$C FMZ PET is used as an example of an application in this study, but the method can be used for the quantitation of regional brain imaging data of any modality within the limits discussed later in this chapter.

### 3.1.3 Methods

The aims were:

- to construct a 3D VOI template, based on MRI anatomy.
- to assess the spatial transformations of this template into individual subjects' MRI space, using manually labeled landmarks.
- using this transformed template, to obtain regional data, in this example of $^{[1]}$C FMZ binding, and to examine its characteristics.
- to compare regional data, using the example of $^{[1]}$C FMZ binding in multiple VOIs, to check the applicability of the method to brain imaging data generated in different orientations.

#### 3.1.3.1 Construction of the template

We first developed an algorithm for the manual subdivision of the brain on T1 weighted 3D MRI datasets into anatomical substructures by a neuroanatomically trained investigator (A.H.). The algorithm was adapted to provide unequivocal guidelines for subdivision even in the presence of anatomical variants. This was assessed by application to five different datasets and the algorithm formalized in text. We then applied this algorithm to the MRI of the MNI single brain (Holmes et al., 1998), which is in the same space as the MNI brain average of 305 subjects scanned with T1 weighted MRI, used as a template in SPM99. This single subject MRI has been widely used as a reference, for example as the template for spatial normalisation in SPM96 or for the construction of the MNI digital brain phantom (Collins et al., 1998) (http://www.bic.mni.mcgill.ca/brainweb).
3.1.3.2 Scanning procedures

We studied 34 healthy volunteers. To test the applicability of the template in different orientations, MRIs and $[^{11}]$C FMZ PET scans of 21 subjects (3 women) were acquired in standard ACPC orientation, and MRIs and $[^{11}]$C FMZ PET scans of 13 subjects (2 women) were acquired in TL orientation. The median age at examination for the two groups was 31 years (range: 20-71 years) and 32 years (range: 23 - 64 years) respectively. They had no history of neurological or psychiatric disorder, were not taking any medication and had normal MRI studies. No individuals consumed alcohol within the 48 hours preceding $^{11}$C-FMZ PET scans.

MRIs were obtained for each subject on a 1 Tesla Picker scanner (Picker, Cleveland, Ohio, USA) using a gradient echo protocol which generated 128 contiguous 1.3 mm thick sagittal images (matrix 256x256 voxels, voxel sizes 1x1x1.3 mm, repetition time (TR) 35 msec; echo time (TE) 6 msec; flip angle 35°). We used a similar PET acquisition techniques as described previously (Koepp et al., 1996; Richardson et al., 1997a). Briefly, PET scans were performed in 3D mode, using a 953B Siemens/CTI PET camera (chapter 2.2). A convolution subtraction scatter correction was used (Bailey and Meikle, 1994) and z-scaling with the inverse of our scanner's axial profile applied to obtain uniform efficiency throughout the field of view (Grootoont, 1995). The acquisition protocol was identical for all scans, only the orientation differed between the groups. Voxel-by-voxel parametric images of $[^{11}]$C FMZ volume of distribution ($[^{11}]$C FMZ-Vd), reflecting binding to GABA$_A$ receptors (Koepp et al., 1991), were produced from the brain uptake and plasma input functions using spectral analysis (Cunningham and Jones, 1993) with correction for a blood volume term.

3.1.3.3 Spatial transformation of the VOI template

The spatial transformations were based on modified software included in SPM99, implemented in Matlab (Mathworks Inc, Sherborn, MA, USA). Image manipulation and measurements were performed on a cluster of Sun Ultra 10 workstations (Sun Microsystems, Mountain View, CA). The VOI template was first transformed into the individual subject’s MRI space. This was achieved by using the MRI of the MNI single brain - from which the template was derived - to estimate the transformation parameters necessary to
transform the MNI single brain into the individual subject’s MRI space. Both linear transformations (translations, rotations and zooms) and nonlinear transformations (7*8*7 basis functions, 12 iterations) were used (Ashburner and Friston, 1999; Meyer et al., 1999). For the spatial transformation, the software processes MRIs with 8mm isotropic smoothing applied. This ensures a globally optimal solution rather than a locally optimal solution is obtained. The calculated transformation parameters were stored and then applied to the VOI template. This resulted in transforming the standard VOI template into that subject’s individual MRI space, in either ACPC or TL orientation. The VOI template was then resliced to have the same matrix as the subject’s MRI, using nearest neighbour interpolation to preserve unequivocal allocation of each voxel to one VOI.

These spatial transformations required approximately 10 minutes of central processing unit (CPU) time on networked Sun Ultra 10 workstations.

3.1.3.4 Assessment of spatial transformation

All spatially transformed templates should occupy the same space as the individual MRIs and therefore landmarks within these images should be very close to each other. To evaluate the accuracy of the automatically determined borders between the multiple VOIs, we identified 11 landmarks that could be readily identified on both the spatially transformed VOI template and the MRI datasets. They were identified manually with a cursor enabling identification of 3D co-ordinates for each landmark. They were chosen so as to be as widely distributed within the image space as possible:

1. superior end of the right and left central sulcus parasagittally;
2. inferior end of the right and left central sulcus adjacent to the sylvian fissure;
3. superior end of parieto-occipital right and left sulci parasagittally;
4. right and left tentorium cerebelli on the same parasagittal slices as 3.;
5. right and left anterior lateral end of the circular sulcus of the insula;
6. inner genu of corpus callosum on midsagittal slice.

The midline was determined on a transaxial slice; the parasagittal slices were defined as being 5 mm either side of the midline. All measurements for this assessment were done by a trained rater at the National Society for Epilepsy's Chalfont MRI centre who was not involved in either template creation or optimisation of the spatial transformation process.
To test the reproducibility (intra-rater reliability) of the landmark positioning, this was repeated three times on four datasets (template transformed to ACPC and TL orientation; MRI in ACPC and TL orientation) and the average of all Euclidean distances across all landmarks taken.

To test the accuracy of the spatial transformation with respect to the target dataset, the 11 landmarks were positioned on 5 randomly chosen datasets (MRI and individualized template) per orientation, and average Euclidean distances between landmarks (MRI:individualized template) were determined.

3.1.3.5 Obtaining regional data

In this chapter, we use FMZ PET as an example for the application of the region template. To analyze the FMZ PET VOI data, the subjects' high resolution volume acquisition MRI scans were automatically segmented into probability images of gray matter (GM), white matter (WM) and CSF using a clustering, maximum likelihood ‘Mixture Model’ algorithm (Hartigan, 1975). Each subject's segmented MRI images were then coregistered with the parametric images of $^{[1]}C$ FMZ-Vd by applying the matrix transformation of the MRI-PET coregistration (Woods et al., 1993). The same matrix transformation was applied to the individualized VOI template. The FMZ-PET VOI data was then corrected for partial volume effect by convolving the former with the 3D PET point spread function of the PET scanner, as previously described in detail (Labbé et al., 1998).

We report the $^{[1]}C$ FMZ-Vd in gray matter for the anatomical regions. 38 out of the 41 VOI values obtained for every brain were analyzed. 16 pertained to temporal lobe subdivisions, 12 to extratemporal neocortex and 10 to the basal ganglia (cf. Table 3.1.1, p. 164). The remaining 3 VOIs, from corpus callosum, ventricular cerebrospinal fluid and brainstem, were not analyzed, as the two former do not have specific FMZ binding and the latter was included to varying degrees in our PET scanner's field of view.

3.1.3.6 Statistical analysis of regional data and comparison between orientations

Normal ranges were established for each orientation separately. For each VOI, the normal range was defined as 3 SD above and below the normal control mean, to account for the multiple comparisons that would be made in the evaluation of a patient's scan against a control set (Hammers et al., 2001a).
There was no significant difference between the partial volume effect corrected $[^{11}\text{C}]$ FMZ-Vd values obtained for the right and left side of the standard anatomical VOIs in either orientation. Both sides were, therefore, considered together, resulting in $38/2=19$ different anatomical areas (Table 3.1.1, p. 164).

Statistical analysis was performed using the Kolmogorov-Smirnov test and Student’s $t$ test with the Bonferroni correction for multiple comparisons for comparison of regional values between orientations.

### 3.1.4 Results

#### 3.1.4.1 Evaluation of spatial transformation

For repeat measures (intra-rater reliability), the average of all Euclidean distances between repeatedly measured landmarks across all landmarks for all datasets was $1.3 \pm 1.0\text{mm}$ (range 0-9.2). The MRI repeat measures yielded very similar average Euclidean distances ($1.3 \pm 0.6\text{mm}$) to the template repeat measures ($1.4 \pm 1.3$).

The assessment of the spatial transformation process yielded average Euclidean distances (between landmarks on the MRI and the same landmarks on the individualised template) of $8.1 \pm 3.7\text{ mm}$ in ACPC and $7.6 \pm 3.7\text{ mm}$ in TL orientation. The range of the individual measurements was 2.2-17.6 mm in ACPC and 1.0-18.9 mm in TL orientation. Some landmarks were associated with smaller variability than others in both orientations, e.g. the corpus callosum landmark only had 4.5mm average distance, whereas the anterior insula had 10mm average distance (Table 3.1.2, p. 165). An example of the individualised template overlying an MRI scan is given in Figure 3.1.1 (p. 166).
<table>
<thead>
<tr>
<th>Region</th>
<th>Temporal lobe orientation (n=13)</th>
<th></th>
<th>ACPC orientation (n=21)</th>
<th></th>
<th>% difference (bold = sign.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (R+L)</td>
<td>SD (R+L)</td>
<td>CV (R+L)</td>
<td>Mean (R+L)</td>
<td>SD (R+L)</td>
</tr>
<tr>
<td>Amygdala</td>
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<td>.19</td>
<td>2.79</td>
<td>0.57</td>
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<td>1.20</td>
<td>.18</td>
<td>6.08</td>
<td>1.08</td>
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<tr>
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<td>0.84</td>
<td>.12</td>
<td>7.22</td>
<td>1.01</td>
</tr>
<tr>
<td>Parahippocampal gyrus</td>
<td>5.25</td>
<td>0.83</td>
<td>.16</td>
<td>4.54</td>
<td>0.71</td>
</tr>
<tr>
<td>Sup. temporal gyrus</td>
<td>6.06</td>
<td>0.52</td>
<td>.09</td>
<td>6.11</td>
<td>0.83</td>
</tr>
<tr>
<td>Middle and inf. temporal gyrus</td>
<td>6.10</td>
<td>0.64</td>
<td>.10</td>
<td>5.94</td>
<td>0.79</td>
</tr>
<tr>
<td>Anterior cingulate gyrus</td>
<td>5.71</td>
<td>1.01</td>
<td>.18</td>
<td>4.66</td>
<td>1.07</td>
</tr>
<tr>
<td>Post. temporal lobe</td>
<td>6.34</td>
<td>0.56</td>
<td>.09</td>
<td>6.12</td>
<td>0.71</td>
</tr>
<tr>
<td>Insula</td>
<td>5.49</td>
<td>0.76</td>
<td>.14</td>
<td>5.09</td>
<td>0.71</td>
</tr>
<tr>
<td>Ant. cingulate gyrus</td>
<td>5.91</td>
<td>0.78</td>
<td>.13</td>
<td>5.60</td>
<td>0.81</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>6.64</td>
<td>0.61</td>
<td>.09</td>
<td>5.81</td>
<td>0.79</td>
</tr>
<tr>
<td>Parietal lobe</td>
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<td>0.49</td>
<td>.08</td>
<td>6.45</td>
<td>0.64</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>7.44</td>
<td>0.64</td>
<td>.09</td>
<td>6.84</td>
<td>0.88</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>3.05</td>
<td>0.64</td>
<td>.21</td>
<td>2.47</td>
<td>0.42</td>
</tr>
<tr>
<td>Putamen</td>
<td>2.51</td>
<td>0.54</td>
<td>.22</td>
<td>2.22</td>
<td>0.40</td>
</tr>
<tr>
<td>Pallidum</td>
<td>2.35</td>
<td>0.48</td>
<td>.20</td>
<td>1.96</td>
<td>0.69</td>
</tr>
<tr>
<td>Thalamus</td>
<td>2.99</td>
<td>0.31</td>
<td>.11</td>
<td>2.61</td>
<td>0.33</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>4.57</td>
<td>0.45</td>
<td>.10</td>
<td>3.88</td>
<td>0.49</td>
</tr>
<tr>
<td>Average CV</td>
<td></td>
<td></td>
<td>.13</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1.1: Partial volume effect corrected gray matter FMZ volume-of-distribution in different acquisition orientations. SD = standard deviation; R = right; L = left; CV = coefficient of variation (SD/Mean); ant = anterior, post = posterior; sup = superior; inf = inferior.
Table 3.1.2: Mean and maximum landmark distances (left and right combined, where applicable). The first two data columns show mean and maximum Euclidean distances for repeat measurements (intra-rater reliability). The remaining four data columns show the mean and maximum Euclidean distances between the landmarks placed on individual MRI datasets and the corresponding individualized templates for ACPC and TL orientation. Max = maximum, sup. = superior. All measurements are in millimeters.
3.1.4.2 Regional values: Individual subjects

In the TL orientation group, none of the regional values of $[^{11}\text{C}]$ FMZ-Vd lay outside the defined normal range. In the ACPC orientation group, a total of two values fell outside the normal range, one value for the left insula ($+3.09$ SD) and one for the right posterior cingulate gyrus ($+3.14$ SD). For the chosen thresholds and the 1292 values obtained (resulting from 38 regions x 34 subjects), less than four values would be predicted to fall outside the defined normal range; the data obtained is therefore in good agreement with the assumption of a normal distribution.
3.1.4.3 Comparison between image orientations

The results (mean, standard deviation, coefficient of variation) for each group are given in Table 3.1.1 (p. 164). The average coefficient of variation (CV, defined as SD/Mean) across all regions was .13 in TL orientation and .16 in ACPC orientation. Average gray matter values of \[^{11}C\] FMZ-Vd were not significantly different in ACPC or TL orientation. There were, however, significant differences between values derived in different orientations in 6/19 anatomical areas (Table 3.1.1, p. 164): parahippocampal gyrus (-13.5% in ACPC compared to TL orientation), fusiform gyrus (-18.3%), posterior cingulate gyrus (-12.5%), caudate nucleus (-18.9%), thalamus (-12.6%) and cerebellum (-15%).

3.1.5 Discussion

In this chapter, we present the implementation, application and assessment of a versatile method for the quantification of brain imaging data.

This method allows for the reliable definition of multiple cortical VOIs. The means and spread of the data obtained are comparable with traditional region-of-interest (ROI) analyses. At the same time, many of the pitfalls of ROI analyses are avoided: The process is entirely automated and therefore avoids intra- and inter-rater subjectivity in the placement of regions. This has the added advantage of allowing repeated measurements over time without the drawbacks associated with user intervention. A further important advantage is speed. It is our experience that it takes a trained observer approximately 15 hours to manually outline all regions to sample an entire brain volume, whereas the spatial transformations undertaken in this study take less than 10 minutes. The method presented here can be used for the quantitation of MRI, magnetic resonance spectroscopy, PET, single photon emission tomography or other brain imaging data on a VOI basis, making it widely applicable.

3.1.5.1 Comparison with previous work

Approaches similar to ours have been described before (e.g. (Bajcsy et al., 1983; Bohm et al., 1991; Christensen et al., 1997; Collins et al., 1999; Evans et al., 1988; Evans et al., 1991; Greitz et al., 1991; Kosugi et al., 1993)). The main difference to most of the earlier approaches are the complete automation of our protocol, with 100% reproducibility and no interobserver variability, and the use of a mature elastic
matching protocol incorporating nonlinear transformations incorporated into SPM99 (Ashburner and Friston, 1999). The spatial transformation module allows for the determination of the necessary warping steps from one image and their application to another one which is in register. As the T1 weighted MRI from which the anatomical template is derived is known, this can be exploited to transform the anatomical template with the same accuracy used in most current voxel-based imaging studies. We have modified existing software from the SPM99 software package to reslice the spatially transformed template into any image space in register with the subject's MRI, e.g. a coregistered functional image, retaining the unequivocality of the assignment of voxels to regions through the use of nearest neighbour interpolation. To our knowledge, this computationally inexpensive and very efficient method has not been previously described.

A sophisticated method has recently been described, using a probabilistic atlas (Collins et al., 1999). Individual data are transformed to the matrix of the atlas and the inverse transformation is used to transform the atlas back to the original image space for segmentation into VOIs. While this ensures that the transformation is atlas-independent, applying a dual transformation process is less efficient than using a single forward transformation from atlas space to individual space as in our approach. The atlas of Collins et al. has the advantage of using probabilistic information, but due to time constraints, the data used to build the atlas was itself automatically generated, with a similar protocol to that being tested. This may have introduced some bias. The only comparison made with manual subdivision was in the superior frontal gyrus. This is a relatively large structure, and no information on accuracy of mapping in other areas of the brain is obtained. It is to be expected, however, that the differences between the two approaches would be small in routine analyses in view of the similarity of the methods.

3.1.5.2 Construction of the template

The algorithm for the manual subdivision of T1 weighted MRI scans was originally intended to be applied to all individual MRIs and therefore tested on five different datasets to provide unequivocal rules for delineation even in the presence of anatomical variants. As this version of the template is being used in studies of patients with epilepsy, we focussed our subdivisions on the temporal lobe (Koepp et al., 2000). As the spatial transformation is independent of the actual atlas used, any other subdivision of the template can be used, and one may define further individual
VOIs as needed for individual cases to address specific questions (Hu et al., 2000) or cluster some VOIs together (Hammers et al., 2001c).

The brain used to define our regional template has been used until recently (in SPM96) by the imaging community as a standard to which individual data has been normalised and therefore currently represents the best available substrate for the definition of standard regions. Ideally, however, such a regional template would not depend on individual anatomy but would incorporate some measure of variability between individuals, i.e. a probabilistic measure of region extent. This remains a desirable goal for the future (Mazziotta et al., 1995) but was not attempted here. One of the reasons is that it proved impossible to reliably outline smaller regions on the composite average brains as provided by the MNI and contained in the SPM99 package. We are currently in the process of manually segmenting a larger number of MRI scans, using the same algorithm, to obtain such an improved template.

3.1.5.3 Accuracy of spatial transformations

We show a 2D representation of a typical example with mean and maximum error ellipses in Figure 3.1.1 (p. 166). The spatial transformations were visually acceptable for all subjects in both orientations. Moreover, the formal evaluation indicated that the accuracy of the spatial transformations did not depend on the different angulation (about 35-40°) of the target images when the region template - created on an MRI in standard ACPC orientation - was transformed to the scans acquired in TL orientation. The parameters chosen for the transformations were thus sufficient to accommodate even relatively large deviations from the standard orientation of the original template. Our assessment of the spatial transformations used a landmark approach rather than measures of overlap. The accuracy data does not, therefore, indicate whether a particular landmark would always be assigned to the same VOI. This might be a problem in functionally distinct but anatomically close structures, as for example pre- and postcentral gyrus. Figure 3.1.1 shows, however, that variability around the landmarks will only marginally affect the region sampled as a whole.

The accuracy of the superimposition of the anatomical regions onto the target brain volume was considered sufficient for cortical VOIs, considering the low resolution of most functional images (e.g. PET, single photon emission tomography or magnetic resonance spectroscopy). It has to be borne in mind that the accuracy data presented here depends not only on the ability of the spatial transformation process to match object template and target MRI but also on intersubject anatomical variability. In
fact, the residual 3D distances between landmarks obtained with our protocol (approximately 7.5 mm on top of the intrarater variability for repeat measures) correspond well to the intersubject anatomical differences found by other workers for scans already registered into stereotaxic space with cross-correlation methods (Collins et al., 1994) or with the AIR (Woods et al., 1993) algorithm (Grachev et al., 1999). The latter method achieved somewhat smaller nominal values through the use of predefined planes for landmark placement, so that only two out of three coordinates could vary. Further refinement of shape matching algorithms such as SPM (Ashburner and Friston, 1999), AIR (Woods et al., 1993) and MNI_AutoReg/ANIMAL (Collins et al., 1994) may therefore not reduce this difference substantially, as it seems due to intersubject differences which are not reducible with these algorithms. It should be noted that the same inexactitude affects voxel-based studies.

We assessed the accuracy of the spatial transformations comparing landmarks determined on the subjects' MRI with those determined on the transformed template. As shown in Figure 3.1.1 (p. 166), the template tends to be slightly larger than the underlying brain. This is due to the way the template was constructed; we did not delineate the depth of intralobar sulci due to their expected positional differences in individual brains. This is likely to have slightly increased the Euclidean distances between landmarks given in Table 3.1.2 (p. 165). When the VOI information from the spatially transformed template is combined with the anatomical information from the MRI to which it has been transformed, e.g. through multiplication of the individualised VOIs with the segmented MRI as in our example, the resulting accuracy will be further improved.

When quantification of brain imaging data in small and specific structures is sought, as for example in the hippocampus, millimetric precision may be required. It is prudent to outline these structures individually and add them onto the individualized template. This approach, however, requires the definition of criteria for the delineation of the anatomical structures in question and the subsequent validation of this protocol (Cook et al., 1992; Niemann et al., 2000a). A combination of speed and accuracy can be obtained by using our template approach for the fast delineation of a large number of regions with satisfactory precision and the individual delineation of small structures on MRI with optimum precision (Hammers et al., 2001c).
3.1.5.4 Characteristics of the regional data obtained

The values of [\(^{11}\text{C}\)] FMZ binding obtained for cortical regions had descriptive characteristics similar to values obtained through traditional VOI analyses (Prevett et al., 1995b). The results obtained for basal ganglia in this study were generally poorer, as reflected by higher CVs. This may partly be due to segmentation into gray and white matter based on one T1 weighted MRI sequence alone; this is particularly relevant for the pallidum. Moreover, even minimal misplacement of the regions contained in the standard template will dramatically increase the variance, as these structures abut cerebrospinal fluid and white matter, with no or only nonspecific [\(^{11}\text{C}\)] FMZ binding. To obviate this problem, the basal ganglia could be individually delineated (Hu et al., 2000). Another possibility would be to develop a PET-to-PET transformation with radioligands yielding a high signal in the basal ganglia, as for example the D2-receptor ligand [\(^{11}\text{C}\)] raclopride. Due to the better statistics with higher counts, this can significantly improve spatial normalisation for the basal ganglia (Meyer et al., 1999).

3.1.5.5 Comparison of regional data obtained from functional datasets in different orientations

Our results indicate that this method can be used for the analysis of brain imaging data acquired in different orientations: The regional values of [\(^{11}\text{C}\)] FMZ-Vd were similar across orientations, although significant differences were observed in six out of 22 anatomical regions (parahippocampal gyrus, fusiform gyrus, posterior cingulate gyrus, caudate nucleus, thalamus and cerebellum). These are most likely due to the fact that our PET scanner produces anisotropic voxel sizes which may lead to different sampling of small and elongated regions like the majority of those for which we found differences (Mazziotta et al., 1981).

We aimed to directly compare regional values obtained and therefore used the absolute values for [\(^{11}\text{C}\)] FMZ-Vd. It is apparent from the mean values that they tended to be higher in the temporal lobe orientation group, reflecting higher global signals. The difference in global signal explains the unidirectionality of the changes observed. As the regional values may be different for different orientations, control and patient data should be acquired in the same orientation.
In summary, in this chapter, we present a region template and a protocol for transforming that template to define anatomical volumes of interest (VOIs). The method described may be used to define anatomical VOIs in individual human brain imaging datasets without operator intervention. It is applicable to brain imaging data of different modalities and/or acquired in different orientations. While generating regional data with characteristics similar to traditional region-based analyses, it is completely observer-independent and faster by a factor of about 90.
3.2 Three-dimensional probabilistic atlas of the human brain in standardised stereotaxic space

3.2.1 Summary

Probabilistic or frequency-based, label-based atlases of neuroanatomy are more representative of population anatomy than single brain atlases. They allow the statistical assessment of normal ranges for structure volumes and extents within the group used for their creation and, ultimately, the comparison of patient groups against this standard. No such manually constructed atlas is currently available for the frequently studied group of young adults.

We investigated 20 normal subjects (10 male, 10 female, median age 31 years) with high resolution MRI scanning. Images were nonuniformity corrected and reorientated along both the anterior-posterior commissure (ACPC) line horizontally and the midsagittal plane sagittally. Building on the work presented in chapter 3.1, we have expanded and refined existing algorithms for the subdivision of MRI datasets into anatomical structures. The resulting algorithm is presented in the appendix to this chapter (chapter 3.2.6). 49 structures were interactively defined as three-dimensional volumes-of-interest (VOIs). The resulting 20 individual atlases were spatially transformed (normalised) into standard stereotaxic space, using standard software (SPM99) and a widely used template (MNI/ICBM 152).

We evaluated volume data for all structures both in native space and after spatial normalisation. We then used the normalised superimposed atlases to create a maximum probability map in stereotactic space which retains quantitative information regarding intersubject variability. Its many potential applications range from the automatic labeling of new scans to the detection of anatomical abnormalities in patients. Further data can be extracted from the atlas for the detailed analysis of individual structures.

3.2.2 Introduction

Tomographic structural and functional neuroimaging is one of the most fruitful techniques for investigating the human brain. Functional imaging parameters (as for example ligand receptor binding, regional blood flow, or spectroscopically measured concentrations of molecules) need to be interpreted in the light of structural imaging data (Duncan and Fish, 1998). For single subjects, correspondence between structure and function can be obtained through coregistration of functional imaging data with high resolution structural imaging, typically magnetic resonance imaging (MRI). There are various coregistration methods available, all of which achieve sub-voxel accuracy [see, for example, (Ashburner and Friston, 1997; Kiebel et al., 1997; Maes et al., 1997; Studholme et al., 1997; van den Elsen et al., 1993; Woods et al., 1993)]. For group studies, this approach is only possible on a subject-by-subject basis, which generally requires time-consuming and observer-dependent region-of-interest analyses. Moreover, for group studies using voxel-based analysis techniques, individual datasets are generally spatially transformed to a common frame of reference, a template in stereotactic space, and statistical tests are applied to these spatially transformed images. The problem of ascribing an anatomical localization to differences thus found between groups is well recognized [see, for example, (Mazziotta et al., 1995)].

Traditional neuroanatomy emphasised systems and their three-dimensional relationships (Lorente de Nó, 1934; Nieuwenhuys et al., 1988; Ramón y Cajal, 1929; Vesalius, 1543). With the advent of Positron Emission Tomography (PET), computed X-ray tomography (CT) and MRI, the emphasis shifted to spatial relationships in the three orthogonal planes, which makes correct labeling of structures noticeably more difficult than when they are viewed three-dimensionally during dissection. Several printed atlases displaying two-dimensional neuroanatomy have been published to aid in correct labeling [for example (Duvernoy, 1991; Jackson and Duncan, 1996; Mai et al., 1997; Roberts and Hanaway, 1970; Schaltenbrand and Wahren, 1977; Talairach et al., 1967; Talairach and Tournoux, 1988). Currently used template coordinates continue to be translated back into "Talairach coordinates" (http://www.mrc-cbu.cam.ac.uk/Imaging/mnispace.html) (Duncan et al., 2000). This printed atlas, together with most others, and many earlier digital atlases [e.g. (Adair et al., 1981; Bajcsy et al., 1983; Bohm et al., 1983; Dann et al., 1989; Evans et al., 1988; Evans et al., 1991; Gee et al., 1993; Greitz et al., 1995)].
1991; Hammers et al., 2002; Miller et al., 1993; Rizzo et al., 1997; Sandor and Leahy, 1997; Seitz et al., 1990; Van Essen and Drury, 1997) share the disadvantage that they are derived from very few brains - typically one - or even, as in the case of the Talairach atlas, from a single hemisphere. Hemispheric asymmetries (Free et al., 2001; Geschwind and Galaburda, 1985; Good et al., 2001; Watkins et al., 2001) are not accounted for when only single hemispheres are used. A wide range of neuroanatomical variation, particularly in phylogenetically or ontogenetically younger structures, is well recognised in primates (Stephan et al., 1988) and will not be taken into account by "oligobrain" atlases.

The recognition of these limitations has led to the development of templates derived from multiple, typically MRJ, datasets (Evans et al., 1992; Evans et al., 1994). They are used principally for spatial transformation purposes. Due to averaging, they do not resolve cortical features well enough to enable anatomical labeling, despite sometimes being called atlases. Related labeled probabilistic maps based on larger numbers of subjects have been developed, but due to the amount of work involved in manual delineation, only automated extraction methods were used (Collins et al., 1999).

Recently, sophisticated gyral pattern matching methods using flow fields in cortical flat maps have been developed (Fischl et al., 1999; Thompson et al., 1997; Thompson et al., 2001). They resolve previously manually outlined gross cortical features well, even after averaging of many subjects. Thus they allow for the comparison of imaging parameters in cortical regions that share a similar relationship to anatomical landmarks, rather than a similar stereotactical location in a three-dimensional coordinate system. An atlas of cortical variability has thus been constructed from MRI data from 20 elderly controls (Thompson et al., 2001) in whom sulci are more easily resolved. This surface-based method does not, however, lend itself to volumetric studies of neuroanatomical structures or the investigation of subcortical structures. No corresponding variability data exists for younger healthy controls.

A further rationale for the development of a probabilistic or frequency-based, label-based atlas of neuroanatomy is to allow the statistical assessment of normal ranges for both structure volumes and spatial extents in native and stereotacthic space, to define a normal range against which patient groups may be compared.
The aims of this study were:

- to refine and expand existing algorithms (Hammers et al., 2002; Niemann et al., 2000a) for the subdivision of MRI datasets into anatomical structures.
- to assess the descriptive statistics of the structures' volumes, both in native and in stereotactic space.
- to create a maximum probability map (probabilistic atlas) in stereotactic space.

### 3.2.3 Methods

#### 3.2.3.1 Subjects

We studied 20 healthy volunteers from the database at the National Society for Epilepsy's MRI Unit. The study group was selected from a cohort of 100 normal controls to contain equal proportions of men and women with age characteristics typically encountered in our imaging research. They had no neurological, medical or psychiatric condition and normal MRI studies as determined by two experienced neuroradiologists. There were 10 women (median/mean age 30.5/31.6 years, range 20-53 years) and 10 men (median/mean age 30.5/31.5 years, range 20-54 years). Two women and three men were determined to be non-right-handed according to a standard questionnaire (Chapman and Chapman, 1987). Ethical approval was obtained from the Joint Medical Ethics Committee of the Institute of Neurology and the National Hospital for Neurology and Neurosurgery, University College London, Queen Square, and all subjects had given written informed consent.

#### 3.2.3.2 MRI acquisition

MRI scans were obtained on the 1.5 Tesla GE Signa Echospeed scanner at the National Society for Epilepsy. A coronal T1 weighted 3D volume was acquired using an inversion recovery prepared fast spoiled gradient recall sequence (GE), TE/TR/NEX 4.2msec (fat and water in phase)/15.5msec/1, time of inversion (TI) 450msec, flip angle 20°, to obtain 124 slices of 1.5mm thickness with a field of view of 18x24cm with a 192x256 matrix. This covers the whole brain with voxel sizes of 0.9375 x 0.9375 x 1.5 mm. Nonuniformity correction was performed using a published method [N3, (Sled et al., 1998)]. The images were then re-orientated with the horizontal line defined by the anterior and posterior commissures (ACPC orientation) and the sagittal planes parallel to the midline as in previous studies.
Images were resliced using windowed sinc interpolation, creating isotropic voxels of 0.9375x0.9375x0.9375mm$^3$. MRI datasets were segmented into gray matter, white matter and CSF using a fully automatic algorithm [Exbrain, (Lemieux, 2001; Lemieux et al., 1999)].

3.2.3.3 Creation of the refined and expanded algorithm for anatomical subdivision
We have previously created a single brain atlas for which a delineation protocol for 39 structures had been developed and tested on five different datasets with voxel sizes of 2x2x2mm$^3$ (Hammers et al., 2002). This delineation algorithm has been expanded to include more structures. It has also been refined to take advantage of the smaller voxel sizes in this study, allowing for more precise anatomical delineation, and improving the definition of some structures. In the creation of the algorithm, we built on our previous work (Hammers et al., 2002; Niemann et al., 2000a) and standard atlases and textbooks of neuroanatomy [e.g. (Duvernoy, 1998; Duvernoy, 1999; Jackson and Duncan, 1996; Kahle, 1986; Mai et al., 1997; Nieuwenhuys et al., 1988)]. The list of all 49 structures and the delineation algorithm can be found in the appendix (chapter 3.2.6).

3.2.3.4 Anatomical subdivision of the datasets
We used Sun Ultra 10 workstations (Sun Microsystems, Mountain View, CA) and Analyze AVW 3.1 (Robb and Hanson, 1991) for the creation of volumes of interest. The region-of-interest-module of the Analyze software allows definition of the borders of structures using a manually controlled cursor. We noted the optimum viewing intensity settings (level and width) for all MRIs, chosen to be comparable among datasets, and applied it every time a given MRI was analyzed. The CSF partition of the segmented MRIs was called up as a related volume to assist in the delineation of the ventricles. Oblique slices, i.e. parallel to the inferior surface of the temporal lobe, were used where appropriate to assist in the delineation of the sulci of the inferior temporal surface. All delineation was performed in native space, i.e. before spatial transformation. All 49 structures were delineated by one investigator on each MRI in turn before the next structure was commenced. After a given structure had been delineated on all 20 MRIs on both the left and right, the structures were reviewed to ensure that there had been no evolution in the interpretation of the protocol. In addition, a separate neuroanatomically trained operator evaluated each structure to ensure that consensus was reached in all difficult cases. Several general
rules applied. For example, when a narrow sulcus was used as a boundary between adjacent structures, this common boundary was drawn along the midline of the sulcus to avoid systematic bias which would favor the apparent volume of the second structure delineated. Where sulci that were more than one voxel wide were used as common boundaries, however, each structure was individually defined up to the pia mater adjoining the sulcus. In delineating larger areas of neocortex, sulci were not followed into their depths.

3.2.3.5 Normalisation of the individual atlases into stereotactic space

The anatomical subdivision of the MRI datasets as described above yielded 20 separate atlases of neuroanatomy in native space, each containing 49 volumes of interest. Within each atlas, each voxel occurring within a volume of interest has a numerical label between 1 and 49, whereas non-brain voxels have a label of zero. The corresponding MRI volumes were spatially normalised to a widely used T1 weighted MRI template in stereotactic space, the Montreal Neurological Institute/International Consortium for Brain Mapping (MNI/ICBM) 152 standard, as contained in the Statistical Parametric Mapping (SPM99) package (Wellcome Department of Imaging Neuroscience, Institute of Neurology, UCL, London, UK, available at http://www.fil.ion.ucl.ac.uk/spm). This template preserves cerebral asymmetries (Evans et al., 1994). Normalisation was performed using the spatial processing routines contained within SPM99, implemented in Matlab version 5 (Mathworks Inc, Sherborn, MA, USA). These represent a mature version of an elastic matching algorithm (Ashburner and Friston, 1997; Ashburner and Friston, 1999). First, an affine linear transformation with 12 parameters (translation, rotation, scaling and shear in each dimension) is performed. This is followed by nonlinear steps utilizing basis functions to accommodate interindividual differences on a smaller scale. The widely used default settings (Ashburner and Friston, 1999; Meyer et al., 1999) of 7x8x7 basis functions (representing x, y and z dimensions in a three-dimensional coordinate system where x increases from left to right, y from posterior to anterior and z from inferior to superior) and 12 iterations were chosen for our study. The spatial transformation module allows for the determination of the necessary warping steps from one image and their application to another one which is in register. As the T1 weighted MRIs from which the individual atlases are derived are known, this can be exploited to transform the individual anatomical atlases. The normalised images were resampled with isotropic voxel sizes of 1x1x1mm3 in a
matrix of x/y/z dimensions of 182/218/182 voxels. We used nearest neighbour interpolation to preserve unequivocal allocation of a given voxel to one VOI. The structures' volumes were automatically extracted both in native and in stereotactic space using Analyze AVW 3.1 (Robb and Hanson, 1991).

3.2.3.6 Creation of the maximum probability map in stereotactic space

The probability of a particular voxel in stereotactic space being occupied by a structure of interest can be ascertained by assessing the frequency of that structure residing at that voxel across the 20 datasets. Each structure delineated is identified by a unique assigned intensity, for example, the right hippocampus was assigned an intensity of one in all datasets, the left, two. We therefore computed the mode for each of the voxels of the normalised individual atlases, revealing the most frequently encountered object at each site and thereby creating a maximum probability map. Where two or more structures occurred with the same frequency at a given voxel, this voxel was randomly assigned one of the corresponding labels (see discussion).

To obtain a visual impression of the improvement through inclusion of more datasets, four maximum probability maps were created, based on 5, 10, 15 and all 20 datasets, respectively.

3.2.4 Results

The final delineation algorithm was successfully applied in all subjects without any alteration.

3.2.4.1 Structure volumes

The results (mean, standard deviation (SD), coefficient of variation - CV, defined as SD/mean) for all structures and the sum of all structures in native space as well as the corresponding values in stereotactic space are shown in Table 3.2.1 (p. 186). As expected, the variation of the total volume of all structures decreases markedly after spatial normalisation (CV from 11% to 2%), whereas the effect of spatial normalisation on the spread of the volumes of the structures is far less marked with an average CV of 18% in native space and 14% in stereotactic space.
3.2.4.2 Maximum probability maps in stereotactic space

There were 37,900 occurrences of multiple modes while calculating the mode for all voxels, compared with a total of 1.9 million brain voxels contained within the normalised regions (2%).

The presentation of the atlas itself is necessarily in the form of a series of representative images. The four maximum probability maps constructed using 5, 10, 15 and all 20 datasets, respectively, are shown in Figure 3.2.1 (p. 182). The smoothness of the boundaries, indicating the precision of the boundary estimate, increases markedly from the first map (five datasets) to the second map (10 datasets) and moderately from the second to the third map (15 datasets), while the improvement from the third to the final map (20 datasets) is subtle. Figure 3.2.1 also shows that easily defined, relatively constant features of human neuroanatomy such as the contours of the brain or the central sulcus appear smooth after inclusion of relatively few datasets, whereas boundaries of structures that are more difficult to define such as the lateral occipital-parietal border only become smooth after inclusion of all 20 datasets. Figure 3.2.2 (p. 183) shows a three-dimensional rendering of the probabilistic atlas revealing some of the internal detail, for example the sylvian aqueduct or the suprachiasmatic recessus of the third ventricle. As another example, Figure 3.2.3 (p. 184) shows the middle genu of the central sulcus and the 'precentral knob' (Yousry et al., 1997) which contains the motor hand area.
<table>
<thead>
<tr>
<th>Region</th>
<th>Original data</th>
<th>Spatially normalised data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (mm³) SD CV</td>
<td>Mean (mm³) SD CV</td>
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<td>R hippocampus</td>
<td>2251 364 .16</td>
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<td>L hippocampus</td>
<td>1996 297 .15</td>
<td>3109 347 .11</td>
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<td>2347 328 .14</td>
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<td>L amygdala</td>
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<td>2572 441 .17</td>
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<tr>
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<td>7465 1343 .18</td>
<td>10735 1912 .18</td>
</tr>
<tr>
<td>L ant medial TL</td>
<td>7148 1113 .16</td>
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<tr>
<td>R ant lateral TL</td>
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<tr>
<td>L ant lateral TL</td>
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<tr>
<td>R PH + ambient gyrus</td>
<td>4775 761 .16</td>
<td>7440 1019 .14</td>
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<tr>
<td>L PH + ambient gyrus</td>
<td>4971 738 .15</td>
<td>7711 768 .10</td>
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<td>R superior temp gyrus</td>
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<td>L superior temp gyrus</td>
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<td>R med + inf temp gyrus</td>
<td>18726 3391 .18</td>
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</tr>
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<td>L med + inf temp gyrus</td>
<td>17522 3106 .18</td>
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<td>7967 1563 .20</td>
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<tr>
<td>L fusiform gyrus</td>
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<td>R insula</td>
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<td>8464 2008 .24</td>
<td>12356 3055 .25</td>
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<td>L caudate nucleus</td>
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<td>R accumbent nucleus</td>
<td>363 97 .27</td>
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<td>L accumbent nucleus</td>
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<td>479 124 .26</td>
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<td>L pallidum</td>
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<td>1995 343 .17</td>
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<td>Corpus callosum</td>
<td>21646 2983 .14</td>
<td>30887 3907 .13</td>
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<td>Body of R lat ventricle</td>
<td>7206 2880 .40</td>
<td>10133 2376 .23</td>
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<tr>
<td>Body of L lat ventricle</td>
<td>7893 2411 .31</td>
<td>11303 2333 .21</td>
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<tr>
<td>R temporal horn</td>
<td>643 128 .20</td>
<td>1018 166 .16</td>
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<td>L temporal horn</td>
<td>559 150 .27</td>
<td>896 198 .22</td>
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<td>Third ventricle</td>
<td>1034 306 .30</td>
<td>1462 366 .25</td>
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<td>Total regional volume</td>
<td>1289661 147973 .11</td>
<td>1886954 37986 .02</td>
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Table 3.2.1: Volumes of the different structures in native space and after normalisation to a stereotactic template. SD = standard deviation; R = right; L = left; CV = coefficient of variation (SD/Mean); ant = anterior, post = posterior; sup = superior; inf = inferior, med = medial, lat = lateral, TL = temporal lobe, PH = parahippocampal.
Figure 3.2.1: Comparison of maximum probability maps obtained after inclusion of five subjects (top left), ten subjects (top right), 15 subjects (bottom left) and all 20 subjects (bottom right). While there is a marked improvement in border definition, indicated through the smoothness of the boundaries, from the five to ten subject version and a moderate further improvement through inclusion of 15 datasets, the difference between the 15 and 20 subject version is minimal.
Figure 3.2.2: Labelled three-dimensional rendering of the probabilistic atlas revealing some of the internal detail.
3.2.5 Discussion

We present the first fully manually constructed, label-based probabilistic atlas of the human brain, constructed for the younger adult age group.

3.2.5.1 Algorithm and anatomical subdivision

The major drawback of any manual labeling method is the strain it puts on human resources. At the resolution used in this study, it took one operator around 30 hours to segment a single MRI dataset. We, therefore, restricted ourselves to the 49 structures presented here. As the individual atlases were created in native space,
there is, however, the possibility of generating new maximum probability maps with further subdivisions (without the major undertaking of recreating all individual atlases) independently of normalisation templates or different warping algorithms. The probabilistic atlas presented here can, therefore, grow with time (Mazziotta et al., 1995). An inclusion of further lobar subdivisions of the frontal, parietal and occipital lobes would be desirable in the future, particularly where anatomical subdivisions correlate with functional specialization.

Non-automatic methods are subject to bias. There is currently no other way, however, to reliably incorporate expert knowledge into the definition of anatomical structures. We have tried to limit subjectivity as far as possible: We have created a detailed, written protocol to which we consistently adhered. Publication of protocols is essential (Bergin et al., 1994) as it allows the comparison of other investigators' results as differences in boundary definition can be assessed and, if necessary, quantified. To avoid bias through different intensity settings, the optimum viewing intensity for all MRIs, chosen to be comparable among datasets, was noted and applied every time a given MRI was analyzed. We measured each structure in turn and reviewed each structure after its delineation was completed on all 20 datasets, to ensure there had been no evolution in the interpretation of the protocol. Difficult structures were noted and a consensus reached between the two main investigators. Segmented images and oblique slices were used where appropriate to ensure optimum reproducibility.

We have not performed formal intra-rater and inter-rater reliability studies. We were, however, able to compare the volumetric results obtained for hippocampus, amygdala and temporal horn with results obtained with the same protocol in a previous study (Niemann et al., 2000a). The volumes for these structures obtained in the current study corresponded well, with the hippocampi being an average of 5.9% and the amygdalae an average of 4.4% larger. This volume increase probably reflects the smaller voxel size used in the current study which minimizes partial volume effects and permits better delineation of the margins of a volume. The right-left-asymmetry of the hippocampus was precisely replicated.

3.2.5.2 Normalisation of the individual atlases into stereotactic space

Both the choice of the target or template which serves to define the stereotactic frame of reference and the choice of software and settings used for the spatial transformations have a direct influence on the results obtained. The MNI152
template was chosen as it is deemed to be representative of normal anatomy through the use of 152 MRI datasets in its construction and preserves asymmetry (Evans et al., 1994). For the spatial transformations, we chose a mature elastic matching protocol incorporating nonlinear transformations as implemented in SPM99, using the widely used default settings and optimizing the process by manually defining the anterior commissure as the starting point for estimation. SPM99 is widely used for spatial transformations, and the current version of the probabilistic atlas can be used in combination. As regions were defined in native space, any future combination of template and spatial transformation software can be used to create further versions.

3.2.5.3 Characteristics of the regional data obtained

The different structures had different variabilities (Table 3.2.1, p. 186). This regional variation has various sources: Firstly, some structures, for example brainstem and cerebellum, are likely to be intrinsically less variable than brain areas that mature later, for example association cortices. Secondly, some structures have more clearly defined boundaries than others. For example, the central sulcus forming the posterior border of the frontal lobe and the outer surface of the brain can be determined with great precision, whereas there is no clear boundary between posterior temporal lobe and occipital/parietal cortex. Thirdly, larger regions tend to have smaller surface-to-volume ratios, allowing less variability through delineation of the surface boundaries. A limitation of the current study is that the various sources for regional variation cannot be precisely distinguished, and we cannot, therefore, comment on the differential interindividually variability of the regions outlined. This would require detailed analysis of much smaller substructures or sulci. Such studies have been performed in several anatomical areas (see below, "Comparison with previous work", chapter 3.2.5.5) but the attempt to investigate this for the whole brain was beyond the scope of this study. Comparison with values obtained previously with the same protocol for some of the structures in this study, however, indicates that our results are both reproducible (see above) and have face validity when compared with ex vivo studies (Niemann et al., 2000a).

3.2.5.4 Creation of the maximum probability map in stereotactic space

In the computation of the mode for each voxel, we used the convention that if two or more values occurred with the same frequency, one of the values would be chosen randomly. Most structures have a small surface/volume ratio, and this convention
was only used in those 2% of voxels for which no unequivocal mode existed. Most of these lie on the outer surface of the brain.

Even with the limited number of subjects included, considerable detail emerges in the maximum probability map, for example the course of the aqueduct (Figure 3.2.2, p. 183), and the position of the middle genu of the central sulcus and the hand knob of the precentral gyrus (Figure 3.2.3, p. 184). Such findings are potentially neurosurgically useful.

3.2.5.5 Comparison with previous work

Collins et al. have described a probabilistic atlas based on a larger number of subjects (Collins et al., 1999). While acknowledging that ideally, manual segmentations of all atlas structures on all subjects should be used, due to time constraints, they used an automatic procedure to segment their datasets into anatomical regions. It would be interesting to compare the maximum probability maps obtained with the two methods to see whether the automatic method yields comparable results in terms of volumes and spatial coordinates. The extension of our work to very large numbers of subjects is hardly feasible, whereas their automatic approach would lend itself to such an extension.

A widely used work of reference has investigated the frequency of occurrence of different sulcal patterns (Ono et al., 1990) based on 25 postmortem brains. While being very useful, this suffers the disadvantages of printed atlases, e.g. difficulty of access and limitation of data shown, and does not investigate the volumes of structures defined by the sulcal boundaries investigated. A variety of studies of small brain regions have been published that investigate volumes, surfaces, sulcal patterns or subcortical structures in more detail [e.g. (Amunts et al., 2000; Andrew and Watkins, 1969; Brierley and Beck, 1959; Chiavaras et al., 2001; Filimonoff, 1932; Geyer et al., 1999b; Geyer et al., 2000; Grefkes et al., 2001; Lohmann et al., 1999; Morosan et al., 2001; Niemann et al., 2000b; Niemann and van Nieuwenhofen, 1999; Paus et al., 1996; Penhune et al., 1996; Rademacher et al., 2001a; Rademacher et al., 1993; Rademacher et al., 2001b; Steinmetz et al., 1990; Thompson et al., 1996; Tomaioulo et al., 1999; Van Buren and MacCubbin, 1962; Westbury et al., 1999; Zilles et al., 1997)]. Such approaches have been shown to be useful for the probabilistic localization of anatomical or functional areas of the brain [e.g. (Fox et al., 2001; Paus et al., 1996; Van Essen et al., 2001)]. Some further studies have investigated volumes of neuroanatomical structures without assessing variability in a
stereotactic reference coordinate system [e.g. (Crespo-Facorro et al., 2000; Kennedy et al., 1998; Lange et al., 1997)].

The development of an atlas incorporating macroscopic in vivo and microscopic in vitro data as well as blood flow activation imaging and other functional information, demographic and genetic information derived from a very large number of subjects by largely automated methods has been pursued since the early 1990s by the International Consortium for Brain Mapping (Mazziotta et al., 1995; Mazziotta et al., 2001). This data collection will be a very useful tool when it becomes available. The work presented here, incorporating manual segmentations of the entire brain, should be regarded as complementary.

A different type of information is being extracted in projects looking at cortical variability using a continuum-mechanical brain template (Thompson et al., 1997; Thompson et al., 2001). This approach achieves exact correspondence of a limited number of previously manually extracted sulci for subsequent measurements of parameters like amount of gray matter at a location defined through its relative distance from the mapped landmarks. In contrast to the approach used here, Thompson et al. achieve "exact" mapping of the sulcal landmarks, with ensuing "crisp" appearance of cortical features even after averaging of many subjects. The approach assumes a one-to-one correspondence, however, and their approach, although generalizable in principle, has so far only been applied to the cortical surface and the hippocampus, with no subcortical or volumetric studies. Again, the approaches should be considered complementary.

3.2.5.6 Future directions

There are several potential applications of the work presented here. First, as the maximum probability map is fully three-dimensional and in register with the ICBM/MNI templates used within SPM99, it can be used to overlay results of group studies, giving probabilistic information based on a sample of 20 healthy controls. This is a significant methodological advance compared to the translation of the coordinates obtained into a stereotaxic coordinate system based on one hemisphere (Talairach and Tournoux, 1988).

Secondly, the maximum probability map can be used as a representative template for VOI studies in native space. The maximum probability map is in register with the ICBM/MNI 152 T1 template and the 152 average. These, or the average of our 20 normalised MRI scans, can, therefore, be transformed to any individual MRI scan.
that may or may not be coregistered to functional data, and the transformation parameters can be applied to the maximum probability map, allowing for the rapid and totally observer-independent segmentation of any given brain imaging dataset into 49 anatomical regions, i.e. their automatic labeling (Hammers et al., 2002).

Thirdly, the stereotactically normalised versions of the individual atlases contain the full probabilistic information for any given voxel. This can be exploited for region-based partial volume correction methods [e.g. (Labbé et al., 1998; Rousset et al., 1995)] in which calculation of absolute parameters of functional imaging data can be based not only on an individual voxel's tissue class probability but also its population-based probabilistic anatomical classification.

A fourth potential application is the use of the probabilistic information obtained here for the detailed analysis of individual structures. By creating "probability shells" corresponding to certain percentile probabilities or by obtaining measures of linear extent or spatial relationship to reference landmarks such as the anterior and posterior commissure, a wealth of information can be obtained on the range of normal anatomy. This information can then be further used for the comparison of patients in whom an alteration of shape or volume of a particular brain structure or region is suspected.

In summary, we present the creation of 20 individual, manually created, label-based atlases of human MRI neuroanatomy. These are used to create a probabilistic atlas of the brain. Current and potential applications include probabilistic anatomical reference for voxel-based studies, automatic labeling of new scans in any modality as long as coregisterable MRI is available, data extraction for the detailed analysis of individual substructures, and the detection of anatomical abnormalities in patients.
3.2.6 Appendix: Delineation Algorithm

The algorithm was developed from a previously defined protocol for the segregation of neuroanatomical structures. Each volume is described in terms of its defining boundaries in each dimension. When this is insufficient, footnotes are used.

Structures 1 and 2: Hippocampus (right; left)\(^1\)
- **Orientation of slices:** Coronal
- **Anterior border:** First slice = most anterior slice where temporal horn loses its slit like appearance, widens and lies next to hippocampus. Include subiculum in measurement anteriorly.
- **Posterior border:** Last slice = slice anterior to that where cella media, temporal horn, and occipital horn fuse. Exclude the fornix on last slice as cannot be separated from the crura fornix.
- **Medial border:** Parahippocampal gyrus; CSF
- **Lateral border:** Anterior -> posterior: Lateral ventricle; WM
- **Superior border:** Anterior -> posterior: Amygdala; lateral ventricle
- **Inferior border:** Parahippocampal gyrus; uncal sulcus; interface of the prosubiculum and cornu ammonis; border between subiculum, and praesubiculum; sulcus hippocampalis
- **Number of slices:** Approximately 25

Structures 3 and 4: Amygdala (right; left)
- **Orientation of slices:** Coronal
- **Anterior border:** End of clear distinction between nucleus corticalis and adjacent cortex
- **Posterior border:** End of amygdala (at this level dorsolaterally to digitatio verticalis hippocampi, in dorsal ventricular wall)
- **Medial border:** Anterior -> posterior: Cisterna chiasmatis and ambiens; previously outlined parts of hippocampus
- **Lateral border:** WM
- **Superior border:** Sulcus endorhinalis
- **Inferior border:** Anterior -> posterior: WM; ventricle / hippocampus
- **Number of slices:** Approximately 15

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\(^1\) See Niemann K, Hammers A, Coenen VA, Thron A, Klosterkötter J. Evidence for smaller left hippocampus and left temporal horn in both patients with first episode schizophrenia and normal controls. Psychiatry Res Neuroimaging 2000a; 99: 93-110. In the coronal orientation, the hippocampus has four distinct shapes when progressing posteriorly. The first is 'boomerang-like'. Three rules were applied to differentiate the hippocampal head from the amygdala in these anterior slices exhibiting shape one: i. the dorso-medial corner of the temporal horn of the lateral ventricle indicated the position of the frontal cleft at the interface of the amygdala and hippocampus; ii. the myelin layer of the alveus of the hippocampus was used as a differentiator; iii. when rules i and ii were insufficient, a region of low signal could frequently be seen defining the border. This has been attributed to either partial volume effect due to a narrow cleft, or small vessels between the two structures. Shape two has been likened to a rabbit with its 'head' medially, and the digitatio verticalis as its 'ears'. When this shape was evident, the uncal sulcus was used as the inferior border. At the lateral end of this sulcus, a line was drawn at 45° to the horizontal in a basolateral direction to approximate the interface of the prosubiculum and cornu ammonis. The third shape of the hippocampus has been compared with binoculars. If the uncal sulcus was no longer visible this far posteriorly, the border between the hypointense subiculum, and the hyperintense praesubiculum was used as the inferior border. The fourth shape is the first through the hippocampal body; at this point both the subiculum and fimbria were included in the measurement, and the hippocampal, not uncal, sulcus was used as the inferior border.
Structures 5 and 6: Anterior temporal lobe, medial part (right; left)\(^2\)
- **Orientation of slices:** Coronal
- **Anterior border:** Temporal pole
- **Posterior border:** First slice = slice anterior to the anterior end of amygdala
- **Medial border:** CSF (cisterna valleculae cerebri -> cisterna ambiens)
- **Lateral border:** Lateral part of anterior temporal lobe
- **Superior border:** CSF; posteriorly, eventually temporal stem (then draw a straight line between most superior lateral & medial border)
- **Inferior border:** CSF
- **Number of slices:** Approximately 25

Structures 7 and 8: Anterior temporal lobe, lateral part (right; left)\(^2\)
- **Orientation of slices:** Coronal
- **Anterior border:** Temporal pole
- **Posterior border:** First slice = slice anterior to the anterior end of amygdala
- **Medial border:** Medial part of anterior temporal lobe
- **Lateral border:** CSF
- **Superior border:** CSF; posteriorly, eventually temporal stem (then draw a straight line between most superior lateral & medial border)
- **Inferior border:** CSF
- **Number of slices:** Approximately 25

Structures 9 and 10: Parahippocampal and ambient gyri (right; left)\(^3\)
- **Orientation of slices:** Coronal
- **Anterior border:** Anterior end of amygdala as previously defined (that slice included)
- **Posterior border:** Posterior border of hippocampus as previously defined (that slice included)
- **Medial border:** Cisterna ambiens
- **Lateral border:** Anterior -> posterior: Sulcus collateralis (not sulcus rhinalis) and superiorly towards amygdala previously defined / most medial extent of temporal horn of lateral ventricle
- **Superior border:** Upper limit of structure remaining after hippocampus and amygdala have been cut out
- **Inferior border:** Cisterna ambiens / sulcus collateralis
- **Number of slices:** Approximately 25

Structures 11 and 12: Superior temporal gyrus (right; left)\(^4\)
- **Orientation of slices:** Coronal
- **Anterior border:** First slice = most anterior slice where amygdala are measured
- **Posterior border:** Last slice = most posterior slice where hippocampus is measured
- **Medial border:** Draw a line radially to the most lateral extent of the inferior horn of the lateral ventricle or, if the ventricle is not discernable, to the most lateral extent of the

\(^2\) The border dividing the medial and lateral portions of the anterior temporal lobe was defined in the original protocol as the sulcus temporalis inferior. The nature of this sulcus was highly variable; it was not present throughout the anterior portion of every temporal lobe analysed. Therefore, it was not possible to rely on this sulcus as a consistent feature for division of the two portions. This observation is supported by Ono et al who reported that the sulcus separates in 16% and 52% of anterior temporal lobes on the right and left respectively, and is absent at the tip of 4% of temporal lobes Ono M, Kubik S, Abernathey CD. Atlas of the cerebral sulci. Stuttgart New York: Georg Thieme Verlag, 1990. The division of the two portions of the anterior lobe was redefined using a ‘radial divider’ tool within the Analyze software. This divides a described region into a predetermined number of segments (in this case two) as radiations from a point defined by the centre of the area of the region. Thus it was possible to provide a consistent definition of the two portions of the temporal lobe.

\(^3\) The course of the sulcus collateralis can be very variable, and may frequently be interrupted and/or duplicated as one progresses from its anterior to posterior extent Ibid.. Consequently, in many of the datasets the path of this sulcus became impossible to determine for a few slices. In such cases, the protocol was refined to include the use of the transverse orientation to extrapolate a projected course over the slices in question.

\(^4\) The use of the sagittal orientation facilitated more accurate determination of the sulci that bound the superior temporal gyrus. Similarly, the transverse orientation was used with the middle and inferior temporal gyri.

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hippocampus (amygdala anteriorly). Line comes from most inferior end of sulcus circularis insulae and most medial end of sulcus temporalis superior, respectively.

- **Lateral border**: CSF
- **Superior border**: Sulcus lateralis, thus including the planum temporale in the structure's posterior portion
- **Inferior border**: Sulcus temporalis superior
- **Number of slices**: Approximately 25

**Structures 13 and 14: Middle and inferior temporal gyri (right; left)**
- **Orientation of slices**: Coronal
- **Anterior border**: First slice = most anterior slice where amygdala are measured
- **Posterior border**: Last slice = most posterior slice where hippocampus is measured
- **Medial border**: Sulcus occipitotemporalis; from superior end draw a line radially to the most lateral extent of the inferior horn of the lateral ventricle or, if the ventricle is not discernable, to the most lateral extent of the hippocampus (amygdala anteriorly).
- **Lateral border**: CSF
- **Superior border**: Sulcus temporalis superior; from medial end draw a line radially to the most lateral extent of the inferior horn of the lateral ventricle or, if the ventricle is not discernable, to the most lateral extent of the hippocampus (amygdala anteriorly).
- **Inferior border**: CSF
- **Number of Slices**: Approximately 25

**Structures 15 and 16: Lateral occipitotemporal gyrus (fusiform gyrus) (right; left)**
- **Orientation of slices**: Coronal
- **Anterior border**: First slice = most anterior slice where amygdala are measured
- **Posterior border**: Last slice = most posterior slice where hippocampus is measured
- **Medial border**: Sulcus occipitotemporalis
- **Lateral border**: Sulcus collateralis
- **Superior border**: From superior ends of sulcus occipitotemporalis and sulcus collateralis draw a line radially to the most lateral extent of the inferior horn of the lateral ventricle or, if the ventricle is not discernable, to the most lateral extent of the hippocampus (amygdala anteriorly).
- **Inferior border**: CSF
- **Number of slices**: Approximately 25

**Structures 17 and 18: Cerebellum (right; left)**
- **Orientation of slices**: Sagittal
- **Anterior border**: Cut cerebellar peduncle parallel to floor of IVth ventricle beginning on the slice where the cerebellar peduncle joins the brainstem (pons)
- **Posterior border**: CSF
- **Medial border**: Midline
- **Lateral border**: CSF / sinus transversus (lateral sinus)
- **Superior border**: CSF / tentorium cerebelli
- **Inferior border**: CSF
- **Number of slices**: Approximately 55

**Structure 19: Brainstem (spans the midline)**
- **Orientation of slices**: Sagittal
- **Anterior border**: CSF
- **Posterior border**: CSF; cut from cerebellum as described under "Cerebellum"
- **Medial border**: No medial border; spans the midline
- **Lateral border**: CSF - pons/midbrain: as soon as the cerebellar peduncle is no longer in contact with the pons, the posterior remainder is measured together with the cerebellum; the superior remainder is measured with the basal ganglia
- **Superior border**: Cut from basal ganglia as soon as pedunculus cerebri enters them using a tangential line following the contours of the basal ganglia
- **Inferior border**: Inferior border of cerebellum
- **Number of slices**: Approximately 30

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5 The anterior border thus defined corresponds to the Spielmeyer cut
Structures 20 and 21: Insula (left; right)
- **Cave:** left, then right
- **Orientation of slices:** Coronal
- **Anterior border:** Last slice on which sulcus circularis insulae is visualized
- **Posterior border:** Last slice on which sulcus circularis insulae is visualized
- **Medial border:** Lateral border of putamen (draw a line from medial end of sulcus circularis insulae); if no longer visible use anterior-lateral border of caput nuclei caudati or lateral border of lateral ventricle, respectively; posteriorly use lateral border of thalamus instead
  - **Lateral border:** CSF in sulcus lateralis
  - **Superior border:** Sulcus circularis insulae
  - **Inferior border:** Sulcus circularis insulae
  - **Number of slices:** Approximately 70

Structures 22 and 23: Occipital lobe (left; right)
- **Orientation of slices:** First sagittal, then transverse
  - **Sagittal cuts (start medially):**
    - **Anterior border:** Sulcus parieto-occipitalis
    - **Posterior border:** CSF
    - **Medial border:** Midline
    - **Lateral border:** Last slice on which sulcus parieto-occipitalis is visible in its full length (then change to transverse cuts)
  - **Superior border:** Sulcus parieto-occipitalis and CSF
  - **Inferior border:** Tentorium cerebelli / CSF
  - **Transverse cuts:**
    - **Anterior border:** Straight line between medial end of sulcus occipitalis anterior, and lateral end of sulcus parieto-occipitalis
    - **Posterior border:** CSF
    - **Medial border:** As previously defined on sagittal cuts
    - **Lateral border:** CSF
  - **Superior border:** Parietal lobe (sulcus occipitalis anterior)
  - **Inferior border:** CSF / tentorium cerebelli
  - **Number of slices:** Approximately 85

Structures 24 and 25: Gyrus cinguli, anterior part (left; right)
- **Orientation of slices:** Transverse, then sagittal, then return to transverse
  - **Transverse cut**
    - **Inferior border:** Define on the most inferior slice on which genu corporis callosi is uninterrupted throughout its width. See Figure 3.2.4, p. 198.
  - **Sagittal Cuts**
    - **Anterior border:** Sulcus cinguli
    - **Posterior border:** Draw vertical line from corpus callosum to sulcus cinguli at the midpoint of the greatest extension of the corpus callosum (measured using coordinates of region of interest module of Analyze AVW) on most medial slice; corpus callosum inferiorly
    - **Medial border:** Midline
    - **Lateral border:** Last slice on which sulcus cinguli is visible in its full length (then change to transverse cuts)
  - **Superior border:** Sulcus cinguli
  - **Inferior Border:** As defined in transverse orientation
  - **Transverse Cuts**
    - **Lateral border:** Re-defined as straight line posteriorly from anterior-lateral end of sulcus cinguli on superior slices.
    - **Anterior border; posterior border; medial border; superior border; inferior border:** As previously defined on sagittal cuts
  - **Number of slices:** Approximately 50

Structures 26 and 27: Gyrus cinguli, posterior part (left; right)
- **Orientation of slices:** Transverse, then sagittal, then return to transverse
  - **Transverse cut**
  - **Inferior border:** Define on the most inferior slice on which splenium corporis callosi is uninterrupted throughout its width. See Figure 3.2.5, p. 198.
  - **Sagittal cuts (see next page)**

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• **Anterior border:** Gyrus cinguli, anterior part
• **Posterior border:** Sulcus subparietalis; inferiorly, sulcus parieto-occipitalis
• **Medial border:** Midline
• **Lateral border:** Last slice on which sulcus cinguli is visible in its full length (then change to transverse cuts)
• **Superior border:** sulcus cinguli
• **Inferior Border:** As defined in transverse orientation
  
  **Transverse Cuts**
• **Lateral border:** Re-defined as straight line anteriorly from posterior-lateral end of sulcus cinguli on superior slices.
• **Anterior border; posterior border; medial border; superior border; inferior border:** As previously defined on sagittal cuts
• **Number of slices:** Approximately 50

Structures 28 and 29: Frontal lobe (left; right)
• **Cave:** Start superiorly to reliably identify central sulcus
• **Orientation of slices:** Transverse
• **Anterior border:** CSF
• **Posterior border:** superior -> inferior: Sulcus centralis -> line orthogonal to midline from medial end of sulcus centralis to interhemispheric fissure / gyrus cinguli / corpus callosum / lateral ventricle / striatum / insula -> CSF in most inferior portion.
• **Medial border:** superior -> inferior: Interhemispheric fissure -> gyri cinguli -> corpus callosum -> lateral border of lateral ventricle -> lateral border of striatum / insula -> interhemispheric fissure
• **Lateral border:** CSF
• **Superior border:** CSF
• **Inferior border:** CSF
• **Number of slices:** Approximately 105

Structures 30 and 31: Posterior temporal lobe (left; right)
• **Orientation of slices:** Transverse
• **Anterior border:** Straight horizontal line marking the last coronal cut of the temporal lobe (see structures 8-11); include temporal operculum up to superior border (see below)
• **Posterior border:** Cerebellum and occipital lobe as previously defined
• **Medial border:** Cerebellum and occipital lobe; cisterna ambiens; cisterna venae cerebri magna; splenium of corpus callosum; lateral ventricle; midline
• **Lateral border:** CSF
• **Superior border:** Last slice on which the posterior border(s) of any of structures 9-16 occupied the majority (greater than 50%) of the space between CSF laterally, and non-temporal lobe structures medially.
• **Inferior border:** CSF
• **Number of slices:** Approximately 45

Structures 32 and 33: Parietal lobe (left; right)
• **Cave:** The parietal operculum, and praecuneus are included in the definition of the parietal lobe

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6 In the more inferior slices where the splenium was absent or non-continuous, the lateral ventricle was included in the definition of the posterior temporal lobe on the medial side. However, in the slices where the splenium was continuous (see Figure B), the lateral ventricle and splenium were excluded.

7 The border with the corpus callosum was defined through a line between the occipital horn of the lateral ventricle and the gyrus cinguli. This line crosses the corpus callosum as it becomes confluent with the WM of the parietal lobe. The posterior border of the insula was defined as the last slice containing sulcus circularis insulae. Sometimes the insula did not extend as far posteriorly as the structures of the mid-part of the temporal lobe (structures 9-16) (see Figure 3.2.6, p. 199). In these circumstances an undefined WM region exists. Superiorly, this area is bounded by, and was included in, the parietal lobe. More inferiorly, however, the region in question was only included in the definition of the parietal lobe when it was continuous with other areas labelled parietal lobe. The coronal orientation was used to ensure the validity of this rule, and revealed that the cut-off point represented the border with the most superior aspects of the temporal lobe (see Figure 3.2.7, p. 199).
• Orientation of slices: transverse
• Anterior border: Previously defined structures
• Posterior border: Previously defined structures; CSF
• Medial border: Midline; previously defined structures; corpus callosum; ventricle; basal ganglia
• Lateral border: CSF
• Superior border: CSF
• Inferior border: Previously defined structures
• Number of slices: Approximately 60

Structures 34 and 35: Caudate nucleus (left, right)
• Cave: This protocol includes the Nucleus accumbens which subsequently will be redefine as a separate structure (see below).
• Orientation of slices: transverse
• Anterior border: superior to inferior: frontal lobe and/or corpus callosum, then lateral ventricle, corpus callosum, frontal lobe
• Posterior border: superior to inferior: lateral ventricle, internal capsule / anterior commissure
• Medial border: superior to inferior: lateral ventricle / corpus callosum, frontal lobe as previously defined, following the intensity gradient of the caudate avoiding the medial gray matter adjacent to the CSF
• Lateral border: superior to inferior: frontal/parietal lobe as previously defined, internal capsule, internal capsule/insula
• Superior border: Start on first slice where on which caudate is visible at the lateral border of the lateral ventricle
• Inferior border: Retaining the medial border, continue defining the caudate until frontal lobe as defined previously is reached. This border is subsequently edited in coronal orientation when defining the accumbens as a substructure of the caudate region as outlined here (see below, structures 36 and 37).
• Number of slices: Approximately 30

Structures 36 and 37: Nucleus accumbens (left, right)
• Cave: This protocol requires the prior definition of accumbens and caudate together on transverse slices as outlined above (see structures 34 and 35). The new regions need to be renumbered.
• Orientation of slices: coronal; start posteriorly.
• Anterior border: Last slice where accumbens can be clearly differentiated from the caudate.
• Posterior border: First slice where the inferior part of the previously defined caudate region is seen (e.g. inferior of the anterior commissure), separated from the superior bulk of the caudate region. This separated section represents the accumbens.
• Medial border: as before, do not change.
• Lateral border: as before, do not change.
• Superior border: Nucleus accumbens is always inferior to the lateral ventricle. Anterior to where the separated parts of the previously defined caudate region merge, the superior border is defined through its shape and a slightly more hypointense appearance than the caudate itself.
• Inferior border: Smooth previously defined border and exclude white matter and the medial gray matter adjacent to the CSF, impinge on previously defined frontal lobe region if necessary.
• Number of slices: Approximately 8

Structures 38 and 39: Putamen (left, right)
• Orientation of slices: transverse
• Anterior border: frontal lobe, internal capsule, insula in varying combinations as previously defined
• Posterior border: internal capsule
• Medial border: superior to inferior: Internal capsule, Lamina medullaris lateralis, substantia perforata anterior
• Lateral border: superior to inferior: frontal lobe/parietal lobe, insula
• Superior border: Most superior slice where putamen is seen
• Inferior border: Frontal lobe. The coronal orientation can be useful to verify the borders.
Number of slices: Approximately 25

Structures 40 and 41: Thalamus (left, right)
- Cave: start posteriorly
- Orientation of slices: coronal
- Anterior border: End of anterior thalamic nucleus at foramen Monrovi
- Posterior border: First slice where pulvinar is visible
- Medial border: posterior to anterior: Cisterna ambiens/laminae tecti, corpus callosum, third ventricle/midline at adhaesio interthalamica
- Lateral border: posterior to anterior: posterior temporal lobe white matter, insula as previously defined, internal capsule
- Superior border: posterior to anterior: white matter/ corpus callosum, lateral ventricle, stria terminalis/vena thalamostriata
- Inferior border: posterior to anterior: cisterna ambiens, temporal lobe as previously defined (include both medial and lateral geniculate body, adjust temporal lobe regions where necessary)
- Number of slices: Approximately 30

Structures 42 and 43: Pallidum (left, right)
- Cave: Do not include lamina medullaris lateralis
- Orientation of slices: coronal
- Anterior border: First slice where visible (pars lateralis, within internal capsule)
- Posterior border: Last slice where visible
- Medial border: Internal capsule
- Lateral border: lamina medullaris lateralis/putamen
- Superior border: Internal capsule
- Inferior border: anterior to posterior: White matter of subcallosal gyrus - anterior commissure - white matter superior to anterior perforated substance/amygdala/hippocampus
- Number of slices: Approximately 20

Structure 44: Corpus callosum
- Cave: Do not include fornix
- Orientation of slices: transverse
- Anterior border, anterior part: superior to inferior: cingulate gyrus and frontal lobe -> frontal lobe
- Anterior border, posterior part: superior to inferior: lateral ventricle, fornix, cisterna fissurae transversae cerebri -> idem thalamus
- Posterior border, anterior part: superior to inferior: lateral ventricle -> caudate/nucleus accumbens
- Posterior border, posterior part: superior to inferior: posterior cingulate gyrus and parietal lobe -> idem and interhemispheric CSF
- Medial border: superior to inferior: cingulate gyri as soon as the corpus callosum appears X-shaped -> frontal/parietal lobe -> idem and posterior temporal lobes -> head of caudate anteriorly
- Lateral border: superior to inferior: frontal and parietal lobes -> idem and lateral ventricle
- Superior border: Defined through remainder after delineation of cingulate gyri and frontal and parietal lobes.
- Inferior border: anteriorly, last slice on which the corpus callosum can be clearly distinguished; posteriorly, inferior end of splenium
- Number of slices: Approximately 35

Structures 45 and 46: Lateral ventricle, frontal horn, central part and occipital horn (right, left)
- Cave: right, then left
- Cave: Use CSF partition of segmented MRI as related volume to aid in delineation, use autotrace function for posterior part inferiorly after CC disappears.
- Orientation of slices: transverse
- Anterior border, anterior part: CC
- Anterior border, posterior part: superior to inferior: thalamus -> unnamed region behind thalamus/posterior of insula -> posterior temporal lobe -> anterior border of posterior temporal lobe region (see structures 47 and 48)
- Posterior border, anterior part: superior to inferior: thalamus ->
thalamus/fornix/capsula interna, caput nuclei caudati

- **Posterior border, posterior part**: superior to inferior: parietal lobe -> corpus callosum -> parietal lobe/posterior temporal lobe/occipital lobe
- **Medial border, anterior part**: superior to inferior: corpus callosum -> septum pellucidum / fornix -> basal forebrain
- **Medial border, posterior part**: superior to inferior: corpus callosum -> medial parietal lobe / posterior temporal lobe
- **Lateral border, anterior part**: superior to inferior: frontal and parietal lobes -> plus corpus nuclei caudati -> caput nuclei caudati -> plus frontal lobe
- **Lateral border, posterior part**: superior to inferior: frontal and parietal lobes -> plus corpus nuclei caudati -> posterior temporal lobe

- **Superior border**: First slice where ventricle visible, include part with partial volume effect
- **Inferior border, anterior part**: End of CSF in frontal lobe
- **Inferior border, posterior part**: End of CSF in posterior temporal lobe that lies posterior to the anterior border of the posterior temporal lobe region

- **Number of slices**: Approximately 35

**Structures 47 and 48**: Lateral ventricle, temporal horn (right, left)

- **Cave**: may only be intermittently visible
- **Cave**: Use CSF partition of segmented MRI as related volume to aid in delineation
- **Orientation of slices**: coronal
- **Anterior border**: first appearance of CSF
- **Posterior border**: Last slice on which hippocampus is still delineated = last slice anterior to posterior temporal regions (see structure 45 and 46)
- **Medial border**: anterior to posterior: parahippocampal gyrus / hippocampus/amygdala -> hippocampus/choroid fissure -> fimbria/crus fornix
- **Lateral border**: anterior to posterior: parahippocampal gyrus or temporal lobe white matter
- **Superior border**: anterior to posterior: amygdala -> temporal white matter (temporal stem)
- **Inferior border**: anterior to posterior: temporal lobe white matter -> plus hippocampus
- **Number of slices**: Approximately 25

**Structures 49**: Third ventricle

- **Cave**: use sagittal for help with posterior extent first
- **Cave**: Use CSF partition of segmented MRI as related volume to aid in delineation
- **Orientation of slices**: coronal
- **Anterior border**: Lamina terminalis
- **Posterior border**: pineal gland, include recessus pinealis/suprapinealis
- **Medial border**: none (single structure)
- **Lateral border**: anterior to posterior: hypothalamus -> thalamus -> nuclei habenularum
- **Superior border**: anterior to posterior: lamina terminalis -> anterior commissure/columna fornicis -> foramen Monroi -> tela choroidea (cave does not exceed height of adhaesio interthalamica posterior to it; do not confound internal cerebral veins superior of tela choroidea)
- **Inferior border**: anterior to posterior: chiasma opticum -> infundibulum -> hypothalamus -> posterior commissure
- **Number of slices**: Approximately 35

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Figure 3.2.4: The genu of the corpus callosum: top, continuous throughout its entire width; bottom, divided in midline (see algorithm for structures 24 & 25, p. 193)

Figure 3.2.5: The splenium of the corpus callosum: left, continuous throughout its entire width; right, interrupted (see algorithm for structures 26 & 27, p. 193, and footnote 6)
Figure 3.2.6: Posterior extent of the insula compared with the structures of the mid-part of the temporal lobe. In some datasets, the insula did not extend as far posteriorly these structures (circled) (see structures 32 & 33, p. 194, and footnote 7)

Figure 3.2.7: Validation of the inferior border of the parietal lobe (circled) (see structures 32 & 33, p. 194, and footnote 7)
3.3 Neocortical abnormalities of $[^{11}\text{C}]$-flumazenil-PET in mesial temporal lobe epilepsy

3.3.1 Summary
We used $[^{11}\text{C}]$ flumazenil (FMZ) PET and complementary voxel-based and quantitative volume-of-interest (VOI) methods to characterize abnormalities in neocortical GABA$_A$ receptor binding in mesial temporal lobe epilepsy (mTLE) patients with unilateral hippocampal sclerosis (HS). We studied thirteen controls and 15 patients with refractory mTLE and unilateral HS with $[^{11}\text{C}]$ FMZ PET. Data were corrected for partial volume effect in the interactively outlined hippocampus and in 28 cortical VOIs using an individualised template. A voxel-based analysis was also performed, using statistical parametric mapping (SPM96).
Fourteen mTLE patients had reduced $[^{11}\text{C}]$ FMZ-Vd in the hippocampus ipsilateral to the EEG focus, extending into the amygdala in four. Five patients showed additional significant neocortical abnormalities of $[^{11}\text{C}]$ FMZ binding: temporal neocortical increases (1), extratemporal decreases (2), extratemporal increases only (1) and temporal and extra-temporal neocortical increases (1). Group VOI analysis revealed significant reductions only in the ipsilateral hippocampus. SPM showed decreased $[^{11}\text{C}]$ FMZ-Vd in the ipsilateral hippocampus in 13 out of 15 patients, extending into the amygdala in eight. Five patients showed additional neocortical abnormalities: temporal neocortical increases only (3), extratemporal decreases (1) or both temporal neocortical and extratemporal decreases (1). Group analysis showed significant reductions in the ipsilateral hippocampus only.
A combination of VOI- and voxel-based analysis of $[^{11}\text{C}]$ FMZ PET detected extrahippocampal changes of GABA$_A$ receptor binding in eight of 15 patients with mTLE due to HS. The finding of abnormalities in patients thought to have unilateral HS only based on MRI suggests more widespread abnormalities being present in HS.

3.3.2 Introduction

Hippocampal sclerosis (HS) is present in 60% of patients with temporal lobe epilepsy (TLE) referred for epilepsy surgery (Babb et al., 1984). After anterior temporal lobe resection, one third of patients with HS continue to have seizures (Berkovic et al., 1995). High quality MRI with measurement of hippocampal volumes (HCV) and hippocampal T2 relaxation times (HCT2) (Jackson et al., 1990; Jackson et al., 1993a; Van Paesschen et al., 1997) reliably detects HS in vivo.

\(\gamma\)-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the brain, acting at the GABA\(_A\) receptor. Flumazenil (FMZ) is a specific, reversibly bound high affinity neutral antagonist (Olsen et al., 1990) at the benzodiazepine binding site of the GABA\(_A\) receptor. \(^{[11]}\text{C}\) FMZ PET provides a useful in vivo marker of GABA\(_A\) receptor binding (Maziere et al., 1984).

In TLE, \(^{[18]}\text{F}\)-fluorodeoxyglucose (FDG) PET studies have revealed a widespread zone of interictal glucose hypometabolism in the region of the epileptogenic focus and surrounds (Arnold et al., 1996; Henry et al., 1993a; Savic et al., 1993) while changes in \(^{[11]}\text{C}\) FMZ binding have been reported to be more restricted (Henry et al., 1993a; Juhász et al., 1999b; Savic et al., 1993; Szelies et al., 1996). Using \(^{[11]}\text{C}\) FMZ PET with statistical parametric mapping (SPM95), we previously reported that a group of patients with unilateral mesial temporal lobe epilepsy due to HS had reductions of GABA\(_A\) receptor binding restricted to the hippocampus (Koepp et al., 1996).

In a similar population, we also demonstrated the importance of performing partial volume effect correction when \(^{[11]}\text{C}\) FMZ binding is being quantified. Partial volume effect arises due to the limited spatial resolution of PET and particularly affects the quantification of signals in structures smaller than twice the full width at half maximum (FWHM) resolution of the scanner used (Hoffman et al., 1979), such as the hippocampus and the cortical ribbon, due to overspill of adjacent radioactivity. Correction for partial volume effect is particularly important when structural abnormalities may be present. The use of partial volume correction increased the sensitivity of \(^{[11]}\text{C}\) FMZ PET for detecting functional abnormalities in both the sclerotic and the contralateral hippocampus and was necessary for absolute quantification of binding changes (Koepp et al., 1997a; Koepp et al., 1997c). Additionally, it demonstrated that GABA\(_A\) receptor binding was reduced over and above hippocampal volume loss (Koepp et al., 1997c).
In patients with TLE, there may also be extratemporal atrophy (DeCarli et al., 1998) and malformations of cortical development not visible on high quality MRI (Desbiens et al., 1993; Kuzniecky et al., 1991). Neocortical changes of GABA_A receptor binding in mTLE with HS have not previously been assessed quantitatively. The aims of the current study were:

1. To determine if there are neocortical changes in GABA_A receptor binding in patients with HS but no extra-temporal structural changes on high resolution MRI, using a template of cortical VOIs and correcting for partial volume effect.
2. To compare the results of the quantitative VOI approach with the complementary voxel-based method of SPM.
3. To correlate these findings with clinical variables.

### 3.3.3 Methods

#### 3.3.3.1 Patients and controls

We studied 15 patients (11 women) with refractory mTLE (Commission on Classification and Terminology of the International League against Epilepsy, 1989). They were recruited as described in Chapter 2.1 (p. 144). The median age at onset of habitual seizures was 7 years (range: 1 - 23 years), the median duration of epilepsy before the PET examination was 24 years (range: 12 - 45 years), and the median age at PET examination was 31 years (range: 19 - 49 years). Eleven of the 15 patients had a history of complex febrile convulsions in early childhood. Their antiepileptic medication was mono- or dual-therapy with carbamazepine (12 patients), lamotrigine (6), gabapentin (5), phenytoin (3), topiramate (1) or sodium valproate (1) (Table 3.3.1, p. 204).

All patients had a clear MRI diagnosis of unilateral HS according to accepted qualitative and quantitative MRI criteria (Jackson et al., 1993a; Van Paesschen et al., 1997) with unilaterally reduced hippocampal volumes (HCVs) corrected for intracranial volume (ICV), abnormal HCV asymmetry indices (HCV-Al), and increased hippocampal T2 times (HCT2). One patient (# 2) had a marginally increased T2 relaxation time in the contralateral hippocampus as well as a marked increase in the ipsilateral hippocampus; he is now seizure free 18 months after surgery. One patient (# 9) had temporal lobe atrophy ipsilateral to the EEG focus on MRI.

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Thirteen healthy volunteers (two women) were studied for comparison. The median age at examination was 32 years (range: 23 - 64 years). They had no history of neurologic or psychiatric disorder, were on no medication and had normal MRI studies.

Voxel-based data has previously been reported in six of the patients (patients 1-6) (Koepp et al., 1996); region-based hippocampal data in 12 of the patients (Koepp et al., 1998a; Koepp et al., 1997a) and in the 13 healthy volunteers.

3.3.3.2 Electroencephalography (EEG)

All 15 patients had prolonged video-EEG recordings with scalp and surface sphenoidal electrodes. Scalp EEGs showed clear, anterior temporal interictal epileptiform activity consisting of sharp and slow wave components in all patients. Seizures were recorded in all patients. Unilateral anterior temporal ictal EEG changes, at or before clinical seizure onset, were recorded in all cases, ipsilateral to the side of the HS. One patient (# 2) had bilateral independent temporal interictal epileptiform activity with a left-sided preponderance (left/right ratio 2:1). Subsequent depth EEG recordings revealed the seizure onset consistently in the sclerotic right hippocampus. Three other patients (# 1, 7, and 12) had bilateral temporal interictal epileptiform activity with a lateralized preponderance and unilateral ictal onset (see Table 3.3.1, p. 204).

3.3.3.3 Epilepsy surgery, histopathology, and surgical outcome

The MRI finding of HS was histologically verified in the excised mesial temporal structures (hippocampus, part of the amygdala, and anterior 3 cm of lateral temporal neocortex) in all patients. Histopathologically the presence of neurons in the white matter of the resected anterior temporal lobe was graded semiquantitatively as few neurons (unlikely microdysgenesis), a moderate amount of neurons (possible microdysgenesis) and many neurons (microdysgenesis) (see Table 3.3.1, p. 204). Median postsurgical follow-up was 3 years (range, 1.5-4 years), and outcome according to the classification of Engel (Engel, 1987) was class IA in nine patients, class IB (occasional auras only) in four patients (3, 9, 13, 15), class IIA (initially seizure-free, one seizure after 3.5 years) in one patient (6) and class IID (nocturnal seizures only) in one patient (12) (Table 3.3.1, p. 204).
<table>
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<th>Patient No.</th>
<th>Age (yr)/Gender</th>
<th>Onset/Duration (yr)</th>
<th>PFC</th>
<th>CPS/yr</th>
<th>Interval CPS → PET (days)</th>
<th>AEDs</th>
<th>EEG (Inter-ictal; Ictal)</th>
<th>MRI Follow-up (years)</th>
<th>WM Neurons</th>
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<td>R HS</td>
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<td>++</td>
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<td>++</td>
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<td>++</td>
</tr>
<tr>
<td>13</td>
<td>31/w</td>
<td>7/24</td>
<td>yes</td>
<td>24</td>
<td>3</td>
<td>G, P</td>
<td>R temp; R temp ant</td>
<td>R ant</td>
<td>1.5</td>
<td>++</td>
</tr>
<tr>
<td>14</td>
<td>34/w</td>
<td>2/32</td>
<td>yes</td>
<td>60</td>
<td>7</td>
<td>C, G</td>
<td>R temp ant; R temp ant</td>
<td>R HS</td>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>15</td>
<td>47/w</td>
<td>2/45</td>
<td>yes</td>
<td>48</td>
<td>5</td>
<td>C, T</td>
<td>L temp ant; L temp ant</td>
<td>L HS</td>
<td>2</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 3.3.1: Clinical, EEG and MRI data in 15 patients with refractory mTLE and HS.

EEG = electroencephalographic; MRI = magnetic resonance imaging; w = woman; m = man; PFC = prolonged febrile convulsions; CPS = complex partial seizures; AEDs = antiepileptic drugs; C = carbamazepine; L = lamotrigine; G = gabapentin; P = phenytoin; V = valproic acid; T = topiramate; CPS → PET = interval between last CPS and PET scan; WM neurons = density of neurons in the resected white matter: + = few, ++ = moderate amount, +++ = many; N/A = not available; R = right; L = left; ant = anterior; HS = hippocampal sclerosis, Outcome = outcome according to the classification of Engel (Engel, 1987)
3.3.3.4 PET methodology

Image acquisition and processing were carried out as described previously (Koepp et al., 1996; Koepp et al., 1997c; Richardson et al., 1996). In summary, PET scans were performed with transaxial images aligned along the long axis of the hippocampus and coronal images orthogonal to it. 370 MBq of high specific activity $[^{11}\text{C}]$ FMZ tracer (Maziere et al., 1984) was injected intravenously. Arterial blood was sampled continuously in order to determine a metabolite-corrected plasma input function. Voxel-by-voxel parametric images of $[^{11}\text{C}]$ FMZ volume of distribution ($[^{11}\text{C}]$ FMZ-Vd), reflecting binding to $\text{GABA}_A$ receptor (Koepe et al., 1991), were produced from the brain uptake and plasma input functions using spectral analysis (Cunningham and Jones, 1993).

3.3.3.5 PET image analysis

Analyze version 7.5 (Robb, 1990) and Matlab (Mathworks Inc, Sherborn, MA, USA) were used to perform image manipulation and measurements.

3.3.3.5.1 Volume-of-interest analysis

The aim was to quantify $[^{11}\text{C}]$ FMZ binding after correction for partial volume effect in hippocampus, amygdala and neocortex. We used the same technique as described previously (Koepp et al., 2000). Briefly, the hippocampus was first delineated on the MRI scan using accepted criteria (Cook et al., 1992) and then geometrically divided along an anterior-posterior axis into three parts of equal length, each 8-10 mm long. A predefined template consisting of 28 extra-hippocampal cortical VOIs, derived from the Montreal Neurological Institute representative brain (SPM96, Wellcome Department of Imaging Neuroscience, London, UK), was first transformed into the individual’s MRI space. The individually outlined hippocampal VOIs (three on each side: anterior, middle and posterior third) were added onto the template. The high resolution volume acquisition MRI scans were then automatically segmented into probability images of gray matter (GM), white matter (WM) and CSF using a clustering, maximum likelihood ‘Mixture Model’ algorithm (Hartigan, 1975). The gray matter, white matter, CSF images and volumes of interest were coregistered with the parametric images of $[^{11}\text{C}]$ FMZ-Vd (Woods et al., 1993) and then ‘blurred’ to the same spatial resolution as PET, by convolving the MRIs with the 3D point spread function of the PET scanner (Labbé et al., 1998). This allows estimates of
partial volume effect within the multiple VOIs of homogenous tracer activity to be obtained. We report only the GM contributions to the activity of neocortical regions.

3.3.3.5.2 Voxel-by-voxel analysis

$[^{11}C]$ FMZ-Vd images were also analyzed using statistical parametric mapping (SPM96, Wellcome Department of Imaging Neuroscience, London, UK) implemented in Matlab (Mathworks Inc, Sherborn, MA, USA). Statistical parametric maps are 3D projections of statistical functions that are used to characterize significant regional brain differences in imaging data (Friston et al., 1991; Friston et al., 1995b; Friston et al., 1994; Worsley et al., 1992). For the purposes of between-group statistical analyses, the $[^{11}C]$ FMZ-Vd images of patients with left sided hippocampal sclerosis were reversed so that the hippocampal sclerosis appeared on the same right side in all patients. The images were then transformed into a standard anatomical space (Friston et al., 1995a). The procedure involves a linear 3D transformation and uses a set of smooth basis functions that allow for normalisation at a finer anatomical scale. Images were smoothed using a 12x12x12 mm (full width at half maximum) isotropic Gaussian kernel as a final pre-processing step. This spatial filter accommodates inter-individual anatomical variability and improves the sensitivity of the statistical analysis (Friston et al., 1991). Each patient’s MRI scan was coregistered with his or her FMZ-Vd image and then transformed into standard space using the transformation matrix derived from the spatial normalisation of that individual’s FMZ-Vd image.

3.3.3.6 Statistical Analysis

3.3.3.6.1 Volume-of-interest analysis

There was no significant difference between the partial volume effect corrected $[^{11}C]$ FMZ-Vd values obtained for the right and left side in controls so these values were considered together. For comparability with other studies, we also calculated asymmetry indices (AI) between partial volume effect-corrected $[^{11}C]$ FMZ-Vd in homotopic regions as $\{(right \ VOI - left \ VOI)\}/\{(right + left \ VOI)/2\}*100$ in controls and as $\{(VOI \ ipsilateral \ to \ EEG \ focus - contralateral \ VOI)\}/\{(ipsilateral + contralateral \ VOI)/2\}*100$ in patients.
We defined the normal range for the PVE corrected absolute hippocampal and amygdaloid \([^{11}\text{C}]\) FMZ-Vd values and the corresponding AIs as 2.5 standard deviations (SD) from the normal control mean, and for all other 26 VOIs and AIs as 3 SD from the mean. These high thresholds were chosen because of the large number of comparisons. The thresholds for mesial temporal VOIs and AIs were lower as we had specific hypotheses for these areas. By analogy, the SPM analysis also employed a lower threshold for mesial temporal structures of \(Z=3.09\) (or \(p<0.001\) without correction for multiple comparisons). Statistical analysis was performed using StatView (Abacus Concepts Inc., Berkeley, CA, USA) (Haycock et al., 1992). Pearson's and Spearman's correlation coefficients (r), \(\chi^2\) square test and Student's t test were used where indicated.

3.3.3.6.2 Voxel-by-voxel analysis

Individual patients were compared with an age-matched group of 13 normal subjects with the design matrix including global cerebral \([^{11}\text{C}]\) FMZ-Vd as a confounding covariate of no interest; this analysis can therefore be regarded as an ANCOVA (Friston et al., 1990). Significant differences between patients and controls were estimated according to the general linear model at each and every voxel (Friston et al., 1995b). Linear contrasts were used to test the hypotheses for specific focal effects. The resulting set of voxel values for each contrast constituted a statistical parametric map of the t statistic SPM \(\{t\}\). The SPM \(\{t\}\) were transformed to a normal distribution, SPM \(\{Z\}\), and thresholded at 3.09 (or \(P = 0.001\) uncorrected). For correction for multiple comparisons, the resulting foci of significant differences were then characterized in terms of peak height and spatial extent. The significance of each region of FMZ-Vd difference was estimated using distributional approximations from the theory of Gaussian fields (Friston et al., 1995b). For neocortical regions, the corrected threshold chosen was \(P<0.05\) for peak height and spatial extent. As hippocampus and amygdala are small structures, below 2 x FWHM of our scanner resolution, FMZ-Vd differences in these regions of special interest were only corrected for peak height but not for spatial extent. Age, gender, number of complex partial seizures (CPS) per year, outcome after epilepsy surgery and interval between last seizure and PET scan were defined as covariates of interest and tested separately for their effect on FMZ binding.
3.3.4 Results

3.3.4.1 Absolute $[^{11}\text{C}]$ FMZ-Vd with correction for partial volume effect

3.3.4.1.1 Controls

The 26 values obtained for each VOI were normally distributed. The results (mean, standard deviation, normal range, asymmetry indices (AI)) for the VOIs in the 13 controls are given in Table 3.3.2 (p. 209). None of the partial volume effect corrected absolute values from individual controls lay outside the normal range; one control had an AI for the anterior third of the hippocampus just outside the normal range.

3.3.4.1.2 Individual patients with hippocampal sclerosis

3.3.4.1.2.1 Mesial temporal structures

After partial volume effect correction, $[^{11}\text{C}]$ FMZ-Vd was unilaterally reduced in the atrophic hippocampus in 14 out of 15 mTLE patients concordant to the side of the EEG focus (Table 3.3.3, p. 211). Thirteen out of these 14 patients showed reductions in the anterior third of the hippocampus, five of 14 in the middle third and five of 14 in the posterior third (Figure 3.3.1, p. 212). These reductions extended into the ipsilateral amygdala in four patients. There were no increases in $[^{11}\text{C}]$ FMZ-Vd in mesial temporal structures (Figure 3.3.1, p. 212). One patient with anterior HS on MRI (# 13) showed reduced $[^{11}\text{C}]$ FMZ binding in the ipsilateral anterior hippocampus with an asymmetry index outside the normal range, but absolute binding was within 2.5 SD of the normal control mean. No contralateral abnormalities were observed.

3.3.4.1.2.2 Temporal neocortex

Temporal neocortical increases in $[^{11}\text{C}]$ FMZ binding were observed in two patients (# 7 and 13) (Figure 3.3.1, p. 212; Figure 3.3.2, p. 213). These involved the anterior lateral temporal lobe and the temporal pole bilaterally in one (#13) and contralateral to the side of the EEG focus in the other (# 7) (Table 3.3.3, p. 211). One of these patients (# 7) had additional increases in the extra-temporal neocortex (see below).
<table>
<thead>
<tr>
<th>Region</th>
<th>Mean (SD)</th>
<th>Normal range (Mean ± 2.5 SD)</th>
<th>Asymmetry Index ±SD</th>
<th>Normal AI range (Mean ± 2.5 SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amyg</td>
<td>4.86 (0.63)</td>
<td>3.27 - 6.44</td>
<td>12.6 ± 10.5</td>
<td>&lt;38.8</td>
</tr>
<tr>
<td>HC ant</td>
<td>5.03 (0.49)</td>
<td>3.80 - 6.26</td>
<td>6.1 ± 4.6</td>
<td>&lt;17.6</td>
</tr>
<tr>
<td>HC mid</td>
<td>4.97 (0.57)</td>
<td>3.55 - 6.40</td>
<td>8.6 ± 5.9</td>
<td>&lt;23.5</td>
</tr>
<tr>
<td>HC post</td>
<td>4.32 (0.56)</td>
<td>2.91 - 5.73</td>
<td>8.3 ± 6.3</td>
<td>&lt;23.9</td>
</tr>
<tr>
<td>Region</td>
<td>Mean (SD)</td>
<td>Normal range (Mean ± 3 SD)</td>
<td>Asymmetry Index ±SD</td>
<td>Normal AI range (Mean + 3 SD)</td>
</tr>
<tr>
<td>TL_AM</td>
<td>6.96 (1.11)</td>
<td>3.63 - 10.29</td>
<td>14.2 ± 7.3</td>
<td>&lt;36.0</td>
</tr>
<tr>
<td>TL_AL</td>
<td>8.13 (0.60)</td>
<td>6.34 - 9.93</td>
<td>4.9 ± 4.6</td>
<td>&lt;18.6</td>
</tr>
<tr>
<td>G_PH</td>
<td>6.01 (0.82)</td>
<td>3.55 - 8.46</td>
<td>11.8 ± 9.5</td>
<td>&lt;40.2</td>
</tr>
<tr>
<td>G_TS</td>
<td>7.45 (0.57)</td>
<td>5.74 - 9.15</td>
<td>8.6 ± 5.3</td>
<td>&lt;24.6</td>
</tr>
<tr>
<td>G_TMI</td>
<td>7.30 (0.70)</td>
<td>5.20 - 9.40</td>
<td>5.3 ± 4.9</td>
<td>&lt;20.1</td>
</tr>
<tr>
<td>G_Fu</td>
<td>6.47 (0.69)</td>
<td>4.41 - 8.52</td>
<td>7.9 ± 5.6</td>
<td>&lt;24.5</td>
</tr>
<tr>
<td>TLpo</td>
<td>7.67 (0.63)</td>
<td>5.77 - 9.57</td>
<td>2.2 ± 1.7</td>
<td>&lt;7.3</td>
</tr>
<tr>
<td>Ins</td>
<td>6.69 (0.68)</td>
<td>4.65 - 8.74</td>
<td>6.2 ± 5.2</td>
<td>&lt;21.9</td>
</tr>
<tr>
<td>G_Can</td>
<td>7.75 (1.06)</td>
<td>4.56 - 10.93</td>
<td>7.0 ± 7.4</td>
<td>&lt;29.3</td>
</tr>
<tr>
<td>G_Cpo</td>
<td>8.51 (0.66)</td>
<td>6.52 - 10.50</td>
<td>5.9 ± 3.0</td>
<td>&lt;14.8</td>
</tr>
<tr>
<td>FL</td>
<td>8.28 (0.59)</td>
<td>6.51 - 10.05</td>
<td>4.2 ± 4.3</td>
<td>&lt;16.9</td>
</tr>
<tr>
<td>PL</td>
<td>8.29 (0.78)</td>
<td>5.95 - 10.62</td>
<td>2.4 ± 1.7</td>
<td>&lt;7.4</td>
</tr>
<tr>
<td>OL</td>
<td>9.08 (0.68)</td>
<td>7.04 - 11.13</td>
<td>3.9 ± 3.9</td>
<td>&lt;15.6</td>
</tr>
</tbody>
</table>

**Table 3.3.2:** Partial volume effect corrected $[^{11}C] FMZ-V_d$ in multiple VOIs in 13 controls.

*SD = standard deviation; Amyg = Amygdala; HC ant/HC mid/HC post = anterior, middle and posterior third of individually outlined hippocampus; TL_AM = anterior medial part of temporal lobe; TL_AL = anterior lateral part of temporal lobe, including temporal pole; G_PH = parahippocampal gyrus; G_TS = superior temporal gyrus; G_TMI = middle and inferior temporal gyrus; G_Fu = fusiform or occipitotemporal gyrus; TLpo = posterior temporal lobe; Ins = insula; G_Can = anterior cingulate gyrus; G_Cpo = posterior cingulate gyrus; FL = frontal lobe; PL = parietal lobe; OL = occipital lobe.*
3.3.4.1.2.3 Extratemporal cortex

Two patients (#1 and 5) had decreases in extra-temporal cortical [11C] FMZ-Vd. These involved the occipital lobe contralateral to the EEG focus in patient 5 and the contralateral frontal lobe in patient 1 (Table 3.3.3, p. 211). Two patients had extratemporal cortical increases (#7 and 8): Patient 7 had increases in the ipsilateral occipital lobe and in the contralateral posterior cingulate gyrus. Patient 8 had increases in the frontal lobe ipsilateral to the EEG focus. (Table 3.3.3, p. 211) No patient had both neocortical increases and decreases in [11C] FMZ binding.

3.3.4.1.3 Between-group analysis

Reductions of [11C] FMZ-Vd in patients compared to controls were found in the following regions: anterior third of the hippocampus (mean reduction -30.7%, p<0.0001, unpaired t-test), middle third (mean reduction -22.2%, p=0.002), posterior third (mean reduction -29%, p<0.0001), and amygdala (mean reduction -14.8%, p=0.01) ipsilateral to the EEG focus. There were no abnormalities in temporal and extratemporal neocortex (at p<0.001, Bonferroni corrected for multiple comparisons).

3.3.4.1.4 Asymmetry indices of partial volume effect corrected [11C] FMZ-Vd

Fourteen mTLE patients had AIs outside the normal range for the hippocampus (12, five and nine patients for the anterior, middle and posterior third), one patient (#9) had a further abnormal AI in the amygdala. Two patients had increased AIs for structures outside the mesial temporal cortex: patient 9 in the fusiform gyrus (lower on side of focus) and posterior cingulate gyrus (higher on side of focus), and patient 7 in the posterior cingulate gyrus. In the latter, the AI outside the normal range corresponded to a contralateral increase in the absolute value for [11C] FMZ-Vd, whereas in patient 9, the AIs outside the normal range did not correspond to any changes in absolute [11C] FMZ-Vd.
Table 3.3.3: Partial volume effect corrected \[^{[T]C}\] FMZ-Vd in 15 patients with unilateral HS.

Values for the individual patients are given for the side ipsilateral to the EEG focus in the upper and for the contralateral side in the lower part of the table. Increases are highlighted in bold, decreases in bold italics. Only regions where at least one patient had significant changes are shown. ipsi/contra = ipsilateral/contralateral to the side of the EEG focus; SD = standard deviation; HC ant/HC mid/HC post = anterior, middle and posterior third of individually outlined hippocampus; TL_AL = anterior lateral part of temporal lobe, including temporal pole; G_Cpo = posterior cingulate gyrus; FL = frontal lobe; OL = occipital lobe. Normal range: Mean ± 2.5 SD for mesial temporal VOIs, mean ± 3 SD for all others (see text).
Figure 3.3.1: Partial volume effect corrected absolute $[^{11}C]$ FMZ-Vd changes in 15 patients with mTLE and unilateral HS. Increases are marked in red, decreases in blue. The intensity of the colours corresponds to the number of patients in whom a change was observed (in grey).

AL = anterior lateral temporal lobe; AM = anterior medial temporal lobe; TMI = middle and inferior temporal gyrus; TS = superior temporal gyrus; Fu = fusiform gyrus; PH = parahippocampal gyrus; Amyg = Amygdala; Han, Hmi, Hpo = anterior, middle and posterior third of the hippocampus; TLpo = posterior temporal lobe; OL = occipital lobe; FL = frontal lobe; GCan = anterior cingulate gyrus; GCpo = posterior cingulate gyrus; PL = parietal lobe.
Figure 3.3.2: Example of a patient (# 7) with decreased $[^{11}\text{C}]$ FMZ-Vd, corrected for PVE, in the ipsilateral (right) middle and posterior hippocampus and increased $[^{11}\text{C}]$ FMZ-Vd in the contralateral anterior lateral temporal neocortex. On the right, a typical control scan is shown. The hippocampal decrease is evident on visual inspection. The anterior lateral temporal neocortical increase is only revealed by quantification.

3.3.4.2 Voxel-by-voxel analysis (SPM)

3.3.4.2.1 Controls
Comparing each individual control against the remaining 12 controls, only one individual had an area of decreased $[^{11}\text{C}]$ FMZ binding, in the right middle and superior temporal gyrus.

3.3.4.2.2 Individual patients with HS

3.3.4.2.2.1 Mesial temporal structures
SPM revealed decreases in $[^{11}\text{C}]$ FMZ-Vd in 13 out of 15 individual patients relative to the control group in mesial temporal structures, ipsilateral to the EEG focus. Out of these 13 patients, 11, 12 and 7 patients showed reductions in the anterior, middle and posterior part of the hippocampus, extending into the region of the amygdala in eight patients. There were no increases in $[^{11}\text{C}]$ FMZ-Vd in mesial temporal structures. Five patients showed additional neocortical abnormalities (see below).
3.3.4.2.2 Temporal neocortex

Temporal neocortical decreases in $[^{11}C]$ FMZ binding were observed in one patient (# 9). These involved the parahippocampal and fusiform gyrus and the posterior temporal lobe, concordant with the side of the EEG focus.

A further three patients (# 2, 7 and 8) showed increases in temporal neocortical $[^{11}C]$ FMZ binding. The ipsilateral middle and inferior temporal gyrus were involved in patients 2 and 7. Patient 7 showed additional increases in the ipsilateral parahippocampal gyrus and superior temporal gyrus as well as in the inferior, middle and superior temporal gyrus contralaterally. Only contralateral increases were observed in patient 8 (anterior lateral temporal lobe and superior temporal gyrus).

3.3.4.2.3 Extratemporal cortex

There were significant decreases in extra-temporal cortical $[^{11}C]$ FMZ binding in two patients with reductions in the occipital lobe ipsilateral to the side of the EEG focus (patient 9), and bilateral reductions in the medial parietal lobe (patient 15). No increases in extra-temporal cortical FMZ binding were observed.

3.3.4.2.3 Covariates of interest

Age, gender, frequency of CPS per year, and interval between last seizure and PET scan had no significant effect on $[^{11}C]$ FMZ binding. There was a positive correlation between increased $[^{11}C]$ FMZ binding in the white matter of the temporal lobe contralateral to the resected temporal lobe with outcome poorer than Engel class IA ($r=0.64$, $p=0.005$, Spearman’s correlation coefficient). There was a positive correlation of the number of white matter neurons in the histology specimen with increased $[^{11}C]$ FMZ binding in the white matter of the resected temporal lobe ($r=0.81$, $p<0.001$) and the contralateral temporal lobe ($r=0.80$, $p<0.001$; Spearman’s correlation coefficient).

3.3.4.2.4 Between-group analysis

There was a highly significant decrease in $[^{11}C]$ FMZ binding in the patient group involving the entire length of the hippocampus on the side of the EEG focus. No other changes were observed.
3.3.4.3 Comparison of PVE-corrected VOI results and SPM results

3.3.4.3.1 Mesial temporal structures
Changes of \([^{11}\text{C}]\) FMZ binding in mesial temporal structures were detected in 14/15 patients using the partial volume effect corrected VOI approach and in 13/15 patients with SPM.

In the two HS cases not identified by SPM (patients 4 and 10), absolute \([^{11}\text{C}]\) FMZ-Vd after partial volume effect correction was significantly reduced with abnormal AIs. In patient 13, no absolute decrease in \([^{11}\text{C}]\) FMZ binding was demonstrated with the VOI approach, but SPM detected significant reductions in voxels located in the anterior hippocampus. AIs for this region were also outside the normal range.

3.3.4.3.2 Temporal neocortical structures
Five patients had significant alterations of \([^{11}\text{C}]\) FMZ binding in the temporal neocortex with VOI and two of these five plus another three patients with SPM analysis. These changes were concordant in that the patients showed either temporal neocortical increases or decreases with both methods. In only one patient (7), however, were these changes significant using both methods. Two patients (# 2 and 8) with significant increases and one (9) with significant decreases in SPM only showed corresponding changes in absolute values when the threshold was lowered. Patient 13 had significant increases in the partial volume effect corrected anterolateral temporal lobe VOI bilaterally, but the corresponding changes in SPM were only significant when uncorrected for spatial extent.

3.3.4.3.3 Extra-temporal neocortical regions
Six patients had significant abnormalities of \([^{11}\text{C}]\) FMZ binding in the extra-temporal neocortex identified with either VOI or SPM analysis. Four patients had abnormalities in the VOI analysis (patients 1, 5, 7, 8; Table 3.3.3, p. 211). In three of them (# 5, 7, 8), VOI detected changes were similar to SPM results when the thresholds were lowered. In patient 1, no corresponding changes were detected using SPM. In two patients (# 9 and 15), SPM showed significant decreases which were not reflected by any binding changes in the VOI analysis.

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3.3.5 Discussion

This is the first study to examine neocortical $^{[11]}$C FMZ binding in patients with histopathologically proven HS using both a quantitative MRI-based multiple VOI approach with correction for partial volume effect and voxel-by-voxel SPM. The main finding of this study was that the combination of both techniques revealed both increases and decreases of $^{[11]}$C FMZ-Vd in eight out of 15 patients with HS outside of the hippocampus and amygdala.

3.3.5.1 Methodological considerations

The use of both a VOI and voxel-based analysis increased the sensitivity of $^{[11]}$C FMZ PET for detecting significant mesial temporal abnormalities in HS to 100%. PVE correction is especially important when structural abnormalities are present. This is clearly the case for mesial temporal structures in mTLE. Extramesial atrophy, however, is well described (DeCarli et al., 1998). In our series, one patient (#9) with temporal lobe atrophy ipsilateral to the HS showed ipsilateral posterior temporal and occipital decreases of $^{[11]}$C FMZ binding on the SPM analysis which were not corroborated by the VOI analysis. As our method corrects for the thickness of the cortical ribbon as well, this and other differences between the two analyses can be explained by normal $^{[11]}$C FMZ binding per unit of GM when PVE correction is used, and underlines the usefulness of PVE correction in extramesial neocortical structures.

For the VOI approach, we used a template of multiple cortical VOIs defined in standard stereotactic space and subsequently transformed into individual MRI and PET space. The use of such a template, its automated coregistration and the subsequent use of PVE correction provides an entirely objective and observer-independent method for defining multiple neocortical VOIs and then quantifying $^{[11]}$C FMZ-Vd (Koepp et al., 1997a; Labbé et al., 1998). This approach works satisfactorily for neocortex, but the software did not segment and normalize the basal ganglia or the hippocampus satisfactorily. The hippocampus was therefore subjectively outlined by hand in all individual subjects. Compared with the VOI approach, SPM localizes significant changes in receptor binding on a voxel level. Thus it may detect abnormalities not identified by the VOI method when these are small in spatial extent and become averaged in large VOIs or do not conform to the anatomical boundaries used in the template. However, due to the large number of
comparisons made, rigorous statistical thresholds for significance must be applied to
the amplitude and extent of changes. Further, the final spatial resolution of SPM is
lower than in a VOI approach due to the necessary smoothing, thus it is more
affected by partial volume effect. SPM cannot distinguish loss of signal due to tissue
atrophy from a true functional abnormality. The two methods are, therefore,
complementary.

3.3.5.2 Comparison with previous findings

We have previously demonstrated, using hippocampal VOIs outlined on high-
resolution MRI and coregistered to PET, that in mTLE due to HS there are decreases
of \[^{11}C\] FMZ binding in the sclerotic hippocampus (Koepp et al., 1997c). Our
previous method, however, used only a box-region around the hippocampus, thus
averaging low temporal and high occipital \[^{11}C\] FMZ binding and leading to an
underestimation of true tracer binding in the anterior hippocampus, and not allowing
the investigation of extrahippocampal regions.

Using SPM 95, we previously found areas of decreased \[^{11}C\] FMZ binding to be
restricted to the sclerotic hippocampus in 12 patients with mTLE due to HS (Koepp
et al., 1996). In the present study, we found abnormalities of \[^{11}C\] FMZ-Vd outside
the hippocampus in five out of 15 HS patients, using a more recent version of SPM
(SPM96) which contains more advanced methods of spatial normalisation and more
stringent criteria for correction of multiple comparisons, assessing both maximal
height and spatial extent, and which is therefore more sensitive. Only one of the six
patients included in both this and the previous study using SPM95 (Koepp et al.,
1996) (patient 2) showed abnormalities in the current SPM analysis that had not been
detected using the previous version of the software. Besides the advanced statistical
methods implemented in SPM96, differences in the patient populations may thus
play a role in the apparent difference in the results obtained.

3.3.5.3 Neurobiological considerations

The exact pathological and pathophysiological mechanisms underlying mTLE are
still under study. Taken as a group, only the reduced \[^{11}C\] FMZ binding in the
epileptogenic hippocampus, extending into the amygdala, was significant. However,
in five of 15 HS patients, there were additional neocortical abnormalities of \[^{11}C\]
FMZ binding. These did not follow a specific pattern in the extratemporal areas to
suggest a predominant involvement of projection areas, in contrast to previous findings (Savic et al., 1998).

The only temporal neocortical changes in the VOI analysis were increases in the anterior lateral temporal VOI including the temporal pole (patient 7 contra- and patient 13 bilaterally). In an autoradiographical study, 11 temporal lobe specimens from patients with HS were compared to six control specimens (Burdette et al., 1995). The authors found decreased $[^3]$H FMZ binding in most hippocampal layers but diffusely increased binding in the lateral temporal neocortex in the patient group which reached statistical significance in cortical layers V and VI. Although our group results for the anterior lateral temporal lobe VOI in the patients did not differ significantly from the control group, two patients had uni- or bilateral increases in $[^1]$C FMZ binding in this VOI (Figure 3.3.1, p. 212). It is possible that chronic deprivation of input leads to a compensatory increase in $\text{GABA}_A$ receptor density, or this may be an adaptive response to epileptic activity.

Other possible explanations for increased $[^1]$C FMZ binding include an increased neuronal density or ectopic neurons bearing $\text{GABA}_A$ receptors, as for example in microdysgenesis. Areas of increased $[^1]$C FMZ-Vd have been demonstrated in cortical dysplasia using SPM (Richardson et al., 1997a; Richardson et al., 1996) and in patients with unremarkable MRI scans (Richardson et al., 1998b).

Recently, postsynaptic increases in the number of $\text{GABA}_A$ receptors underlying inhibitory potentiation in the kindling model have been described (Nusser et al., 1998). Such an increase in available binding sites (or Bmax) will lead to an increase in $[^1]$C FMZ-Vd. Our method does not distinguish between changes of Bmax and changes of the receptor affinity (Kd). Autoradiographic and histopathological studies of sclerotic human hippocampi obtained during epilepsy surgery have showed reduced neuron counts and $\text{GABA}_A$ receptor densities (Hand et al., 1997; Otis et al., 1994) and reduced $\text{GABA}_A$ receptor density per remaining neuron while receptor affinity was increased in hilus, dentate gyrus and subiculum (Hand et al., 1997). This suggests that functional abnormalities may be greater than structural ones in human mTLE. Similarly, a recent study using the pilocarpine model has found pre- and postsynaptic changes of GABA transmission (Brooks-Kayal et al., 1998) involving changes of $\text{GABA}_A \beta$ receptor subunit composition. Thus, increased density or affinity of available receptors per neuron, either on abnormal nerve cells or as an
adaptive response to the abnormal neuronal activity in mTLE, may explain the observed extramesial increases of $[^{11}\text{C}]$ FMZ binding.

### 3.3.5.4 Clinical considerations

Earlier studies showed decreases in mesiotemporal $[^{11}\text{C}]$ FMZ binding in eight of eight patients with TLE (Savic et al., 1993) or found mesiotemporal decreases in 10/10 patients with mTLE, but detected no neocortical changes (Henry et al., 1993a). These studies concentrated on the mesial temporal lobe and used ROI-based analyses placing the regions directly on the PET images.

Using the same methods as described here, we have recently found increases and decreases in mesial temporal structures and in the neocortex ipsi-and contralateral to the EEG focus in eight of 10 patients with TLE and normal structural imaging (Koepp et al., 2000). Recently, $[^{11}\text{C}]$ FMZ-PET findings in a series of 100 patients evaluated for epilepsy surgery were reported (Ryvlin et al., 1998). Out of 35 patients with hippocampal sclerosis, reduced $[^{11}\text{C}]$ FMZ binding in the mesial temporal lobe extended towards the temporal pole or lateral temporal neocortex in seven. Extra-temporal areas were not examined in these patients. We found the mesial temporal decreases to extend into the amygdala in four of 15 patients in our volume-of-interest analysis and in eight of 15 patients in the SPM voxel-by-voxel analysis. We only found one patient (# 9) with decreased $[^{11}\text{C}]$ FMZ binding in temporal neocortex using SPM, which was not corroborated by the VOI analysis. This is most likely explained by partial volume effect due to the temporal lobe atrophy in this patient.

Increases in $[^{11}\text{C}]$ FMZ binding in the white matter of the temporal lobe contralateral to the side of the resection found on the voxel-based assessment were correlated with a poorer chance of an Engel class IA outcome and may indicate epileptogenic abnormalities in the contralateral temporal lobe. Temporal lobe white matter increases of $[^{11}\text{C}]$ FMZ binding were found to be correlated with the presence of an increased number of white matter neurons in the specimen and may therefore indicate microdysgenesis. There was, however, no significant correlation between presence of white matter neurons and outcome.

In the long term, one third of patients will continue to have seizures after epilepsy surgery for HS (Berkovic et al., 1995). A longer follow-up than the median of 3 years in this study and a larger series may show more clearly whether patients with
extrahippocampal neocortical abnormalities of $[^{11}\text{C}]$ FMZ binding have a poorer prognosis.

Using quantitative post-processing of preoperative structural MRI, extrahippocampal abnormalities have been reported in 14/27 patients with subsequently histologically proven HS, 10 of whom did not become seizure-free after anterior temporal lobectomy (Sisodiya et al., 1997). Our findings of neocortical functional abnormalities of the GABAergic transmission give further evidence that abnormalities may extend beyond the mesial temporal lobe.

A positive correlation of seizure frequency with the degree of GABA$_A$ receptor reduction has been described in patients with daily, severely disabling CPS compared to patients with weekly or less frequent seizures (Savic et al., 1996). We did not replicate this finding, and $[^{11}\text{C}]$ FMZ binding was not correlated with the interval between the last seizure and the PET scan. However, all our patients were medically refractory and may have been too homogenous to find any such correlation.
3.4 Central benzodiazepine receptors in malformations of cortical development. A quantitative study

3.4.1 Summary
We calculated \[^1\text{C}]\text{ flumazenil-volume-of-distribution ([^1\text{C}] FMZ-Vd)} after partial volume effect (PVE) correction in 10 patients with malformations of cortical development (MCD) and partial seizures, to quantify benzodiazepine binding to GABA\textsubscript{A} receptors. Abnormal grey matter (GM) and adjacent or overlying cortex were individually outlined and added to an individualised anatomical template for correction for PVE. Nine of 10 patients showed single or multiple increases or decreases of \[^1\text{C}]\text{ FMZ-Vd} in or around MCD. Two of three patients with laminar (band) heterotopia showed multiple increases in overlying cortex. In three of four patients with subependymal nodular heterotopia, nodules had lower \[^1\text{C}]\text{ FMZ-Vd} than the overlying cortex, which was normal. Decreases in \[^1\text{C}]\text{ FMZ-Vd} were found in two of three clefts and one of six adjacent regions in one schizencephalic patient; another had normal \[^1\text{C}]\text{ FMZ-Vd} in the thickened cortex itself but increases in all adjacent regions. Binding was reduced within focal cortical dysplasia but increased in adjacent cortex. \[^1\text{C}]\text{ FMZ-Vd} was normal within one patient’s polymicrogyric cortex but increased in one of six adjacent VOIs. Localisation of abnormalities correlated with EEG and clinical data in cortical MCD. FMZ binding was decreased in some MCD with increased GM volume and increased in some adjacent or overlying areas of normal appearing cortex, suggesting functional abnormalities beyond MRI-detectable structural changes.


3.4.2 Introduction
Malformations of cortical development (MCD) are due to disorders of neuronal and glial proliferation, abnormal neuronal migration, or abnormal postmigrational cortical organisation and are present in 15-20% of adults with intractable partial seizures, some of whom are candidates for epilepsy surgery (Kuzniecky and Jackson,
Surgical resection of areas of MCD in patients with drug resistant epilepsy, however, results in only about 20%-40% of patients becoming seizure free (Cascino et al., 1993a; Sisodiya, 2000), compared with 70% in patients with hippocampal sclerosis (Berkovic et al., 1995). A possible explanation is that the area of functional cortical abnormality may be greater than the structural abnormality shown by conventional magnetic resonance imaging (MRI) techniques (Richardson et al., 1997a; Richardson et al., 1996; Sisodiya et al., 1995).

Animal models have shown functional abnormalities in cortex adjacent to MCD, possibly due to formation of aberrant thalamocortical connections subsequent to the presence of MCD in the original projection area (Jacobs et al., 1999a; Jacobs et al., 1999b). Abnormal adjacent cortex might in part explain the low surgical success rate in MCD and the observation that lesionectomies tend to have a less good outcome than more extended resections (Raymond et al., 1995).

\(\gamma\)-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the brain, acting at the GABA\(_A\) receptor, and plays an important role in the genesis of partial seizures. Flumazenil (FMZ) is a specific, reversibly bound high affinity neutral antagonist at GABA\(_A\) receptors (Olsen et al., 1990) containing \(\alpha1\), \(\alpha2\), \(\alpha3\), and \(\alpha5\) subunits, and \(^{[11]}\)C FMZ positron emission tomography (PET) provides a useful in vivo marker of GABA\(_A\) receptor binding (Maziere et al., 1984).

The limited spatial resolution of PET results in partial volume effect that particularly affects the quantification of signals in structures smaller than twice the full width at half maximum (FWHM) resolution of the scanner used (Hoffman et al., 1979), such as the cortical ribbon or small heterotopic nodules, due to tissue averaging effects. Correction for partial volume effect is particularly important when structural abnormalities are present. Subtle changes in GM content in MCD may not be detected on visual inspection of high quality MRI (Desbiens et al., 1993; Kuzniecky et al., 1991). Without correction for partial volume effect, it is not possible to distinguish if an abnormality detected using PET represents a true functional abnormality due to a change of receptor density or affinity per neuron, a structural abnormality due to an increase or decrease of GM, or both of these together (Labbé et al., 1998; Müller Gartner et al., 1992; Rousset et al., 1993).

Voxel-based methods of analysis (Statistical Parametric Mapping, SPM) have been applied to \(^{[11]}\)C FMZ-PET and MR images (Richardson et al., 1997a; Richardson et al., 1996) and have shown abnormalities of \(^{[11]}\)C FMZ-\(V_d\) which were frequently
more extensive than structural changes seen on MRI. These findings indicated that some of the PET abnormalities could be accounted for by abnormalities of cortical grey matter volume. The voxel-based SPM approach, however, does not give quantitative estimates of $[^1]^C$ FMZ binding to GABA$_A$ receptors and was not suited to specifically test hypotheses about normal appearing cortex adjacent to or overlying MCD.

The aims of the current study were:
1. To determine if there were changes in $[^1]^C$ FMZ-V$_d$ after correction for partial volume effect, within areas of abnormal grey matter due to MCD, within the overlying and adjacent areas of cortex that appeared structurally normal on high resolution MRI, and within distant cortical areas.

2. To correlate clinical and EEG abnormalities with PET findings.

### 3.4.3 Material and Methods

#### 3.4.3.1 Patients and Controls

We studied 10 patients (six women) with partial seizures and MCD that was diagnosed on high resolution MRI. They were recruited as described in Chapter 2.1 (p. 144). The median age at onset of habitual seizures was 13 years (range: 1-21 years), the median duration of epilepsy before the PET examination was 17 years (range: 5-33 years), and the median age at PET examination was 30 years (range: 18-47 years). The antiepileptic medication was carbamazepine (8 patients), phenytoin (2), lamotrigine (1), vigabatrin (1), sodium valproate (1) or ethosuximide (1) alone or in combination; six patients were taking carbamazepine monotherapy.

Four patients had subependymal nodular heterotopia (SNH; one had an additional parieto-occipital schizencephaly), three had laminar (band) heterotopia (BHT), one had schizencephaly with three clefts (left frontal, right central and right temporoparietal), one had focal cortical dysplasia (FCD) in the temporal lobe and one had bilateral perisylvian polymicrogyria (PMG).

Twenty-one healthy volunteers (three women) were studied for comparison. The median age at examination was 31 years (range: 20-71 years). They had no history of neurological or psychiatric disorder, were on no medication and had normal MRI studies.
Non-quantitative voxel-based data has previously been reported for eight of the patients and for seven of the controls (Richardson et al., 1997a; Richardson et al., 1996).

Clinical details for all 10 patients are shown in Table 3.4.1 (p. 227). None of our patients has undergone epilepsy surgery.

3.4.3.2 PET Technique

We used the same acquisition technique as described previously (Richardson et al., 1997a; Richardson et al., 1996). Briefly, PET scans were performed in 3D mode with the septa retracted, using a 953B Siemens/CTI PET camera with a reconstructed image resolution of about 8mm x 8mm x 4mm at FWHM for 31 simultaneously acquired planes (Spinks et al., 1992). Scans were performed with transaxial images obtained parallel to the plane defined by the anterior and posterior commissures and coronal images orthogonal to this. An eight-channel EEG was recorded during the PET studies to ensure that the scans were interictal. 370 MBq of high specific activity [11C] FMZ tracer (Maziere et al., 1984) was injected intravenously. Arterial blood was sampled continuously in order to determine a metabolite-corrected plasma input function. A dynamic 3D series, consisting of 20 frames over 90 minutes, was acquired for the brain volume. A convolution subtraction scatter correction was used (Bailey, 1992). The 20 time frames of the dynamic image were realigned with one another by an automated "least-squares" technique, to minimize any movement artifact during the scan (Friston et al., 1995a). Parametric images of [11C] FMZ-Vd, reflecting binding to the GABA\textsubscript{A} receptor at the voxel-level (Koeppe et al., 1991), were produced from the brain uptake and plasma input functions using spectral analysis (Cunningham and Jones, 1993).

3.4.3.3 PET image analysis

Analyze® version 7.5 (Robb, 1990) and Matlab (Mathworks Inc, Sherborn, MA, USA) were used to perform image manipulation and measurements on Sun Ultra 10 workstations (Sun Microsystems, Mountain View, CA). The spatial transformations were based on software included in the SPM99 package (Wellcome Department of Imaging Neuroscience, London, UK).

The aim was to quantify [11C] FMZ binding after correction for partial volume effect in areas of abnormal GM due to MCD, and in normal appearing cortex overlying the
subcortical MCDs or adjacent to the cortical MCDs. These areas were outlined manually on the MRI scans as follows:

- Areas containing only thickened GM, representing the MCD itself, were defined first.
- In the cortical forms of MCD, adjacent VOIs, consisting of cortex which appeared normal on MRI, were then defined on transverse planes. Extrapolating from findings in animal models, we outlined approximately 1.5 cm of normal appearing brain, comprising both GM and white matter (WM), for later automatic segmentation.
- In the case of discontinuous GM abnormalities (patients 8 and 10, see Table 3.4.1, p. 227), more than two adjacent VOIs were defined.
- In the subcortical forms, the VOIs of overlying cortex were defined in the dependent migrational zone overlying the SNHs, by dropping a perpendicular from the SNH to the cortical surface and allowing for about 20° of lateral migration. These sections included GM and WM. The subependymal heterotopia in patient 5 and the two anteriorly located band-like subependymal heterotopia in patient 6, however, were too small to be themselves accurately delineated on the MRI images. This did not prevent the delineation of overlying cortex (cf. Table 3.4.1, p. 227). Due to the widespread nature of the BHT, all cortical VOIs of the convexity were considered overlying cortex.

We used an MRI scan of a single brain obtained at high resolution at the Montreal Neurological Institute (MNI) (Holmes et al., 1998) as delivered with the SPM99 package and an interactive algorithm to create a brain template consisting of 29 VOIs (Hammers et al., 2000) which is a modification of the template described in chapter 3.1 (p. 156). This was first transformed into the individual patient’s MRI space. The individually outlined additional VOIs (i.e., MCDs and overlying or adjacent cortex) were added onto the template. The high resolution volume acquisition MRI scans were then automatically segmented into probability images of GM, WM and CSF (Hartigan, 1975), excepting the individually outlined areas of GM within the MCDs. The GM, WM and CSF images and the VOIs were first coregistered with the PET data (Woods et al., 1993) and then ‘blurred’ to the same spatial resolution as PET, by convolving each segmented probabilistic MRI with the 3D point spread function of the PET scanner. A least squares weighted fit of these ‘blurred’ images to the observed PET images was then calculated (Labbé et al., 1998). This allowed
estimates of PVE within the multiple VOIs of homogenous tracer activity to be obtained. To obtain a control range for $^{[11]}C$ FMZ-V$_d$ in equivalent cerebral areas to the MCD and surrounding cortex, the individualised template for each patient was spatially normalised to all 21 controls' MRI scans, and partial volume effect corrected $^{[11]}C$ FMZ-V$_d$ values were obtained for the corresponding GM areas in healthy volunteers (Figure 3.4.1, p. 230).

### 3.4.3.4 Statistical Analysis

We defined the normal range for the absolute $^{[11]}C$ FMZ-V$_d$ values after correction for PVE as 2.5 standard deviations (SD) from the normal control mean for the areas corresponding to individually outlined MCDs and adjacent/overlying VOIs, and for all other standard VOIs as 3 SD from the mean. These strict thresholds were chosen to allow for the large number of comparisons. The lower threshold was used for MCDs and surrounding cortex as we had specific hypotheses that these would be abnormal. The rationale behind the choice is a calculation of the numbers of false positives expected for the number of comparisons (1.56 for an average of 6 regions with specific hypotheses, a threshold of ±2.5SD and 21 controls; and a very similar value, 1.58, for 29 regions without specific hypotheses, a threshold of ±3SD and 21 controls). There was no significant difference between the corrected $^{[11]}C$ FMZ-V$_d$ values obtained for the right and left side of the standard anatomical VOIs in controls so these values were considered together. Statistical analysis was performed using Pearson's and Spearman's correlation coefficients ($r$, $r_s$), Student’s t test, Wilcoxon's rank-sum test, $\chi^2$-square test and the Kolmogorov-Smirnov test where indicated.
Table 3.4.1: Clinical, MRI and EEG data and $[^{11}C]$ FMZ-V_d abnormalities in 10 patients with MCD

<table>
<thead>
<tr>
<th>Pat. No.</th>
<th>MRI: MCD type</th>
<th>Age (yrs)/Gender</th>
<th>Age of onset (yrs)</th>
<th>Seizure type</th>
<th>EEG (inter-ictal)</th>
<th>Region</th>
<th>$[^{11}C]$ FMZ-V_d GM mean, % difference from control mean</th>
<th>Control $[^{11}C]$ FMZ-V_d: Mean ± SD, normal range (mean ± 2.5 SD)</th>
<th>MRI: MCD (black) and surrounding/overlying cortex (grey)</th>
<th>FMZ-PET: increases (red) and decreases (blue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>bil BHT</td>
<td>33/m</td>
<td>2</td>
<td>CPS</td>
<td>irreg gen spikes</td>
<td>R FL</td>
<td>10.22, +32%</td>
<td>7.75 ± 0.76, 5.84-9.65</td>
<td><img src="image1.png" alt="MRI image" /></td>
<td><img src="image2.png" alt="PET image" /></td>
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<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>L FL</td>
<td>9.88, +27%</td>
<td>7.75 ± 0.76, 5.84-9.65</td>
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<td><img src="image4.png" alt="PET image" /></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>R PL</td>
<td>9.94, +26%</td>
<td>7.89 ± 0.82, 5.85-9.93</td>
<td><img src="image5.png" alt="MRI image" /></td>
<td><img src="image6.png" alt="PET image" /></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>L OL</td>
<td>10.56, +35%</td>
<td>7.82 ± 1.00, 5.32-10.33</td>
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<td><img src="image8.png" alt="PET image" /></td>
</tr>
<tr>
<td>2</td>
<td>bil BHT</td>
<td>30/m</td>
<td>12</td>
<td>SPS: motor R arm</td>
<td>L hemi slow</td>
<td>R FL</td>
<td>9.96, +28%</td>
<td>7.75 ± 0.76, 5.84-9.65</td>
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<td><img src="image10.png" alt="PET image" /></td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>L FL</td>
<td>9.69, +25%</td>
<td>7.75 ± 0.76, 5.84-9.65</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td>R PL</td>
<td>10.13, +28%</td>
<td>7.89 ± 0.82, 5.85-9.93</td>
<td><img src="image13.png" alt="MRI image" /></td>
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<tr>
<td>3</td>
<td>bil BHT</td>
<td>22/f</td>
<td>18</td>
<td>CPS</td>
<td>normal</td>
<td>none</td>
<td>none</td>
<td>n/a</td>
<td><img src="image15.png" alt="MRI image" /></td>
<td><img src="image16.png" alt="PET image" /></td>
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Table 3.4.1, continued: Clinical, MRI and EEG data and $[^{11}C]$ FMZ-V_d abnormalities in 10 patients with MCD

<table>
<thead>
<tr>
<th>Pat. No.</th>
<th>MRI: MCD type</th>
<th>Age (yrs)/Gender</th>
<th>Age of onset (yrs)</th>
<th>Seizure type</th>
<th>EEG (inter-ictal)</th>
<th>Region</th>
<th>$^{11}C$ FMZ-V_d GM mean, % difference from control mean</th>
<th>Control $^{11}C$ FMZ-V_d: Mean ± SD, normal range (mean ± 2.5 SD)</th>
<th>MRI: MCD (black) and surrounding/overlying cortex (grey)</th>
<th>FMZ-PET: increases (red) and decreases (blue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>bil Schizencephaly</td>
<td>34/f</td>
<td>1</td>
<td>SPS: motor L arm 2° gen</td>
<td>no defin. abnormality</td>
<td>R centr cleft R temp-par cleft adj to L front cleft</td>
<td>4.41, -41% 5.47, -24% 5.07, -29%</td>
<td>7.52 ± 0.99, 5.04-10.00 7.24 ± 0.70, 5.50-8.97 7.19 ± 0.72, 5.40-8.98</td>
<td>[Image]</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>L post SNH</td>
<td>29/f</td>
<td>15</td>
<td>SPS: motor L arm 2° gen</td>
<td>bil SW</td>
<td>L post SNH</td>
<td>2.77, -61%</td>
<td>7.13 ± 0.81, 5.10-9.17</td>
<td>[Image]</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>SNH</td>
<td>47/f</td>
<td>21</td>
<td>CPS 2° gen</td>
<td>bil temp foci</td>
<td>R ant SNH R post SNH L ant SNH L post SNH</td>
<td>3.41, -54% 4.72, -36% 1.06, -86% 4.22, -45%</td>
<td>7.48 ± 0.74, 5.63-9.33 7.34 ± 0.73, 5.51-9.18 7.43 ± 0.77, 5.50-9.36 7.67 ± 0.80, 5.68-9.65</td>
<td>[Image]</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>R par-occ SNH</td>
<td>26/f</td>
<td>19</td>
<td>SPS 2° gen.</td>
<td>no defin. abnormality</td>
<td>R par-occ SNH</td>
<td>5.31, -26%</td>
<td>7.19 ± 0.70, 5.43-8.96</td>
<td>[Image]</td>
<td></td>
</tr>
<tr>
<td>Pat. No.</td>
<td>MRI: MCD type</td>
<td>Age (yrs)/ Gender</td>
<td>Age of onset (yrs)</td>
<td>Seizure type</td>
<td>EEG (inter-ictal)</td>
<td>Region</td>
<td>Control $[^{11}C]$ FMZ-$V_d$: GM mean, % difference from control mean</td>
<td>MRI: MCD (black) and surrounding/ overlying cortex (grey)</td>
<td>FMZ-PET: increases (red) and decreases (blue)</td>
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<tr>
<td>8</td>
<td>bil occ SNH + R par-occ schiz-encephaly</td>
<td>29/f 2</td>
<td>CPS (visual) 2° gen</td>
<td>R post hemisphere adj to R par-occ cleft: ant middle post</td>
<td>9.68, +38% 9.85, +41% 11.79, +69%</td>
<td>6.99 ± 0.99, 4.51-9.47</td>
<td><img src="image1.png" alt="MRI Image" /> <img src="image2.png" alt="PET Image" /></td>
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<td></td>
</tr>
<tr>
<td>9</td>
<td>R temp FCD</td>
<td>18/m 7</td>
<td>CPS 2° gen</td>
<td>R frontotemp R temp FCD adj to R temp FCD</td>
<td>3.40, -51% 9.36, +35%</td>
<td>6.93 ± 0.87, 4.76-9.09</td>
<td><img src="image3.png" alt="MRI Image" /> <img src="image4.png" alt="PET Image" /></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>bil peri-sylvian PMG</td>
<td>30/m 14</td>
<td>L focal motor Tonic R mid-temp slow 1/3 adj VOIs on R</td>
<td></td>
<td></td>
<td>8.58, +23%</td>
<td>6.95 ± 0.62, 5.40-8.50</td>
<td><img src="image5.png" alt="MRI Image" /> <img src="image6.png" alt="PET Image" /></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.4.1: Clinical, MRI and EEG data and $[^{11}C]$ FMZ-$V_d$ abnormalities in 10 patients with MCD. MCD = malformation of cortical development; BHT = band heterotopia; SNH = subependymal nodular heterotopia; FCD = focal cortical dysplasia; PMG = polymicrogyria; f = female; m = male; S/CPS = simple/complex partial seizures; EEG = electroencephalogram; SW = spike-wave; MRI = magnetic resonance imaging; R = right; L = left; bil = bilateral; temp/par/occ = temporal/parietal/occipital; FL/PL/OL = frontal/parietal/occipital lobe; adj = adjacent; n/a = not applicable. Imaging findings: Black = MCD itself (band heterotopia not shown), grey = cortex cortex overlying the subcortical MCD or adjacent to the cortical MCD; red = increased $[^{11}C]$ FMZ-$V_d$; blue = decreased $[^{11}C]$ FMZ-$V_d$ binding, compared with controls.
Figure 3.4.1: Method for VOI definition: The standard anatomical template is first normalised into the patient's MRI space. The lesion and adjacent or overlying cortex are outlined on the patient's MRI and combined with the normalised template to create an individualised template for each patient. This is subsequently transformed to all 21 controls, and PVE corrected $[^{11}C]$ FMZ-$V_d$ values are calculated for all of them, thus giving a normal range for each VOI for each patient.
3.4.4 Results

3.4.4.1 Absolute [$^{11}$C] FMZ-$V_d$ with correction for partial volume effect

3.4.4.1.1 Controls
In each set of 21 controls individualised for each of the 10 patients, the values obtained for the VOIs were normally distributed, including the corrected [$^{11}$C] FMZ-$V_d$ values for regions derived from the manually outlined MCDs and overlying/adjacent cortex. In nine of the 10 control sets, one value for the left insula was >3 SD above the mean. In one control set, the value for the left cingulate gyrus fell >3 SD above the mean. This is not unexpected given the approximately 600 control values obtained for comparison with each patient.

3.4.4.1.2 Patients

3.4.4.1.2.1 MCDs and adjacent/overlying VOIs.
- Laminar (band) heterotopia (BHT)
  Of the three patients with BHT, one (patient #3) had a normal FMZ-PET scan, and two (patients #1 and 2) showed multiple areas of increased [$^{11}$C] FMZ-$V_d$ in the overlying cortex (Table 3.4.1, p. 227).
- Subependymal heterotopia (SNH)
  Of the four patients with SNH (patients #5-8), three (patients 5-7) showed significantly less FMZ binding in the heterotopic nodules compared to the overlying cortex; the overlying cortex itself was normal in all four (Table 3.4.1, p. 227).
- Schizencephalies
  PVE corrected FMZ-Vd was less in two of the three clefts in one schizencephalic patient (patient #4) than in equivalent normal GM with an additional decrease in 1/6 adjacent regions; the other patient (patient #8) had normal [$^{11}$C] FMZ-$V_d$ in the thickened cortex itself but significant increases in all adjacent regions (Table 3.4.1, p. 227).
- Focal cortical dysplasia (FCD)
  Binding was increased in cortex immediately adjacent to the FCD (patient #9) which itself showed significantly reduced PVE corrected [$^{11}$C] FMZ-$V_d$ (Table 3.4.1, p. 227).
Bilateral perisylvian polymicrogyria (PMG)

After PVE correction, the patient with bilateral PMG (patient #10) showed normal \[^{[11}C\] FMZ-V_d within the polymicrogyric cortex but a significant increase in one of the six adjacent VOIs (Table 3.4.1, p. 227).

3.4.4.1.2.2 Standard anatomical VOIs.

None of the standard anatomical VOIs lay outside the individual control ranges for any of the patients.

3.4.4.2 Correlations with EEG and clinical data

Localisation of maximum abnormalities (both decreases and increases) correlated with EEG and clinical data in both schizencephalic patients, in the patient with FCD and in the patient with PMG, i.e. in the cortical forms of MCD:

Schizencephalies

Patient 4 suffered from partial motor seizures affecting the left arm and secondarily generalised seizures. Correspondingly, the maximum decreases in \[^{[11}C\] FMZ-V_d were found in the thickened cortex in the right central cleft (-41%), with additional significant decreases in the thickened cortex in the right temporoparietal cleft and in one of two VOIs adjacent to the left frontal cleft (-24% and -29%, respectively).

Patient 8 had visual complex partial seizures (CPS) and secondarily generalised seizures. Her bilateral occipital SNHs showed normal binding compared to overlying cortex, as did the thickened cortex within her right parieto-occipital schizencephalic cleft. In all three neighbouring VOIs, however, \[^{[11}C\] FMZ-V_d was significantly increased (by 38, 41 and 69%, respectively). Interictal EEG abnormalities were localised in the right posterior hemisphere.

Focal cortical dysplasia

Patient 9 had temporal-lobe type CPS with secondary generalisation. \[^{[11}C\] FMZ-V_d showed a significant decrease within the right temporal FCD (-51%) with significant increases (+35%) in one of the two neighbouring cortical areas in the right temporal lobe. Interictal EEG abnormalities were localised in the right frontotemporal region.

Bilateral perisylvian PMG

Patient 10 had partial motor seizures affecting the left side of his body as well as tonic seizures. After PVE correction, the areas of polymicrogyric cortex were shown to have normal \[^{[11}C\] FMZ-V_d binding. One of the VOIs of adjacent cortex in the central region on the right that appeared normal on MRI showed a significant
increase in $[^{11}\text{C}]$ FMZ-$V_d$ (+23%). Interictal EEG changes were localised in the right mid-temporal region.

There were no significant correlations of $[^{11}\text{C}]$ FMZ-PET and clinical and EEG data in the patients with subcortical forms of MCD (BHT and SNH, see Table 3.4.1, p. 227).

3.4.5 Discussion

This is the first study to apply a quantitative MRI-based multiple VOI approach with correction for PVE to parametric images of cerebral $[^{11}\text{C}]$ FMZ binding of patients with MCD. Abnormalities of PVE corrected $[^{11}\text{C}]$ FMZ-$V_d$ were detected in 9/10 patients.

The main novel finding was a general pattern of decreased FMZ binding in areas of increased GM volume, e.g. heterotopic nodules or FCD, and increased FMZ binding in adjacent or overlying areas of normal cortex across the various subtypes of MCD, compared with normal neocortex. Moreover, the localisation of abnormal FMZ binding correlated with EEG and clinical data in the cortical forms of MCD (schizencephaly, FCD and PMG). Using this methodology, absolutely quantified PET data, obtained in vivo, are in good accordance with experimental and in vitro data.

3.4.5.1 Methodological considerations

PVE are a consequence of the limited spatial resolution of PET. PVE correction is necessary to accurately quantify changes and distinguish between functional changes merely reflecting structural changes (atrophy or increased GM content) and functional changes per se, e.g. changes in binding to receptors on neurons (Frost et al., 1995; Koepp et al., 1998a; Labbé et al., 1996; Rousset et al., 1995).

In the current study, we used a region-template of multiple cortical VOIs defined in standard stereotactic space and subsequently transformed this into each subject's MRI and PET space. The use of such a template, its automated coregistration and the subsequent use of PVE correction provided an entirely objective and observer-independent method for defining multiple neocortical VOIs and then quantifying $[^{11}\text{C}]$ FMZ-$V_d$ in them (Koepp et al., 2000; Labbé et al., 1998). This approach works satisfactorily for neocortex if there is no structural abnormality (Hammers et al.,
1998; Koepp et al., 2000), but the presence of MCDs of different shapes and sizes in each individual patient necessitated that the structures of particular interest, in this case the areas of abnormal GM due to MCD and the adjacent/overlying cortex, were individually outlined by hand. By applying this individualised template, specific for one patient, to all 21 controls for each patient, we were able to define normal ranges of equivalent areas of the brain for each of the individual VOIs. This method is, however, computationally very demanding, as each patient was evaluated individually against all 21 controls.

The method used depends on an automatic segmentation algorithm for the areas outside the MCD itself, whereas within the MCD, abnormal GM was outlined by hand and not segmented. Inspection of the segmented GM images of the VOIs outside the MCD showed that the segmentation was visually acceptable. Although a small error due to misclassification of some voxels cannot be entirely ruled out, this is very unlikely to have influenced our results as probability images were used, i.e. the probability of a given voxel to belong to the tissue class of GM was used in the calculations rather than simple binary decisions (Ashburner and Friston, 1997; Hartigan, 1975). Even changed characteristics of the GM/WM interface should be accommodated by this method.

Using voxel-based analyses (Statistical Parametric Mapping, SPM) applied to parametric images of $^{[1]}$C FMZ binding in MCD, we previously demonstrated areas of abnormal $^{[1]}$C FMZ-$V_d$ in 10/12 patients (Richardson et al., 1996). These areas frequently extended beyond the lesions visible on MRI and consisted of increases as well as decreases of $^{[1]}$C FMZ-$V_d$. With this approach, however, some of the $^{[1]}$C FMZ binding changes could simply have reflected changed GM content, i.e. merely structural changes. In a second step, we combined the information from statistical parametric maps of MCD patients' $^{[1]}$C FMZ-$V_d$ images with SPMs of these patients' structural MRIs (Richardson et al., 1997a). This approach showed areas of disproportionate abnormal $^{[1]}$C FMZ binding in 6/10 patients, including areas which appeared functionally normal on examination of PET data alone. The method could not, however, quantitate absolute binding abnormalities, and areas of SNH were neglected by this study which was confined to the neocortical shell of GM.

Of those seven patients included in both our current and the previous study (Richardson et al., 1997a) (patients #1, 2, 4-8), findings of abnormal $^{[1]}$C FMZ-$V_d$ in the neocortical shell were very similar for three (# 1, 4, 5). Additional areas of
abnormalities were found in two (# 2, 8), most likely attributable to our working with absolutely quantified values in patient #2 and to the correction for PVE in #8. Relatively small abnormalities seen with the SPM-based method in areas remote from the MCD could not be replicated in two patients (# 6, 7), most likely because the detected small areas of abnormalities were averaged in the larger VOIs employed by the present study. In both these cases, abnormalities were found within or adjacent to the MCD instead in the current study.

None of our patients were taking benzodiazepines prior to the PET studies. One patient each, however, was taking vigabatrin and sodium valproate. Vigabatrin inhibits the GABA transaminase irreversibly and therefore elevates brain GABA concentrations up to threefold. This does not, however, lead to any alteration of GABA_A receptor binding as assessed by [123I] iomazenil SPECT (Verhoeff et al., 1999). One of the mechanisms of action of sodium valproate is via GABAergic properties. Again, this does not alter GABA_A receptor binding in man in vivo (Koepp et al., 1997b). Moreover, our two patients on vigabatrin and sodium valproate did not show global changes of [11C] FMZ binding as would be expected if the drugs acted on the benzodiazepine binding site or led to an up- or downregulation of GABA_A receptors (Savic et al., 1991). Neither the perilesional increase in patient 8 (on sodium valproate) nor in patient 10 (on vigabatrin) is therefore likely to be explained by their medication.

3.4.5.2 Clinical and neurobiological considerations

The exact pathological and pathophysiological mechanisms underlying epileptogenesis in MCD are still under study. While MCDs can be intrinsically epileptogenic (Mattia et al., 1995; Palmini et al., 1995; Sisodiya et al., 1999a), the generally poor results after epilepsy surgery (Engel, 1993), quantitative MRI findings (Sisodiya et al., 1995), PET studies (Richardson et al., 1997a; Richardson et al., 1996; Ryvlin et al., 1998; Van Bogaert et al., 1998a) as well as animal models (Jacobs et al., 1999b) indicate that structural and functional abnormalities are more widespread than the structural lesion visualised by MRI.

Epileptogenic foci have generally been reported to exhibit decreased [11C] FMZ binding (Henry et al., 1993a; Koepp et al., 2000; Koepp et al., 1997a; Muzik et al., 2000; Richardson et al., 1998b; Ryvlin et al., 1998; Savic et al., 1993; Savic et al., 1988; Savic et al., 1995; Szalies et al., 1996), so the finding of cortical areas of decreased [11C] FMZ binding may indicate the localisation of the focus. Some
studies, however, have found localised increases of the number or affinity of GABA_A receptors (Brooks-Kayal et al., 1998; Hand et al., 1997), and it is also possible that areas of increased [¹¹C] FMZ-V_d mark the epileptogenic zone in some forms of focal epilepsy.

3.4.5.2.1 Subcortical forms of MCD (BHT, SNH)

We did not detect any cortical decreases of [¹¹C] FMZ binding in the patients with heterotopia (patients #1-3, 5-7), i.e. subcortical forms of MCD. Two of the three patients with band heterotopia (patients #1-3) showed widespread bilateral significant increases of [¹¹C] FMZ-V_d in VOIs overlying the band heterotopia, in keeping with the generalised nature of these MCDs. In contrast to the band heterotopia, the subependymal nodular heterotopia (patients #5-7) were big enough to be outlined individually. In each case, PVE corrected [¹¹C] FMZ-V_d within the nodules was significantly less than the normal values for the overlying cortex, but the overlying cortex appeared normal.

This result is in keeping with the immunocytochemical finding of less morphologically complex GABAergic interneurons within nodules in autopsy and surgical material. These interneurons showed less neuropeptide Y binding, indicative of fewer synapses being present and implying that these neurons were less mature than their counterparts in the overlying cortex. This conclusion is also reinforced by a study using proton magnetic resonance spectroscopy (Marsh et al., 1996). The decrease in inhibitory function in these nodules as shown by this study, together with the fact that there are projections out of the nodules (Hannan et al., 1999), may explain the high epileptogenicity of these lesions.

An in-vitro study (Hannan et al., 1999) has described clusters of abnormal GABAergic interneurons in the cortex overlying subcortical heterotopic nodules, but did not report whether there was associated increased FMZ binding (see below).

An interesting observation is that within the group of three patients with BHT, more widespread [¹¹C] FMZ-V_d abnormalities in the cortex correlated with more severe epilepsy (as defined by younger age at onset, more widespread EEG changes, more medication and more frequent seizures).

3.4.5.2.2 Cortical forms of MCD (schizencephaly, FCD, PMG)

Some of the patients with cortical forms of MCD (patients #4, 8-10) showed normal [¹¹C] FMZ binding, corrected for PVE, within the thickened cortex, indicating
normal GABA<sub>A</sub> receptor binding per volume of GM (patients #8, 10). Two others showed decreases in the thickened cortex (patients #4, 9). This is in keeping with histopathological evidence for low numbers of calcium binding protein immunopositive GABAergic interneurons within abnormal cortex in FCD in surgical specimens (Spreafico et al., 1998) and with significantly reduced binding to GABA<sub>A</sub> receptors in dysplastic tissue and, albeit to a lesser degree, in the exofocal cortex in an animal model (Zilles et al., 1998), as in our patient #4.

In the current study the surrounding cortex, which appeared normal on high resolution MRI, showed significant increases of [<sup>11</sup>C] FMZ-V<sub>d</sub> in three of these four patients, implying a pattern of decreased inhibition inside the MCD with increased inhibition in the adjacent areas. From the freeze microgyrus model, there is evidence of enhanced inhibitory function in perilesional areas (Prince and Jacobs, 1998) and of abnormalities outside the actual structural lesion as well as epileptogenesis in the paramicrogyral zone (Jacobs et al., 1999a). This MCD also leads to widespread disruption of cortical organisation, probably due to aberrant thalamocortical projections (Jacobs et al., 1999c). Human data is scarce, partly due to the small numbers of patients with MCD who undergo surgery. A case report has shown large numbers of neurons in the white matter underlying the normal cortex close to PMG (Battaglia et al., 1996) which could explain our observation of a perilesional increase in our case with PMG.

The localisation of the peak binding abnormalities was in keeping with EEG and clinical data in all four patients with cortical MCDs, providing additional lateralising information in those patients who had bilateral MCDs on MRI (patients #4, 8 and 10). This information may prove useful in those patients with diffuse or bilateral MCD for whom surgery is being considered (Raymond et al., 1995). A caveat is the unavailability of intracranial recordings in our study, as the interictal scalp EEG data may be equivocal (Raymond et al., 1995).

There are several possible explanations for increased [<sup>11</sup>C] FMZ binding in cortex adjacent to or overlying MCDs. These include an increase in the number of available receptors per neuron; or an increased neuronal density or ectopic neurons bearing GABA<sub>A</sub> receptors, as for example in microdysgenesis. It is conceivable that cortex in the immediate vicinity to visible lesions may exhibit microdysgenesis not visible on high resolution MRI (Desbiens et al., 1993) or detected by the MRI segmentation.
program. This would be in keeping with our recent finding of a correlation of neuronal density in the resected white matter of the temporal lobe, i.e. microdysgenetic changes, with $^{[11]}\text{C}$ FMZ binding in patients with unilateral hippocampal sclerosis (Hammers et al., 2001a) (see chapter 3.3, p. 200). It is unlikely that increased $^{[11]}\text{C}$ FMZ binding represents an adaptive upregulative response to epileptic activity, as increases in $^{[11]}\text{C}$ FMZ binding were not seen in patients with acquired lesions that underlay epileptic activity (Richardson et al., 1998b).

Our method does not distinguish between changes of the number of available binding sites ($B_{\text{max}}$) and changes of receptor affinity ($K_d$). Changes in both parameters have been described (Nagy et al., 1999). In this paper, spiking neocortex in a wide variety of pathologies was examined, compared to perilesional cortex and tissue from patients with severe head trauma. In a more homogenous group of patients, all with hippocampal sclerosis and compared against autopsy controls, however, we found decreases of $B_{\text{max}}$, over and above neuronal loss, in the CA1 region, and increases of affinity, i.e. decreases of $K_d$, in some regions (Hand et al., 1997). Recently, postsynaptic increases in the number of GABA$_A$ receptors underlying inhibitory potentiation in the kindling model have been described (Nusser et al., 1998). As not all GABA$_A$ receptors bind benzodiazepines, increases in GABA binding will only be paralleled by increased $^{[11]}\text{C}$ FMZ binding if more GABA$_A$ receptors containing the $\alpha$ subunits 1,2,3 or 5 are present. Using the pilocarpine model, pre- and postsynaptic changes of GABA transmission have been found (Brooks-Kayal et al., 1998) involving changes of affinity-mediating GABA$_A$ $\beta$ receptor subunit composition. Thus, increased density or affinity of available receptors per neuron, either on abnormal nerve cells or as a response to the abnormal circuitry in MCD, may explain the observed increases of $^{[11]}\text{C}$ FMZ binding. Despite few patients with MCD being good surgical candidates and the resulting scarcity of tissue available, correlative in vitro quantitative neuropathological and autoradiographic studies are in hand to address these questions.
3.5 Abnormalities of grey and white matter $[^{11}C]$ flumazenil binding in temporal lobe epilepsy with normal MRI

3.5.1 Summary

In 20-30% of potential surgical candidates with refractory focal epilepsy, standard MRI does not identify the cause. GABA is the principal inhibitory neurotransmitter in the brain, and GABA$_A$ receptors are expressed by most neurons. $[^{11}C]$ flumazenil (FMZ) PET images the majority of GABA$_A$ receptor subtypes. We investigated abnormalities of FMZ binding in grey and white matter in 18 patients with refractory temporal lobe epilepsy (TLE) and normal quantitative MRI. Parametric images of $[^{11}C]$ FMZ-volume-of-distribution (FMZ-$V_d$) were calculated. 21 healthy controls were scanned for comparison. Statistical parametric mapping (SPM99) was used to localise significant changes in FMZ-$V_d$ in individual patients and between groups, specifically including the entire white matter in all subjects through explicit masking. Sixteen of 18 patients showed single or multiple abnormalities of FMZ-$V_d$. Six had hippocampal decreases. Eleven patients showed FMZ-$V_d$ increases in the temporal lobe white matter (TLWM). Outside the mesial temporal structures, seven showed multiple areas of increase or decrease and only one a single area of decrease. In seven of the 16 patients with abnormalities, findings were concordant with EEG and clinical data, enabling further presurgical evaluation. Group findings were: 1.) decreased FMZ-$V_d$ in the ipsilateral ($Z=3.01$) and contralateral ($Z=2.56$) hippocampus; 2.) increased FMZ-$V_d$ in the ipsilateral ($Z=3.71$) and contralateral TLWM (two clusters, $Z=3.11$ and 2.79) and 3.) increased FMZ-$V_d$ in the ipsilateral FLWM between the superior and medial frontal gyrus ($Z=3.80$) with similar changes contralaterally ($Z=4.87$). No changes were found in the thalamus and basal ganglia. ROI analyses indicated an average increase of FMZ binding of 16% in the TLWM ipsilateral to the epileptic focus. PET findings were corroborated by invasive EEG or pathology in five cases. FMZ PET analysed using SPM with explicit masking was sensitive in patients with normal MRI, and hippocampal abnormalities were detected in a third of these patients. Furthermore, TLWM increases of FMZ binding, indicating microdysgenesis, were detected in the majority of these patients and may represent the structural basis for their epilepsy.
3.5.2 Introduction

In patients with medically refractory partial seizures, surgery offers the possibility of a lasting suppression of seizures. The majority of patients referred for epilepsy surgery have temporal lobe epilepsy (TLE), and 60% of these have hippocampal sclerosis (HS) (Babb et al., 1984). MRI has been reported to be normal in 15-30% of patients with TLE, even when histopathological examination of resected specimens detect HS, focal cortical dysplasia or other pathologies (Chugani et al., 1990; Desbiens et al., 1993; Kuzniecky et al., 1991; Van Paesschen et al., 1997). In the absence of identifiable pathology on imaging, surgery in TLE patients has a less favourable outcome (Berkovic et al., 1995). Thus, while patients with TLE and normal MRI represent a relatively large and important group in epilepsy centres, they are less likely to undergo surgery.

$\gamma$-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the brain, acting at the GABA$_A$ receptor. Flumazenil (FMZ) is a specific, reversibly bound high affinity neutral antagonist at the benzodiazepine site of GABA$_A$ receptors (Olsen et al., 1990) containing the $\alpha$ subunits 1,2,3 or 5, expressed by most neurons. $^{[1]}C$FMZ provides a useful in vivo marker of GABA$_A$ receptor binding (Maziere et al., 1984).

In TLE, we have shown using partial volume effect corrected FMZ-PET that FMZ binding is reduced over and above hippocampal volume loss in HS (Koepp et al., 1997c). Correlational analysis of autoradiography and quantitative neuropathology in resected hippocampi also revealed a greater reduction of FMZ binding than neuronal cell density (Hand et al., 1997) and good correlation between in-vivo $^{[1]}C$FMZ PET and $^{[3]}H$FMZ autoradiography (Koepp et al., 1998a). These results suggested that $^{[1]}C$FMZ-PET may be more sensitive than MRI in the identification of subtle hippocampal abnormalities, and therefore clinically useful when MRI is unremarkable.

Malformations of cortical development (MCD) are increasingly recognised as underlying medically intractable epilepsy (Kuzniecky and Jackson, 1997). Microdysgenesis, a minimal form of MCD, is not detectable on conventional MRI
and a common form is an increased density of heterotopic neurons in the white matter (WM) (Raymond et al., 1995). We have recently demonstrated a strong and highly significant correlation between in vivo temporal lobe white matter (TLWM) FMZ binding and WM neuron number in patients with HS who underwent anterior temporal lobe resection (Hammers et al., 2001a). FMZ PET is uniquely suited to the study of microdysgenesis in white matter due to its highly specific binding to neurons and the resulting high contrast-to-noise ratio. Previous PET studies of patients with TLE and normal MRI have not considered FMZ binding to GABA_A receptors in the WM.

The aims of the current study were to:

1. Determine the ability of FMZ-PET to localise abnormalities in TLE patients with normal, high resolution, quantitative MRI, using a more advanced version of statistical parametric mapping (SPM99) than previously (Koepp et al., 2000) in a different and larger series of patients.

2. Investigate with SPM99 whether TLWM FMZ binding in these patients is increased, using explicit masking.

3. Assess common abnormalities using group comparisons.

### 3.5.3 Material and Methods

#### 3.5.3.1 Patients and Controls

We studied 18 patients (11 women) with medically refractory TLE. The diagnosis and focus lateralisation was based on a comprehensive assessment. This included seizure semiology obtained from eye witnesses or video recordings, interictal EEG abnormalities in all, ictal Video-EEG findings in 10 patients (Table 3.5.1, p. 246) and detailed neuropsychological assessment. They were recruited as described in Chapter 2.1 (p. 144). The median age at onset of habitual seizures was 19 years (range: 7-54 years), the median duration of epilepsy before the PET examination was 13 years (range: 1-34 years), and the median age at PET examination was 37 years (range: 17-64 years). None had a history of prolonged febrile convulsions. Patients had a median of 62 complex partial seizures per year (range: 4-1270). The antiepileptic medication was lamotrigine (11 patients), phenytoin (7), carbamazepine (5), topiramate (3), vigabatrin (2), sodium valproate (2) or gabapentin (1) mostly combining two antiepileptic drugs; one patient was not taking any medication, two
were on lamotrigine monotherapy and one on carbamazepine monotherapy. Patients who were treated with benzodiazepines or barbiturates within two months of the PET examination were not included in the study as these drugs could possibly interfere with $[^{11}\text{C}]$ FMZ binding. Vigabatrin has been shown not to influence benzodiazepine binding (Hammers et al., 2001c; Verhoeff et al., 1999).

Twenty-one healthy volunteers (three women) were studied for comparison. The median age at examination was 31 years (range: 20-71 years). They had no history of neurological or psychiatric disorder, were on no medication and had normal MRI studies.

Clinical data for all 18 patients are shown in Table 3.5.1 (p. 246). At the time of writing, four patients have undergone epilepsy surgery (patients #8, 15, 17 and 18), and one (#7) has been evaluated with depth electrodes.

3.5.3.2 PET

We used the same acquisition technique as described previously (Hammers et al., 2002). PET scans were performed in 3D mode with the septa retracted, using a 953B Siemens/CTI PET camera with a reconstructed image resolution of about 4.8 x 4.8 x 5.2 mm full width half maximum (FWHM) in air at the centre of the scanner field of view. Images were displayed as 31 transaxial planes (Bailey, 1992) with voxel sizes of 2.09 x 2.09 x 3.42 mm. Scans were acquired with transaxial planes parallel to the plane defined by the anterior and posterior commissures (AC-PC line) and coronal images orthogonal to this. A transmission scan using three rotating $^{68}\text{Ga}/^{68}\text{Ge}$-rotatory line sources was performed to enable emission scans to be corrected for attenuation. An eight-channel EEG was recorded during the PET studies to ensure that the scans were interictal. 370 MBq of high specific activity $[^{11}\text{C}]$ FMZ (Maziere et al., 1984) was injected intravenously. Arterial blood was sampled continuously in order to determine a metabolite-corrected plasma input function (Lammertsma et al., 1993). A dynamic 3D series, consisting of 20 frames over 90 minutes, was acquired for the brain volume. A convolution subtraction scatter correction was used (Bailey, 1992) and z-scaling with the inverse of our scanner’s axial profile applied to obtain uniform efficiency throughout the field of view (Grootoonk, 1995). The 20 time frames of the dynamic image were realigned with one another by an automated “least-squares“ technique, to minimise any movement artefact during the scan (Friston et al., 1995a). Parametric images of $[^{11}\text{C}]$ FMZ-$V_d$, reflecting binding to $\text{GABA}_\text{A}$ receptors at the voxel-level (Koepp et al., 1991), were produced from the
brain uptake and plasma input functions using spectral analysis (Cunningham and Jones, 1993) with correction for blood volume.

3.5.3.3 MRI

MRIs were obtained with a 1 Tesla Picker scanner (Picker, Cleveland, Ohio, USA) using a gradient echo protocol which generated 128 contiguous 1.3 mm thick sagittal images (matrix 256x256 voxels, voxel sizes 1x1x1.3 mm, repetition time (TR) 35 msec; echo time (TE) 6 msec; flip angle 35°). These high resolution volume acquisition MRI scans were coregistered to the parametric images of [¹¹C] FMZ binding. In addition, all patients also had hippocampal volumetric studies with a 1.5T GE Signa scanner (Milwaukee, USA). An inversion recovery-prepared 3D Spoiled Gradient Echo (IRP-SPGR) sequence (TR/TE/TI/NEX 17.4/4.2/450/2, flip angle 20, matrix size 256 x 192, 24 x 18 cm FOV) with 124 contiguous coronal slices and a slice thickness of 1.5 mm was used for volumetric studies as described previously (Woermann et al., 1998). Hippocampal T2 (HCT2) were obtained as described previously (Duncan et al., 1996b; Woermann et al., 1998). All MRIs were reported after the electroclinical diagnosis of TLE was made, and particular attention was paid to the temporal lobes. They were found to be normal on inspection by experienced neuroradiologists. In addition, quantitative analysis revealed normal hippocampal volumes normalised to intracranial volume, no asymmetry, and normal HCT2 in all patients.

3.5.3.4 PET image analysis

[¹¹C] FMZ-V₅ images were analysed using statistical parametric mapping (SPM99, Wellcome Department of Imaging Neuroscience, London, UK) implemented in Matlab (Mathworks Inc, Sherborn, MA, USA). The images were volumetrically normalised to a symmetrical reference FMZ-V₅ template created in our unit that occupies the same space as the SPM99 MRI templates. The normalisation procedure involves a linear 3D transformation and uses a set of smooth basis functions that allow for normalisation at a finer anatomical scale (Ashburner and Friston, 1999). Images were smoothed using a 12x12x12 mm (full width at half maximum) isotropic Gaussian kernel as a final pre-processing step. This spatial filter accommodates inter-individual anatomical variability and improves the sensitivity of the statistical analysis (Friston et al., 1991). Each subject’s MRI scan was coregistered with his or her FMZ-V₅ image (Ashburner and Friston, 1997; Woods et al., 1993) and then
transformed into standard space using the transformation matrix derived from the spatial normalisation of that individual’s FMZ-\(V_d\) image.

### 3.5.3.5 Statistical analysis

Significant differences between patients and control subjects were estimated according to the general linear model at each and every voxel of the normalised and smoothed images (Friston et al., 1995b). Parametric images of FMZ-\(V_d\) were interrogated with SPM99 implemented in Matlab5 (Mathworks Inc. Sherborn MA, USA). Statistical parametric maps are 3D projections of statistical functions that are used to characterise significant regional differences in imaging data. We have described the use of SPM in \([^{11}C]\) FMZ PET studies of patients with unilateral HS (Koepp et al., 1996), in patients with malformations of cortical development (Richardson et al., 1996) and in patients with TLE and normal MRI (Koepp et al., 2000). SPM99 combines the general linear model to create the statistical map and random field theory to make statistical inferences about regional effects (Friston et al., 1995b; Worsley et al., 1996).

Individual patients were compared with the 21 normal control subjects using a design matrix designating global cerebral FMZ-\(V_d\) differences as a confounding covariate (Friston et al., 1990). For the purposes of between-group statistical analyses, the \([^{11}C]\) FMZ-\(V_d\) images of the six patients with right sided TLE were reversed before spatial manipulation so that the focus was on the same left side in all patients. The three patients with bilateral foci (Table 3.5.1, p. 246) were not included in the group analyses. Linear contrasts were used to test the hypotheses for specific focal effects. The resulting set of voxel values for each contrast constitutes a statistical parametric map of the \(t\) statistic SPM \(\{t\}\). The SPM \(\{t\}\) were thresholded at \(p=0.001\) uncorrected. The significance of foci of relative FMZVD changes is estimated using Random Field Theory, correcting for multiple comparisons using the number of resolution elements (resels) in the statistical image (Worsley et al., 1992; Worsley et al., 1996). This looks at the probability that the observed cluster of voxels could have occurred by chance, given its extent and peak height. The threshold chosen for the corrected \(p\) values of the extramesial clusters was \(p<0.05\). As hippocampus, amygdala and temporal lobe white matter are small structures, below twice the FWHM of our scanner resolution, FMZ-\(V_d\) differences in these regions of special \textit{a priori} interest were regarded as significant if the value of the \(t\) statistic, transformed to the unit normal distribution, exceeded a Z-score of >2.5, as in our previous studies.
(Hammers et al., 2001a; Koepp et al., 2000; Koepp et al., 1997a; Koepp et al., 1997c). Age, gender, age at onset of epilepsy, duration of epilepsy, frequency of seizures, time between scan and last seizure and hippocampal volume were defined as covariates of interest and tested separately for their effect on FMZ-V_d.

The aim was to localise abnormalities of FMZ-V_d in individual patients, compared to the control group, and between the control and patient groups, in both grey and white matter. In SPM analyses, white matter, as a region of relatively low signal, is usually excluded through thresholding. This is done to include all areas of high signal, while restricting the number of multiple comparisons through exclusion of areas that are not of interest in blood flow studies. To specifically include the entire white matter in the statistical analysis of all subjects, we, therefore, created an anatomical mask in template space, encompassing the grey matter in the cortex, the basal ganglia and the white matter.

In order to demonstrate the effect size, quantitative region-of-interest analyses were performed on unnormalised parametric images in the ipsilateral TLWM. An average of $2235 \text{ mm}^3$ was sampled with circular regions of interest, using Analyze AVW software (Robb and Hanson, 1991), in all 15 patients with a unilateral focus, and in corresponding areas in all 21 controls.
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (yr)/ Sex</th>
<th>Handed- ness</th>
<th>Onset of epilepsy (yrs)</th>
<th>Duration (yrs)</th>
<th>CPS/yr</th>
<th>Interval last CPS → PET (days)</th>
<th>Medication</th>
<th>EEG (Inter-ictal)</th>
<th>EEG (Ictal)</th>
<th>Focus &amp; PET: temporal increases</th>
<th>Focus &amp; PET: temporal decreases</th>
<th>Focus &amp; PET: extratemporal abnormalities (1/1)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>38/ m</td>
<td>left</td>
<td>27</td>
<td>11</td>
<td>200</td>
<td>2</td>
<td>LTG</td>
<td>l&gt;r temp</td>
<td>l temp</td>
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<td>ipsilateral GTS/GYM: Z3.04 k445 p.002 (52/-54/3/-2) and Z3.97 k240 p.029 (52/-52/-22)</td>
<td>ipsilateral G frontalis medius: Z3.95 k291 p.013 (46/52/-8)</td>
</tr>
<tr>
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<td>right</td>
<td>12</td>
<td>34</td>
<td>4</td>
<td>13</td>
<td>LGT/PHT</td>
<td>l&gt;r temp</td>
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<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
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<td>right</td>
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<td>5</td>
<td>125</td>
<td>7</td>
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<td>bil temp</td>
<td>l temp</td>
<td>none</td>
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<td>none</td>
</tr>
<tr>
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<td>18</td>
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<td>30</td>
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<td>none</td>
</tr>
<tr>
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<td>right</td>
<td>15</td>
<td>32</td>
<td>1270</td>
<td>1</td>
<td>CBZ/LTG</td>
<td>r&gt;l temp</td>
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<tr>
<td>6</td>
<td>24/ m</td>
<td>right</td>
<td>10</td>
<td>14</td>
<td>50</td>
<td>10</td>
<td>LTG/PHT</td>
<td>bil (fronto) temp indep r&gt;l</td>
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<td>none</td>
<td>none</td>
<td>none</td>
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<td>right</td>
<td>7</td>
<td>20</td>
<td>100</td>
<td>1</td>
<td>CBZ/LTG</td>
<td>bil temp</td>
<td>4 (r)-temp depth: 5</td>
<td>HC, 4 r HC</td>
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<td>none</td>
</tr>
<tr>
<td>8**</td>
<td>17/f</td>
<td>right</td>
<td>13</td>
<td>4</td>
<td>220</td>
<td>3</td>
<td>TPM/PHT</td>
<td>l temp</td>
<td>1ा temp</td>
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<tr>
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<td>64/ f</td>
<td>left</td>
<td>54</td>
<td>10</td>
<td>72</td>
<td>6</td>
<td>none</td>
<td>l temp</td>
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<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
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<td>right</td>
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<td>19</td>
<td>8</td>
<td>1</td>
<td>LTG/PHT</td>
<td>l&gt;r temp</td>
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<td>ipsilateral HC: Z3.47 k17552 (42/-10/-24), Z3.88 k476 (-40/0/-28)</td>
<td>ipsilateral HC: Z3.88 k291 (-16/10/-22)</td>
<td>ipsilateral WM inferolateral of posterior temp horns: r Z3.05 k223 p.037 (18/44/2), r Z4.69 k301 p.012 r Z4.43 k291 p.012 r Z4.44 k232 p.028 (28/48/-6)</td>
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<tr>
<td>11</td>
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<td>l temp</td>
<td>l temp</td>
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<td>right</td>
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<td>8</td>
<td>24</td>
<td>8</td>
<td>CBZ/LTG</td>
<td>r temp</td>
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<td>21</td>
<td>208</td>
<td>1</td>
<td>VPA/LTG</td>
<td>bil indep r&gt;t non localising</td>
<td>bil</td>
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<td>right</td>
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<td>15</td>
<td>26</td>
<td>2</td>
<td>CBZ/LTG</td>
<td>l&gt;r temp</td>
<td>N/A</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
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<td>26/ m</td>
<td>right</td>
<td>20</td>
<td>6</td>
<td>52</td>
<td>14</td>
<td>PHT/GEM/ GBP</td>
<td>r&gt;l temp</td>
<td>subdural grid: r temp</td>
<td>ipsilateral TLWM: Z4.18 k841 (-32/-18/-34), r Z3.79 k286 (50/-14/26)</td>
<td>ipsilateral GYM: Z3.29 k332 p.032 (70/-16/-14)</td>
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</tr>
<tr>
<td>16</td>
<td>55/ m</td>
<td>left</td>
<td>54</td>
<td>1</td>
<td>24</td>
<td>30</td>
<td>LTG</td>
<td>r temp</td>
<td>r temp</td>
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<td>none</td>
<td>ipsilateral FLWM: Z3.35 k264 p.02 (34/36/-14)</td>
</tr>
<tr>
<td>17**</td>
<td>46/f</td>
<td>right</td>
<td>26</td>
<td>20</td>
<td>72</td>
<td>4</td>
<td>GVG/LTG</td>
<td>r temp</td>
<td>r temp</td>
<td>none</td>
<td>none</td>
<td>none</td>
</tr>
<tr>
<td>18**</td>
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<td>right</td>
<td>32</td>
<td>10</td>
<td>120</td>
<td>3</td>
<td>PHT/VPA</td>
<td>r hemisph., non localising</td>
<td>contralateral TLWM: Z3.30 k149 (-34/-10/-24)</td>
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Table 3.5.1: Clinical, EEG and imaging data in 18 patients with refractory TLE and normal MRI. f=female, m=male, yr(s)=year(s), PFC=prolonged febrile convulsions, CPS=complex partial seizures, GTCS=secondarily generalised tonic-clonic seizures, EEG=electroencephalography, N/A=not available, HC=hippocampus, LTG=lamotrigine, PHT=phenytoin, CBZ=carbamazepine, TPM=topiramate, GVG=Vigabatrin, VPA=valproic acid, GBP=gabapentin, bil/contralateral/ipsilateral, temp=seizure localisation, HC=hippocampus, Amyg=amygdala, GM/WM=grey/white matter, TLWM=temporal lobe white matter, G+gyrus, GTS=gyrus temporals superior, GTM=gyrus temporals medius, FL=frontal lobe, OL=occipital lobe, Z=Z-score compared with 21 controls, k=number of voxels in cluster. Coordinates in brackets are MNI coordinates, referring to our symmetrical FMZ template which is in register with the MNI/ICBM152 T1 template as supplied with SPM99. Focus determination was based on comprehensive assessment including seizure semiology, interictal and ictal Video-EEG findings where available as well as detailed neuropsychological assessment. ** Patient underwent operation; histopathology available (see text).
3.5.4 Results

3.5.4.1 Controls

3.5.4.1.1 Hippocampus, amygdala and TLWM
Comparing each individual control subject against the remaining 20 controls, none had white matter, hippocampal, or amygdala abnormalities of FMZ-V_d.

3.5.4.1.2 Extramesial structures
One individual had an increase in the anterior pallidum (Z=4.67, k=390, p=0.003) and another had increases of FMZ-V_d, localised to the left middle frontal gyrus (Z=4.30, k=288, p=0.011) and the right orbitofrontal gyri (Z=4.25, k=278, p=0.013). Given the chosen thresholds and the number of comparisons resulting from 21 controls x 2 contrasts, two positive results would be predicted by chance.

3.5.4.2 Individual patients
(see Table 3.5.1, p. 246)

3.5.4.2.1 Hippocampus and amygdala
Six patients had abnormally low FMZ-V_d in the hippocampus. In three of them (#8, 10, 11), the decrease was ipsilateral to the EEG focus, one of these (#10) had additional extramesial increases and one (#11) had an additional TLWM increase (see below). Two others (#7, 17) had bilateral decreases, and the sixth (#6) had a unilateral decrease but bilateral EEG abnormalities. The decreases extended into the amygdala in patients #8 (Figure 3.5.1, p. 248), #7 and #17. No increases in hippocampal or amygdala FMZ-V_d were observed.
**Figure 3.5.1:** Example of a patient (#8) with decreased FMZ-V_d in the ipsilateral hippocampus, extending into the amygdala. Colourscale: Z score (maximum Z=3.71). Statistical results are overlaid onto the patient's coregistered MRI. Only the mesial temporal decrease, highlighted with a crosshair, is significant. Left side of the coronal image and upper part of the transverse image correspond to the left side of the brain.

**Figure 3.5.2:** Example of a patient (#5) with increased FMZ-V_d in the TLWM bilaterally. Colourscale: Z score (maximum Z=4.45). Statistical results are overlaid onto the patient's coregistered MRI. Only the ipsilateral TLWM increase (highlighted with a crosshair) and the contralateral TLWM increase are significant. Left side of the coronal image and upper part of the transverse image correspond to the left side of the brain.
Isolated ipsilateral neocortical decrease of FMZ-Vd of patient #15. Pathology showed a small malformative lesion similar to a Taylor type focal cortical dysplasia. Statistical results (maximum Z=5.29) are shown overlaid onto the patient's normalised FMZ-Vd image on the right. The left of the image shows that this decrease is visible even before statistical analysis. Left side of the images corresponds to the left side of the brain.

3.5.4.2.2 Temporal lobe white matter

Eleven of the 18 patients had FMZ-Vd increases in the TLWM. In four, they were ipsilateral, two contralateral, four bilateral and one unilateral with bilateral EEG changes. Of the four patients with ipsilateral TLWM increases (#1, 11, 12, 16), three had additional abnormalities, ipsilateral hippocampal decrease in one (#11), multiple neocortical decreases in one (#1) and an ipsilateral frontal WM increase in another (#16). Of the two patients with contralateral TLWM increases (#4, 18), one (#4) also had a contralateral temporal neocortical increase. Of the four patients with bilateral TLWM increases (#3, 5, 10, 15; see Figure 3.5.2, p. 248), three had additional abnormalities, a contralateral frontal increase in one (#3), an ipsilateral temporal neocortical decrease in another (#15; see Figure 3.5.3, p. 249) and multiple white matter increases in one (#10, see below).
3.5.4.2.3 Extramesial structures

Seven patients showed abnormalities outside the mesial temporal structures. Only one patient (#9) had extramesial abnormalities only (increases in the orbitofrontal white and grey matter bilaterally); the other six had extramesial abnormalities in addition to their temporal lobe changes described above. Extramesial decreases were found in two patients: Patient 15 (Figure 3.5.3, p. 249) had a single temporal neocortical decrease and bilateral TLWM increases; patient 1 had decreases in the ipsilateral temporal neocortex and ipsilateral FL in addition to ipsilateral TLWM increases. Extramesial increases were found in five patients. Three had one extramesial increase only: Patient 3 in the ipsilateral FL (in addition to bilateral TLWM increases), patient 4 in the contralateral posterior TL (in addition to a contralateral TLWM increase) and patient 16 in the ipsilateral FL white matter (in addition to the ipsilateral TLWM increase). Two had multiple extramesial increases. Patient 9 had bifrontal increases. Patient 10 had multiple white matter increases infero-laterally of both posterior temporal horns and in both FLs, in addition to bilateral TLWM increases and an ipsilateral hippocampal decrease.

3.5.4.3 Between-group analyses

3.5.4.3.1 Hippocampus/Amygdala

There was a decrease of FMZ-V_d in the ipsilateral hippocampus in the patient group, extending into the amygdala (Z=3.01), as well as in the contralateral hippocampus (Z=2.56) (Figure 3.5.4, p. 251).

3.5.4.3.2 Temporal lobe white matter

The patient group showed ipsilateral (Z=3.71) and contralateral (two clusters, Z=3.11 and Z=2.79) increases in FMZ-V_d (Figure 3.5.4).

In order to demonstrate the magnitude of the effect, quantitative ROI analyses were performed on unnormalised parametric images. The ipsilateral TLWM was sampled in all 15 patients with a unilateral focus, and corresponding areas were sampled in all 21 controls. These showed an average increase of FMZ-V_d in ipsilateral TLWM in the patients of 16% which was highly significant (two-tailed t test, p<0.007).
3.5.4.3.3 Extramesial structures

We found increased FMZ-V$_d$ in the ipsilateral FLWM between the superior and medial frontal gyrus (Z=3.80, k=293, p<0.025) with similar changes contralaterally (Z=4.87, k=510, p<0.0004). There were no decreases outside of the mesial temporal lobe.

3.5.4.4 Correlations with clinical data

There were no correlations anywhere in the brain between FMZ-V$_d$ and age, gender, age at onset of epilepsy, duration of epilepsy, frequency of seizures, time between scan and last seizure, or hippocampal volume.

3.5.4.4 Surgery and histological results

So far, four patients have had surgery. One patient (#15) with a marked unilateral temporal neocortical decrease of FMZ-V$_d$ in addition to bilateral TLWM increases has had a focal cortical resection. Histology showed a small malformative lesion with some features similar to a Taylor type focal cortical dysplasia (Taylor et al., 1971). There were dysplastic neurons in the cortex and gliosis. The demarcation between the grey and white matter was poorly defined. There were abnormal, large neurons
present in the white matter, but only a small amount of white matter was resected, and the neuronal density could not be quantified. The patient remains seizure free 25 months after the operation.

Three patients (#8, 17 and 18) underwent modified anterior temporal lobe resections. One patient (#8) with a left hippocampal decrease of FMZ-V_d extending into the amygdala had a left anterior temporal lobe resection including amygdala and hippocampus at another centre. Unfortunately, tissue was not preserved for detailed histological analysis, and the hippocampal formation could not be identified in the resection specimen. The resected cortex showed moderate subpial and white matter gliosis. The patient remains seizure free 26 months after the operation but suffered a significant verbal memory decline. Patient #17 had an ipsilateral (right) decrease of FMZ binding in hippocampus and amygdala. A decrease in contralateral hippocampal FMZ binding was less marked in terms of both Z score and spatial extent. Pathology showed amygdala gliosis (Figure 3.5.5c, d, p. 253) and mild end folium sclerosis (Figure 3.5.5e). Immunohistochemistry with neuronal nuclear antigen (NeuN) and quantification showed low TLWM neuronal density (Figure 3.5.5b) at 1010/mm^3 (control mean 1660±772mm^3 (Thom et al., 2001)). The patient has been seizure free for 4 months after the operation but recently had a single seizure within two weeks of starting fluoxetine. Patient #18 had no ipsilateral (right) abnormalities on FMZ PET but contralateral TLWM increases of FMZ binding. On pathology, the right hippocampus was not fully represented, but parts of CA1, CA4 and subiculum were available. There was mild gliosis but no evidence to support classical hippocampal sclerosis. TLWM neuronal density on quantitative immunohistochemistry with NeuN was moderately high (Figure 3.5.5a) at 2368/mm^3. The patient currently remains seizure free 4 months after the operation.
One patient (#7) with bilateral hippocampal FMZ-V$_d$ decreases, more marked on the left, has been evaluated with bilateral hippocampal depth electrodes. Nine seizures were recorded; five started within the right and four within the left hippocampus.

**Figure 3.5.5: Histological results.** (a) White matter neurons labelled with neuronal marker NeuN in subject 18 (a) and subject 17 (b) which showed a lower number of both large and small neurons in a similar size field in (b) than (a). (c) Luxol Fast Blue stained section of the amygdala resection from subject 17 shows some preservation of neurons, but marked diffuse gliosis is seen in the adjacent GFAP immuno-stained section (d). (e) GFAP immuno-staining in subject 17 also demonstrated marked gliosis in the end folium (lower half of the field) extending into the granule cell layer and compatible with the diagnosis of end folium sclerosis. Bars = 50 microns.
3.5.5 Discussion

Patients with TLE and normal high resolution MRI represent a challenge in epilepsy surgery referral centres. This is the largest study of such a group so far and the first to explicitly examine white matter changes in FMZ-V_d in these patients. The main novel finding is the high proportion (61%) of patients with increased white matter FMZ binding.

In total, we found abnormalities in 16/18 patients with TLE and normal high resolution MRI which included volumetric hippocampal studies and HCT2 quantitation, confirming that [¹¹C] FMZ PET detects functional abnormalities over and above structural abnormalities revealed by optimal MRI (Koepp et al., 2000; Koepp et al., 1997c). Moreover, in this study the abnormalities found were surgically relevant in the six patients with unilateral or asymmetric bilateral decreases of hippocampal FMZ-V_d and in the one patient with a single temporal neocortical decrease.

3.5.5.1 Methodological considerations; comparison with previous findings

SPM is a voxel-based approach that examines the entire 3D brain volume dataset and has been validated for the interpretation of ligand PET scans in epilepsy (Koepp et al., 2000; Koepp et al., 1996; Richardson et al., 1997a; Richardson et al., 1998b; Richardson et al., 1996). While SPM localises receptor binding abnormalities, it cannot differentiate between structural and functional abnormalities. The effect of anatomical abnormalities can be accounted for using both voxel-based (Richardson et al., 1997a) and region-based analyses (Hammers et al., 2001a; Koepp et al., 2000; Koepp et al., 1997a). In this study, we only included patients whose MRI was normal on inspection and in whom quantitative analysis of the hippocampi, including volumetry and T2 mapping, was entirely normal. In this situation, SPM is superior to region-based analyses as the entire brain volume can be studied and no assumptions about number, localisation and extent of abnormalities need to be made. Interpolation and smoothing necessarily decrease the spatial resolution of the resulting statistical map to about 13 mm FWHM. In contrast, the final spatial resolution of region-based analyses depends on the number and size of regions chosen (typically 10-50 for a given brain imaging data set). SPM examines the data at our level of smoothing with
about 500 independent comparisons and without *a priori* hypotheses about the exact localisation of neocortical abnormalities.

SPM does, however, need rigorous correction for the multiple comparisons made to avoid spurious false positives. If such a correction is applied to the entire volume, mesial temporal abnormalities, which are small in extent, may remain undetected. For mesial temporal structures (hippocampus and amygdala) and temporal white matter, we had *a priori* hypotheses. Within this defined small search volume (approximately 4 resolution elements), we did not correct for the spatial extent of the clusters of abnormal FMZ binding, but only for height, as in our previous studies (Hammers et al., 2001a; Koepp et al., 2000; Koepp et al., 1997a; Koepp et al., 1997c). The validity of this approach was tested by investigating each control against the remaining 20 controls, with no mesial temporal false positives detected. This rigorous exclusion of detection of artefactual increases or decreases amounts to a random effect analysis on our normal material.

In a previous study, we compared 10 different patients with normal quantitative MRI whose scans were acquired in temporal lobe orientation with 13 different controls acquired in temporal lobe orientation, using both an earlier version of SPM (SPM96) and partial volume effect correction (Koepp et al., 2000). Although FMZ-\(V_d\) abnormalities were shown in eight of 10 patients, unilateral decreases were concordant with EEG data in only two, and most TLWM was excluded from the analysis by the use of standard thresholding methods.

The larger control group in this study makes the analysis more sensitive. Further, the process of spatial normalisation has been much improved in SPM99 (Ashburner and Friston, 1997; Ashburner and Friston, 1999). Better registration, particularly of the small mesial temporal structures, contributes to increased sensitivity and specificity. We previously found a strong correlation (\(r>0.8, p<0.001\)) between preoperative white matter FMZ-\(V_d\) in both temporal lobes and the WM neuron number determined in the resected ipsilateral specimen of patients with hippocampal sclerosis (Hammers et al., 2001a). Increased TLWM neuron number is a hallmark of microdysgenesis. In this study, we investigated the hypothesis that microdysgenesis, manifesting itself as increased TLWM FMZ-\(V_d\), could underlie some of the cases of TLE and normal MRI. SPM was originally devised for the study of grey matter, and thresholding normally excludes white matter from the analysis. Lowering this
blanket threshold to include white matter signal gives inconsistent results due to the interindividual variability in global binding and increases the number of voxels included in the analysis, thereby accentuating the multiple comparisons problem. We, therefore, created an anatomical mask in template space, encompassing the grey matter in the cortex and basal ganglia as well as central white matter. This enabled us to explicitly study, for the first time, white matter changes while restricting the number of voxels inspected. There is a theoretical concern that the error distribution could become positively skewed in areas of low binding due to the non-negativity constraint. The actual values derived from ROI studies, however, indicate that mean values were more than 10 standard deviations away from zero in the TLWM, and the error distribution can therefore be approximated by parametric measures of variability.

3.5.5.2 Neurobiological considerations

There is now extensive evidence that decreased FMZ-binding can localise the epileptogenic focus both in mesial TLE and in neocortical epilepsy (Henry et al., 1993a; Koepp et al., 1996; Koepp et al., 1997c; Muzik et al., 2000; Ryvlin et al., 1998; Savic et al., 1993; Savic et al., 1988; Savic et al., 1995; Szelies et al., 1996).

In earlier studies that included both patients with hippocampal sclerosis and patients with normal MRI, there was a clear negative correlation between hippocampal volume and FMZ binding (Henry et al., 1993a; Lamusuo et al., 2000). Hippocampal FMZ binding is, however, reduced over and above volume loss in hippocampal sclerosis (Hammers et al., 2001a; Henry et al., 1993a; Koepp et al., 1997a; Koepp et al., 1997c), indicating functional abnormalities over and above evident structural abnormalities. Our results suggest that the same is true for TLE with normal quantitative MRI: Both the group of patients and 6/18 individual patients showed significant hippocampal FMZ-Vd decreases despite normal quantitative MRI. Moreover, there was no correlation between hippocampal volume and hippocampal FMZ-Vd, further indicating that our results were not due to atrophy or partial volume effect.

FMZ binds with high affinity to the benzodiazepine binding site of GABA_A receptors containing α1, α2, α3 and α5 subunits, and our study can, therefore, only look at the sum of effects of changes in these subunits. TLE is associated not only with changes in GABA_A receptor number but also with differential changes in subunit composition (Brooks-Kayal et al., 1998; Loup et al., 2000; Sperk et al., 1998) which may precede
the development of epilepsy in rats (Brooks-Kayal et al., 1998). With the development of subunit-specific tracers, it may be possible in the future to study epileptogenesis in vivo.

We found increases of FMZ-\(V_d\) in the TLWM in 11/18 patients with TLE and normal MRI. We suggest that these are due to microdysgenesis, i.e. an increased number of neurons in the white matter. There is a large body of direct and indirect evidence to support this claim: (i) We have previously shown a strong correlation between TLWM FMZ-\(V_d\) and histologically determined neuron number in the white matter in patients with HS (Hammers et al., 2001a) (see chapter 3.3, p. 200). (ii) In the three patients (#15, 17, and 18) in this present series, where TLWM was available for pathology, the prediction about presence or absence of heterotopic neurons based on FMZ PET was confirmed, and quantified immunohistochemically with NeuN in two cases (#17 and 18), directly validating our results. (iii) FMZ-\(V_d\) increases were frequently seen in malformations of cortical development (Richardson et al., 1997a; Richardson et al., 1996) but not in acquired lesions (Richardson et al., 1998b). (iv) Histopathological studies of human MCDs have frequently shown increased WM neuron numbers (Battaglia et al., 1996; Burdette et al., 1995; Hannan et al., 1999; Spreafico et al., 1998).

Theoretically, an increase in TLWM FMZ binding could be due to an apparent, relative rather than absolute increase in heterotopic neuronal density due to atrophy. We do not believe this can explain our results for the following reasons. (i) After the electroclinical diagnosis of TLE was made, all MRIs were carefully reviewed with particular attention to the temporal lobes. No abnormality was found on inspection by experienced neuroradiologists, in both the quantitative volumetric studies and in the T2 quantification studies of the hippocampi. The MRIs can therefore be regarded as normal by best current standards (Commission on Neuroimaging of the International League Against Epilepsy, 1997). (ii) Even minimal temporal lobe atrophy will be detected by SPM as a widespread decrease of FMZ binding following the temporal neocortex (Hammers et al., 2001a), and this was not seen in this study. (iii) Neuronal density in the white matter does not correlate with three-dimensional volume of the white matter (Bothwell et al., 2001) or white matter gliosis (Kasper et al., 1999; Thom et al., 2001).

FMZ-PET is well suited to reflect abnormal neuronal density in WM in vivo. Most neurons express GABA\(_A\) receptors, and FMZ can, therefore, be regarded to a certain
extent as a neuronal marker. Whereas clusters of heterotopic neurons need to be at least 1mm\(^3\) in size to be evident on MRI, the signal-to-noise-ratio for heterotopic neurons in the WM is very high for PET. The highly specific nature of FMZ binding makes it very improbable that these findings are functional in nature, in contrast to some abnormalities in grey matter FMZ binding (Ryvlin et al., 1999) or on metabolic ([\( ^{18}\)F]-fluorodeoxyglucose PET) imaging.

Heterotopic WM neurons can contribute to epileptogenesis by providing additional or abnormal circuitry. This has been shown experimentally for heterotopic CA1 neurons in the rat model of methylazoxymethanol-(MAM-) induced cortical malformations (Chevassus-au-Louis et al., 1998). The finding of heterotopic WM neurons in TLE with normal MRI can, therefore, be a reasonable explanation for the pathogenesis of epileptic circuits in the face of normal structural imaging studies. The relevance of our findings for TLE is further underlined by the fact that 11/18 patients but 0/21 controls showed TLWM increases (\( \chi^2 \)-test: p<0.0001).

Taken together as a group, the patients showed significant increases of FMZ-V\(_d\) in the FLWM bilaterally. Widespread disturbances of cerebral structure have been demonstrated in malformations of cortical development (e.g. (Richardson et al., 1997a)). The most tenable explanation for these white matter increases of FMZ-V\(_d\) is again the presence of heterotopic white matter neurons. We do not, however, have histological material to prove this. Moreover, in the individual analysis, FLWM increases of FMZ-V\(_d\) in a localisation closely related to the group finding were only found in one patient with very widespread white matter increases (patient #10). It would be reasonable to assume that a diffuse disturbance of migration can manifest itself principally in the TLWM but have some smaller effect elsewhere. Frontal lobe white matter contains approximately six times fewer neurons in controls than TLWM (Rojiani et al., 1996), and our finding is compatible with a small effect size which reaches significance in SPM due to its large spatial extent and the large number of subjects studied.

### 3.5.5.3 Clinical considerations

Patients with normal or nondiagnostic MRI and medically refractory TLE represent an important and difficult subgroup of patients who undergo investigations for epilepsy surgery. Accordingly, there have been various studies using FMZ-PET centered on or including "MRI-negative" TLE patients (Debets et al., 1997; Henry et al., 1993a; Koepp et al., 2000; Lamusuo et al., 2000; Ryvlin et al., 1998; Savic et al., 2000;...
1993; Szclies et al., 1996). In most earlier studies, however, MRI was only assessed qualitatively for signs of hippocampal sclerosis, and quantitative hippocampal MRI data was only available in three studies (Koepp et al., 2000; Lamusuo et al., 2000; Ryvlin et al., 1998). Also, most of the earlier studies relied on measures of asymmetry for both hippocampal volumes and FMZ binding and could not therefore detect bilateral abnormalities.

In our series, amongst other findings, there were patients with hippocampal decreases of FMZ binding, most likely representing hippocampal damage, subjects with TLWM increases, most likely representing microdysgenesis, and subjects with both. This heterogeneity is hardly surprising as the group of "MRI-negative" patients will include patients with any pathology not detectable with current optimal MRI. Both hippocampal decreases and white matter increases in FMZ binding were detected in the group comparison despite subject heterogeneity. We, therefore, believe that both are common mechanisms in MRI-negative TLE.

Our decision to include patients was based on the electroclinical diagnosis of temporal lobe epilepsy. Ictal video-EEG was not obtained in seven, but we do not believe that overemphasis should be placed on ictal EEG in this situation. A case in point is patient #7 (Table 3.5.1, p. 246) who would have been misclassified as having right temporal lobe epilepsy on the basis of four ictal surface EEG recordings but was correctly classified as having bitemporal foci on the basis of the remainder of the electroclinical data. FMZ PET subsequently showed bilateral mesial temporal decreases of FMZ-Vd, and depth recordings confirmed the bilateral independent onset of seizures. In suspected bitemporal epilepsy, FMZ-PET may, therefore, help to confirm the bilateral origin (Ryvlin et al., 1998).

In our series, three patients (#8, 17, and 18) have undergone modified anterior temporal lobe resections to date. Two (#8 and 18) are currently seizure free 26 and 4 months after the operation, respectively, and one (#17) has had a single seizure within two weeks of starting fluoxetine therapy after four months without seizures, compared with six seizures/month before the operation. Despite the short follow-up in the latter two cases, this suggests that the decrease in hippocampal FMZ-Vd detected by SPM indeed reflected the epileptic focus.

Two of the operated cases (patients #8 and 17) had ipsilateral hippocampal decreases of FMZ-Vd, accompanied by amygdala decreases in the case of patient #17. For the patient operated in our centre (#17), pathology was available and showed amygdala
sclerosis as well as mild hippocampal end folium sclerosis, confirming that end folium sclerosis and amygdala sclerosis may be associated with decreases of FMZ-V_d, even if high resolution MRI is unremarkable.

In two of the operated cases (patients #17 and 18), sufficient amounts of TLWM were available to quantify TLWM neuronal density immunohistochemically with NeuN, a specific neuronal marker, as described recently (Thom et al., 2001). In patient #17, no TLWM increases were detected on FMZ PET, and immunohistochemistry confirmed low neuronal densities (1010/mm^3). In patient #18, contralateral but not ipsilateral increases of TLWM FMZ binding were seen. Immunohistochemistry showed ipsilateral neuronal densities in the high normal range (2368/mm^3). Ipsilateral high normal neuronal densities are compatible with the concept of microdysgenesis as a diffuse process, while the fact that they were not significantly elevated corroborates our PET finding.

Another patient (#15) with a small area of significant FMZ-V_d decrease in the ipsilateral temporal neocortex and bilateral TLWM FMZ-V_d increases was operated upon and found to have a malformative lesion similar to a Taylor type focal cortical dysplasia (Taylor et al., 1971). Pathology showed abnormal white matter neurons although the amount of resected white matter was not sufficient to quantify them. He remains seizure free 25 months after the operation. This case confirms that focal cortical malformations may be associated with decreases of FMZ-V_d, even if MRI is unremarkable, and represents further evidence for the pathological equivalent of increases of FMZ-V_d detected in vivo. Further, besides the confirmation that malformations may indeed be associated with white matter increases of FMZ-V_d, in this single case at least, bilateral white matter changes did not seem to adversely affect the prognosis after surgery in the limited duration of available follow-up.

This would be in keeping with an early study of temporal lobe surgery, before MRI diagnosis of hippocampal sclerosis in vivo became widely available (Hardiman et al., 1988). 50 patients were treated with anterior temporal lobe resections, sparing the hippocampus and the amygdala. Severe neuronal ectopia was present in 42% of patients, and 28% showed neuronal clustering. Interestingly, both were predictive of good clinical outcome. The rate of seizure free patients, 52%, was lower than in contemporary series with removal of the hippocampus and amygdala. The study does, however, raise the intriguing possibility that in some cases with microdysgenesis, resection of heterotopic WM neurons may be sufficient to achieve
freedom from seizures and suggests the possibility that seizures may arise from epileptic networks that involve WM.

A caveat when considering patients with bilateral TLWM increases are reports of worse postoperative outcome after surgery in patients with intractable temporal lobe epilepsy (mostly found to have hippocampal sclerosis) when there were high numbers of white matter neurons (Kasper et al., 1999) and our own finding of a positive correlation between increased FMZ-Vd in the TLWM contralateral to the lobe resected for hippocampal sclerosis with outcome poorer than Engel class IA (Hammers et al., 2001a).

In contrast, Choi et al. found a better surgical outcome for hippocampal sclerosis patients with MRI detectable TLWM changes which correlated with heterotopic neuron number (Choi et al., 1999). The groups were, however, defined through the MRI findings before WM neurons were counted, and there may be a contamination with the fact that patients with more damage tend to have a better outcome (Jack et al., 1992). Finally, Thom et al. used immunohistochemistry and a rigorous quantitative approach using three dimensional cell counting methods in 31 patients with MRI-diagnosed and pathologically confirmed hippocampal sclerosis (Thom et al., 2001). The 17/26 patients with sufficient follow-up who became seizure free had significantly higher TLWM neuronal densities and other microdysgenetic features than the 9/26 who did not. As with all surgical studies, only tissue ipsilateral to the hippocampal sclerosis could be studied.

These findings need not be contradictory. It is conceivable that microdysgenesis may sometimes be due to generalised disturbances of neuronal migration or cell death. In these cases heterotopic neurons are merely a marker of more widespread disturbance, and consequently, prognosis may be adversely affected (Hammers et al., 2001a). In other cases, however, when migration or cell death are disturbed only locally, surgical interruption of abnormal circuits involving heterotopic neurons (Chevassus-au-Louis et al., 1998) may be sufficient to achieve seizure freedom, and in these cases microdysgenesis may indicate a better prognosis (Choi et al., 1999; Hardiman et al., 1988; Thom et al., 2001).

In summary, we have optimised the analysis of FMZ-PET scans by adapting SPM99 to the analysis of grey and white matter changes in TLE with normal high resolution and quantitative MRI. We were able to increase the yield of both noninvasively
detected abnormalities and surgically relevant abnormalities. Inclusion of the white matter in the analysis provides new and interesting insights into the pathophysiology of "MRI-negative" TLE. FMZ-PET provides useful information in a significant proportion of potential surgical candidates.
3.6  Gray and white matter flumazenil binding in neocortical epilepsy with normal MRI. A PET study of 44 patients.

3.6.1  Summary
In 20-30% of potential surgical candidates with refractory focal epilepsy, standard MRI does not identify the cause. GABA is the principal inhibitory neurotransmitter in the brain. $[^{11}	ext{C}]$ flumazenil (FMZ) PET images the GABA$_A$ receptor, present on most neurons. We investigated GABA$_A$ receptor binding in gray and white matter in 16 normal controls and in 44 patients with refractory neocortical epilepsy and normal MRI. Fourteen patients had unilateral frontal lobe epilepsy, five occipital lobe epilepsy (OLE), six parietal lobe epilepsy (PLE), and 19 neocortical epilepsy that was not clearly lobar. Parametric images of FMZ-volume-of-distribution (FMZ-$V_d$) were calculated. Statistical parametric mapping (SPM99) with explicit masking, including the white matter, was used to analyze individual patients and groups. Thirty-three of the 44 patients showed focal abnormal FMZ-$V_d$; increases in 16, decreases in eight and both in nine. In seven patients, the increases in FMZ binding were periventricular, in locations normally seen in periventricular nodular heterotopia on MRI. There were frontal and parietal increases in gray and white matter in the PLE group and decreases in the cingulate gyrus in the OLE group. FMZ binding increases, particularly periventricular increases, were a prominent feature of MRI negative partial epilepsies and may represent microdysgenesis and/or migration disturbances.

(Gray and white matter flumazenil binding in neocortical epilepsy with normal MRI. A PET study of 44 patients. Hammers A, Koepp MJ, Richardson MP, Hurlemann R, Brooks DJ, Duncan JS. Submitted to Brain, 08/2001)

3.6.2  Introduction
In patients with partial seizures whose seizures have not been suppressed by optimal doses of two standard antiepileptic drugs (AEDs), the chance of becoming seizure free with medication is less than 5% (Kwan and Brodie, 2000). In patients with medically refractory partial seizures, surgery offers the possibility of a cure if the epileptic focus can be defined. Advances in MRI have allowed the identification of
structural abnormalities that are presumed to be the seizure focus in 70-80% (Duncan, 1997a). The remaining 20-30% in whom no lesion is identified by high quality MRI present a particular challenge to epilepsy surgery centers. Surgery has a less favorable outcome when the presumed epileptogenic region is removed in the absence of identifiable pathology on imaging or the resected specimen (Berkovic et al., 1995; Jack et al., 1992). MRI may be normal even when histopathological examination of resected specimens detects focal cortical dysplasia, hippocampal sclerosis or other pathologies (Chugani et al., 1990; Desbiens et al., 1993; Kuzniecky et al., 1991; Van Paesschen et al., 1997).

γ-aminobutyric acid (GABA) is the principal inhibitory neurotransmitter in the brain, acting at the GABA_A receptor. Flumazenil (FMZ) is a specific, reversibly bound high affinity neutral antagonist at the benzodiazepine site of GABA_A receptors (Olsen et al., 1990) containing the α subunits 1,2,3, or 5. [1^1C] FMZ PET provides a useful in vivo marker of GABA_A receptor binding (Maziere et al., 1984).

Epileptogenic foci have generally been reported to exhibit decreased [1^1C] FMZ binding (Henry et al., 1993a; Koepp et al., 2000; Koepp et al., 1997a; Muzik et al., 2000; Richardson et al., 1998b; Ryvlin et al., 1998; Savic et al., 1993; Savic et al., 1988; Savic et al., 1995; Szelies et al., 1996). Some studies, however, have found localized increases of the number or affinity of GABA_A receptors (Brooks-Kayal et al., 1998; Hand et al., 1997), and it is also conceivable that areas of increased [1^1C] FMZ-V_d mark the epileptogenic zone in some forms of focal epilepsy. In vivo, we have previously shown that malformations of cortical development (MCD) are frequently associated with increases of FMZ-V_d (Richardson et al., 1997a; Richardson et al., 1996), whereas no increases of FMZ binding were seen in acquired lesions (Richardson et al., 1998b).

MCD are increasingly recognized as underlying medically intractable epilepsy (Kuzniecky and Jackson, 1997). Microdysgenesis, a minimal form of MCD, is not detectable on current MRI and is usually defined as an increased density of heterotopic neurons in the stratum molecular or the white matter (WM) (Raymond et al., 1995). Some WM neurons are found in healthy controls, and the distinction is quantitative (Emery et al., 1997; Hardiman et al., 1988; Kasper et al., 1999; Thom et al., 2001) with very marked regional variation (Rojiani et al., 1996). On the other side of the spectrum, when a critical density and extent are reached, heterotopic neurons can be identified macroscopically or by MRI as heterotopic gray matter.
(Raymond et al., 1995), whereas microdysgenesis is, by definition, not visible macroscopically.

We have recently demonstrated a strong and highly significant correlation between in vivo temporal lobe white matter (TLWM) FMZ binding and WM neuron number, determined semiquantitatively ex vivo, in patients with HS who underwent anterior temporal lobe resection (Hammers et al., 2001a) and similar increases in TLE with normal MRI (Hammers et al., 2001b) (see chapter 3.5, p. 238).

The aims of the current study were:
1. To determine the ability of quantitative FMZ-PET to localize abnormalities in a large series of patients with medically refractory focal epilepsies and normal, high resolution MRI.
2. To investigate WM FMZ binding in these patients as a marker of heterotopic WM neurons.
3. To assess common abnormalities by making comparisons of homogenous groups of subjects.

3.6.3 Materials and Methods

3.6.3.1 Patients and Controls

We studied 44 patients (18 women) with medically refractory focal epilepsy. They had either unilateral frontal lobe epilepsy (FLE, n=14; numbered F1-F14), parietal lobe epilepsy (PLE, n=6; numbered P1-P6) or occipital lobe epilepsy (OLE, n=5; numbered O1-O5). The remainder (n=19; numbered X1-X19) were classified as neocortical epilepsies without clear lobar origin. Diagnosis was based on seizure semiology, prolonged interictal EEG in all, and ictal Video-EEG findings in 31/44 patients (Table 3.6.1, p. 272). They were recruited as described in Chapter 2.1 (p. 144).

The median age at onset of habitual seizures was 10 years (range: 1-34), the median duration of epilepsy before the PET examination was 17 years (range: 4-58 years), and the median age at PET examination was 26 years (range: 18-61 years). None had a history of prolonged febrile convulsions.

Patients had a median of 96 complex partial seizures per year (range: 2-1100). The antiepileptic medication was carbamazepine (32 patients), lamotrigine (13), gabapentin (8), phenytoin (8), sodium valproate (7), topiramate (6), remacemide (2),
and one each was treated with vigabatrin, oxcarbazepine and tiagabine. One patient was treated with a vagal nerve stimulator. Two were on no medication, 12 on monotherapy, 26 on two antiepileptic drugs, and five on three.

Sixteen healthy volunteers (four women) were studied for comparison. The median age at examination was 46 years (range: 26-61 years). They had no history of neurological or psychiatric disorder and were on no medication.

Clinical data for all 44 patients are shown in Table 3.6.1 (p. 272). So far, one patient has undergone epilepsy surgery (patient #F11).

3.6.3.2 PET

We used the same acquisition technique as described previously (Hammers et al., 2002) (see chapter 3.1, p. 156).

3.6.3.3 MRI

MRIs were obtained as described previously (see chapter 3.5.3.3, p. 243), with the exception of hippocampal volumes and hippocampal T2 which were only obtained in those patients in whom a temporal origin of their seizures was considered possible.

3.6.3.4 PET image analysis

\[^{11}C\] FMZ-V\(_d\) images were analyzed within the framework of statistical parametric mapping as described previously (see chapter 3.5.3.4, p. 243).

3.6.3.5 Statistical analysis

Significant differences between patients and control subjects were estimated according to the general linear model at each and every voxel of the normalized and smoothed images (Friston et al., 1995b) as described previously (see chapter 3.5.3.5, p. 244), with the following differences:

Each control was compared against the remaining 15 controls, using the same design matrices as for the patients, with the design matrix designating global cerebral FMZ-V\(_d\) differences and age as nuisance covariates (Friston et al., 1990). The same type of analysis was performed for the post hoc analysis of the magnitude of the difference of FMZ binding between patients and controls in the periventricular area (see below). Individual patients were then compared with the 16 normal control subjects For the purposes of between-group statistical analyses, the \[^{11}C\] FMZ-V\(_d\) images of patients with unilateral lobar epilepsy were reversed before spatial manipulation so that the
focus was on the same side in all patients. The datasets of patients with seizures arising from the side most frequently affected within each group were left unflipped so that the smallest possible number of datasets needed reversing.

Linear contrasts were used to test the hypotheses for specific focal effects. The resulting set of voxel values for each contrast constitutes a statistical parametric map of the t statistic SPM $\{t\}$. The SPM $\{t\}$ were thresholded at $p=0.001$ uncorrected. The significance of foci of relative FMZ-V$_d$ changes is estimated using Random Field Theory, correcting for multiple comparisons using the number of resolution elements (resels) in the statistical image (Worsley et al., 1992; Worsley et al., 1996). This examines the probability that the observed cluster of voxels could have occurred by chance, given its extent and peak height. The threshold chosen for the corrected cluster $p$ values was $p<0.05$. No extent thresholding was applied. This is referred to as "conventional threshold" in the results and discussion sections.

Initial exploration of the data revealed significant periventricular increases in a localization typical for subependymal periventricular nodular heterotopia (Dubeau et al., 1995; Raymond et al., 1994b) in a number of patients. In a post hoc analysis, therefore, we examined periventricular areas in all patients and controls with a mask encompassing approximately 5mm of tissue around the ventricular system as obtained from segmentation of the MNI T1 MRI template. We used the framework of SPM to search for effect sizes exceeding 2.5SD of the control mean in a precisely defined search volume, as we were testing a specific hypothesis in a restricted area (Hammers et al., 2001a; Koepp et al., 2000).

Age at onset of epilepsy, duration of epilepsy, frequency of seizures, and interval between scan and last seizure were defined as covariates of interest and tested separately for their effect on FMZ-V$_d$. Age was included as a nuisance variable in the design matrix in all comparisons.

The aim was to localise abnormalities of FMZ-V$_d$ in individual patients, compared to the control group, and between the control and patient groups, in both gray and white matter. We, therefore, created an anatomical mask in template space, encompassing the gray matter in the cortex, the basal ganglia and the white matter, to include all these areas in the statistical analysis (Figure 3.6.1, below).
To exclude the possibility that the overall composition of the control group could influence results, we reanalysed one of the patients (#F4) with periventricular increases of FMZ binding, substituting the current control group with a different one that has been used in a study of temporal lobe epilepsy (Hammers et al., 2001b). This second control group had been scanned in the same orientation as the current control group but centered on the temporal lobe. Therefore, the vertex was not always included in the field of view, and it could not be used for this study. The periventricular areas, however, were present in the center of the field of view in all cases, allowing the verification of the finding of periventricular increases of FMZ binding.

In the biggest homogenous group (unilateral FLE), with 7 right sided and 7 left sided cases, the group analysis was repeated after flipping the other half of datasets to ensure that the reversing and subsequent normalisation to our symmetrical template had no, or only a minor, influence on the results. The same procedure was performed in the PLE group with three patients having a right sided and three patients having a left sided seizure focus.

To exclude the possibility that our findings were due to normalisation artifacts, we performed a ROI analysis on the raw, unnormalised parametric Vd map of a patient who had shown periventricular increases of FMZ binding (patient #X4). Circular ROIs sampling approximately 2000mm³ were placed on three adjacent slices in the appropriate periventricular areas in all 16 controls and the patient, and FMZ Vd values measured directly.
Table 3.6.1: Clinical, EEG and imaging data in 44 patients with refractory partial epilepsy and normal MRI

<table>
<thead>
<tr>
<th>Pat. No</th>
<th>Age (yr)/ Sex</th>
<th>Duration (yr)</th>
<th>Interval last CPS to PET</th>
<th>EEG (Inter-ictal)</th>
<th>EEG (Ictal)</th>
<th>Seizures</th>
<th>Probable focus localization</th>
<th>FMZ-PET: increases</th>
<th>FMZ-PET: decreases</th>
<th>FMZ-PET: PV WM increases &gt;2.5 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>36/F</td>
<td>7/29</td>
<td>360 16h</td>
<td>PHT</td>
<td>Generalized attenuation only</td>
<td>R FL</td>
<td>L inferior frontal G</td>
<td>NS</td>
<td>bilateral central/posterior</td>
<td></td>
</tr>
<tr>
<td>F2</td>
<td>22/M</td>
<td>1/21</td>
<td>36 42d</td>
<td>CBZ/PH/ VPA</td>
<td>Continuous R mid-front slow, occ sharp activity in same area</td>
<td>none</td>
<td>R FL</td>
<td>NS</td>
<td>L lat. middle frontal G, L medial occipital G</td>
<td>none</td>
</tr>
<tr>
<td>F3</td>
<td>21/M</td>
<td>4/17</td>
<td>480 16h</td>
<td>CBZ/GBP</td>
<td>L central sylvian high amplitude fast and spike activity</td>
<td>L FL</td>
<td>L central sylvian high amplitude fast and spike activity at seizure onset</td>
<td>L FL</td>
<td>NS</td>
<td>bilateral widespread with anterior emphasis</td>
</tr>
<tr>
<td>F4</td>
<td>18/F</td>
<td>7/11</td>
<td>24 21d</td>
<td>LTG</td>
<td>Bilateral anteriorly predominant nonspecific abnormalities, more localized sharp waves in L frontal region</td>
<td>none</td>
<td>L FL</td>
<td>L central PV ext to L anterior PV, L superior PL, L paracentral lobule, R anterior PV ext to central PV</td>
<td>R FL middle frontal G, R lateral inferior TL widespread, L inferior posterior TL, L middle occipital G</td>
<td>bilateral widespread with anterior emphasis</td>
</tr>
<tr>
<td>F5</td>
<td>26/M</td>
<td>5/21</td>
<td>480 2h</td>
<td>CBZ/PH/ REM</td>
<td>Dystonic asymmetrical arm posturing, head looks down</td>
<td>R front slow and sharp</td>
<td>R frontal fast activity</td>
<td>R FL</td>
<td>superior temporal G, occipitotemporal WM</td>
<td>bilateral posterior and L anterior/central</td>
</tr>
<tr>
<td>F6</td>
<td>28/F</td>
<td>11/17</td>
<td>48 28d</td>
<td>LTG</td>
<td>CPS with head turning to L, wanders around and may run, 2°GTCS</td>
<td>Low amplitude slow and sharp R fronto-temp</td>
<td>no significant change with self-reported attack</td>
<td>R FL</td>
<td>NS</td>
<td>bilateral posterior</td>
</tr>
<tr>
<td>F7</td>
<td>24/F</td>
<td>6/18</td>
<td>144 3d</td>
<td>CBZ/GBP</td>
<td>Tingling in mouth, R arm posturing, evolving to 2°GTCS</td>
<td>L fronto-temporal spikes</td>
<td>generalized attenuation, then rhythmic discharge L fronto-central</td>
<td>L FL</td>
<td>NS</td>
<td>bilateral posterior, L central, R anterior</td>
</tr>
<tr>
<td>F8</td>
<td>18/M</td>
<td>11/7</td>
<td>96 14d</td>
<td>CBZ/PH</td>
<td>Frequent nocturnal, head turns to L, L arm rises</td>
<td>L fronto temporal spikes</td>
<td>none</td>
<td>L FL</td>
<td>R FL, precentral G &gt; L PL, postcentral G, L central PV WM</td>
<td>R medial PL, R middle frontal G, L central &gt; anterior &gt; posterior</td>
</tr>
<tr>
<td>F9</td>
<td>24/F</td>
<td>6/18</td>
<td>360 1.5d</td>
<td>CBZ/GBP</td>
<td>L face postures with L limb movements</td>
<td>irregular widespread rhythmic activity</td>
<td>R FL</td>
<td>R postcentral &gt; precentral G WM</td>
<td>NS</td>
<td>bilateral central</td>
</tr>
<tr>
<td>F10</td>
<td>30/M</td>
<td>6/24</td>
<td>30 28d</td>
<td>LTG/ VPA</td>
<td>Loss of awareness, stretches out, staring ahead, purposeful movements of arms, L leg kicking out</td>
<td>Some delta R&gt;L irregular slow &amp; sharp waves at FP2</td>
<td>R FL</td>
<td>R superior and middle frontal gyri, widespread, ext to L, R precentral G (2 cluster), R middle frontal G WM &gt; GM, L middle precentral G, L anterior cingulate G</td>
<td>R FL, superior and middle frontal gyri, widespread, ext to L, R precentral G (2 cluster), R middle frontal G WM &gt; GM, L middle precentral G, L anterior cingulate G, bilateral medial FL</td>
<td>R superior and bilateral posterior</td>
</tr>
</tbody>
</table>

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Table 3.6.1, continued: Clinical, EEG and imaging data in 44 patients with refractory partial epilepsy and normal MRI

<table>
<thead>
<tr>
<th>Pat. No.</th>
<th>Age (yr)</th>
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<th>Last CPS on PET</th>
<th>Treatment</th>
<th>Seizures</th>
<th>EEG (Inter-ictal)</th>
<th>EEG (Ictal)</th>
<th>Probable focus localization</th>
<th>FMZ-PET: increases</th>
<th>FMZ-PET: decreases</th>
<th>FMZ-PET: PV WM increases &gt;2.5 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>F12</td>
<td>26/M</td>
<td>22/4</td>
<td>72</td>
<td>14d</td>
<td>CBZ</td>
<td>CPS: loss of awareness, fiddling with clothes, standing up/falls, confused</td>
<td>epileptiform activity bitemporal L&gt;R or widespread over L hemisphere in sleep</td>
<td>none</td>
<td>L FL</td>
<td>L. middle frontal G WM/GM</td>
<td>NS</td>
<td>R posterior</td>
</tr>
<tr>
<td>F13</td>
<td>37/M</td>
<td>20/7</td>
<td>96</td>
<td>2d</td>
<td>none</td>
<td>mostly nocturnal, stiffening or thrashing of all 4 limbs</td>
<td>irregular sharp theta bursts with L temporal emphasis, sharp wave trains prominently over vertex and mid-parietal areas</td>
<td>L FL, maximum F3/C3</td>
<td>L FL</td>
<td>NS</td>
<td>NS</td>
<td>R central</td>
</tr>
<tr>
<td>F14</td>
<td>23/M</td>
<td>4/19</td>
<td>540</td>
<td>22h</td>
<td>CBZ/LTG/TPM</td>
<td>CPS with straightening, turning to L, may giggle; tonic seizures; 2 GTCS with R sided Todd’s paresis</td>
<td>frequent bifronto-temporal spike wave activity, more marked over L.</td>
<td>bilateral attenuation, then semi-rhythmic 5Hz activity L anterior area</td>
<td>L FL</td>
<td>R FL</td>
<td>middle frontal G &gt; superior and medial G GM/WM</td>
<td>NS</td>
</tr>
<tr>
<td>P1</td>
<td>37/M</td>
<td>4/33</td>
<td>120</td>
<td>14d</td>
<td>OXC/PHT</td>
<td>L leg sensory SPS evolving to 2 GTCS</td>
<td>Theta more marked R hemisphere</td>
<td>High voltage polyspike/ wave, no clear lat</td>
<td>R PL</td>
<td>NS</td>
<td>L OL WM R OL WM</td>
<td>none</td>
</tr>
<tr>
<td>P2</td>
<td>26/M</td>
<td>6/20</td>
<td>52</td>
<td>2d</td>
<td>CBZ/GBP</td>
<td>numbness or pain R arm, sometimes spreading to R leg, aphasia</td>
<td>non-specific bitemporoparietal abnormalities</td>
<td>none</td>
<td>L PL</td>
<td>L FL anterior superior frontal G L frontal operculum G L superior frontal G GM/WM R middle frontal gyrus GM/WM</td>
<td>NS</td>
<td>L posterior</td>
</tr>
<tr>
<td>P3</td>
<td>55/M</td>
<td>7/48</td>
<td>168</td>
<td>60d</td>
<td>CBZ/PHT</td>
<td>sensation of numbness and twitchiness R arm</td>
<td>L frontoocentromtemporal sharply formed waves, some theta/delta bilaterally</td>
<td>none</td>
<td>R PL</td>
<td>NS</td>
<td>L middle frontal G</td>
<td>none</td>
</tr>
<tr>
<td>P4</td>
<td>19/F</td>
<td>1/18</td>
<td>21</td>
<td>120d</td>
<td>CBZ</td>
<td>paraesthesia and numbness L arm, then jerking L arm</td>
<td>none</td>
<td>none</td>
<td>R PL</td>
<td>NS</td>
<td>R posterior</td>
<td></td>
</tr>
<tr>
<td>P5</td>
<td>27/M</td>
<td>5/22</td>
<td>1095</td>
<td>12h</td>
<td>VPA</td>
<td>SPS: throbbing feeling R arm, shatters</td>
<td>possible attenuation L&gt;R, no clear lateralizing or localizing features</td>
<td>L PL</td>
<td>R postcentral G &gt; L precentral G L middle frontal G R superior PL</td>
<td>NS</td>
<td>R central</td>
<td></td>
</tr>
<tr>
<td>P6</td>
<td>34/F</td>
<td>25/9</td>
<td>100</td>
<td>7d</td>
<td>CBZ/LTG</td>
<td>L sensorimotor SPS, may evolve to 2 GTCS</td>
<td>R sided continuous slow activity + infrequent epileptiform discharges, max frontocentral or posterior temporal/occipital</td>
<td>rhythmic discharge over R hemisphere</td>
<td>R PL</td>
<td>L inferior PL GM/WM R PL/WM ext. to posterior lateral ventricle</td>
<td>R &gt; L posterior, bilateral central</td>
<td></td>
</tr>
<tr>
<td>O1</td>
<td>25/M</td>
<td>8/17</td>
<td>24</td>
<td>7d</td>
<td>CBZ/GBP</td>
<td>SPS with R visual field phenomena, may evolve to 2 GTCS, R-L</td>
<td>Spikes L postero-temporo-occipital region, maximum T5/01.</td>
<td>L post temp/occ repetitive sharp waves</td>
<td>L OL</td>
<td>NS</td>
<td>L medial OL extending to R medial OL (large cluster)</td>
<td>R posterior</td>
</tr>
<tr>
<td>O2</td>
<td>32/M</td>
<td>10/22</td>
<td>7</td>
<td>14d</td>
<td>CBZ/LTG</td>
<td>SPS with bil flashing lights, colored shapes and formed images</td>
<td>fairly frequent bursts of spikes or sharp waves bilateral posteriorly</td>
<td>none</td>
<td>OL ?lateralization</td>
<td>NS</td>
<td>NS</td>
<td>R posterior</td>
</tr>
<tr>
<td>O3</td>
<td>33/M</td>
<td>16/17</td>
<td>12</td>
<td>61d</td>
<td>CBZ</td>
<td>SPS with feeling of brightness and fear, head &amp; eyes to L, blinking eyelids</td>
<td>bursts of theta waves with no consistent side emphasis</td>
<td>none</td>
<td>OL ?lateralization</td>
<td>L inferior PL R middle frontal G</td>
<td>NS</td>
<td>R central</td>
</tr>
<tr>
<td>O4</td>
<td>39/M</td>
<td>34/5</td>
<td>105d</td>
<td>3</td>
<td>CLB</td>
<td>blurred vision, eyelid blinking, sweating forehead</td>
<td>normal</td>
<td>none</td>
<td>OL ?lateralization</td>
<td>NS</td>
<td>NS</td>
<td>L central R posterior</td>
</tr>
<tr>
<td>O5</td>
<td>26/F</td>
<td>19/16</td>
<td>2</td>
<td>90d</td>
<td>TPM</td>
<td>distorted vision L upper quadrant or flashing lights L hemisphere, decreased awareness</td>
<td>intermittent theta, sometimes of sharp morphology, in the temporal regions</td>
<td>none</td>
<td>R OL</td>
<td>NS</td>
<td>L precentral G</td>
<td>R posterior</td>
</tr>
</tbody>
</table>
Table 3.6.1, continued: Clinical, EEG and imaging data in 44 patients with refractory partial epilepsy and normal MRI

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<th>Seizures</th>
<th>EEG (Inter-ictal)</th>
<th>EEG (Ictal)</th>
<th>Probable focus localization</th>
<th>FMZ-PET: increases</th>
<th>FMZ-PET: decreases</th>
<th>FMZ-PET: PV WM increases &gt;2.5 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1</td>
<td>23/M</td>
<td>6/17</td>
<td>480 4h</td>
<td>CBZ/ LTG</td>
<td>R sensory SPS; CPS with L arm automatisms; 2&quot;GTCS</td>
<td>indep L &amp; R post temp spikes</td>
<td>gen atten, late R temp delta in one attack</td>
<td>L &amp; R post temp</td>
<td>NS</td>
<td>bilateral OL</td>
<td>L central and temporal</td>
</tr>
<tr>
<td>X2</td>
<td>26/M</td>
<td>15/11</td>
<td>144 7d</td>
<td>CBZ/ PHT</td>
<td>frequent neck jerking arms &amp; legs. CPS with no clear lateralization</td>
<td>ant dominant runs of sharp delta</td>
<td>diffuse high voltage sharp, then diffuse slow</td>
<td>L &amp; R post temp</td>
<td>NS</td>
<td>L, central PV</td>
<td>L, central &gt; anterior</td>
</tr>
<tr>
<td>X3</td>
<td>18/F</td>
<td>8/10</td>
<td>36 1d</td>
<td>CBZ/ REM</td>
<td>SPS (auditory phenomena); may progress to CPS: head turning to L. Further pattern with R arm dystonia &amp; head turning to R</td>
<td>Bifrontal sharpened slow wave transients; independent sharp/slow wave in frontotemporal regions; runs of semi-rhythmic 3-5Hz independently over both hemispheres (anterior predominance); occasional independent bitemporal spikes</td>
<td>First pattern: R ant temp theta. Second pattern: L post temp sharp</td>
<td>R ant temp and L post temp</td>
<td>NS</td>
<td>L, superior PL</td>
<td>R, central/posterior</td>
</tr>
<tr>
<td>X4</td>
<td>40/M</td>
<td>27/13</td>
<td>48 2d</td>
<td>CBZ/ LTG</td>
<td>CPS: stands, claps hands, rocks back and forward if sitting</td>
<td>Frequent high amplitude sharp waves over both frontal regions</td>
<td>none</td>
<td>FL lateralization</td>
<td>L, superior PL</td>
<td>NS</td>
<td>R central &gt; anterior</td>
</tr>
<tr>
<td>X5</td>
<td>47/M</td>
<td>10/37</td>
<td>72 2d</td>
<td>CBZ/ GBP</td>
<td>CPS with repetitive facial movements, head turning to L; may evolve to 2&quot;GTCS</td>
<td>Spikes during sleep, max. at F8 and R superficial sphenoidal. Rare similar independent runs on L.</td>
<td>Fast activity R posterior temporal occipital (16/02)</td>
<td>R post temp</td>
<td>R superior PL</td>
<td>R GL/FL WM</td>
<td></td>
</tr>
<tr>
<td>X6</td>
<td>39/F</td>
<td>9/30</td>
<td>192 12h</td>
<td>CBZ/ LTG</td>
<td>Mainly nocturnal CPS: arms held rigidly straight in front; may evolve to 2&quot;GTCS</td>
<td>Bil ant slow</td>
<td>Bilateral central rhythmic activity and distorted spikes and sharp. No clear lat.</td>
<td>FL lateralization</td>
<td>NS</td>
<td>NS</td>
<td>R posterior and anterior</td>
</tr>
<tr>
<td>X7</td>
<td>24/M</td>
<td>19/5</td>
<td>32 90d</td>
<td>CBZ</td>
<td>SPS: numbness in mouth, L side of face pulled upwards, drooling; L wrist flexing; may evolve to 2&quot;GTCS</td>
<td>Frequent sharp waves/spike discharges in R centro-temporal region</td>
<td>Artifact; possible slow wave rhythmic discharge and distorted spikes and sharp</td>
<td>R centrotemp</td>
<td>NS</td>
<td>NS</td>
<td>bilateral central</td>
</tr>
<tr>
<td>X8</td>
<td>20/F</td>
<td>11/9</td>
<td>10 7d</td>
<td>CBZ/ VPA</td>
<td>Speech disturbance, stares, may put R hand on chest</td>
<td>predominately bifrontal sharp waves</td>
<td>L &gt; R anterior rhythmic discharge + spikes</td>
<td>Neocortical</td>
<td>NS</td>
<td>R medial posterior PL</td>
<td>R FL/WM</td>
</tr>
<tr>
<td>X9</td>
<td>42/M</td>
<td>33/5</td>
<td>264 1d</td>
<td>GV/ PHT</td>
<td>Mostly nocturnal CPS: walking around or falls, leg/arm movements</td>
<td>7-10 sec after clin onset: semi-rhythmic slow R frontal region, wide field, no clear localization</td>
<td>Neocortical L hemisphere</td>
<td>R, medial orbitofrontal gyr R FL WM/GM between superior and medial frontal G</td>
<td>NS</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>X10</td>
<td>18/F</td>
<td>7/11</td>
<td>12 75d</td>
<td>LTG/ VPA</td>
<td>head -&gt; L, stiffening and posturing of the limbs, nocturnal 2&quot;GTCS</td>
<td>some nonspecific changes in posterior temporal areas</td>
<td>Semi-rhythmic 7-8Hz discharge, maximum R centrotemporal-parietal</td>
<td>Neocortical R hemisphere</td>
<td>L FL WM under inferior frontal G</td>
<td>R medial posterior PL</td>
<td>bilateral central</td>
</tr>
</tbody>
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<th>EEG (Ictal)</th>
<th>Probable Focus Localization</th>
<th>FMZ-PET: increases</th>
<th>FMZ-PET: decreases</th>
<th>FMZ-PET: PVE WM increases &gt; 2.5 SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>X11</td>
<td>19/F</td>
<td>130</td>
<td>48</td>
<td>7d</td>
<td>LTG/TPM</td>
<td>L hand postured, head &amp; eyes to R</td>
<td>spikes and sharp/low wave transients in R posterior temporal region (sleep)</td>
<td>semi-rhythmic slow activity over the R, maximum T6</td>
<td>R posterior temporal/occipital</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>X12</td>
<td>39/F</td>
<td>33/26</td>
<td>1000</td>
<td>1d</td>
<td>CBP/TPM</td>
<td>starts with mv R hand, then irregular thrashing of all limbs</td>
<td>small amplitude sharp waves in both anterior quadrants</td>
<td>movement artifacts, no clear EEG change</td>
<td>FLE % lateralization</td>
<td>R orbitofrontal gyrri L &gt; R medial FL &amp; frontal poles widespread R superior frontal G</td>
<td>L medial posterior FL bilateral posterior and L anterior</td>
</tr>
<tr>
<td>X13</td>
<td>38/F</td>
<td>10/28</td>
<td>220</td>
<td>5d</td>
<td>CBZ/TPM</td>
<td>limb automatisms, vocalization, head turning</td>
<td>normal</td>
<td>bilateral (frontal) rhythmic changes without clear localization</td>
<td>FLE % lateralization</td>
<td>R FL WM/GM between medial and middle frontal G R FL WM/GM between medial and middle frontal G</td>
<td>NS none</td>
</tr>
<tr>
<td>X14</td>
<td>25/F</td>
<td>17/8</td>
<td>1100</td>
<td>2.5h</td>
<td>CRZ/LTG</td>
<td>SPS, CPS: loss of awareness, tearing at clothing, fiddling, confused</td>
<td>widespread independent epileptiform discharges over both temp regions, sometimes more ant</td>
<td>R fronto-centro-temporal</td>
<td>Neocortical R hemisphere</td>
<td>NS</td>
<td>L medial PL and medial posterior TL bilateral posterior + bilateral central</td>
</tr>
<tr>
<td>X15</td>
<td>26/F</td>
<td>3/22</td>
<td>250</td>
<td>4d</td>
<td>CBZ/TGB/TPM</td>
<td>CPS: crying out, fiddling, coughing</td>
<td>predominantly anterior bilateral epileptiform activity, sometimes L sided emphasis</td>
<td>bilateral, start in L hemisphere</td>
<td>L hemisphere</td>
<td>NS</td>
<td>NS R posterior and L central</td>
</tr>
<tr>
<td>X16</td>
<td>37/M</td>
<td>24/13</td>
<td>300</td>
<td>1d</td>
<td>CBZ/VPA</td>
<td>blank stare, head jerking</td>
<td>independent mid-temp spikes and slow</td>
<td>bilateral, initially R fronto-temp-central rhythmic discharge</td>
<td>R hemisphere</td>
<td>L anterior superior frontal gyrus</td>
<td>NS</td>
</tr>
<tr>
<td>X17</td>
<td>24/M</td>
<td>13/11</td>
<td>170</td>
<td>2d</td>
<td>CBZ/LTG</td>
<td>Eyes &amp; head to R, R arm and leg extended and jerking, bimanual automatisms</td>
<td>bilfrontal/generalized semi-rhythmic sharp and slow activity, left sided predominance</td>
<td>generalized attenuation followed by generalized, anterior predominant, semi-rhythmic slow</td>
<td>Neocortical ?L FL</td>
<td>Neocortical ? Localization</td>
<td>NS</td>
</tr>
<tr>
<td>X18</td>
<td>18/M</td>
<td>11/7</td>
<td>150</td>
<td>4d</td>
<td>CBZ/VNS</td>
<td>no warning, loss of awareness, derealization, abnormal space &amp; voice perception, R arm &gt; leg may twitch</td>
<td>bilateral nonspecific abnormalities, bilateral sharp waves/ spikes posteriorly, maximum R centro-temporal</td>
<td>none</td>
<td>Neocortical ? localization</td>
<td>L anterior middle frontal G</td>
<td>NS</td>
</tr>
<tr>
<td>X19</td>
<td>61/M</td>
<td>3/58</td>
<td>12</td>
<td>120d</td>
<td>CBZ</td>
<td>complete amnusor, head thrown back</td>
<td>isolated epileptiform discharges in parietal and central regions bilaterally, R&gt;L, bilateral spike-wave discharge after hyperventilation</td>
<td>none</td>
<td>Neocortical ? Localization</td>
<td>R medial orbitofrontal gyrri R &gt; L, bilateral frontal pole</td>
<td>L medial frontal G L lateral PL R medial PL ext. to R central PV L anterior OL/posterior PL L inferior occipital G L posterior G</td>
</tr>
</tbody>
</table>

Table 3.6.1: Clinical, EEG and imaging data in 44 patients with refractory partial epilepsy and normal MRI. No=Number, sz=seizures, yr(s)=year(s), R=right, L=left, CPS=complex partial seizure(s), 2°GTCS=secondarily generalized tonic-clonic seizures, EEG=electroencephalography, CBZ=carbamazepine, CLB=clobazam, GBP=gabapentin, GVG=Vigabatrin, LTG=lamotrigine, OXC=oxcarbazepine, PHT=phenytoin, REM=Remacemide, TPM=topiramate, VNS=vagus nerve stimulator, VPA=valproic acid, prn=pro re nata, FL=frontal lobe, OL=occipital lobe, PL=parietal lobe, TL=temporal lobe, PV=periventricular, >=more than/ extending to. Increases and decreases of FMZ-Vd, compared with the control group, are sorted by height of Z scores. 272
3.6.4 Results

3.6.4.1 Controls
Comparing each individual control subject against the remaining 15 controls, one individual showed an increase of FMZ-Vd in the left anterior pallidum ($Z=4.55$, $k=407$, $p<0.001$) and another in the left parietal lobe ($Z=4.14$, $k=189$, $p=0.027$). Given the chosen thresholds and the number of comparisons resulting from 16 controls x 2 contrasts, 1.6 positive results would be predicted by chance. Thus, our control group did not contain outliers, and we could verify our expected false positive rate empirically. No control had any significant increases around the ventricles at the conventional threshold.

Comparing each individual control subject's periventricular region against the corresponding area in the remaining 15 at the lower threshold of $p<0.01$ uncorrected for the post hoc analysis, 5/16 showed increases within the periventricular mask.

There was a significant negative correlation between age and FMZ binding in the right middle and superior frontal lobe, the left precentral gyrus and the right parietal lobe, leading to the inclusion of age as a nuisance variable in all tests. There were no significant correlations with gender.

3.6.4.2 Unilateral frontal lobe epilepsy

3.6.4.2.1 Individual patients
(cf. Table 3.6.1, p. 272)
Four patients (#F3, F6, F7, F13) had no significant changes at conventional thresholds. Another four had single areas of increased FMZ binding (#F1, F9, F12, F14); two of these (in patients #F9 and F12) were in the ipsilateral frontal lobe. Two more had multiple areas of increased FMZ-Vd (#F10 and F11); in both these included large areas of the ipsilateral frontal lobe. One patient (#F2) had only areas of decreased FMZ binding; these were located in the contralateral hemisphere. Three patients had multiple areas of increased as well as multiple areas of decreased FMZ-Vd (#F4, F5, F8). In all three, increases included the ipsilateral frontal lobe, and represented the only increases in two of them (#F4, F8). All three had decreases outside the ipsilateral frontal lobe. Three patients (#F4, F5, F8) had clusters of increased FMZ binding around the ventricles at conventional thresholds. The
periventricular locations at conventional thresholds were bilateral central, bilateral anterior and contralateral posterior in one (#F4, Figure 3.6.2, p. 274), contralateral central and posterior in another (#F5) and ipsilateral central in the third (#F8). The post hoc analysis revealed periventricular increases in 13/14 patients, including the four in whom no abnormality had been found previously (see Table 3.6.1, p. 272). The only patient who did not show any periventricular increases was the only one with areas of decreased FMZ-V_d but no areas of increased FMZ-V_d (#F2). The periventricular increases in the post hoc analysis included, alone or in combination, the anterior horn in 8/13, central periventricular areas in 7/13 and the posterior horn in 9/13.

3.6.4.2.2 Group analysis

All 14 patients could be included in the group analysis. Datasets of patients with right sided foci were flipped to the left before normalization. There were no significant changes at conventional thresholds. In the post hoc analysis, ipsilateral frontal lobe white matter increases were seen, extending to the ipsilateral central lateral ventricle.

Figure 3.6.2: Example of a patient (#F4) with increased FMZ-V_d around the posterolateral ventricles bilaterally. Colourscale: Z score (maximum Z=4.87). Statistical results are overlaid onto the patient's coregistered MRI on the left of the figure; the right shows a 3D rendering of the statistical results (in red) overlain onto the patient's ventricles (in white) to show their spatial relationship.
3.6.4.3 Parietal lobe epilepsy

3.6.4.3.1 Individual patients

(cf. Table 3.6.1, p. 272)

All six patients had significant abnormalities at conventional thresholds. Three patients (#P2, P3, P5) had multiple areas with increased FMZ binding. These were bifrontal in all three and included the ipsilateral parietal lobe in two (#P2, P5). Two patients (#P1, P4) showed only areas of decreased FMZ-Va. These were bilateral occipital in one (#P1), and the other one (#P4) had a single area of decrease in the frontal lobe contralateral to the presumed seizure focus. The remaining patient (#P6) had biparietal increases as well as an area of significantly decreased FMZ-Va in the ipsilateral orbitofrontal cortex. This patient also had periventricular increases (Z=4.37, k=435, p=0.001) around the ipsilateral posterosuperior lateral ventricle. These were connected with the ipsilateral parietal increase.

The post hoc analysis revealed periventricular increases in 4/6 patients (see Table 3.6.1, p. 272); only the two patients with areas of decreased FMZ-Va but no areas of increased FMZ-Va did not show any periventricular increases (#P1, P4). The periventricular increases in the post hoc analysis included, alone or in combination, the anterior horn in none, central periventricular areas in 2/4 and the posterior horn in 3/4.

3.6.4.3.2 Group analysis

There were two significant clusters of FMZ-Va increases, in the ipsilateral anterior middle frontal gyrus (Z=4.53, k=356, p=0.005) and in the contralateral angular gyrus (Z=4.12, k=298, p=.012), both at the gray matter/white matter interface. In the post hoc analysis, a small area of increased binding was seen medial to the contralateral ventricle centrally.

3.6.4.4 Occipital lobe epilepsy

3.6.4.4.1 Individual patients

(cf. Table 3.6.1, p. 272)

Two patients (#O2, O4) had no significant abnormalities at conventional thresholds. One patient (#O3), without clear lateralization of her focus, had an area of increased FMZ-Va in one inferior parietal lobe and another one in the frontal lobe on the other
side. Two patients (#01, 05) showed decreases only, one (#01) in the ipsilateral and contralateral medial occipital lobe and the other (#05) in the contralateral precentral gyrus. No patient had periventricular increases at conventional thresholds.

The *post hoc* analysis revealed periventricular increases in all 5/5 patients, including the two in whom no abnormality had been found previously (see Table 3.6.1, p. 272). The periventricular increases in the *post hoc* analysis included, alone or in combination, the anterior horn in one, central periventricular areas in 3/5 and the posterior horn in 4/5.

### 3.6.4.4.2 Group analysis

As only two patients (#01, 05) had clearly lateralized occipital lobe epilepsy, all patients' unflipped normalized and smoothed scans were included in the group analysis. There was one cluster of significantly decreased FMZ-V₄ in the left middle cingulate gyrus (Z4.05, k228, p=0.26). The *post hoc* analysis showed bilateral central and right posterior periventricular increases.

### 3.6.4.5 Neocortical epilepsy, not clearly unilobar

#### 3.6.4.5.1 Individual patients

(cf. Table 3.6.1, p. 272)

Five patients (#X6, X7, X11, X15, X17) had no significant abnormalities at conventional thresholds.

Six had areas of increases FMZ-V₄ only; two had single areas of increased FMZ binding (#X16, X18) and four had more than one area of increased FMZ-V₄ (#X4, X9, X13, X19). The latter included two of the three patients in this group with periventricular increases at conventional thresholds (#X2, X4, X19). Three patients had areas of decreased FMZ binding only; one had a single area of decreased FMZ-V₄ (#X14), and two had more than one area (X1, X8). Five patients showed both increases and decreases (#X2, X3, X5, X10, X12). In total, in nine of the 14 patients, abnormalities included the area of presumed seizure onset.

The *post hoc* analysis revealed periventricular increases in 16/19 patients, including four of the five in whom no abnormality had been found previously (see Table 3.6.1, p. 272). One patient's (#X11) FMZ PET did not show any abnormality at all, and the two others (#X9, X13) had areas of increased FMZ binding elsewhere which included areas of white matter. The periventricular increases in the *post hoc* analysis
included, alone or in combination, the anterior horn in 7/16, central periventricular areas in 14/16 and the posterior horn in 5/16.

3.6.4.6 Correlation with clinical data

There was no correlation between duration of epilepsy, age at onset of seizures, frequency of seizures, interval between last seizure and PET scan and FMZ binding anywhere in the brain.

3.6.4.7 Exclusion of artifacts

3.6.4.7.1 Exclusion of chance effects through composition of the control group

Comparing patient #F4, with significant periventricular increases of FMZ binding at conventional thresholds, against a different control group as described in the methods section showed essentially the same significant clusters around the ventricles, with very similar Z scores, extents and p values. Our finding of periventricular increases was not therefore dependent on the composition of the control group.

3.6.4.7.2 Exclusion of flipping artifacts in the group analyses

In both groups (unilateral FLE and PLE) in which the group analysis was repeated after flipping the other half of the raw images before normalization to our symmetrical template, we obtained the same results. Thus, potential flipping artifacts did not influence our results.

3.6.4.7.3 Exclusion of normalization artifacts through verification with ROI analysis

ROI analysis in patient #X4, with significant periventricular increases of FMZ binding at conventional thresholds in the SPM analysis, showed a significant increase in bilateral periventricular ROIs. This was +114% for the left periventricular area and +102% for the right periventricular area (control mean ± SD ROI value 0.57 ± 0.10, patient ROI value 1.15 ± 0.75 on the right and 1.22 ± 0.5 on the left). Our findings are therefore not due to artifacts arising from the manipulation of datasets for analysis with SPM99 (see also Figure 3.6.3, p. 278).
Figure 3.6.3: Example of an SPM of a patient with unilateral subependymal periventricular nodular heterotopion on MRI (patient #7 from (Hammers et al., 2001c)), using exactly the same technique as in the remainder of the patients in this study. The patient showed the expected ipsilateral (right; left on image) periventricular increase of FMZ-V<sub>d</sub> corresponding to the periventricular nodular heterotopion confirmed on MRI. Furthermore, SPM reveals a contralateral (left; right on image) area of increased periventricular FMZ-V<sub>d</sub> which had no correlate on MRI but corresponds equally well to the appearance of periventricular increases in the current study.

3.6.4.8 Surgical and histological results; outcome

So far, one patient had surgery. Patient #F11 with an electroclinical diagnosis of right frontal lobe epilepsy had multiple areas of increased FMZ-V<sub>d</sub> including the right frontal lobe. Diffusion tensor imaging, analyzed with SPM99 against a control group of 30 subjects, showed an increase in mean diffusivity in the right orbitofrontal WM (Rugg-Gunn et al., 2001a). Depth electrodes in orbitofrontal and inferior lateral frontal cortex demonstrated very localized onset of ictal activity. This area was subsequently surgically removed, and the patient has had a >90% reduction of seizures over the 10 months of postoperative follow-up. Pathology showed marked WM gliosis. One section was selected randomly, sectioned at 20 microns and stained with the neuronal marker NeuN. All NeuN-positive cells, i.e. all neurons, were counted in radial columns in white matter using a three dimensional cell counting technique as described recently (Thom et al., 2001). WM neuronal density was estimated at 1598/mm<sup>3</sup> (see discussion).
3.6.5 Discussion

This is the first study to explicitly examine both gray and white matter changes in FMZ-$V_d$ in patients with neocortical focal epilepsies and normal high resolution MRI. The main novel finding is the high proportion of patients with increased white matter FMZ binding, notably around the ventricles, and we suggest that this finding represents a disorder of neuronal migration which is etiologically and clinically relevant.

In total, we found abnormalities in 33/44 patients with partial epilepsy and normal optimal MRI, confirming that $[^{11}\text{C}]$ FMZ PET detects abnormalities over and above structural MRI-visible abnormalities.

3.6.5.1 Methodological considerations

SPM is a voxel-based analysis technique that examines the entire dataset and has been validated for the interpretation of ligand PET scans in epilepsy (Koepp et al., 2000; Koepp et al., 1996; Richardson et al., 1997a; Richardson et al., 1998b). SPM cannot differentiate between abnormalities due to structural alterations or purely functional abnormalities. Therefore, in this study, we only included patients whose high-quality MRI including various sequences was normal on inspection by two experienced neuroradiologists. In this situation, SPM has advantages over region-based analyses: the entire brain volume can be studied, and no assumptions about number, localization and extent of abnormalities need to be made. A disadvantage is the decrease of the spatial resolution of the resulting statistical map to about 13 mm FWHM, owing to the necessary interpolations following spatial manipulation and the smoothing procedure to accommodate inter-individual anatomical differences. The final spatial resolution of region-based analyses, however, depends on the number and size of regions chosen (typically 10-50 for a given brain imaging data set), whereas at our level of smoothing, SPM examines the data with about 500 independent comparisons. Furthermore, no a priori hypotheses about the exact localization of neocortical abnormalities need to be made. As in our previous studies, the validity of our approach was tested empirically through investigation of each control against the remaining controls. This gives a good empirical verification of the false positive rate expected as all healthy controls should a priori have normal FMZ-$V_d$, and the expected number of false positives was found.
SPM needs rigorous correction for the multiple comparisons made. If such a correction is applied to the entire volume, abnormalities of small magnitude or extent will remain undetected. We have found periventricular increases in a substantial number of patients at conventional thresholds but in none of the controls. To further explore periventricular changes we defined an additional mask for the periventricular areas for a post hoc analysis. This mask was defined as the average location of the ventricles in the stereotactic space used threedimensionally dilated by 5 mm and therefore allowed an objective search procedure for such changes. Within this defined search volume, we did not correct for the spatial extent of the clusters of abnormal FMZ binding, but only for height, as in our previous studies (Hammers et al., 2001a; Koepp et al., 2000). With this approach, 5/16 controls showed periventricular increases compared with 38/44 patients ($\chi^2$-test: $p<0.0001$).

We took great care to exclude potential artifacts as the source of our findings and are confident that methodological issues are not responsible for them.

FMZ PET revealed periventricular binding increases which did not correspond to MRI changes, even after the MRIs were reexamined in the light of the FMZ PET findings. Even though the signal-to-noise ratio is generally lower for PET than for MRI, the greater ability of FMZ PET to detect abnormalities in this situation with its femtomolar sensitivity can be explained through the better contrast-to-noise ratio.

FMZ binds highly specifically to GABA$_A$ receptors containing the $\alpha$ subunits 1,2,3 or 5, present on most neurons. Even slightly or diffusely increased average numbers of neurons per unit of white matter in the periventricular area will thus lead to a detectable increase of FMZ-$V_d$, whereas clusters of neurons smaller than 1 mm$^3$ would not be detectable on current MRI.

### 3.6.5.2 Comparison with previous findings

Earlier studies have provided extensive evidence that decreased FMZ binding can localize the epileptogenic focus both in mesial TLE and in neocortical epilepsy (Henry et al., 1993a; Juhász et al., 2001; Koepp et al., 1996; Koepp et al., 1997c; Muzik et al., 2000; Ryvlin et al., 1998; Savic et al., 1993; Savic et al., 1988; Savic et al., 1995; Szelies et al., 1996). Decreased FMZ binding may be related to structural pathology as found in areas of past acute and nonprogressive cerebral injury (Richardson et al., 1998b). Decreased FMZ binding can, however, indicate functional abnormalities over and above evident structural abnormalities (Hammers et al., 2001a; Henry et al., 1993a; Koepp et al., 2000; Koepp et al., 1997a; Koepp et al., 2000).
which represents the rationale for using FMZ-PET in patients with normal structural imaging, as in the present study.

Increased FMZ binding is difficult to detect on region-based analyses using measures of asymmetry and may be more difficult to see on visual inspection (Hammers et al., 2001a). Using quantified parametric images of FMZ binding and comparisons with control groups, however, we have previously shown areas of increased binding in patients with malformations of cortical development (Hammers et al., 2001c; Richardson et al., 1997a; Richardson et al., 1996). Similar increases were found in patients with neocortical epilepsy and normal MRI but in none of six patients with epilepsy clearly due to acquired nonprogressive lesions (Richardson et al., 1998b), leading to the hypothesis that malformations not evident on MRI may be the basis of increased FMZ binding.

We have recently found a strong correlation ($r>0.8$, $p<0.001$) between preoperative white matter FMZ-$V_d$ in both temporal lobes and the WM neuron number determined in the resected ipsilateral specimen in patients with hippocampal sclerosis (Hammers et al., 2001a) (see chapter 3.3, p. 200). Increased neuron number in the temporal lobe white matter is a hallmark of microdysgenesis. Using the same technique as described here, we subsequently found temporal lobe white matter increases, presumably indicating microdysgenesis, in a group of 18 patients with temporal lobe epilepsy and normal qualitative and quantitative MRI, as well as in 11/18 individual patients (Hammers et al., 2001b) (see chapter 3.5, p. 238). We found a high frequency (25/44) of increases of FMZ binding in the current study in patients with neocortical epilepsy and normal MRI, often including WM or, as in the case of the periventricular increases, located exclusively within the WM.

To keep the number of voxels studied to a minimum, in SPM, white matter is normally excluded from the analysis through thresholding. If this blanket threshold is simply lowered to include white matter signal, results are inconsistent due to the interindividual variability in global binding. Further, the number of voxels included in the analysis is unnecessarily increased around the outer border of the brain image, thereby accentuating the multiple comparisons problem. We, therefore, created an anatomical mask in stereotactic space, encompassing the gray matter in the cortex and in the basal ganglia as well as central white matter. This enabled us to explicitly study, for the first time, white matter changes while restricting the number of voxels inspected.
3.6.5.3 Neurobiological considerations

Our finding of areas of decreased FMZ binding, alone in 8/44 patients and in combination with areas of increased FMZ binding in a further 9/44, replicates earlier findings both in mesial TLE and in neocortical epilepsy (Henry et al., 1993a; Juhász et al., 2001; Koepp et al., 1996; Koepp et al., 1997c; Muzik et al., 2000; Ryvlin et al., 1998; Savic et al., 1993; Savic et al., 1988; Savic et al., 1995; Szelies et al., 1996). Such decreases may indicate a loss of GABAA receptors, loss of GABAA receptor bearing cells, or a change in subunit composition (Brooks-Kayal et al., 1998; Loup et al., 2000; Sperk et al., 1998) with ensuing reduction of affinity (Nagy et al., 1999).

Cortical increases of FMZ binding may be due to an increase in GABAA receptor density, an increased neuronal density or ectopic neurons bearing GABAA receptors, as for example in microdysgenesis. The dysgenesis hypothesis is supported by the fact that cortical increases of FMZ-Vd have only been observed in patients with malformations of cortical development, including areas of cortex appearing normal on MRI (Richardson et al., 1997a; Richardson et al., 1996), in "MRI-negative" patients (Koepp et al., 2000; Richardson et al., 1998b) and in patients with hippocampal sclerosis with associated microdysgenesis (Hammers et al., 2001a) but not in acquired epilepsies (Richardson et al., 1998b).

Subcortical WM increases of FMZ binding are best explained by increased numbers of WM neurons (Hammers et al., 2001a). FMZ-PET is well suited to determine abnormal nerve cell content in the WM in vivo: Most neurons express GABA<sub>A</sub> receptors. FMZ can therefore be regarded to a certain extent as a neuronal marker, and the contrast-to-noise-ratio for ectopic neurons in the WM is very high for FMZ PET.

Ectopic WM neurons might contribute to epileptogenesis by providing additional or abnormal connections between nerve cells, as shown experimentally for ectopic CA1 neurons in the rat model of methylazoxymethanol-(MAM-) induced cortical malformations (Chevassus-au-Louis et al., 1998). Human tissue is scarce as patients with malformations are less suitable for epilepsy surgery (Sisodiya, 2000), but there is direct evidence for connectivity of ectopic gray matter from both autopsy cases (Hannan et al., 1999), functional imaging studies (Pinard et al., 2000; Richardson et al., 1998a; Spreer et al., 2001) and evidence for epileptogenicity of periventricular neuronal clusters (Francione et al., 1994; Mattia et al., 1995; Palmini et al., 1995; Sisodiya et al., 1999a). The finding of ectopic WM neurons in neocortical focal
epilepsy with normal MRI is therefore a tenable explanation for the pathogenesis of epileptic circuits. Further, the periventricular increases tended to be more anterior in the cases of FLE and more posterior in the PLE and OLE groups.

Microdysgenesis has been defined in different ways, and this accounts partly for the controversies surrounding its relevance in epilepsy (Lyon and Gastaut, 1985; Meencke and Janz, 1984; Meencke and Janz, 1985). Increased WM neuron numbers, however, are regularly included in the definition (Raymond et al., 1995). It is evident that there is a rather wide range of normal variation (Emery et al., 1997; Hardiman et al., 1988; Kasper et al., 1999; Rojiani et al., 1996; Thom et al., 2001), there is regional variation with approximately six times more ectopic neurons found in the temporal lobe WM compared with either frontal or occipital lobe WM (Rojiani et al., 1996), and more neurons are found in periventricular areas compared to subcortical WM (Thom et al., 2001). In the current study, increased FMZ binding in periventricular WM was present in 7/44 patients and 0/16 controls at conventional thresholds. In the post hoc analysis of the periventricular areas 38/44 patients versus 5/16 controls showed such increases (χ²-test: p<0.0001).

The quantitative ROI analysis showed an approximately 100% increase in FMZ-Vd around the posterior horns in a case of periventricular increases, compared with the corresponding area in controls. As we determined the effect size in a very clear-cut case, the order of magnitude of the changes is compatible with previous pathological studies of average increases in WM neurons in temporal lobe specimens obtained at surgery for refractory temporal lobe epilepsy which showed increases of 30-75% (Emery et al., 1997; Thom et al., 2001).

Only one patient has been operated upon so far. One cluster of significantly increased FMZ binding in the right orbitofrontal WM was included in the resection. Quantitative immunohistochemistry (Thom et al., 2001) with NeuN estimated the neuronal density in a randomly selected section of the WM at 1598/mm³. This would be near the mean neuronal WM density in temporal lobe in controls as determined with the same method (Thom et al., 2001). We do not yet have other frontal lobe specimens. A previous study looking at the distribution of heterotopic neurons in normal hemispheric white matter, however, found 6.2 times higher neuronal densities in temporal lobe WM compared with frontal lobe WM, with no overlap of the normal ranges (Rojiani et al., 1996). Therefore, the finding of a neuronal density in the middle of the normal range for temporal lobe in our frontal lobe specimen is likely to
represent a large increase in neuronal density compared to controls. This would then confirm one aspect of microdysgenesis as the neurobiological basis for our PET findings.

3.6.5.4 Clinical considerations

Patients with normal or nondiagnostic MRI and medically refractory neocortical focal epilepsy represent an important and difficult subgroup of patients who undergo investigations for epilepsy surgery. Accordingly, there have been various FMZ-PET studies including MRI-negative patients (Debets et al., 1997; Henry et al., 1993a; Juhász et al., 2001; Koepp et al., 2000; Lamusuo et al., 2000; Muzik et al., 2000; Richardson et al., 1998b; Ryvlin et al., 1998; Savic et al., 1993; Savic et al., 1988; Savic et al., 1995; Szelies et al., 1996). Most of the earlier studies used region-of-interest approaches with limited numbers of VOIs or relied on measures of asymmetry for FMZ binding and could not therefore detect bilateral abnormalities. No previous studies have explicitly included WM.

If our finding of frequent and mostly bilateral periventricular abnormalities can be confirmed, this would represent a very important addition to the noninvasive presurgical evaluation and would be a cogent reason for not pursuing the evaluation further as results of focal resection are likely to be poor (Li et al., 1997; Sisodiya, 2000).

The only case in this series who has so far been operated upon (#F11) might be a case in point. On electroclinical grounds, she was suspected to have right frontal lobe epilepsy. Diffusion tensor imaging showed right orbitofrontal abnormalities (Rugg-Gunn et al., 2001a). Intracranial recordings showed onset of typical attacks from just one electrode over the right orbitofrontal cortex, and she underwent a partial right frontal lobectomy. She had a 90% reduction of seizures over the 10 month of postoperative follow-up so far with a seizure-free period postoperatively, a pattern that has been observed before in patients with periventricular nodular heterotopia (Li et al., 1997). The result of the present study which became available later suggested widespread abnormalities of FMZ binding, involving mainly both frontal lobes and consisting of increases involving both gray and white matter. In the post hoc analysis but not in the standard analysis, she showed bilateral periventricular increases of FMZ binding, possibly indicating more widespread abnormalities.

9/44 cases had single abnormalities of FMZ binding at conventional thresholds, but in only one of these cases no periventricular increases of FMZ binding were seen in
the post hoc analysis. Single abnormalities are potential targets for invasive EEG monitoring to confirm if this is the site of seizure onset and represent the other end of the spectrum from bilateral increases. Clinically, single cortical increases or decreases may encourage to proceed with the implementation of depth electrodes or subdural grids, whereas the finding of multiple or periventricular abnormalities will argue against pursuing potentially harmful investigations.

In summary, we have optimized the analysis of FMZ PET scans by adapting SPM99 to the analysis of gray and white matter changes in neocortical focal epilepsy with normal high resolution MRI. We were able to increase the yield of noninvasively detected abnormalities. Inclusion of the white matter in the analysis of FMZ-PET provides additional information in a significant proportion of patients with MRI-negative neocortical focal epilepsy.
3.7 \[^{11}C\]-diprenorphine PET in malformations of cortical development

3.7.1 Summary

MCD are present in up to 15% of adult patients with medically refractory epilepsy referred for epilepsy surgery. Surgery in these patients has a less favourable outcome than if there is a discrete lesion, probably due to the presence of abnormalities beyond those appreciated on standard MRI. \[^{11}C\] diprenorphine (DPN) PET images all subtypes of opioid receptors. There is evidence that release of endogenous opioids accompanies partial seizures.

In total, we studied 15 patients with partial epilepsy due to MCD. Four had subcortical forms of MCD: 2 laminar heterotopia (LH), 2 subependymal nodular heterotopia (SNH) and one subcortical heterotopia (SH). Ten had cortical or mixed forms: 3 focal cortical dysplasias (FCD), 2 dysembryoplastic neuroepithelial tumours (DNT), 1 schizencephaly, 1 perisylvian polymicrogyria (PMG) and 3 tuberous scleroses (TS). 20 healthy controls were studied for comparison. All had quantitative \[^{11}C\] DPN PET, and all patients and 13 controls had high resolution MRI. Spectral analysis was used to produce parametric images of \[^{11}C\] DPN volume-of-distribution (Vd). SPM99 with explicit masking was used for the comparison of 14 individual patients and controls (one patient with SNH could not be studied). Absolute quantification of \[^{11}C\] DPN-Vd with partial volume effect correction (PVC) was performed in the areas of abnormal grey matter (GM), surrounding or overlying cortex, and multiple other volumes of interest (VOI) in 10 patients compared with 13 controls (only 13 controls had high resolution MRI, and one patient with SNH, one with DNT, the patient with schizencephaly and two patients with TS could not be studied).

All 14 patients who could be included in the SPM analysis showed abnormal DPN-Vd. Two patients had increases of DPN-Vd, five had decreases and six both increases and decreases. Within subgroups, findings tended to be similar. In 3 of 4 patients with subcortical forms, the heterotopic GM was detected as an increase. The two LH patients showed widespread cortical decreases. The patients with SNH and SH had decreases of DPN-Vd in the vicinity of the lesions. Of the three patients with FCD, one had a decrease within the lesion; one had decreases in the vicinity of the lesion, and one patient had bitemporal increases. Of the two patients with DNT, one showed increases including the lesion, and the other contralaterally. The patient with
schizencephaly had increases within the thickened cortex in the clefts but decreases in the adjacent areas. The patient with PMG had widespread increases and decreases. The three patients with TS had no cortical increases, and, despite multiple tubers, few decreases, some of which were confined to single cortical tubers.

Eight of the 10 patients who could be included in the VOI/PVC analysis had abnormalities of $[^{11}\text{C}]$ DPN binding per unit of GM. Of the four patients with subcortical forms, two LH patients had largely normal binding; the patient with SNH had widespread cortical increases but normal binding per unit of GM within the heterotopion; and the patient with SH had decreased binding in one of the two heterotopia and increases in some cortical regions in the vicinity. Of the six patients with cortical forms, one of three patients with FCD showed decreased binding within the lesion, another one showed an increase in the vicinity, and the third had normal binding per unit of GM. The patient with DNT had multiple decreases, within the lesion, adjacent to the lesion, and more remotely. The patient with PMG showed normal binding per unit of GM in the lesion and its surroundings but increases in two nearby regions. One patient with TS had a decrease in one of multiple tubers only, with otherwise normal binding.

With both analyses, there was a broad correlation between electroclinical and imaging data in most patients.

In conclusion, DPN-PET showed abnormalities in the vast majority of MCD patients. In some forms, abnormalities may be confined to the lesions seen on MRI, but particularly the subcortical forms show more widespread disturbances in the SPM analysis. Quantitative region-based analyses with correction for tissue type suggest that some apparent abnormalities are due to structural changes, but there were also functional abnormalities over and above structural abnormalities. Both techniques are complementary. Clinical usefulness is possible in TS where a minority of tubers showed abnormalities.

3.7.2 Introduction

Malformations of cortical development (MCD) are increasingly recognised as a cause of medically refractory epilepsy. A widely used system classifies them according to time or mechanism of origin: disorders of neuronal and glial proliferation, abnormal neuronal migration, or abnormal postmigrational cortical organisation (Barkovich et al., 1996). MCD are present in 15-20% of adults with intractable partial seizures, some of whom are candidates for epilepsy surgery (Kuzniecky and Jackson, 1997). Surgical resection of areas of MCD in patients with drug resistant epilepsy, however, results in at best about 40% of patients becoming seizure free (Cascino et al., 1993a; Sisodiya, 2000), compared with 70% in patients with hippocampal sclerosis (Berkovic et al., 1995). A possible explanation is that the area of functional cortical abnormality may be greater than the structural abnormality shown by conventional magnetic resonance imaging (MRI) techniques ((Hammers et al., 2001c; Richardson et al., 1997a; Richardson et al., 1996; Sisodiya et al., 1995), and see chapter 3.4).

Animal models have shown functional abnormalities in cortex adjacent to MCD, possibly due to formation of aberrant thalamocortical connections subsequent to the presence of MCD in the original projection area (Jacobs et al., 1999a; Jacobs et al., 1999b). Abnormal adjacent cortex might in part explain the low surgical success rate in MCD and the observation that lesionectomies tend to have a less good outcome than more extended resections (Raymond et al., 1995).

Opiate receptors are G-protein coupled receptors and are widely distributed throughout the human CNS (Hiller and Fan, 1996; Pilapil et al., 1987). There are three main subtypes, µ, κ and δ, and while the net effect of their ligands depends on the precise experimental setting, species and anatomical location, the main effect is inhibitory (Tortella, 1988a). Diprenorphine (DPN) is a high affinity opiate receptor ligand with similar in vivo affinities for the 3 main receptor subtypes: µ, κ and δ (Pfeiffer et al., 1982). It acts predominantly as an antagonist, but, at higher doses in man, acts as a weak partial agonist and leads to mild sedation. [11C] DPN is established as a useful opioid receptor PET ligand and has been used in the study of normal subjects and patients (Jones et al., 1988; Koepp and Duncan, 2000; Mayberg et al., 1991).

Endogenous opioids are released in response to high-frequency firing and have been shown to displace [3H] DPN (Neumaier and Chavkin, 1989). Opioids are released at
the time of seizures in rodent models (Tortella and Long, 1985; Tortella and Long, 1988), and, in certain forms of generalised epilepsy (Bartenstein et al., 1993) and partial epilepsy (Koepp et al., 1998b), such evidence has been found in humans as well.

Voxel-based methods of analysis (Statistical Parametric Mapping, SPM) have been applied to $^{[11]}$C flumazenil PET and MR images of MCD (Richardson et al., 1997a; Richardson et al., 1996). PET abnormalities were frequently more extensive than structural changes seen on MRI. These findings indicated that some of the PET abnormalities could be accounted for by abnormalities of cortical grey matter volume. The voxel-based SPM approach, however, did not lend itself to yield quantitative estimates of ligand binding and was not suited to specifically test hypotheses about normal appearing cortex adjacent to or overlying MCD. Furthermore, the limited spatial resolution of PET results in partial volume effect that particularly affects the quantification of signals in structures smaller than twice the full width at half maximum (FWHM) resolution of the scanner used (Hoffman et al., 1979), such as the cortical ribbon or small MCD, due to tissue averaging effects. Correction for partial volume effect is particularly important when structural abnormalities are present. Subtle changes in GM content in MCD may not be detected on visual inspection of high quality MRI (Desbiens et al., 1993; Kuzniecky et al., 1991). Without correction for partial volume effect, it is not possible to distinguish if an abnormality detected using PET represents a true functional abnormality due to a change of receptor density or affinity per neuron, a structural abnormality due to an increase or decrease of GM, or both of these together (Labbé et al., 1998; Müller Gartner et al., 1992; Rousset et al., 1993). Using a volume-of-interest based method with partial volume effect correction (PVC) applied to $^{[11]}$C flumazenil PET and MRI datasets in patients with MCD, we have previously shown that some areas of increased GM have reduced $^{[11]}$C flumazenil binding and that cortex adjacent to or overlying MCD is frequently abnormal ((Hammers et al., 2001c) and see chapter 3.4, p. 221).

In this study, we aimed to investigate $^{[11]}$C DPN binding in MCD using a voxel-based analysis as implemented in SPM99 as well as a complementary volumes-of-interest based technique with PVC, and to correlate clinical and EEG abnormalities with PET findings.
3.7.3 Methods

3.7.3.1 Patients and Controls

We studied a total of 15 patients (seven women) with partial epilepsy and an MRI diagnosis of MCD. They were recruited as described in Chapter 2.1 (p. 144), and additional patients were referred from the Zentrum Epilepsie Erlangen, Germany. The median age at onset of habitual seizures was 7 years (range, 0-24 years), the median duration of epilepsy before the PET examination was 20 years (range: 13-28 years), the median age at PET examination was 29 years (range: 20-51 years), and the median interval between last seizure (excluding SPS) was 2 days (range 1-3650 days). The antiepileptic medication was carbamazepine (10 patients), lamotrigine (6), sodium valproate (5), topiramate (3), clobazam (3), gabapentin (2), clonazepam (1), tiagabin (1), or vigabatrin (1), alone or in combination; 5 patients were taking carbamazepine monotherapy. Five had subcortical forms of MCD: 2 laminar heterotopia (LH), 2 subependymal nodular heterotopia (SNH) (one each included in the SPM and VOI study), and one subcortical heterotopia (SH). Ten had cortical or mixed forms: 3 focal cortical dysplasias (FCD), 2 dysembryoplastic neuroepithelial tumours (DNT), 1 schizencephaly, 1 perisylvian polymicrogyria (PMG) and 3 tuberous scleroses (TS).

One DNT was not available for the SPM study, and one DNT, the schizencephaly and 2 TS were not available for the VOI based study (see Table 3.7.1, p. 296).

20 healthy controls (2 women) were studied for comparison. They had no history of neurological or psychiatric disorder, were on no medication and had normal MRI studies. 13 of the 20 controls had high resolution MRI studies and were available for the VOI based study.

Clinical details for all 15 patients are shown in Table 3.7.1 (p. 296).

3.7.3.2 PET Technique

We used the same acquisition technique as described previously (Koepp et al., 1998b; Prevett et al., 1994). Briefly, PET scans were performed in 3D mode with the septa retracted, using a 953B Siemens/CTI PET camera with a reconstructed image resolution of about 8mm x 8mm x 4mm at FWHM for 31 simultaneously acquired planes (Spinks et al., 1992). Scans were performed with transaxial images obtained parallel to the plane defined by the anterior and posterior commissures and coronal
images orthogonal to this. An eight-channel EEG was recorded during the PET studies to ensure that the scans were interictal. 370 MBq of high specific activity $^{11}$C DPN tracer (Luthra et al., 1991) was injected intravenously. Arterial blood was sampled continuously, with discrete samples, in order to determine a metabolite-corrected plasma input function. A dynamic 3D series, consisting of 27 frames over 90 minutes, was acquired for the brain volume. Scatter was estimated and corrected for using a "dual window" method (Grootoonk et al., 1996). Parametric images of $^{11}$C DPN-Vd, reflecting specific binding to opioid receptors (Cunningham et al., 1991; Cunningham et al., 1993), were produced from the brain uptake and plasma input functions using spectral analysis (Cunningham and Jones, 1993).

3.7.3.3 PET image analysis

Analyze AVW (Robb and Hanson, 1991) and Matlab 5 (Mathworks Inc, Sherborn, MA, USA) were used to perform image manipulation and measurements on Sun workstations (Sun Microsystems, Mountain View, CA, USA).

3.7.3.3.1 Volume-of-interest analysis

The aim was to quantify $^{11}$C DPN binding after correction for partial volume effect in areas of abnormal GM due to MCD, and in normal appearing cortex overlying the subcortical MCDs or adjacent to the cortical MCDs. These areas were outlined manually on the MRI scans as in our previous study (see chapter 3.4.3.3, p.224). We used our probabilistic brain atlas (see chapter 3.2, p. 173) to automatically define the other regions of interest. The maximum probability map was first transformed into the individual patient's MRI space. The individually outlined additional VOIs (i.e. MCDs and overlying or adjacent cortex) were added onto the template. The high resolution volume acquisition MRI scans were then nonuniformity corrected (Sled et al., 1998) and automatically segmented into probability images of GM, WM and CSF (Lemieux, 2001; Lemieux et al., 1999), excepting the individually outlined areas of GM within the MCDs. The GM, WM and CSF images and the VOIs were first coregistered with the PET data using mutual information coregistration as implemented in SPM99 (Maes et al., 1997) and then ‘blurred’ to the same spatial resolution as PET, by convolving each segmented probabilistic MRI with the 3D point spread function of the PET scanner. A least squares weighted fit of these ‘blurred’ images to the observed PET images was then calculated (Labbé et al., 1998) using a new and computationally more efficient algorithm (Aston et al., 2002).
This allowed estimates of PVE within the multiple VOIs of homogenous tracer activity to be obtained. To obtain a control range for $[^{11}C]$ DPN-Vd in equivalent cerebral areas to the MCD and surrounding cortex, the individualised template for each patient was spatially normalised to all 13 controls' MRI scans, and partial volume effect corrected $[^{11}C]$ DPN-Vd values were obtained for the corresponding GM areas in healthy volunteers, using a recently developed automated method (Aston et al., 2002). As in our previous study ((Hammers et al., 2001c), see chapter 3.4, p. 221), only the GM contribution of the multiple cortical VOIs was examined.

3.7.3.3.2 Voxel-by-voxel analysis

$[^{11}C]$ DPN-Vd images were also analyzed using statistical parametric mapping (SPM99, Wellcome Department of Imaging Neuroscience, London, UK) implemented in Matlab 5 (Mathworks Inc, Sherborn, MA, USA). The images were volumetrically normalised to a PET template that occupies the same space as the SPM99 MRI templates. These were an average of 152 scans supplied by the Montreal Neurological Institute that have been linearly matched to the brain in the atlas of Talairach and Tournoux (Talairach and Tournoux, 1988). The normalisation procedure involves a linear 3D transformation and uses a set of smooth basis functions that allow for normalisation at a finer anatomical scale (Ashburner and Friston, 1999). Images were smoothed using a 10x10x10 mm (full width at half maximum) isotropic Gaussian kernel as a final pre-processing step. This spatial filter accommodates inter-individual anatomical variability and improves the sensitivity of the statistical analysis (Friston et al., 1991). Each patient's MRI scan was coregistered with their $[^{11}C]$ DPN-Vd image (Ashburner and Friston, 1997; Woods et al., 1993) and then transformed into standard space using the transformation matrix derived from the spatial normalisation of that individual's $[^{11}C]$ DPN-Vd image.

3.7.3.4 Statistical Analysis

3.7.3.4.1 Volume-of-interest analysis

We defined the normal range for the PVE corrected absolute $[^{11}C]$ DPN-Vd values as 2.5 standard deviations (SD) from the normal control mean for the areas corresponding to individually outlined MCDs and adjacent/overlying VOIs, and for all other standard (automatically defined) VOIs as 3 SD from the mean in all other VOIs, as in our previous study ((Hammers et al., 2001c), see chapter 3.4, p. 221).
These high thresholds were chosen because of the large number of comparisons. The lower threshold was used for MCDs and surrounding cortex as we had specific hypotheses that these would be abnormal. No correction for global $[^{11}\text{C}]$ DPN binding was applied.

3.7.3.4.2 Voxel-by-voxel analysis

Significant differences between patients and control subjects were estimated according to the general linear model at each and every voxel of the normalized and smoothed images (Friston et al., 1995b). Statistical parametric maps are 3D projections of statistical functions that are used to characterise significant regional differences in imaging data. We have described the use of SPM in $[^{11}\text{C}]$ FMZ PET studies of patients with unilateral hippocampal sclerosis (Koepp et al., 1996, Hammers, 2001 #997), in patients with malformations of cortical development (Richardson et al., 1997a) and in patients with partial epilepsy and normal MRI ((Koepp et al., 2000; Richardson et al., 1998b) and see chapters 3.5 and 3.6). SPM99 combines the general linear model to create the statistical map and random field theory to make statistical inferences about regional effects (Friston et al., 1995b; Worsley et al., 1996).

Each patient was compared against the 20 controls, with the design matrix designating global cerebral DPN-Vd differences as a nuisance covariate (Friston et al., 1990). Linear contrasts were used to test the hypotheses for specific focal effects. The resulting set of voxel values for each contrast constitutes a statistical parametric map of the t statistic SPM $\{t\}$. The SPM $\{t\}$ were thresholded at $p=0.001$ uncorrected. The significance of foci of relative DPN-Vd changes is estimated using Random Field Theory, correcting for multiple comparisons using the number of resolution elements (resels) in the statistical image (Worsley et al., 1992; Worsley et al., 1996). This examines the probability that the observed cluster of voxels could have occurred by chance, given its extent and peak height. The threshold chosen for the corrected cluster $p$ values was $p<0.05$. No extent thresholding was applied.
<table>
<thead>
<tr>
<th>No.</th>
<th>MRI-MCD type</th>
<th>Age (yrs) / sex</th>
<th>AEDs</th>
<th>Age of onset (yrs)</th>
<th>Interval CPS/2°gen -&gt; PET (days)</th>
<th>Seizure type(s)</th>
<th>EEG</th>
<th>PVC analysis: Region</th>
<th>PVC analysis: [(^{11})C] DPN-Vd: % difference from controls</th>
<th>PVC analysis: Control [(^{11})C] DPN-Vd: Mean ± 2.5/3SD, normal range</th>
<th>SPM analysis: Increases of DPN-Vd</th>
<th>SPM analysis: Decreases of DPN-Vd</th>
</tr>
</thead>
<tbody>
<tr>
<td>4188</td>
<td>bil LH</td>
<td>27/m CBZ, LTG, VPA</td>
<td>6</td>
<td>1</td>
<td>N/A</td>
<td>SPS, TL-type CPS, 2° gen</td>
<td>mild to moderate backgr abn, focal discharges L temp region; more widespread ep discharges</td>
<td>LG TMI</td>
<td>25.1, -30%</td>
<td>35.8±3.2, 26.2-45.4</td>
<td>R occ band: Z4.52, k257, p&lt;.014</td>
<td>L front operculum: Z6.31, k837, p&lt;.001</td>
</tr>
<tr>
<td>4494</td>
<td>bil LH</td>
<td>27/f GVG, CLB, TGB, TPM</td>
<td>12</td>
<td>1</td>
<td>N/A</td>
<td>CPS trance-like, sometimes handwringing, 2° gen</td>
<td>normal</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>bil post cing G: Z4.82, k372, p&lt;.003</td>
<td>R orbitofront cortex: Z4.31, k739, p&lt;.002</td>
</tr>
<tr>
<td>4304</td>
<td>R post SNH</td>
<td>51/m CBZ</td>
<td>24</td>
<td>540</td>
<td>N/A</td>
<td>CPS: pain L arm, L arm raised</td>
<td>slow background, no ep features</td>
<td>R TLAL</td>
<td>67.3, +81%</td>
<td>37.2±6.5, 17.6-56.8</td>
<td>R front operculum: Z5.20, k524, p&lt;.001</td>
<td></td>
</tr>
<tr>
<td>4330</td>
<td>R post SNH</td>
<td>32/f CBZ</td>
<td>19</td>
<td>3650</td>
<td>N/A</td>
<td>hot flush, loss of awareness, 2° gen</td>
<td>No definite abn</td>
<td>L TLAL</td>
<td>67.4, +87%</td>
<td>36.0±4.2,23.6-48.5</td>
<td>L fusiform G: Z5.28, k672, p&lt;.001</td>
<td></td>
</tr>
<tr>
<td>4290</td>
<td>R SH (x2)</td>
<td>23/m CBZ, GBP, CLN</td>
<td>0</td>
<td>26</td>
<td>SPS L arm tingling, CPS head drops, jerking R arm, eye flickering; 2° gen, non-epileptic</td>
<td>R temp and parasagittal single and repetitive focal spikes, max T6 and P4</td>
<td>R amyg</td>
<td>61.7, +100%</td>
<td>30.8±9.3,3.2-9.58.8</td>
<td>R frontal-par SH: Z5.66, k1093, p&lt;.001</td>
<td></td>
<td></td>
</tr>
</tbody>
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### Table 3.7.1 (continued): Clinical, EEG, MRI and $[^{11}C]$ DPN PET data in 15 patients with MCD

<table>
<thead>
<tr>
<th>No.</th>
<th>MRI type</th>
<th>MCD type</th>
<th>Age (yrs)/sex</th>
<th>Age of onset (yrs)</th>
<th>Interval CPS/2°gen -&gt; PET (days)</th>
<th>Seizure type(s)</th>
<th>EEG</th>
<th>PVC analysis: Region</th>
<th>PVC analysis: $[^{11}C]$ DPN-Vd, % difference from controls</th>
<th>PVC analysis: Control $[^{11}C]$ DPN-Vd, Mean ± 2.5/3SD, normal range</th>
<th>SPM analysis: Increases of DPN-Vd</th>
<th>SPM analysis: Decreases of DPN-Vd</th>
</tr>
</thead>
<tbody>
<tr>
<td>4258</td>
<td>R Amyg</td>
<td>FCD</td>
<td>21/m CBZ, TPM</td>
<td>4</td>
<td>2</td>
<td>TL type CPS, 2°gen</td>
<td>bitemp spikes, R&gt;L, ictal: R hemisphere, not well localised</td>
<td>R FCD</td>
<td>5.23, -84%</td>
<td>32.8±5.5, 19.1-46.6</td>
<td>none</td>
<td>R Amyg (FCD): Z5.61, k395, p&lt;002</td>
</tr>
<tr>
<td>4382</td>
<td>R media</td>
<td>OL FCD</td>
<td>30/m VPA, TPM, CLB</td>
<td>7</td>
<td>1</td>
<td>CPS with severe disorientation, 2° gen</td>
<td>normal, ictal: spikes R parietal, R temporal and R occipital areas</td>
<td>R GPH/A</td>
<td>43.0, +123%</td>
<td>19.3±5.2, 3.7-34.9</td>
<td>none</td>
<td></td>
</tr>
<tr>
<td>4595</td>
<td>R ant TL FCD</td>
<td>20/m VPA, LTG</td>
<td>1</td>
<td>2</td>
<td>SPS: L arm dystonia, 2° gen</td>
<td>mild gen abn, spikes R&gt;L fronto-temp, ictal: R temp-front-central</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>none</td>
<td>R orbitofront: Z4 94, k890, p&lt;001 R post HC: Z3.67, k296, p&lt;008</td>
<td></td>
</tr>
<tr>
<td>4463</td>
<td>L TL DNT</td>
<td>32/m LTG, GBP</td>
<td>18</td>
<td>10</td>
<td>TL type CPS, 2° gen</td>
<td>L ant temp spikes during drowsiness, ictal: L temp onset</td>
<td>L TLDNT ant to DNT post to DNT</td>
<td>L TLAL</td>
<td>9.3, -70%</td>
<td>30.9±3.9, 21.2-40.7</td>
<td>R uncus region: Z4.65, k193, p&lt;041</td>
<td>none</td>
</tr>
<tr>
<td>4492</td>
<td>R TL pole DNT</td>
<td>31/f CBZ</td>
<td>14</td>
<td>1</td>
<td>TL type SPS (déjà vu/vécu) and CPS, Hx of 2° gen</td>
<td>R TL spikes, ictal: R temp onset</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>R temp to R basal forebrain (partly in lesion): Z4.48, k2625, p&lt;001</td>
<td>none</td>
<td></td>
</tr>
</tbody>
</table>

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**Table 3.7.1 (continued): Clinical, EEG, MRI and [11C] DPN PET data in 15 patients with MCD**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Clinical Manifestations</th>
<th>EEG</th>
<th>MRI</th>
<th>PET</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>4084</td>
<td>39f</td>
<td>CBZ</td>
<td>21</td>
<td>SPS: motor L arm 2° gen</td>
<td>no definite abnormality</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>4291</td>
<td>PMG</td>
<td>34f</td>
<td>VPA, LTG, CLB</td>
<td>CPS sensory R arm -&gt; dystonic posturing R arm, fidgeting L, 2° gen</td>
<td>normal, ictal: some attenuation only</td>
<td>L G TS</td>
<td>L G TMI</td>
<td>30.9, +39% 51.3, +40%</td>
</tr>
<tr>
<td>4467</td>
<td>TS</td>
<td>29f</td>
<td>VPA, CBZ, LTG</td>
<td>SPS/CPS: throbbing in mouth/both cheeks, autonomic features</td>
<td>sharpened theta transients over R temp region; very rare sharp waves L ant temp in sleep ictal: muscle artefact, no clear changes</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>4653</td>
<td>TS</td>
<td>20f</td>
<td>CBZ, LTG</td>
<td>SPS itching nose, feeling of breathlessness, speech arrest; 2° gen</td>
<td>mild nonspecific disturbances</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>4666</td>
<td>TS</td>
<td>29m</td>
<td>CBZ</td>
<td>TL type SPS, CPS: motor twitches, clenching teeth</td>
<td>excess irreg slow, few sharp waves over TL, l/r</td>
<td>L FL tuber 24.3, -40%</td>
<td>40.6±5.6, 26.6-54.6</td>
<td>L ant inf front G (tuber): Z5.93, k503, p&lt;0.001</td>
</tr>
</tbody>
</table>

**Table 3.7.1:** Clinical, EEG, MRI and [11C] DPN PET data in 15 patients with MCD. MCD=malformation of cortical development; LH=laminar (band) heterotopia; SNH=subependymal nodular heterotopia; SH=subcortical heterotopia; FCD=focal cortical dysplasia; DNT=dysembryoplastic neuroepithelial tumour; PMG=polymicrogyria; TS=tuberous sclerosis; f/m=female/male; S/CPS=simple/complex partial seizures; R=right; L=left; bil=bilateral; ant/post=anterior/posterior; sup=superior; temp/par/occ=temporal/parietal/occipital; FL/PL/OL=frontal/parietal/occipital lobe; adj=adjacent; TM=middle and inferior temporal lobe; cinc=cingulate; TLAL/TLAM=anterior lateral/medial temporal lobe; GPH/A=parahippocampal/ambiens gyrus; TLpo=posterior temporal lobe; ins=insula; GC/an=anterior/posterior cingulate gyrus; NC=neocortex; Cereb=cerebellum; Hx=history; gen=generalised; G=gyrus; N/A=not available.
3.7.4 Results

3.7.4.1 Voxel-by-voxel SPM analysis

3.7.4.1.1 Subcortical forms

- Laminar heterotopia (LH)
  In both patients with LH (#1 and 2), the band was visible on the statistical maps as an increase compared to the white matter encountered in this location in controls. The increase reached significance in patient #1 in the right posterior quadrant. Both patients showed several areas of decreased $[^{11}\text{C}]$ DPN binding, including the cortex overlying the laminar heterotopia (Table 3.7.1, p. 296).

- Subependymal nodular heterotopia (SNH)
  In the patient with SNH who underwent analysis with SPM99 (#4), the SNH itself showed increased binding compared to the white matter and CSF encountered in controls in this location. There was one area of decreased $[^{11}\text{C}]$ DPN binding, in the ipsilateral inferior insula (Table 3.7.1.).

- Subcortical heterotopia (SH)
  In the one patient with SH (#5), increases of $[^{11}\text{C}]$ DPN binding relative to the same location in controls were seen in the bigger one of two SH and in the ipsilateral parahippocampal gyrus. Three areas of decreased $[^{11}\text{C}]$ DPN binding were seen, all were in the vicinity of the lesions (Table 3.7.1).

3.7.4.1.2 Cortical or mixed forms

- Focal cortical dysplasia (FCD)
  Three patients with FCD were studied (#6, 7 and 8). One (#6) had a highly significant decrease in the lesion only, with no other abnormality. One (#7) did not have a decrease in the lesion, which was located in the medial occipital lobe which shows little DPN binding, but had bitemporal neocortical increases of $[^{11}\text{C}]$ DPN binding. The third patient had decreases in the orbitofrontal cortex and posterior hippocampus ipsilateral to the temporal FCD (Table 3.7.1, p. 296).

- Dysembryoplastic neuroepithelial tumour (DNT)
  Of the two patients with DNT studied (#9 and 10, both temporal), neither had any decreases of $[^{11}\text{C}]$ DPN binding. One (#9) had an increase in the uncus region
contralateral to the lesion; the other (#10) had an increase in the ipsilateral temporal lobe, extending to the basal forebrain and partly enclosing the lesion (Table 3.7.1).

- Schizencephaly
  The patient with schizencephaly (#11) showed increases of $[^{11}\text{C}]$ DPN binding in areas of increased GM in all three clefts, compared to the same location in controls. Decreases were seen bilaterally in the posterior cingulate gyrus and adjacent to the right central cleft (Table 3.7.1).

- Polymicrogyria (PMG)
  The patient with PMG (#12) had multiple areas of increases $[^{11}\text{C}]$ DPN binding. These included the thickened polymicrogyric cortex itself, both lateral temporal neocortices, the ipsilateral thalamus, and the ipsilateral orbitofrontal cortex. Areas of decreased $[^{11}\text{C}]$ DPN binding included an apparent decrease in the widened ipsilateral sylvian fissure, the contralateral cingulate gyrus, and the ipsilateral thalamus. Three areas of decreased $[^{11}\text{C}]$ DPN binding were seen in different periventricular areas, both ipsilaterally and contralaterally (Table 3.7.1).

- Tuberous sclerosis (TS)
  Of the three patients with TS (#13, 14 and 15), only one (#14) showed a single area of increased $[^{11}\text{C}]$ DPN binding, in the left thalamus. All showed at least one area of decreased $[^{11}\text{C}]$ DPN binding; this included single tubers but not all tubers (Table 3.7.1).

3.7.4.2 Volume-of-interest analysis with partial volume correction

3.7.4.2.1 Subcortical forms

- Laminar heterotopia (LH)
  Of the two patients with LH (#1 and 2), one (#2) did not have any abnormalities in the PVC analysis, and the other (#1) had one area of decreased $[^{11}\text{C}]$ DPN binding per unit of GM, in the left middle and inferior temporal gyrus (Table 3.7.1, p. 296).

- Subependymal nodular heterotopia (SNH)
  In the patient with SNH who could be studied with PVC (#3), multiple (16) regions with increases in $[^{11}\text{C}]$ DPN binding were found, with the magnitude of increases ranging between +39% and +93% (Table 3.7.1). There was no clear clustering of abnormalities in one lobe or even in one hemisphere. This patient showed the highest global $[^{11}\text{C}]$ DPN binding of all subjects studied (+2.2 SD from the patient mean and +3.9 SD from the control mean).
• Subcortical heterotopia (SH)
The patient with SH (#5) had four regions with abnormally high $[^{11}\text{C}]$ DPN binding in the PVC analysis, with the magnitude of increases ranging from +42 to +116% (Table 3.7.1). All four areas were located in the right temporal or parietal lobe, i.e. in the vicinity of the lesions and in the area of electroencephalographic abnormalities. Binding per unit of GM was, however, normal in the lesions themselves and in the immediately adjacent areas.

3.7.4.2.2 Cortical or mixed forms

• Focal cortical dysplasia (FCD)
Of the three patients studied in the PVC analysis (#6, 7 and 8), one (#8) did not have any abnormalities. One (#6) had a 84% decrease of $[^{11}\text{C}]$ DPN binding within the lesion, with no abnormalities elsewhere. The third patient (#7) with the FCD in the medial occipital lobe, which shows little DPN binding, did not have a decrease in the lesion, but had one increase of 123% in the ipsilateral parahippocampal/ambient gyrus (Table 3.7.1, p. 296).

• Dysembryoplastic neuroepithelial tumour (DNT)
One patient with a DNT (#9) could be studied with PVC. He showed multiple (17) regions with decreased $[^{11}\text{C}]$ DPN binding, with the magnitude ranging between -41% and -81%. The areas included the DNT itself and both adjacent regions, and 8/14 of the remaining areas were ipsilateral to the lesion (Table 3.7.1). This patient showed the lowest global $[^{11}\text{C}]$ DPN binding of all subjects studied (-2.1 SD from the patient mean and -2.9 SD from the control mean).

• Polymicrogyria (PMG)
The patient with left perisylvian PMG (#12) showed two areas of increased $[^{11}\text{C}]$ DPN binding per unit of GM, in the ipsilateral superior temporal gyrus and the ipsilateral middle and inferior temporal gyrus, i.e. in the vicinity of the lesion but not immediately adjacent to it. The magnitude of these decreases was 39% and 40%, respectively (Table 3.7.1).

• Tuberous sclerosis (TS)
One patient with TS (#15) could be studied with PVC. He had a 40% decrease of $[^{11}\text{C}]$ DPN binding in a left frontal tuber. There were no other abnormalities.
3.7.4.3 Correlations with EEG and clinical data

(see also Table 3.7.1, p. 296)

3.7.4.3.1 Subcortical forms

One patient with LH (#1) had temporal-lobe type CPS and focal epileptiform discharges in the left temporal region; both SPM and PVC analyses showed decreased $[^{11}\text{C}]\text{DPN}$ binding in the left temporal lobe. SPM analysis showed two further regions of decreased binding which were not corroborated in the PVC analysis. The patient with SH (#5) had mainly SPS involving tingling of the left arm; EEG showed spikes in the right temporoparietal area with maxima over T6 and P4. PVC analysis showed increases of $[^{11}\text{C}]\text{DPN}$ binding in four right temporoparietal regions, and SPM analysis similarly showed increases within the frontoparietal SH and one right temporal region. Three further abnormalities in the SPM analysis, all decreases of $[^{11}\text{C}]\text{DPN}$ binding, were not corroborated in the PVC analysis.

The remaining three patients with subcortical MCDs (#2, 3 and 4) had no or only nonspecific EEG changes. Only in patient #3 did the clinical features suggest a specific onset zone, with pain in the left arm and elevation of the left arm. Only PVC analysis was available, and abnormalities included increases of $[^{11}\text{C}]\text{DPN}$ binding in both the right parietal and right frontal lobe, but there were multiple other increases as well.

3.7.4.3.2 Cortical or mixed forms

In all four patients with temporal lesions (two FCD, patients #6 and 8, and two DNT, patients #9 and 10), there was broad concordance between clinical semiology, ipsilateral temporal epileptiform EEG changes and ipsilateral temporal abnormalities of $[^{11}\text{C}]\text{DPN}$ binding. The latter was confined to the lesion in one FCD patient (#6) in both SPM and PVC analysis, included the ipsilateral orbitofrontal cortex in the SPM analysis in the other FCD patient (#8) in whom no abnormalities were seen on PVC analysis, included and exceeded the lesion in one DNT patient (#10) in whom no PVC analysis was available, and included and exceeded the lesion in the other DNT patient (#9) on PVC analysis which also revealed multiple other decreases, while these abnormalities were not seen on SPM analysis.

The patient with right medial occipital FCD (#7) did not have a specific seizure semiology; EEG was normal interictally, and ictal EEG showed widespread spiking
over the ipsilateral parietal, temporal and occipital areas. Both PVC and SPM analyses were concordant insofar as they showed an ipsilateral temporal increase of $[^{11}\text{C}]$ DPN binding, with a similar change contralaterally in the SPM analysis.

The patient with bilateral schizencephaly (#11) but mainly left arm motor SPS had her maximum abnormalities localised in the right motor areas; her EEG did not show definite abnormalities. Seizures were compatible with involvement of ipsilateral temporal and parietal areas in the patient with left perisylvian PMG (#12), but multiple ictal surface EEGs were non-localising. Both PVC and SPM analyses detected increases in the ipsilateral temporal lobe; SPM analysis showed further abnormalities which were not corroborated in the PVC analysis.

All three patients with TS (#13, 14 and 15) were remarkable for the paucity of abnormalities of $[^{11}\text{C}]$ DPN binding, despite multiple tubers being present in all of them. In all, EEG, clinical and imaging data were broadly concordant. Both SPM and PVC analyses were available in only one of them (patient #15); both showed a clearcut decrease of $[^{11}\text{C}]$ DPN binding in one left frontal tuber only, with a further decrease in the right temporal lobe seen on SPM analysis only. Seizure semiology and EEG features were compatible with (bi)temporal and frontal epileptogenic foci. Patient #13 had a seizure semiology compatible with a (peri)insular epileptogenic zone; interictal EEG showed maximum abnormalities in the R temporal region, and $[^{11}\text{C}]$ DPN PET analysed with SPM showed only one area of decreased $[^{11}\text{C}]$ DPN binding, in the right frontal operculum. Patient #14 had SPS with speech arrest as a salient feature, and nonspecific EEG changes; all three decreases of $[^{11}\text{C}]$ DPN binding detected with SPM were localised in the left frontal lobe.

### 3.7.5 Discussion

This is the first study to investigate in vivo opioid receptor binding in patients with MCD. The main finding was the high proportion of patients who showed abnormalities of opioidergic neurotransmission; 13/14 in the voxel-based analysis with SPM99 and 8/10 in the VOI-based analysis with PVC. The localisation of abnormal $[^{11}\text{C}]$ DPN binding correlated broadly with EEG and clinical data in the majority of patients.
3.7.5.1 Methodological considerations

Partial volume effects arise due to the limited spatial resolution of PET. PVC is necessary to accurately quantify changes and distinguish between functional changes merely reflecting structural changes (atrophy or increased GM content) and functional changes per se, e.g. changes in binding to receptors on neurons (Frost et al., 1995; Koepp et al., 1998a; Labbé et al., 1996; Rousset et al., 1995).

In the current study, we used a probabilistic region-template of multiple cortical VOIs. To create the probabilistic template, multiple VOIs were defined in native space on 20 MRIs, of 10 men and 10 women with a similar age range to the subjects in this study, and spatially normalised into standard stereotactic space. To be unequivocal, a maximum probability map was calculated, assigning each and every voxel to one VOI only (see chapter 3.2). This maximum probability map was subsequently transformed this into each subject's MRI and PET space. The use of such a template, its automated coregistration and the subsequent use of PVC provided an entirely objective and observer-independent method for defining multiple neocortical VOIs and then quantifying $[^{11}\text{C}]$ DPN binding in them (Hammers et al., 2002; Koepp et al., 2000; Labbé et al., 1998). This approach works satisfactorily for neocortex if there is no structural abnormality (Hammers et al., 2001a; Koepp et al., 2000), with an average misplacement of single point landmarks of approximately 7-8mm in Euclidean distances (Hammers et al., 2002). As the MCDs were of different shapes and sizes in each individual patient, we outlined the structures of particular interest, in this case the areas of abnormal GM due to MCD and the adjacent/overlying cortex, individually by hand to achieve millimetric precision. By applying this individualised template, specific for one patient, to all 13 controls for each patient, we were able to define normal ranges of equivalent areas of the brain for each of the individual VOIs. As each patient was evaluated individually against all 13 controls, this method is computationally still very demanding, even after the algorithm improvements (Aston et al., 2002) made compared to our previous study ((Hammers et al., 2001c) and see chapter 3.4). It also requires high quality MRI data which was only available for 13 controls.

The method used depends on an automatic segmentation algorithm (Lemieux, 2001; Lemieux et al., 1999) for the areas outside the MCD itself, whereas within the MCD, abnormal GM was outlined by hand and not segmented. Inspection of the segmented GM images of the VOIs outside the MCD showed that the segmentation was visually
acceptable. Although a small error due to misclassification of some voxels cannot be ruled out, this is very unlikely to have influenced our results as probability images were used, i.e. the probability of a given voxel to belong to the tissue class of GM was used in the calculations rather than simple binary decisions (Hartigan, 1975; Lemieux, 2001; Lemieux et al., 1999). Even altered characteristics of the GM/WM boundary which are recognised in MCD (Bernasconi et al., 2001b) should be accommodated by this method.

A fundamental disadvantage of VOI-based analyses, however, is that the number, size and position of VOIs is necessarily predetermined. In our case, approximately 35 VOIs were investigated for each patient. If alterations of $[^{11}\text{C}]$ DPN binding do not correspond roughly to these predefined boundaries, they may be averaged out within large VOIs or not be detected because the effect is spread over parts of several neighbouring VOIs. Voxel-based analysis, with SPM99 in the example of our study, overcomes this limitation as no VOIs need to be specified in advance. An important disadvantage, however, is the additional smoothing introduced through the spatial normalisation process and the smoothing step inherent in the method, with a resulting smoothness of about 13-14mm FWHM of the final statistical map. In the case of MCDs, an even more important limitation is working in standard stereotactic space. With the presence of structural abnormalities, non-corresponding parts of the brain will be compared. This is clearly the reason why heterotopic GM is detected by SPM as an increase of $[^{11}\text{C}]$ DPN binding, for example in the subcortical forms or in the patient with PMG - GM in patients is compared with white matter in controls. Similarly, the normalisation process itself may be altered through the presence of structural abnormalities even in remote areas (Brett et al., 2001). In the future, one way of overcoming such limitations may be to use the areas of abnormality detected by SPM99, reverse the normalisation process to spatially transform these areas back into native space and use them for subsequent confirmatory PVC analysis. Software to allow such "reverse normalisation" is currently being created.

Some further differences between both analyses can be explained through differences in the statistical models used. While the SPM analysis used ANCOVA to remove the effect of varying global levels of binding, the PVC analysis used absolute raw values. While the means of global binding values were not significantly different in the patient and control groups, the spread of the global binding values was greater in the patient group. Accordingly, the patient with the highest global binding value (#3)
showed multiple areas of increased $[^{11}\text{C}]$ DPN binding, and the patient with the lowest global binding value (#9) showed multiple areas of decreased $[^{11}\text{C}]$ DPN binding. In the future, quantitative autoradiographic studies may be able to show whether such global excursions from the mean have a biological basis or are due to technical vagaries in fully quantitative PET scanning.

3.7.5.2 Comparison with previous findings

There were no significant side-to-side differences in $[^{11}\text{C}]$ DPN binding in patients with unilateral temporal lobe epilepsy in two early studies (Bartenstein et al., 1994; Mayberg et al., 1991). In contrast, higher binding of the $\mu$ subtype selective agonist $[^{11}\text{C}]$ carfentanyl was seen in ipsilateral temporal neocortex, while binding in the ipsilateral amygdala was decreased (Mayberg et al., 1991). The latter finding might be due to partial volume effect which could be corrected for in contemporary studies (Meltzer et al., 1996), but the finding of lateral neocortical increases confirmed an earlier study in 13 patients (Frost et al., 1988). It has been speculated that an increase in $\mu$ receptors in the temporal neocortex may be a manifestation of a tonic antiepileptic system that serves to limit the spread of epileptiform activity from other temporal lobe structures.

Using $[^{18}\text{F}]$ cyclofoxy which is a specific antagonist at both $\mu$ and $\kappa$, but not $\delta$ receptor subtypes, increased binding was seen in the ipsilateral temporal lobe in some of the 14 temporal lobe epilepsy patients studied, compared with 14 normal controls, but there was no overall asymmetry in the group (Theodore et al., 1992a). Considering the other available studies, this could be explained through a decrease of affinity or number of $\kappa$ receptors and would also be consistent with decreased availability of $\kappa$ receptors through occupation by an endogenous ligand.

The $\delta$ receptor subtype selective antagonist $[^{11}\text{C}]$ methylnaltrindole has been used in temporal lobe epilepsy patients who were also investigated with $[^{11}\text{C}]$ carfentanyl and $[^{18}\text{F}]$DG PET (Madar et al., 1997). As expected, $[^{18}\text{F}]$DG PET showed ipsilateral widespread decreases, while both opioid tracers showed more localised increases in the ipsilateral temporal cortex. Increases in the $\delta$- and $\mu$-receptor binding showed different regional patterns. Increases in $\mu$ receptor binding were confined to the middle aspect of the inferior temporal cortex, whereas binding of delta receptors increased in the mid-inferior temporal cortex and anterior aspect of the middle and superior temporal cortex.
Differences between antagonist ([11C] methylnaltrindole, [18F] cyclofoxy) or partial agonist binding ([11C] DPN) on the one hand and agonist binding ([11C] carfentanyl) on the other may explain some of the differences found (Koepp and Duncan, 2000), as agonist-driven internalisation of receptors may play a role for G-protein coupled receptors (see chapter 1.5.2.3.5, p. 89). Further, quantitative data of macroscopic and microscopic opioid receptor subtype distribution in humans is now becoming available (see chapter 1.5.2.3.1, p. 85, chapter 1.5.2.3.2, p. 86, and chapter 1.5.2.3.3, p. 87), and such data should aid in the interpretation of seemingly disparate results.

No studies of [11C] DPN binding in MCD have been made available.

Ictal studies have provided evidence for focal release of endogenous opioids in certain forms of generalised epilepsy (Bartenstein et al., 1993) and partial epilepsy (Koepp et al., 1998b) in humans. The patients in our study had a wide range of intervals between last seizure and PET scan. As expected, there was no clear relationship between this interval and global [11C] DPN binding. An analysis of regional influences was neither possible in individual patients due to the single-scan design nor as a correlational analysis in the group due to the heterogeneity of structural changes.

A comparison of this study to our previous study of [11C] flumazenil binding in MCD which used the same principle as the PVC analysis here ((Hammers et al., 2001c), see chapter 3.4, p. 221) is limited due to the disparity in structural alterations. Some generalisations, however, seem possible. Firstly, while heterotopic GM generally showed decreased [11C] flumazenil binding per unit of GM, no such changes of heterotopic GM were seen for [11C] DPN, but cortical [11C] DPN binding in the subcortical forms tended to be less extensively altered in LH and more extensively altered in SNH (and SH). Secondly, in the cortical or mixed forms, the general pattern of predominance of abnormalities of receptor binding in the lesion and its surroundings found in the earlier study was broadly replicated, but abnormalities tended to be found in the wider vicinity rather than in the individually outlined immediately adjacent VOIs. This would correspond to the earlier studies in temporal lobe epilepsy where changes were seen remotely from the structural abnormality as well (Mayberg et al., 1991).

### 3.7.5.3 Clinical and neurobiological considerations

The exact pathological and pathophysiological mechanisms underlying epileptogenesis in MCD are still under study. MCDs can be intrinsically...
epileptogenic (Mattia et al., 1995; Palmini et al., 1995; Sisodiya et al., 1999a), but the generally poor results after epilepsy surgery (Engel, 1993; Sisodiya, 2000), quantitative MRI findings (Sisodiya et al., 1995), PET studies (Hammers et al., 2001c; Richardson et al., 1997a; Richardson et al., 1996; Ryvlin et al., 1998; Van Bogaert et al., 1998a) as well as animal models (Jacobs et al., 1999b) indicate that structural and functional abnormalities are more widespread than the structural lesion visualised by MRI.

The current study provides further evidence for this general concept, with abnormalities of $[^{11}C]$ DPN binding seen both within the lesions and more remotely. This was particularly true for the patients with subcortical forms of MCD in the SPM analysis. Many of the decreases of $[^{11}C]$ DPN binding in the overlying cortex, however, were not confirmed on PVC analysis. This may indicate subtle thinning of the overlying cortex, with preserved numbers of opioid receptors per unit of GM. Similarly and in accordance with a limited number of autopsy studies available (reviewed in chapter 3.4, p. 221) and animal models of PMG (Jacobs et al., 1999c; Prince and Jacobs, 1998), the two patients with PMG and schizencephaly had abnormalities that exceeded the lesions themselves and, in the case of the patient with PMG (#12), PVC analysis demonstrated that these were not merely due to structural changes.

In contrast, some patients had restricted abnormalities of $[^{11}C]$ DPN binding, in particular those with FCD and DNT (not considering patient #9 with the very low global binding), lesions known to be more amenable to surgical treatment (Sisodiya, 2000).

Surprisingly, the three patients with TS, all with multiple tubers demonstrated on MRI, had relatively few abnormalities, all of which were decreases (except for one thalamic increase). In the one patient (#15) in whom both analyses were available, their results were concordant, indicating decreases in one of many tubers only. If these results can be confirmed, they would be in accordance with the results of PET studies using alpha-methyl-L-tryptophan (AMT) which have shown abnormalities of serotoninergic neurons in few tubers in children with TS, and those tubers seemed to be the site of seizure onset (Chugani et al., 1998). It is conceivable that epileptogenic tubers show more derangements of neuronal composition and receptor numbers than others, and that $[^{11}C]$ DPN PET may be clinically useful in this setting.
4 DISCUSSION

4.1 Summary of main findings

The main themes of the investigations outlined in this thesis were advancement of methodologies and their application to the study of patients with epilepsy due to HS and MCD on the one hand, and to patients with epilepsy but without structural MRI abnormalities on the other hand.

The main findings of this thesis were:

- It is possible to create an anatomical template from a single subject and to use this to automatically subdivide individual MRI and PET datasets into anatomical areas. This anatomical template was made more representative by using a larger number of subjects, thus incorporating probabilistic information.

- In patients with epilepsy due to HS studied with $[^{11}C]$ flumazenil PET, the combination of voxel-based analysis and region-based analysis with partial volume effect correction showed extramesial abnormalities in 8/15 patients. Correlation with histopathology showed that increases in the temporal lobe white matter were due to increased numbers of heterotopic neurons, an indicator of microdysgenesis, and there was a positive correlation between increased $[^{11}C]$ flumazenil binding in the white matter of the temporal lobe contralateral to the resected temporal lobe with outcome poorer than Engel class I A.

- In patients with malformations of cortical development studied with $[^{11}C]$ flumazenil PET, abnormalities were seen in the majority of patients. There was a general pattern of decreased $[^{11}C]$ flumazenil binding within the lesion and increased $[^{11}C]$ flumazenil binding in overlying or adjacent cortex.

- Sixteen of 18 patients with temporal lobe epilepsy and normal MRI had abnormalities on $[^{11}C]$ flumazenil PET; these were useful for the presurgical evaluation in seven and could be corroborated by invasive EEG or pathology in five. As a group, these patients showed bilateral hippocampal decreases and
bilateral temporal white matter increases of $[^{11}\text{C}]$ flumazenil binding, the latter indicating microdysgenesis. This was also a prominent feature in 11/18 individual patients.

- Of 44 patients with neocortical epilepsy and normal MRI, 33 had abnormalities on $[^{11}\text{C}]$ flumazenil PET. While there was a variety of abnormalities, $[^{11}\text{C}]$ flumazenil binding increases, particularly in periventricular locations, were a prominent feature of MRI negative partial epilepsies, most likely representing microdysgenesis and/or migration disturbances.

- More than three quarters of patients with epilepsy due to malformations of cortical development, studied with $[^{11}\text{C}]$ diprenorphine PET, had abnormalities of opioidergic neurotransmission. In comparison to the GABA-ergic system, heterotopic grey matter did not show abnormalities of opioid receptors per unit grey matter, and extralesional abnormalities tended to be more remote. Patients with multiple tubers had comparatively few receptor abnormalities.

4.2 Implications

The development of a standard anatomical template or atlas has enabled the automatic subdivision of imaging datasets into a large number of VOIs with reasonable accuracy for cortical regions, and has sped up this process by a factor of about 90. For small brain regions, however, manual delineation following a previously established protocol is still the gold standard.

The creation of a probabilistic atlas has overcome some of the limitations of the previous atlas which had been derived from a single brain. Its many current uses include the anatomical labelling of group studies in stereotactic space, the rough anatomical segmentation of dynamic PET studies of novel ligands for modelling purposes, probabilistic description of spatial localisation and extent of small structures, and automatic anatomical subdivision of individual imaging datasets using the maximum probability map. As different brains may exhibit different patterns, however, the assumption of a point-to-point correspondence does not always hold which remains a fundamental limitation of all such approaches.
The use of combined methodologies allowed us to detect extramesial abnormalities in 8/15 patients with HS. *Ex vivo* and *in vivo* correlations were crucial in assigning the origin of the vast majority of $[^{11}]C$ flumazenil signal in the temporal white matter to (heterotopic) white matter neurons, a finding which has led us to develop methods to investigate white matter $[^{11}]C$ flumazenil binding in other patient groups (see below). White matter $[^{11}]C$ flumazenil binding in the contralateral temporal lobe was correlated with poorer outcome. While the patient numbers were too small to reach firm conclusions, our findings suggest that microdysgenesis contralateral to the hippocampal sclerosis may be one of the reasons why approximately a third of these patients do not become seizure free after anterior temporal lobectomy.

Patients with MCD may exhibit functional abnormalities beyond MRI-visible lesions. Our new approach for the absolute quantification of changes of $[^{11}]C$ flumazenil volume-of-distribution in these patients showed decreases within and next to a variety of lesions, and frequent increases in the surrounding or overlying cortex, even when the lesion itself did not show altered GABA$_A$ receptor binding, corrected for grey matter content. The new methodology allowed, for the first time, the quantification of $[^{11}]C$ flumazenil binding to heterotopic grey matter, and all our findings are in good agreement with *ex vivo* studies and animal models. Recent publications suggest a close relationship of $[^{11}]C$ flumazenil binding decreases and the epileptogenic zone, and it may be possible to use these results in guiding invasive electrophysiological studies in those patients in whom surgery is considered.

The ipsilateral hippocampal decreases of $[^{11}]C$ flumazenil binding shown in our studies of patients with temporal lobe epilepsy and normal MRI have provided further evidence that $[^{11}]C$ flumazenil binding abnormalities may be over and above structural changes. Such abnormalities were present, to a lesser degree, in the contralateral hippocampus, something which was not seen in the group of patients with HS, but which may be one of the reasons why MRI-negative patients fare less well after temporal lobectomy than those in whom a lesion is demonstrated on MRI. The most intriguing finding was that of ipsilateral and contralateral increases of $[^{11}]C$ flumazenil binding in the white matter of the temporal lobes, which was demonstrated in the group analysis as well as in 11/18 individual patients. Similarly
to our study in patients with HS, the correlation of white matter $[^{11}\text{C}]$ flumazenil binding and white matter neuron number could be confirmed in those few patients in whom tissue was available for histological analysis. Animal models suggest that such heterotopic neurons can provide bridges between the hippocampus and neocortical neurons, and they may indeed represent the pathophysiological basis for these patients' epilepsy. The mechanism of additional neuronal connections could also explain why such increases in binding to an inhibitory receptor are seen in patients with epilepsy, a finding which would otherwise be difficult to reconcile with the concept of cortical hyperexcitability.

We applied the same methodology to the study of a further 44 patients with neocortical epilepsy. With ongoing improvements in MRI methodology, fewer and fewer patients are considered "MRI-negative", and our study population was highly selected. This is likely the reason why less single decreases in the lobe of presumed seizure onset were found than might have been expected from older studies. Again, the most important novel finding was that of frequent increases of $[^{11}\text{C}]$ flumazenil binding, often encompassing both grey and white matter, and often situated in a periventricular location. There is no histological material to directly prove that these periventricular increases in $[^{11}\text{C}]$ flumazenil binding correspond to mature neurons, but the high specificity of $[^{11}\text{C}]$ flumazenil, our findings in patients with HS and MRI-negative temporal lobe epilepsy, and simulation studies on imaging datasets from patients with neuronal migration disorder demonstrated on MRI submitted to an identical analysis, all provide evidence pointing towards this explanation. Patients with periventricular migrational abnormalities are known to be poor surgical candidates, and our finding may contribute to the high surgical failure rate in patients with neocortical partial epilepsies and normal MRIs.

Our preliminary study in patients with MCD using $[^{11}\text{C}]$ diprenorphine yielded results which are more difficult to interpret than the $[^{11}\text{C}]$ flumazenil PET findings. This is largely due to the existence of three major subtypes of opioid receptors which are all labelled by $[^{11}\text{C}]$ diprenorphine. The most important finding, however, is the existence of abnormalities of opioid neurotransmission in such a high proportion of patients with MCD. Whether these are aetiologically relevant or represent compensatory mechanisms, the finding of abnormalities in interictal scans in this
group of patients adds to the evidence suggesting that opioid receptors may be a useful pharmacological target in epilepsy. In tuberous sclerosis, cortical abnormalities consisted of decreases only and were restricted to some of the multiple tubers; this finding might be surgically useful if intracranial electrophysiology can confirm that these are the epileptogenic tubers. The studies with $^{11}\text{C}$ diprenorphine await histological and autoradiographic confirmation.
5 FUTURE STUDIES

All functional neuroimaging studies ultimately rely on the correlation with the underlying anatomy. While our work on anatomical templates or atlases can assist in correct anatomical labelling and is currently being used for this purpose in a number of ways, the frontal, parietal and occipital lobe are not or not sufficiently subdivided. A higher number of subjects would also be desirable. Work is in progress to address these issues.

Work in this thesis has focused on PET tracers for two inhibitory systems of neurotransmission. Most hypotheses about basic mechanisms of epileptic seizures agree on an imbalance of excitation and inhibition. The development of PET tracers for excitatory amino acid receptors is desirable. There are various potential targets for such tracers. If, for example, the phencyclidine site within the NMDA receptor could be targeted with an irreversibly bound tracer, it is conceivable that PET might be able to specifically highlight areas of abnormal activity, i.e. areas of spiking. Such work could be carried out in parallel with studies combining EEG and fMRI to investigate regional cerebral blood flow changes.

Different tracers give complementary information. For example, studies with $[^{18}\text{F}]$ fluorodeoxyglucose show an area of altered metabolism which is usually bigger than the epileptogenic zone but usually encompasses it. The combination with $[^{11}\text{C}]$ flumazenil has been found to be useful. Studies with different receptor ligands would likely yield complementary information if they could be used in the same individual. The development of higher sensitivity scanners and instrumentation will allow to perform multiple tracer experiments in the same subject.

PET is uniquely suited to the study of events in the synaptic cleft. A promising approach to functional neuroimaging is to use paired PET studies to study focal neurotransmitter changes associated with cognitive or behavioural changes, or focal or generalised seizure activity. The study of the brain's response to seizures, although methodologically challenging, will be a fascinating task for future PET studies. It may ultimately be possible to study areas of seizure generation and seizure termination, and even response to therapy.
Finally, the combination of *in vivo* quantitative neuroimaging in humans with similar work in animal models, the ongoing correlation of *in vivo* PET findings with histological and autoradiographical data obtained from surgical specimens, and the correlation of PET findings with genetic studies will cross-fertilise all areas of research involved and help to elucidate the biological basis of the data obtained.
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OWN CONTRIBUTION

In general, contribution to the individual chapters is reflected in the author lists of the published or submitted papers (see summaries of individual results chapters). Contributions by others, for example neurosurgical procedures or pathological examination, are detailed in the Acknowledgements (p. 12).

1. Data acquisition and creation of parametric images

I have recruited most of the patients in this study myself. I have personally performed (i.e. arterial and venous cannulation, EEG where applicable, positioning, help in the blood sampling etc.) the $[^{11}C]$ flumazenil PET scans of 15 controls, 18 patients with TLE and normal MRI (chapter 3.5), and 44 patients with neocortical localisation-related epilepsy and normal MRI (chapter 3.6). I have personally performed the $[^{11}C]$ diprenorphine PET scans in all 20 controls and 15 patients with MCD (chapter 3.7). Raw PET data for 15 patients and 13 controls used in chapter 3.3, as well as for 10 patients used in chapter 3.4, was acquired by Drs Matthias Koepp and Mark Richardson before I started my work. The same applies to a proportion of the controls used in chapters 3.4 - 3.6 whose data could be used again, avoiding further irradiation of healthy controls. All raw data has, however, been completely reanalysed by myself, incorporating all methodological improvements available at the time.

I have assisted in the acquisition of the MRI data for all of the controls scanned by myself, as well as some patients.

I have contributed to many methodological improvements regarding data acquisition and calculation of the parametric images. To name but a few, the use of a hand-defined mask to include white matter already during the calculation of the parametric images was reintroduced following my investigation of the matter; lack of $z$-scaling in the images using convolution subtraction scatter correction was detected by myself, and the corrections applied to the recalculation of around 145 image data sets by myself, taking about 5 months.

2. Data analysis

All >300 voxel-based (SPM) analyses were performed by myself, and many methodological refinements introduced by myself. To name but a few, defining the anterior commissure prior to spatial normalisation; use of explicit masking in the
analysis; validation of small volume correction for the study of mesial temporal structures; introduction of mutual information coregistration into routine use; move away from primarily p-value-led analyses to primary analysis of effect sizes.

All region-based (VOI) analyses were performed by myself. This included the rough atlas-based segmentation of all 13+21+20 control, 15 HS and 10+15 MCD MRI datasets (chapters 3.3, 3.4 and 3.7, respectively), individual definition of 56 hippocampi (chapter 3.3), MCDs and overlying/adjacent areas in 10 patients studied with $^{11}$C flumazenil PET (chapter 3.4) and 15 patients studied with $^{11}$C diprenorphine PET (chapter 3.7).

All regions used for the original single-subject atlas were defined by myself (chapter 3.1), as well as the algorithm for their delineation, which was subsequently used for the probabilistic brain atlas as well (chapter 3.2). All 980 regions (consisting of about 8-105 slices each, with an estimated total of $\sim$20,000 slices) used for the probabilistic brain atlas (chapter 3.2) have been checked by myself.

3. Study design

All creative research ideas I had during the work summarised in this thesis were at least facilitated by working in and discussions with an excellent group, and it is difficult to ascribe ownership of ideas to single persons.

The idea of a template- (atlas-) based VOI analysis method arose within the group around the time when I started work on my PhD; whereas the protocol / algorithm was independently devised and implemented by myself. The idea of extending the method to a probabilistic atlas, and the detailed planning of its creation (chapter 3.2) were my own responsibility.

The inclusion criteria for the PET studies were set by my supervisor, Professor John S. Duncan. My main contributions were methodological for the VOI studies; and the idea to study the white matter in detail has been pursued through my initiative.

4. Statistical analysis and data interpretation

The interpretation of all of the data was originated by me. All results were internally presented by myself and critically reviewed with Professor John S. Duncan and Dr Matthias Koepp. I was responsible for the final presentation and interpretation of the data.