

Thesis submitted for the
Degree of Doctor in Philosophy

PET Studies of Neurotransmission in Temporal Lobe Epilepsy

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Declaration of Originality

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ABSTRACT

PET Studies of Neurotransmission in Temporal Lobe Epilepsy

Introduction

Epilepsy, defined as the recurrence of unprovoked seizures, is one of the commonest serious conditions in neurology. The World Health Organisation (WHO) estimates a prevalence of 50 million people worldwide, with a temporal lobe focus being the commonest cause of complex partial seizures. Patients with temporal lobe epilepsy (TLE) often have reduced GABA_A receptors at their seizure focus, and poor memory performance. Blockade of GABA_A receptors containing $\alpha 5$ subunits is promnestic. Animal models have also demonstrated alterations in cannabinoid type 1 (CB₁) receptor availability in response to seizures. The studies presented in this thesis were designed to first determine the reliability of measurement with two novel PET tracers for the expression of $\alpha 5$ subunits of GABA_A receptors ([¹¹C]Ro15 4513) and CB₁ receptors ([¹¹C]MePPEP). These were then used in human TLE to try and elucidate mechanisms of memory impairment and minimally invasive characterisation of the seizure focus.

Methods

Adult healthy volunteers underwent paired scans with [¹¹C]Ro15 4513 (GABA_A $\alpha 5$ receptor partial inverse agonist) and [¹¹C]MePPEP (CB₁ receptor mixed inverse agonist and antagonist). Test–retest variability was characterised for both radiotracers with quantification in regions spanning high and low receptor concentrations by regional compartmental modelling and a variety of regional and voxel-wise model-free analyses (spectral analysis

and variants). Semiquantification with modified standard uptake values (mSUVs), in widespread clinical use, was also explored.

In the clinical studies, healthy volunteers were compared with TLE patients using Statistical Parametric Mapping software. Paired post-ictal and interictal studies were obtained with [^{11}C]MePPEP to determine changes in response to seizures. Single PET scans were obtained with [^{11}C]Ro15 4513 to determine changes in relationship to memory impairments.

Results

[^{11}C]Ro15 4513 could be reliably quantified with voxel-wise spectral analysis, and the simplified reference tissue model, but not mSUVs or compartmental models. For [^{11}C]MePPEP, voxel-wise spectral analysis, a one tissue compartment model and simple mSUVs were the most reliable methods in controls, while preserving between-region differences.

CB₁ availability in TLE was higher, at the group level, in the ipsilateral temporal lobe in post-ictal scans than in controls, and negatively correlated with time since last seizure. In individual patients, however, focal increases were not consistently found in the epileptogenic temporal lobe.

[^{11}C]Ro15 4513 scans could be obtained in 12 patients but have not yet been fully analysed.

Discussion

I demonstrated that two novel PET tracers can be reliably quantified, using a much larger cohort and a much greater variety of methods than available in the literature in the case of [^{11}C]MePPEP, and performing such an analysis for the first time for [^{11}C]Ro15 4513.

This laid the foundation for the clinical study of CB₁ receptor availability in TLE patients. The hypothesis of an upregulation of this inhibitory G-protein coupled receptor type in response to single spontaneous seizures could be confirmed, but the method was not so far useful in individual patients.

[¹¹C]Ro15 4513 PET holds promise for the investigation of the mechanisms of memory impairment in TLE.

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ABBREVIATIONS

--	Unavailable data
(+)-TACP	(+)-trans-(1S,3S)-3-aminocyclopentane-1-carboxylic acid,
α -EMGBL	α -ethyl- α -methyl- γ -butyrolactone
β -EMGBL	β -ethyl- β -methyl- γ -butyrolactone
μ g	Micrograms
μ mol	Micromoles
μ V	MicroVolt
nmol	Nanomoles
1c	One tissue compartment
2-AG	2-arachidonoylglycerol
2c	Two tissue compartment
2D	Two dimensional
2k	Two rate-constants model
2kbv	One brain compartment, two rate-constants model
4k	Four rate-constants model
4kbv	Two brain compartments, four rate-constants model
4-PIOL	5-(4-piperidyl) isoxazol-3-ol
3 α -OH-DHP	5 α -pregnan-3 α ,21-diol-20-one
3D	Three dimensional
^{3} H	Hydrogen-3
3T	Three Tesla
5 α -THDOC	Tetrahydrodeoxycorticosterone
^{11} C	Carbon-11
^{13} N	Nitrogen-13

^{15}O	Oxygen-15
^{18}F	Fluoride-18
^{123}I	Iodine-123
^{137}Cs	Caesium-137
^{35}S	Sulfur-35
A	Anterior
AC	Adenylate cyclase
AC-PC	Anterior - posterior commissure
ACG	Anterior cingulate gyrus
ADC	Apparent diffusion coefficient
ADD	Summed radioactivity-weighted image(s)
AEA	Arachidonylethanolamide
AED	Antiepileptic drug(s)
AMT	alpha-methyl-L-tryptophan
ANCOVA	Analysis of covariance
ant	Anterior aspect
ARSAC	Administration of Radioactive Substances Advisory Committee
AVM	Arterio-venous malformation(s)
b	Bilateral
Ba^{2+}	Barium
BGO	Bismuth germanate on-line
Bil	Bilateral
B_{max}	Receptor density
BMI	Body-mass index
BP	Binding potential
BP-SRTM	Binding potential on specified reference tissue model
BPND	Binding potential

Bq	Bequerels
Br ⁻	Bromine
BS	Between-subjects
BSCV	Between-subject coefficients of variation
bv	Variable blood component
BZ	Benzodiazepine(s)
C	Central region
C _f	Free concentration in brain tissue
C _p	Plasma concentration
C _t	Tissue concentration
Ca ²⁺	Calcium
CB ₁	Cannabinoid receptor type 1
CB ₂	Cannabinoid receptor type 2
CBF	Cerebral blood flow
CBZ	Carbamazepine
Cd ²⁺	Cadmium
CFS	Complex focal seizures
Cl ⁻	Chloride
CL-218,872	3-methyl-6-[3-(trifluoromethyl)phenyl]-[1,2,4]triazolo[3,4-f]pyridazine
CLB	Clobazam
cm	Centimetre
cm ³	Cubic centimetre
CNS	Central nervous system
cps	Counts per second
CSF	Cerebrospinal fluid
CSC	Clinical Sciences Centre
CV	Coefficient of variation

DBI	Diazepam binding inhibitor
df	Degrees of freedom
DPGL	α,α -di-isopropyl- γ -butyrolactone
DPN	Diprenorphine
DTI	Diffusion tensor imaging
DWI	Diffusion weighted imaging
ECI	Electro-Cap International
EEG	Electroencephalography
ES	Epilepsy Society
ESA	Exponential spectral analyses
ETLE	Extra-temporal lobe epilepsy
f	Focal seizure(s)
F	Frontal
F	Frontal lobe
F	Female
FDG	Fluoro-deoxyglucose
Fe	Female
FL	Frontal Lobe
FLAIR	Fluid-attenuated inversion recovery
FLE	Frontal lobe epilepsy
fMRI	Functional magnetic resonance imaging
FMZ	Flumazenil
FORE	Fourier rebinning algorithm
FOV	Field of view
FWHM	Full width at half-maximum
Fz	Reference electrode
g	Grams

GABA	gamma-Aminobutyric acid
GABOB	Gamma-Amino-beta-hydroxybutyric acid
GBq	Gigabequerels
GI	Global intensity
GM	Grey matter
GP	General practitioner
GTCS	Generalised tonic-clonic seizures
HC	Hippocampus
HPLC	High-performance liquid chromatography
hr	Hour(s)
HS	Hippocampal sclerosis
Hz	Hertz
ICBM	International Consortium for Brain Mapping
ICC	Intraclass Correlation Coefficient
ICL	Imperial College London
ICS 205-930	Tropisetron
ID	Identification
IEDs	Interictal epileptiform discharges
IF	Input function
IFE	Idiopathic focal epilepsy
IFG	Inferior frontal gyrus
ILAE	International League Against Epilepsy
inf	Inferior aspect
Inj	Injected
ins	Insula
iqr / i.q.r.	Interquartile range
KA	Kainic acid

KBq	Kilobecquerels
K_D	Dissociation constant
keV	Kilo-electron-Volts
Kg	Kilograms
K_i	Influx trapping constant
L	Left
L-655,708	ethyl(13aS)-7-methoxy-9-oxo-11,12,13,13a-tetrahydro-9H-imidazo[1,5-a]pyrrolo[2,1-c][1,4]benzodiazepine-1-carboxylate
L-838,417	3-(2,5-Difluorophenyl)-7-(1,1-dimethylethyl)-
La^{3+}	Lanthanum
LAC	Lacosamide
LEV	Levetiracetam
L_i	Length of frame i
Log	Logarithm
LS-193,268	3-(5-methylisoxazol-3-yl)-6-[(1-methyl-1H-1,2,3-triazol-4-yl)methoxy] [1,2,4]triazolo[3,4-a]phthalazine
LTG	Lamotrigine
m	Mesial / medial
M	Male
MAPER	Multi-atlas propagation with enhanced registration and decision fusion
Max	Maximum
MBq	Megabecquerels
MCD	Malformation(s) of cortical development
MEG	Magnetoencephalography
MePPEP	((3R,5R)-5-(3-methoxy-phenyl)-3-((R)-1-phenyl-ethylamino)-1-(4-trifluoromethyl-phenyl)-pyrrolidin-2-one)
Mg^{2+}	Magnesium

Min	Minimum
mins	Minutes
ml	Millilitres
mm	Millimetres
Mn ²⁺	Manganese
MNI	Montreal Neurological Institute
MR	Magnetic resonance
MRC	Medical Research Council
MRI	Magnetic resonance imaging
MS	Mean sum of squares
mSUV	Modified standardized uptake value
mSv	miliSievert
mTLE	Medial/mesial temporal lobe epilepsy
mTLE-HS	Medial/mesial temporal lobe epilepsy with hippocampal sclerosis
n	Negative
NAD	No abnormality demonstrated
NH ₄ ⁺	Ammonium
NHNN	National Hospital for Neurology and Neurosurgery
NHS	National Health System
NMDA	N-methyl-D-aspartate
nMRI	Negative /normal magnetic resonance imaging
nTLE	Temporal lobe epilepsy with normal magnetic resonance imaging
O	Occipital lobe
ORG 20599	(2β,3α,5β)-21-chloro-3-hydroxy-2-morpholin-4-ylpregnan-20-one
OXC	Oxcarbazepine
<i>p</i>	<i>p</i> -value / probability-value
P	Parietal lobe

P	Posterior
PET	Positron emission tomography
PGB	Pregabalin
pH	Power of hydrogen
PLE	Parietal lobe epilepsy
prn	<i>Pro re nata (as required)</i>
PVE	Partial-volume effect
PWZ-029	8-chloro-3-(methoxymethyl)-5-methyl-4H-imidazo[1,5-a][1,4]benzodiazepin-6-one
QH-ii-066	7-ethynyl-1-methyl-5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one
R	Regularization
R	Right
rCMr _{glu}	Regional cerebral metabolic rate of glucose;
REC	Research ethics committee
RMM	Relative molecular mass/weight
RNA	Ribonucleic acid
Ro15 1788	Flumazenil
Ro15 4513	Ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-1,4-benzodiazepine-3-carboxylate
Ro19 4603	5,6-Dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a]thieno[2,3-f][1,4]diazepine-3-carboxylic acid 1,1-dimethylethyl ester
ROI	Region-of-interest
RPM	Receptor parametric mapping
RS	Rank-shaping
RS-SA	Orthogonalized-functional-base / Rank-shaping regularisation of spectral analyses
RU5135	3 α -hydroxy-16-imino-5 β -17-aza-androstan-11-one

s	Second
SA	Spectral Analysis
SA-maps	Spectral analysis applied at the voxel level
sd / SD	Standard deviation
SFS	Simple focal seizure
SFS-RR	Structural and Functional Synergistic - Resolution Recovery
SGS	Secondary generalised seizures
SISCOM	Subtraction ictal SPECT co-registered to MRI
SL-651,498	6-fluoro-9-methyl-2-phenyl-4-(pyrrolidin-1-yl-carbonyl)-2,9-dihydro-1H-pyrido[3,4-b]indol-1-one
SpAct	Specific activity of the radiotracer at the time of injection
SPECT	Single photon emission computed tomography
Sr ²⁺	Strontium
SR95531	4-[6-imino-3-(4-methoxyphenyl)pyridazin-1-yl] butanoic acid hydrobromide
SRTM	Simplified reference tissue model
SUV	Standardized uptake value
T	Temporal lobe
t _{1/2}	Half-life
TAC	Time-(radio)activity curve
TB-21007	6,6-dimethyl-3-(2-hydroxyethyl)thio-1-(thiazol-2-yl)-6,7-dihydro-2-benzothiophen-4(5H)-one
THC	Tetrahydrocannabinol
Thio-THIP	4,5,6,7-tetrahydroisoxazolo [5,4-c]pyridin-3-ol
THIP	Tetrahydroisoxazolo [5,4-c]pyridin-3-ol
T _i	Rate of true coincidences
TL	Temporal lobe

TLE	Temporal lobe epilepsy
TPM	Topiramate
u	Unilateral
UCL	University College London
UF	Uncinate fasciculus
UK	United Kingdom
Un	Unavailable or unhelpful
USA	United States of America
V_T	Volume-of-distribution
WHO	World Health Organisation
w_i	Weight for frame i
WM	White matter
WS	Within-subjects
ZAPA	(Z)-3-[(aminoiminomethyl)thio]-2-propenoic acid hydrochloride
ZK93426	ethyl 4-methyl-5-propan-2-yloxy-9H-pyrido[5,4-b]indole-3-carboxylate
Zn^2	Zinc

PREFACE

The epilepsies are a heterogeneous group of neurological conditions characterised by recurrent, unprovoked seizures. Unfortunately the pathophysiology of the epilepsies is still not fully understood. At present the main therapies used for those afflicted with the condition are pharmacotherapy and surgical resection. Pharmacotherapy is not without side effects, and often polypharmacy is required. In some cases even the use of multiple antiepileptic drugs (Asai et al.) do not stop seizures completely. In these cases resective surgery is of use but not without risk. Further difficulties arise when the epileptogenic focus is not readily identifiable. Investigative modalities such as electroencephalography (Panayiotopoulos et al., 2004), magnetic resonance imaging (Kapur et al., 1994), magnetoencephalography (MEG), positron emission tomography (PET) and single-photon emission tomography (SPECT) are used in the diagnosis of epilepsy, and in the localisation of epileptogenic brain tissue. These modalities have their own limitations and are complementary.

This thesis presents data generated via PET. Two novel PET radiotracers, [^{11}C]Ro15 4513 and [^{11}C]MePPEP, were used in an attempt to measure GABA_A $\alpha 5$ receptor and cannabinoid type 1 receptor (CB₁) availability (respectively) in participants with temporal lobe epilepsy (TLE). Success would demonstrate an investigation capable of providing novel *in vivo* data of potential pathophysiological and clinical relevance, for both epilepsies and other neuropsychiatric conditions.

Research Objectives

The objectives of the research reported in this thesis were:

1. To evaluate the potential of a novel PET radiotracer, [^{11}C]Ro15 4513, for the quantification of GABA_A receptor $\alpha 5$ subunit availability *in vivo* in:

- a. Healthy controls
 - b. Participants with temporal lobe epilepsy (TLE)
2. To evaluate the potential of a novel PET radiotracer, [^{11}C]MePPEP, for the quantification of CB₁ receptor availability *in vivo* in;
 - a. Healthy controls
 - b. Participants with TLE

Author's Contributions

I was accountable for:

- Acquiring approval from regulatory authorities (National Health Service (NHS) local research ethics committee (Cheng et al.), NHS Research and Development Departments, the Administration of Radioactive Substances Advisory Committee (ARSAC)).
- Identification, liaison and recruitment of participants. Including: healthy controls from general population and participants with TLE from outpatient epilepsy clinics at the National Hospital for Neurology and Neurosurgery (NHNN) and the Epilepsy Society (ES)
- Obtaining relevant medical data for participants. Including from general practitioners (GP) and from neurological notes and investigations for participants with TLE.
- Data acquisition including: Informed consent, arterial and venous cannulations, EEG data acquisition, r administration and monitoring of movement and completion of scans.
- Basic acute emergency treatment of seizures occurring in participants with TLE while in the premises.
- Health and safety of participants advice prior and post studies.
- Data processing and analyses

- Dissemination of results

Thesis Structure

This thesis presents a body of work addressing the objectives listed above. The thesis is organised as follows:

- In Chapter 1 I concisely summarise the state of the art, describing the various forms of the epilepsies, with a review of existing investigative (particularly neuroimaging) methodologies and receptor systems relevant to the thesis.
- In Chapter 2 I detail the common materials and methods used in the research.
- In Chapter 3 I characterise [^{11}]Ro15 4513 PET in healthy control participants, detailing the cohort demographics, methodology, findings and implications.
- In Chapter 4 I characterise [^{11}]MePPEP PET in healthy control participants, detailing the cohort demographics, methodology, findings and implications.
- In Chapter 5 I describe the application of [^{11}]MePPEP PET imaging to participants with TLE, detailing the demographics, methodology, findings and clinical implications.
- In Chapter 6 I provide an overall discussion of the research, and a review of the main findings, implications and limitations.
- In Chapter 7 I suggest further work, identifying the next logical steps in the research. This includes the future application of [^{11}]Ro15 4513 PET imaging to participants in TLE and the determining a relationship between both radiotracers concentration binding to memory impairments in TLE participants.

CHAPTER 1

Introduction and Background

1.1 The Epilepsies

Second to migraine, epilepsy is the most common condition in neurology (Sander, 2003). A heterogeneous syndrome whose cardinal feature is a predisposition to recurrent, unprovoked seizures (Beghi, 2007, Duncan, 2007b, Chang and Lowenstein, 2003), the WHO defines it as “a chronic disorder characterized by recurrent seizures, which may vary from a brief lapse of attention or muscle jerks, to severe and prolonged convulsions. The seizures are caused by sudden, usually brief, excessive electrical discharges in a group of brain cells (neurones)” (www.who.int/topics/epilepsy/en/, 29 April 2010). The net excitation of neurons in an epileptic focus stems from lack of inhibition or excess excitation, but the understanding of the molecular mechanisms underlying the generation and cessation of seizures is still incomplete.

Conservative estimates suggest an approximate prevalence of 5 per 1000 people, with an incidence of approximately 50 per 100,000 people per year, irrespective of race, age or social status (Sander, 2003, Forsgren et al., 2005). Up to a third of those suffering with the condition in developed countries fail to achieve remission of seizures (Sander, 2003, Kwan and Sander, 2004).

Patients with epilepsy battle with bio-psychosocial co-morbidities and stigma of the condition (Zaccara, 2009, Gaitatzis et al., 2004c, Hermann et al., 2008), and have a reduced life expectancy (Gaitatzis et al., 2004b).

Seizures manifestations vary depending on the brain area affected. Awareness, movement, function and perception have all been reported as altered during seizure activity. Identifying the need for a standardised classification, the International League Against Epilepsy (ILAE), in 1981, divided epilepsies in two major groups based on their origin. Discharges arising focally from the cortex were called 'partial', while discharges arising from synchronously from both hemispheres were called 'generalised' (ILAE, 1981). Further subgroups have since been described and integrated in this classification ((ILAE, 1989); Table 1.1); amongst these, focal (previously known as partial) epilepsies are the most common seizure disorder in adults (Hart and Sander, 2008).

Localization-related epilepsies and syndromes

Idiopathic

Benign childhood epilepsy with centro-temporal spike

Childhood epilepsy with occipital paroxysms

Primary reading epilepsy

Symptomatic

Chronic progressive epilepsia continua of childhood

Syndromes characterized by seizures with specific modes of precipitation

Cryptogenic

Generalized epilepsies and syndromes

Idiopathic

Benign neonatal familial convulsions

Benign neonatal convulsions

Benign myoclonic epilepsy in infancy

Childhood absence epilepsy (pyknolepsy)

Juvenile absence epilepsy

Juvenile myoclonic epilepsy

Epilepsy with grand mal (GTCS) seizures on awakening

Other generalized idiopathic epilepsies not defined above

Epilepsies with seizures precipitated by specific modes of activation

Cryptogenic or symptomatic

West syndrome

Lennox-Gastaut syndrome

Epilepsy with myoclonic-astatic seizures

Epilepsy with myoclonic absence

Symptomatic

Nonspecific aetiology

Early myoclonic encephalopathy

Early infantile epileptic encephalopathy

Other symptomatic generalized epilepsies not defined above

Specific syndromes

Epilepsies and syndromes undetermined whether focal or generalized

With both generalized and focal seizures

Neonatal seizures

Severe myoclonic epilepsy in infancy

Epilepsy with continuous spike-waves during slow wave sleep

Acquired epileptic aphasia

Other undetermined epilepsies not defined above

Without unequivocal generalized or focal features

Special syndromes

Situation-related seizures

Febrile convulsions

Isolated seizures or isolated status epilepticus

Seizures occurring only when there is an acute metabolic or toxic event

Table 1.1. ILAE classification of epilepsies and epileptic syndromes. ((ILAE, 1981))

Symptoms of focal epilepsies can reflect the area of the brain involved where the abnormal activity leads to positive or negative phenomena. However, propagated depolarization of neighbouring neurons, by synaptic and extra-synaptic mechanisms, can result in symptoms which are unrelated to the common functions of the area where the epileptogenic focus is located (Tatum et al., 1995). Focal seizures are classified as 'simple' when consciousness is not affected and 'complex' when consciousness is altered or lost. Focal seizures can also evolve into generalised seizure activity, this type is known as 'partial seizures with secondarily generalisation' (Hart and Sander, 2008, ILAE, 1981).

Temporal lobe foci have been identified as the commonest cause of adult, complex focal seizures (Engel, 1996b), accounting for approximately 60% of cases (Duncan and Shorvon, 1995). According to the localization of the epileptic focus, temporal lobe epilepsy (TLE), can be subdivided in medial (mTLE; also known as mesial or mesio-basal) and lateral (also known as neocortical). These subtypes can further be described by their aetiology; 'symptomatic' when a suspected aetiology has been determined, and 'cryptogenic' when its aetiology is unknown (Walczak, 1995, Engel, 1996b, Theodore, 2004). In 20-30% of patients a symptomatic aetiology is suspected but aetiology is not known; such cases are described as 'cryptogenic epileptic syndromes' (ILAE 1989) or 'magnetic resonance imaging (Kapur et al., 1994) –negative' (nMRI) epilepsies. An accurate diagnosis is often difficult to achieve and extensive investigations including physical examination, blood tests,

electroencephalography (Panayiotopoulos et al., 2004), neuropsychological tests and neuroimaging techniques such as MRI, positron emission tomography (PET) and single photon emission computed tomography (SPECT) are often needed (Duncan, 1997).

1.1.1 Temporal lobe epilepsy (TLE)

As mentioned above, seizures arising in the temporal lobe can, broadly speaking, start in either the medial temporal lobe (mesio-basal type TLE) or in the lateral temporal neocortex (lateral neocortical type TLE).

In patients with the mesio-basal type, a past history of febrile convulsions is often elicited and underlying hippocampal sclerosis (Craven et al.) is frequently identified (Malmgren and Thom, 2012). Classical clinical, electroencephalographic and imaging features include (Duncan, 2009):

- Seizures lasting longer than two minutes with slow evolution and gradual onset/offset.
- Auras (visceral, cephalic, gustatory, dysmnestic, affective, perceptual or autonomic).
- Partial awareness commonly preserved.
- Prominent motor arrest or absence (“motionless stare”).
- Post-ictal confusion and dysphasia.
- Autonomic changes (e.g. pallor, redness and tachycardia).
- Automatisms, either oro-alimentary (lip-smacking, chewing, swallowing), or gestural (e.g. fumbling, fidgeting, repetitive motor actions, undressing, walking, running). Vocalisation is also common. Other motor automatisms can occur.

- Anterior or mid-temporal spikes on electroencephalography (Panayiotopoulos et al.); best shown on sphenoidal electrodes) and other focal discharges in temporal lobe regions.
- Hippocampal sclerosis (demonstrable by unilateral decrease in hippocampal volume and increase in signal on T2-weighted or FLAIR MRI scan) and/or other structural lesions such as tumours, arterio-venous malformations (AVM) and neuronal migration defects (dysplasia).

The initial neurological examination is usually unremarkable, however some evidence of memory deficits is found in neuropsychological testing. Approximately 25% of cases with mTLE become refractory to medical treatment (Semah et al., 1998).

Features of the lateral temporal neocortical type which contrast with the mesio-basal type include (Duncan, 2009):

- History of febrile convulsions is absent.
- Hallucinatory and/or illusionary auras.
- Spikes often smaller and not prominent at sphenoidal electrodes.
- Structural changes (especially malformation of cortical development and tumours) shown on imaging studies.

However, these features do not occur in many cases (Duncan, 2009), and some clinical features such as aura and post-ictal amnesia show substantial overlap between medial and lateral TLE (Williams et al., 1987).

The association between TLE and HS (Margerison and Corsellis, 1966), and the latter with prolonged complex febrile convulsions in childhood has long been identified (VanLandingham et al., 1998). However, the nature of the association between HS in mTLE has been disputed between those who consider HS the cause of recurrent seizures

(VanLandingham et al., 1998) and those who consider it a result of prolonged episodes (Meldrum et al., 1974).

Considering that the hippocampus and the surrounding temporal lobe are fundamental to long-term episodic memory especially to episodic memory encoding (Squire, 1992, Squire et al., 1992), it is unsurprising that HS and temporal lobe abnormalities caused by TLE can therefore translate into life-changing memory difficulties.

Memory complaints (Tellez-Zenteno et al., 2007) and psychiatric co-morbidities are frequent among patients with epilepsy (Gaitatzis et al., 2004a), particularly when mesial structures are involved (Guimarães et al., 2006). The nature of the memory deficit varies from verbal to visual memory impairment depending on injury to the dominant or non-dominant hemisphere. Classical models point to lesions of the left hippocampus as a source of verbal memory deficits (Hermann et al., 1995), while lesions of the right hippocampus are a source of visual memory impairment (Kimura, 1963). Nonetheless, this association is less clear for right-sided hippocampal lesions than left-sided ones (Alessio et al., 2004).

Collinson et al. showed that in the GABA_A $\alpha 5$ receptor knock out animal model, behaviour compatible with hippocampal learning abilities was enhanced by 30% when compared to the knock in animal model (Collinson et al., 2002). Other studies have shown that the performance in hippocampus-independent delay conditioning was not improved (Crestani et al., 2002, Yee et al., 2004)

Patients with focal epilepsies may be amenable to surgical intervention as a treatment option. However, this is dependent on the epileptogenic focus being located in a surgically-accessible region. Structural imaging such as MRI may be used to accurately extrapolate the location of this pathology. Nonetheless, 20 – 30% of TLE patients have no obvious

structural defect on MRI (nTLE) (Duncan, 1997). Nuclear imaging modalities, such as fludeoxyglucose (FDG) PET, can locate the epileptogenic focus in approximately 85% of patients with TLE (Pillai et al., 2007) and is particularly useful for the detection of abnormalities in the nTLE subgroup.

At present, MRI and transcranial EEG are the methods of choice used in the assessment of patients with TLE. MRI is used primarily to identify structural abnormalities that might be the cause of the epileptic seizures (e.g. benign tumours such as dysembryoplastic neuroepitheliomas, cavernous angiomas, gliomas, malformations of cortical development including focal cortical dysplasias, and gliosis secondary to cerebral infection (Duncan, 2007a, Craven et al., 2011)).

The commonest finding in TLE is mesial temporal sclerosis, identified on T1 MRI images as: a reduction in hippocampal volume of 15-35% in comparison to the contralateral hippocampus and to controls (Cendes et al., 1993, Fuerst et al., 2003); with higher signal intensity on T2 MRI images and FLAIR; and atrophy of its internal structure (Craven et al., 2011, Deblaere and Achten, 2008). These MRI abnormalities can be confirmed histopathologically (Cascino et al., 1991).

Transcranial EEG, on the other hand, allows the identification of abnormal electrical discharges through a two-dimensional representation of the three-dimensional cortical neural activity (Plummer et al., 2008). EEG studies can be performed during (ictal) or between (interictal) seizure episodes.

Ictal EEG patterns are distinguished from interictal epileptiform discharges (IED) in that the discharges of the former evolve in frequency and amplitude, and are coupled with clinical symptoms. The most distinguishing EEG feature of focal seizures is their progression to

high-amplitude activity with slower frequencies. Simple partial seizures are usually associated with cessation of interictal spikes; while in complex partial seizures low-voltage fast (5 - 7 Hertz) rhythmic activity is observed in one sphenoidal electrode (Risinger et al., 1989). However, more than 70% of simple focal seizures do not clearly correlate with transcranial studies (Engel, 1996a).

Usual findings with intracranial EEG in mTLE are anterior or mid-temporal spikes and spike-wave discharges, that can be unilateral or bilateral and independent (Engel, 1996b), and frequently disseminate to electrodes on the frontal pole or posterior temporal area (Emerson et al., 1995). In contrast, neocortical lateral TLE spikes are usually smaller, and less prominent on sphenoidal electrodes. They are frequently over the entire temporal lobe, and maximal along the lateral convexity (Duncan, 2007b).

1.1.2 Lateralization of the epileptogenic focus

The lateralization of epileptogenic focus by imaging techniques, EEG or memory tests are crucial for curative surgical intervention of patients with epilepsy. As mentioned above, coarse hemispheric lateralization can be obtained from EEGs when abnormal anterior or mid-temporal spikes are unilateral. The presumed epileptogenic focus can be localised on MRI in approximately 80% of patients (Duncan, 1997), for example by the identification of sclerosis or other visible lesions of the hippocampus.

In FDG PET, the epileptogenic focus is inferred when a hypometabolic area with decreased activity is found (Pillai et al., 2007). Diffusion weighted imaging (Gaitatzis et al.) has shown that lateralization can also be achieved by inspection of apparent diffusion coefficient (ADC) images (Yoo et al., 2002). Findings in diffusion tensor imaging (DTI) studies point to increased diffusivity and decreased fractional anisotropy in occult epileptogenic cerebral lesions (Rugg-Gunn et al., 2001). Furthermore, *de novo* organization of connecting

pathways between distant epileptogenic foci has been identified with DTI-based tractography (Bhardwaj et al., 2010). DTI findings suggest that memory and language impairments in TLE could be associated with an increased mean diffusivity (McDonald et al., 2008), particularly in the left uncinate fasciculus (UF) when poor auditory memory is present; and in the right UF when visual memory is impaired in left TLE (Diehl et al., 2008).

During the ictal period there is an increase of glucose metabolism and cerebral blood flow (CBF) in epileptogenic foci (Engel et al., 1983). There is also evidence to suggest an increase in glucose metabolism during the first 24 – 48 hours post-ictally (Leiderman et al., 1994). However, both parameters decrease during the interictal period (Tai and Piccini, 2004).

1.2 Positron emission tomography (PET), receptors, and ligands

Positron emission tomography (PET) scan is a nuclear medicine imaging technique that is used to produce a 3D image of functional processes in organs and tissues following the injection of the subject with a ligand labelled with a positron-emitting radionuclide, such as fluorine-18 (^{18}F) or carbon-11 (^{11}C) (Blokland et al., 2002, Frackowiak, 1989). A PET camera is then used to detect the release of a pair of γ -rays that results from the annihilation between an emitted positron with a nearby electron (Frackowiak, 1989).

Some of the clinical applications of PET in neurology include the measurement of CBF and the quantification of specific binding of the radiotracer to target receptors. Cameras can detect radiation in absolute terms (Becquerel [Bq] per pixel) and images are reconstructed from the acquired data. These quantitative images can then be analysed; for instance, the concentration of radioactivity (Bq/ml) within different areas of the brain can be determined in defined regions of interest (ROIs) and normalised by the concentration of the injected dose (Bq/g). The resulting ratio is known as the standard uptake value (SUV) and denotes the

ligand distribution (Innis et al., 2007). Furthermore, measured radiation distributions can be transformed into functional parameters using compartmental models and a suitable input function, derived either from arterial plasma concentration of the radiotracer (which also allows to account for metabolism) or from image data, i.e. the time course of radioactivity in a reference region devoid of specific binding.

Four concepts are integral to understanding the analysis methods of PET:

- *Specific binding*: This is binding to the target receptor by the radiotracer, where the binding to other macromolecular components and radiotracer that is not bound at all (free) in the tissue sample is not included (Innis et al., 2007).
- *Binding potential (BP)*: This is the result of the quantification of the equilibrium concentration of specific binding as a ratio with another reference concentration (Innis et al., 2007).
- *Volume-of-distribution (V_T)*: is the ratio at equilibrium of the concentration of the parent radiotracer in a tissue/an organ of interest (e.g. the brain) to that of the parent radiotracer in plasma, separated from metabolites (Innis et al., 2007). V_T is strictly unitless, as $1\text{cm}^3 = 1\text{ml}$ (Innis et al., 2007). This (macro-) parameter is a linear function of free receptor concentration and is less sensitive to noise than microparameters such as individual rate constants (e.g. K_1 , k_2 ; see Figure 1.1), which are difficult to quantify accurately. This latter quality is particularly of use when quantifying at the voxel level, rather than within a region-of-interest.

- *Compartment models:* These are theoretical models that assume constant homogeneity of tracer concentration in physiological “spaces”. Classical compartment modelling is limited by the inference of a correct number of compartments. For example, in a region with specific binding, it can often be assumed that there are six fraction rate constants (fraction of the concentration moving between compartments per unit of time, [k]) which describe movement between the following four compartments: plasma, an intracerebral space where radiotracer is free, a space where the radiotracer is bound to nonspecific targets, and a space where the radiotracer is bound to a specific target (Frost et al., 1989, Slifstein and Laruelle, 2001) (Figure 1.1).

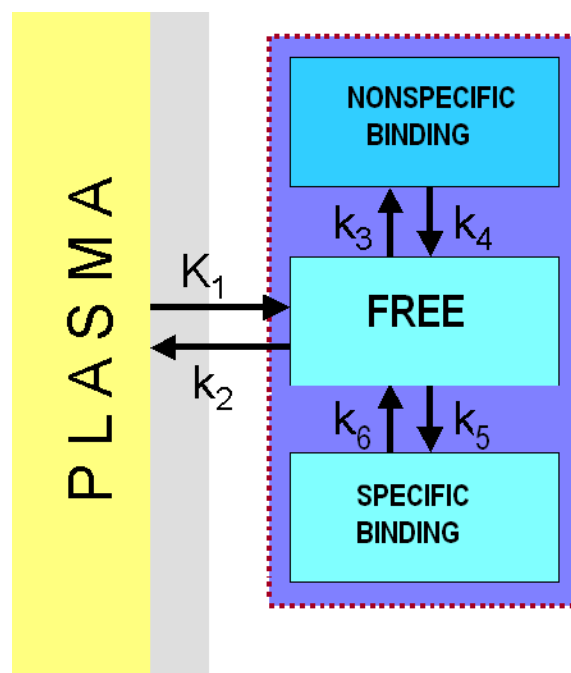


Figure 1.1. Four compartments model (plasma, free radiotracer space, bound to nonspecific targets space and bound to a specific targets space); where k are the fraction rate constants.

PET employs radioisotope with short-life such as carbon-11 (^{11}C), oxygen-15 (^{15}O), nitrogen-13 (^{13}N) and fluoride-18 (^{18}F). Due to its 20-minute half-life ($t_{1/2}$), versatility in synthesis and

labelling potential, ^{11}C is an ideal radioisotope for research studies (Paans et al., 2002, Långström et al., 2007). For radioisotopes to bind to a target, synthetic molecules or tracers are designed to incorporate radionuclide (label) and to bind to specific targets, for example receptor systems.

Receptors are proteins that act as specific binding sites for ligands such as neurotransmitters. This process of linkage between ligand and receptor induces a conformational change in the receptor that initiates a cellular signal. Ligands are classified according to their binding site and by the types of cellular signal they induce; if they produce the maximum stimulatory effect by binding to the natural (endogenous agonist) ligands' receptor binding-site, they are described as *full agonists* (Figure 1.2) (Blumenthal and Garrison, 2005).

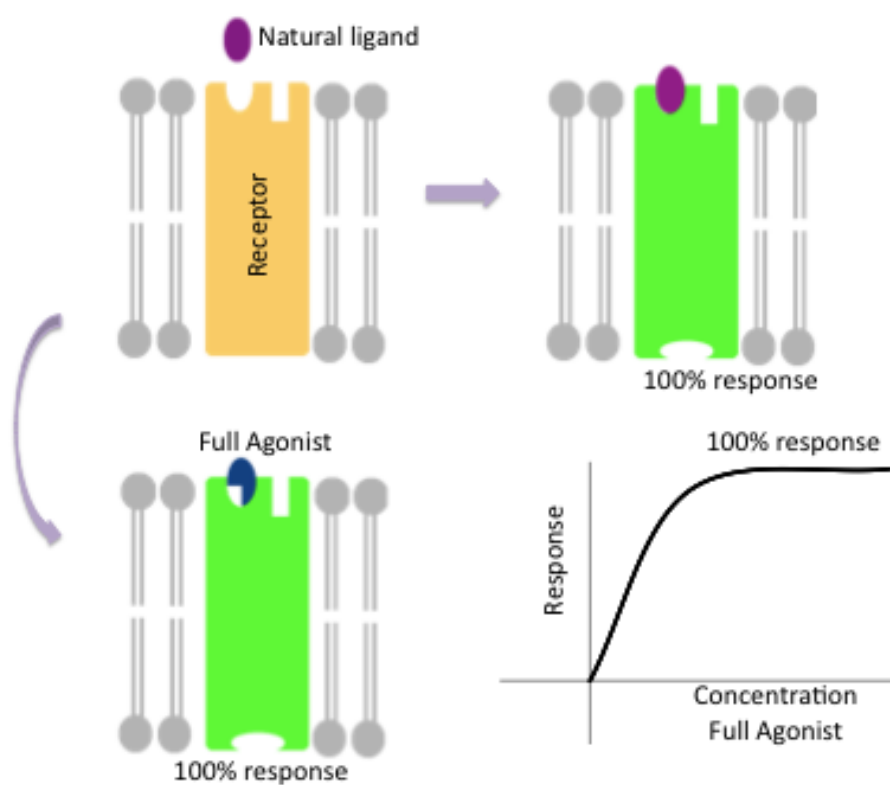


Figure 1.2. Full receptor agonists.

Partial agonists exert an incomplete stimulatory effect while still binding to the natural ligands' binding-site (Figure 1.3) (Blumenthal and Garrison, 2005).

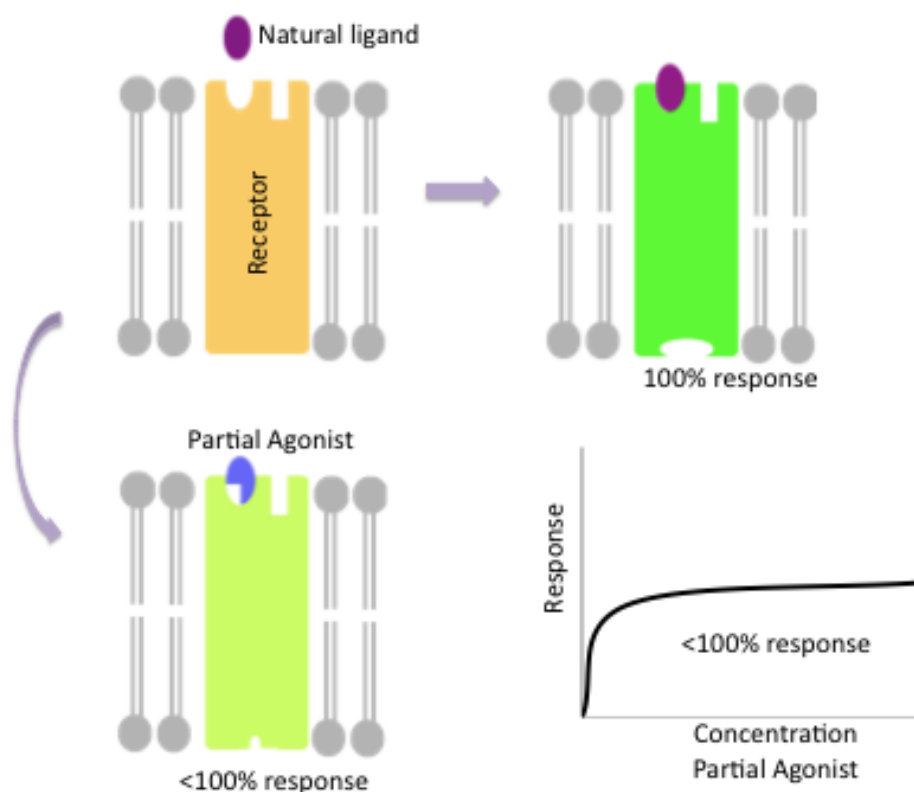


Figure 1.3. Partial receptor agonists.

Antagonists, in contrast, inhibit the agonist-mediated response. Antagonistic ligands are divided in two subtypes: *competitive* and *non-competitive*. *Competitive antagonists* bind and block the natural ligand's binding-site; disrupting the binding of agonists, whereas *non-competitive antagonists* bind to a different binding-site from that of the natural ligands, yet inhibit the agonist-mediated response (Figure 1.4) (Blumenthal and Garrison, 2005).

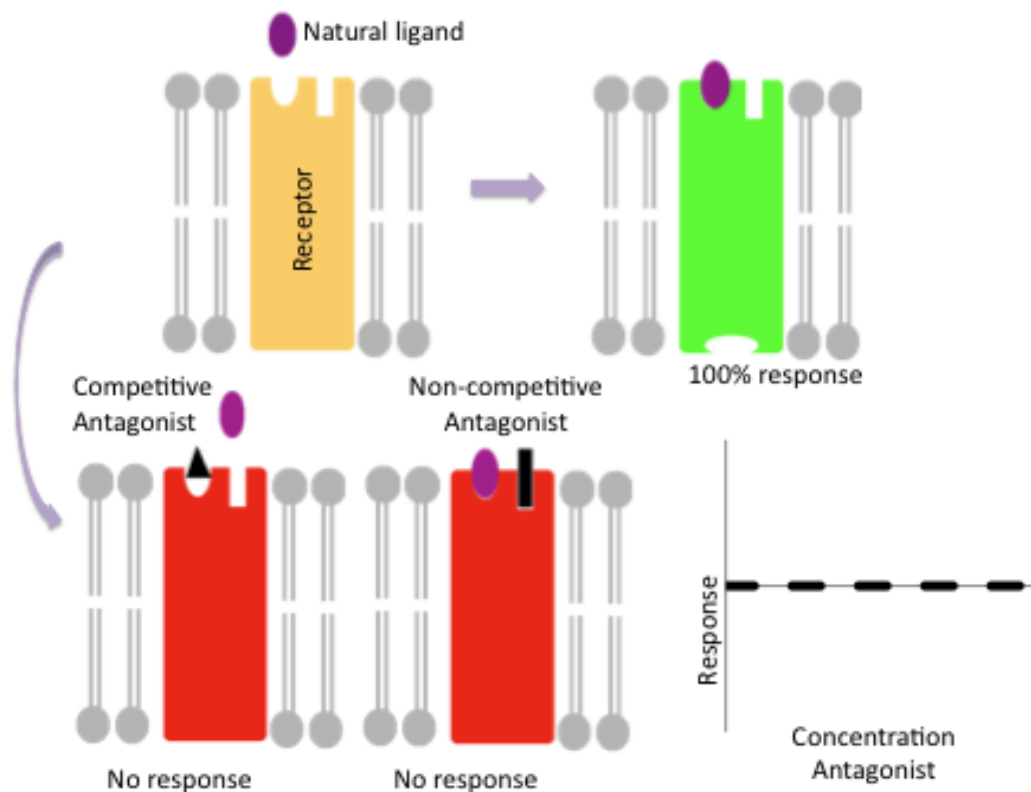


Figure 1.4. Receptor antagonists.

Inverse agonists are ligands which bind to the agonists' binding-site in a receptor that has a basal level of activity in the absence of any ligand, triggering a signal which is the opposite to that mediated by the agonist (Figure 1.5) (Blumenthal and Garrison, 2005)

Finally, *allosteric modulators* can influence the effect of agonists and inverse agonists by co-binding to a distinct site of that of the endogenous ligand. This modulation can be *positive* when the effect is amplified, *negative* when the effect is reduced and silent or *neutral* (Chang et al., 2010).

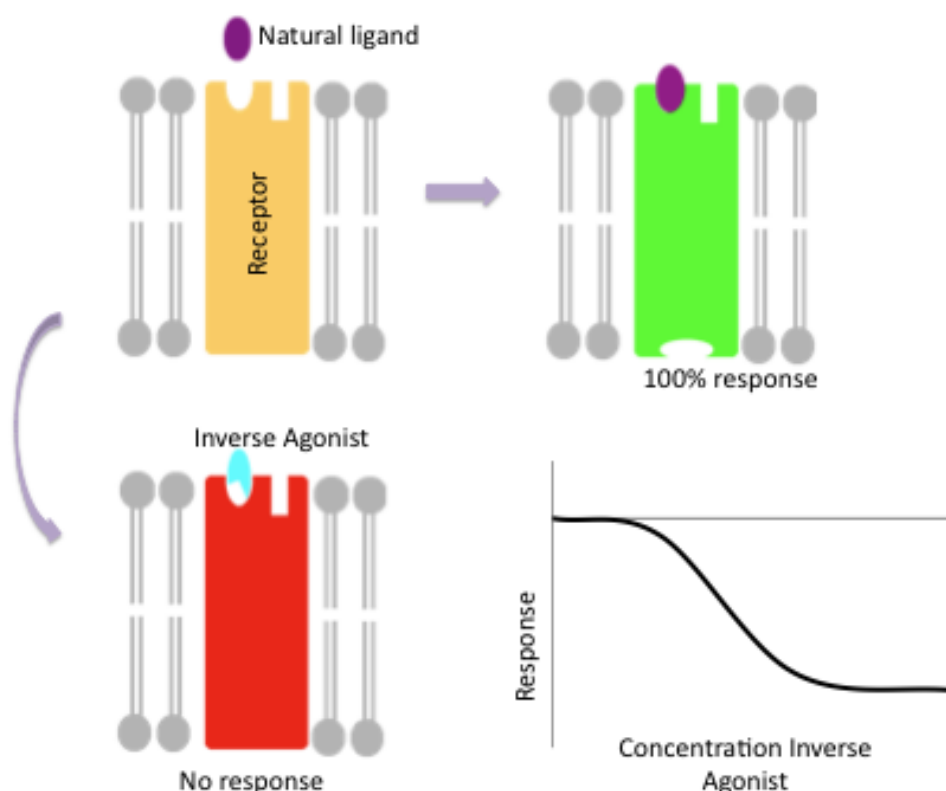


Figure 1.5. Inverse agonists.

1.2.1 PET in temporal lobe epilepsy

In focal epilepsy, [^{18}F]fluoro-deoxyglucose (FDG) PET is used in the pre-surgical assessment of interictal cerebral metabolism in subjects. The presumed epileptogenic focus is determined by the identification of areas of hypometabolism by comparison with the neighbouring cortex and/or databases of normal controls (Henry and Votaw, 2004).

Interictal [^{18}F]FDG PET can uncover ipsilateral temporal lobe hypometabolism in approximately 60-90% within TLE (Henry et al., 1993c, Rugg-Gunn, 2009, Gaillard et al., 1995) and also reveal hypometabolism in the ipsilateral insula (Bouilleret et al., 2002). Carne was able to lateralise 87% nTLE (Carne et al., 2004), however, lateralisation is less reliable

in cases where the area of hypometabolism found in PET is not related to the area of abnormal neuronal discharge on EEG (Hajek et al., 1993).

Reductions in uptake similar to those seen with [^{18}F]FDG PET have been found with various radiotracers targeting different receptors such as: [^{11}C]flumazenil (FMZ) PET (Savic et al., 1993, Henry et al., 1993b) targeting the benzodiazepine (BZ) receptor and [^{11}C]ketamine PET (Kumlien et al., 1999) targeting the N-methyl D-aspartate (NMDA) receptor. In contrast, [^{11}C]carterafil PET (Mayberg et al., 1991) and [^{11}C]diprenorphine (DPN) PET (Hammers et al., 2007a), which image opioid receptors, and [^{11}C]alpha-methyl-L-tryptophan (AMT) PET (Natsume et al., 2003) which images serotonin synthesis, have shown increased binding in the ipsilateral temporal neocortex of subjects with TLE.

1.3 Receptor systems in the epilepsies

1.3.1 GABA receptors

γ -aminobutyric acid (GABA) is one of the principal inhibitory neurotransmitters in the brain (Curtis et al., 1970). Two main types of GABA receptors have been described; two ligand-gated ion channel receptors (GABA_A including GABA_{A-p}) and one G protein-coupled receptor (GABA_B) (Lobo and Harris, 2008).

GABA_A receptors are described in detail in the following section. In contrast to GABA_A receptors, GABA_B receptors are dimers that consist of two subtypes, GABA_{B1} and GABA_{B2} (Bettler et al., 2004). Two heteromeric receptors exist, GABA_{B(1a, 2)}} and GABA_{B(1b, 2)}}. Binding of GABA to the venus fly trap-like domain of the B1 subtype induces an allosteric change in B2, which in turn activates the G protein.

Activation of GABA_B receptors inhibits voltage-gated Ca^{2+} channels, opens K^{+} channels, and inhibits adenylyl cyclase. Presynaptic GABA_B receptors suppress neurotransmitter release by

inhibiting Ca^{2+} channels and via secondary messenger effects on vesicle priming (Sakaba and Neher, 2003). The effect can actually give a net disinhibitory effect when the receptors are located presynaptically on an inhibitory neuron terminal. Most GABA_B receptors, however, are located distant to GABAergic terminals. Postsynaptic GABA_B receptors induce a slow inhibitory postsynaptic potential (IPSP_B) by activating K^+ channels, resulting in membrane hyperpolarisation.

Presynaptic GABA_B receptors located in GABAergic terminals promote long-term potentiation (LTP) by disinhibition of the postsynaptic neuron (Davies and Collingridge, 1996, Davies et al., 1991), whereas postsynaptic GABA_B receptors diminish LTP via hyperpolarisation.

1.3.1.1 GABA_A receptors and its subunits

GABA_A receptors are members of the wider Cys loop family, which also includes nicotinic and glycine receptors (Barnard, 2000). The receptors are formed by a combination of subunits in a circular arrangement, with a central pore (Wang et al., 1994, Cutting et al., 1991). So far, nineteen GABA_A receptors subunits isoforms are known in humans including $\alpha 1 - \alpha 6$, $\beta 1 - \beta 3$, $\gamma 1 - \gamma 3$, δ , π , ϵ , $\rho 1 - \rho 3$ and θ (Barnard et al., 1998). Each subunit consists of a hydrophilic extracellular N-terminal domain containing a Cys loop, and four transmembrane sequences (one of which, M2, lines the ion channel). For the assembly of a GABA-gated ion channel a minimum inclusion of five subunits (pentameric structure) including two α 's subunits, two β 's subunits, and (for benzodiazepine site activity) one γ subunit is needed (Connolly et al., 1996, Bormann et al., 1987). It is believed that the most common isoforms in human adults are $\alpha 1\beta 2\gamma 2$, $\alpha 2\beta 3\gamma 2$, and $\alpha 3\beta 1-3\gamma 2$ (McKernan and Whiting, 1996). The combination of the different subunits creates a diversity of GABA_A isoforms with differing pharmacology and physiology (Johnston, 1996, Chang et al., 2010),

consequently allowing a wide variety of ligands to interact with specific sites on the receptor (Table 1.2) (Johnston, 1996).

Examples of Agents acting on GABA _A	
Competitive Antagonists	Bicuculine, (+)-Hydrastine, SR95531, Pitrazepin, Securinine, RU5135, Benzyl penicillin, (+)-Tubocurarine
Non-competitive Antagonists	Picrotoxinin, δ -Guanidinovaleric acid, m-Benzenesulfonic acid diazonium chloride, Cunaniol, Dopamine sulfate, Dimeflin, Enoxacin, Norfloxacin, Pentylenetetrazole, Furosemide
Agonists (Endogenous)	GABA, GABOB, Imidazole-4-acetic acid, β -Alanine, Taurine
Agonists (Exogenous)	Muscimol, THIP, Isoguvacine, ZAPA, (+)-TACP, Pentobarbitone, SL-651,498 (at $\alpha 2$ and $\alpha 3$), CL-218,872, QH-ii-066, Gaboxadol, Ibotenic acid, Muscimol, Progabide
Partial Agonists	4-PIOL, Thio-THIP, SL-651,498 (at $\alpha 1$ and $\alpha 5$), Bretazenil, Imidazenil
Positive Allosteric Modulators (Endogenous)	3 α -OH-DHP, 5 α -THDOC, Arachidonic acid, Interleukin-1, H ⁺ , NH ₄ ⁺ , Mg ²⁺
Positive Allosteric Modulators (Exogenous)	Pentobarbitone, Etomidate, Diazepam, α -EMGBL, Halothane, Diethylether, Enflurane, Isoflurane, Alphaxolone, Ketamine, Propofol,

	Ethanol, Trichloroethanol, ORG 20599, Cd ²⁺ , Mn ²⁺ , La ³⁺ , Br ⁻ , Dinatin, Chrysin, Amentoflavon, Miltirone, L-838,417
Negative Allosteric Modulators (Endogenous)	DBI, Butyl β -carboline-3-carboxylate, Cortisone, Ca ²⁺ , Zn ²⁺ , Phosphatidylethanolamine, Purines
Negative Allosteric Modulators (Exogenous)	Ro19 4603, β -carbolines, β -EMGBL, Cortisone, Dieldrin, Lindane, Deltamethrin, Sr ²⁺ , Ba ²⁺ , Colchicine, Nocodazole, Vinblastine, Taxol
Bidirectional Allosteric Modulators (Endogenous)	Pregnenolone, Pregnenolone sulfate, Cortisol
Bidirectional Allosteric Modulators (Exogenous)	Avermectin B _{1a} , ICS 205-930, Amitriptyline, Forskolin, 8-Bromo-cAMP, Mefenamic acid, Flufenamic acid
Neutralising Allosteric Modulators	Ro151788, ZK93426, DPGL, Epipregnanolone
Inverse Agonists	TB-21007, PWZ-029, L-655,708, LS- 193,268, Ro15-4513

Table 1.2 Agents acting on GABAA receptors (Johnston, 1996).

GABA_A receptor binding sites are located in the extracellular surface of the receptor. The quantity of the various sites that interact with GABA_A agents is unknown. However some of the likely binding sites already recognised include: the agonists/ competitive antagonist recognition sites, the benzodiazepine site (dependent of the presence of γ 2 subunit. Figure 1.6), the sedative-hypnotic barbiturates sites, the picrotoxin sites, the neuroactive steroid

sites, ethanol sites (dependent of the presence of a phosphorylated $\gamma 2_L$ subunit), stereoselective sites for inhalation of anaesthetics (e.g. Isoflurane), sites for furosemide, sites for Zn^{2+} , sites for a variety of divalent cations (such as Ca^{2+} , Sr^{2+} , Ba^{2+} , Cd^{2+} , Mn^{2+} and Mg^{2+}) and sites for La^{3+} among others (Johnston, 1996).

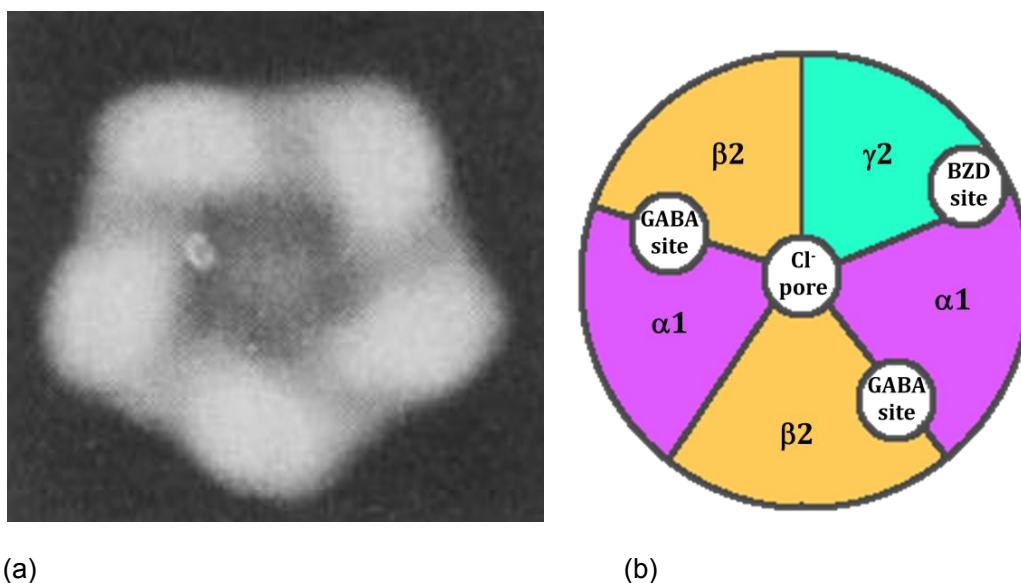


Figure 1.6 Pentameric structure of GABA receptors (a) visualized by electron microscopy (Nayeem et al., 1994). (b) Diagram of a GABA_A receptor isoform $\alpha 1\beta 2\gamma 2$, illustrating the five subunits, the chloride (Cl^-) ion channel and three binding sites: two GABA binding sites at the $\alpha 1$ and $\beta 2$ interfaces and one allosteric benzodiazepine binding site at the $\gamma 2$ and $\alpha 1$ interface.

GABA_A receptor subunits exhibit markedly heterogeneous but overlapping distributions within the brain (Elster et al., 1995, Sieghart and Sperk, 2002). For example, the $\alpha 1$ subunits are the most abundant overall and predominate in the cortex, while the $\alpha 5$ subunits are expressed in limbic regions (Pirker et al., 2000). The structure with the most abundant $\alpha 5$ receptors in the human brain is the hippocampus, where this subtype is extrasynaptic and accounts for almost 28% of binding sites in [^3H]Ro15–1788 PET studies (Sur et al., 1998).

GABA_A receptors are usually located in the postsynaptic membrane and mediate fast inhibition (within milliseconds). The binding of agonists to GABA_A receptors leads to changes in the conformation of the receptor and opening of the integral anion-selective channel with subsequent Cl⁻ influx and hyperpolarisation of the postsynaptic membrane, which reduces the cell's excitability. In contrast, extrasynaptic GABA_A receptors are activated by GABA that has diffused from its site of release, and therefore mediate long-term (tonic) inhibition (Farrant and Nusser, 2005).

The role of GABA_A receptors changes during development from excitatory to inhibitory; this is also the case with certain pathologies (Galanopoulou, 2008, Charych et al., 2009). *In vivo* studies suggest that abnormalities in GABA_A receptors can predispose to epilepsy (Lloyd et al., 1986, Galanopoulou et al., 2002). Moreover, low GABA concentrations have been associated with continued seizure activity (Petroff et al., 1996) and poor seizure control in complex partial seizures (Petroff et al., 2001). Additionally, it has been proposed that under certain conditions GABA_Aergic signalling increases neuronal synchronization, contributing to interictal discharges (Kohling et al., 1998).

Marco et al. reported a decrease in the number of GABAergic inhibitory interneurons in epileptogenic cortices (Marco et al., 1996). With the use of benzodiazepine antagonists like FMZ in PET studies, it has been possible to confirm changes in GABA_A receptor concentrations (see overleaf; (Chugani et al., 2001)). Some antiepileptic drugs (Asai et al.) target GABA_A receptor in order to achieve seizure suppression. Other pharmaceutical modulators of GABA_A receptors typically used include BZ and barbiturates (Barnard et al., 1998).

1.3.1.2 GABA_A receptors PET in epilepsy

[¹¹C]FMZ, also known as [¹¹C]Ro15 1788, is a PET radiotracer that is selective for the GABA_A receptor (Maziere et al., 1984, Olsen et al., 1990) which is predominantly expressed on neurons (Savic et al., 1988). FMZ targets an allosteric site on the receptor complex with a consequent decreases in the efficiency of the main site and indirectly the conductance of Cl⁻ (Palaoglu and Ayhan, 1986, Haefely, 1988). It is likely that given this negative allosteric modulation, FMZ can exert an anti-epileptic effect (Savic et al., 1991, Hart et al., 1991). FMZ's allosteric binding to GABA_A receptor has been used to localise the probable epileptogenic focus. An example of this can be found in the studies performed by Maziere et al. (1984) and Olsen et al. (1990), using [¹¹C]FMZ PET in 10 participants with refractory focal epilepsy, six of whom were localised to mesial temporal regions by EEG. Participants with epilepsy had significantly lower receptor density (B_{max}) in the presumed epileptogenic zone than in contralateral homologues or than in controls.

In a study of 15 participants with refractory mTLE-HS and after correction for the partial volume effect (PVE), V_T was reduced in the ipsilateral hippocampus in 14 participants, additionally extending into the amygdala for four, (relative to the V_T of 13 controls; (Hammers et al., 2001a)). Neocortical abnormalities were additionally observed in five participants. In the same study, a strong positive correlation was observed between temporal white matter [¹¹C]FMZ V_T and white matter neuron number.

Hammers et al., reported a study of [¹¹C]FMZ PET in 44 participants with refractory, MRI-normal neocortical epilepsy (none of whom 'temporal', 19 'not clearly lobular') and 16 healthy control participants. Decreases in [¹¹C]FMZ V_T were observed for eight participants with refractory epilepsy, whereas increases were observed for 16 participants, and both decreases and increases were observed for nine participants. For seven participants, the increases were periventricular, suggestive of neuronal migration disorders (Hammers et al.,

2003b). The same group subsequently reported a study of [^{11}C]FMZ PET in 15 patients with refractory mTLE and histologically-verified unilateral HS. Comparison of the seven participants who continued to have seizures with the eight participants with Engel IA outcome after surgery revealed increased [^{11}C]FMZ V_T around the posterior horns of the ipsilateral and contralateral ventricles (Hammers et al., 2005).

The synthesis of novel tracers such as Ro15 4513 (ethyl-8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo-1,4-benzodiazepine-3-carboxylate; F. Hoffmann–La Roche Ltd., Basel, Switzerland) promises the further understanding of GABA receptors. Ro15 4513 is an imidazodiazepine that behaves as an inverse agonist and can decrease the threshold for seizures at high doses (Bonetti et al., 1984, Lister and Nutt, 1988), manifesting on EEG as a state of alertness (Marrosu et al., 1989). Competition studies in the rat *in vivo* revealed that radiolabeled Ro15 4513 uptake was reduced to nonspecific levels only by drugs that have affinity for the $\alpha 5$ subtype (flunitrazepam, RY80, Ro15 4513, L655,708), but not by the $\alpha 1$ selective agonist, zolpidem. Moreover, receptors that express $\alpha 5$ subunits have 10 to 20 times more affinity for Ro15 4513 than BZ type 2 receptors (Hadingham et al., 1993, Luddens et al., 1994).

[^{11}C]Ro15 4513 PET offers greater potential to investigate GABA_A receptor binding in the hippocampus in particular than [^{11}C]FMZ PET and [^{123}I]iomazenil PET which are mainly indicative of the distribution of $\alpha 1$ subtype. Lingford-Hughes' research team evaluated [^{11}C]Ro15 4513 in three healthy men. V_T images were produced via voxel-wise exponential spectral analysis and compared on a regional basis with [^{11}C]FMZ V_T derived from six healthy volunteers (Lingford-Hughes et al., 2002), and indicated relatively greater binding in the hippocampus for [^{11}C]Ro15 4513. Taken together with blocking studies in the rat with compounds selective for the $\alpha 1$ or $\alpha 5$ subunit, it was concluded that $\alpha 5$ binding was the dominant source of [^{11}C]Ro15 4513 V_T . [^{11}C]Ro15 4513 binding has also been quantified in

eight healthy men using both compartmental modelling and linear graphical analyses (Asai et al., 2009); the simplified reference tissue model (SRTM) with pons as a reference emerged as a suitable method based on resilience to noise, but test-retest studies were not performed. More recently, pre-scan administration of the α_1 -selective agonist zolpidem did not significantly decrease [^{11}C]Ro15 4513 V_T (Myers et al., 2012)

[^{11}C]Ro15 4513 PET has recently been used to demonstrate alterations in GABA_A $\alpha 5$ availability/distribution in alcohol dependence (Lingford-Hughes et al., 2012), tobacco smoking (Stokes et al., 2013a), autism spectrum disorder (Mendez et al., 2013), schizophrenia (Asai et al., 2008), and temporal lobe epilepsy (Barros et al., 2010). In order to facilitate the interpretation of these and future studies, it is necessary to document the test-retest reproducibility of [^{11}C]Ro15 4513 PET parameters.

1.3.2 Endocannabinoid system

The endocannabinoid system is an ubiquitous group of lipids and their receptors, which is believed to have developed early in the evolution of mammals and which modulates the release and action of neurotransmitters (Di Marzo et al., 1998). Through presynaptic inhibition of voltage-sensitive calcium (Ca^{2+}) channels and adenylate cyclase (AC) (Felder et al., 1995, Mackie et al., 1993, Mackie et al., 1995), this system has an important role in neuronal functions such as pain, cognition, memory, appetite, mood and addictions; as well as inflammatory processes (Calignano et al., 1998, Rodriguez de Fonseca et al., 2001, Rodriguez de Fonseca et al., 1997, Navarro et al., 1997, Marsicano et al., 2002, Wolf and Ullrich, 2008).

The first endocannabinoid, which was isolated and identified as arachidonylethanolamide (AEA), was named anandamide (Mechoulam et al., 1995) (named after the Sanscrit word 'ananda' translated as supreme joy (Mechoulam, 2007)). Another endogenous

arachidonate-based eicosanoid that was found was 2-arachidonoylglycerol (2-AG)(Sugiura et al., 1995); both are physiological ligands of the cannabinoid receptors (Pertwee, 2006).

Two types of cannabinoid receptors have been found to date in mammalian tissues: cannabinoid receptor 1 (CB₁) (Matsuda et al., 1990) and cannabinoid receptor 2 (CB₂) (Munro et al., 1993).

On presynaptic cannabinoid receptors, endocannabinoids act by inducing either suppression of inhibition or suppression of excitation in response to postsynaptic depolarisation (Ohno-Shosaku et al., 2002).

1.3.2.1 Cannabinoid receptors

CB₁ is the most abundant G-protein coupled receptor in the brain (Yasuno et al., 2008). CB₁ is extensively expressed in the central nervous system (Devane et al., 1992); particularly on presynaptic nerve terminals followed by microglia, astrocytes and oligodendrocytes (Di Marzo et al., 1998) CB₁ can also be encountered in the periphery including pituitary gland, immune cells, reproductive tissues, gastrointestinal tissues, sympathetic ganglia, heart, lung, urinary bladder and adrenal gland (Pertwee, 1997). Despite their inhibitory nature, cannabinoid receptors have been found to enhance the release of stimulatory neurotransmitters including glutamate (Ferraro et al., 2001), dopamine (Chen et al., 1990), acetylcholine (Acquas et al., 2001) and dynorphin (Houser et al., 2000). In the hippocampus, CB₁ receptors are present on both excitatory glutamatergic neurons and inhibitory GABAergic interneurons (Hammers et al., 2003b). In contrast, CB₂ is expressed almost exclusively in immune system cells (Munro et al., 1993), particularly in B-cells and natural killer cells (Pertwee, 1997). Activation of presynaptic CB₁ can therefore inhibit the release of

central and peripheral neurotransmitter whilst activation of CB₂ can facilitate or inhibit the release of cytokine on immune cells (Molina-Holgado et al., 1999).

Abundantly expressed in presynaptic glutamatergic and GABAergic terminals (Katona and Freund, 2008), as mentioned above, CB₁ receptors have a heterogeneous central nervous system (CNS) distribution. High concentrations are found in the cerebral cortex, hippocampus, caudate nucleus and putamen, substantia nigra pars reticulata, globus pallidus, entopeduncular nucleus, the molecular layer of the cerebellum and in pain pathways of brain and spinal cord (Herkenham et al., 1990, Irving et al., 2002). However, the thalamus and most of the brainstem show low concentrations (Herkenham et al., 1990).

In animals, a wide range of CB₁-selective radiotracers have been used successfully, such as [³H]CP-55,940 (Devane et al., 1988, Devane et al., 1992), (-)-5'-[¹⁸F]-Δ⁸-THC (Charalambous et al., 1991), [¹²³I]AM251 (Gatley et al., 1996), [³H]SR141716A (Petitet et al., 1996), [¹²³I]AM281 (Gatley et al., 1998), [³⁵S]GTPγS (Griffin et al., 1998), [¹¹C]OMAR ([¹¹C]JHU75528) (Horti et al., 2006), [¹¹C]MePPEP (Yasuno et al., 2008), [¹¹C] / [¹⁸F]-PipISB (Finnema et al., 2009) and [¹¹C]CB-119 (Hamill et al., 2009).

MePPEP ((3R,5R)-5-(3-methoxy-phenyl)-3-((R)-1-phenyl-ethylamino)-1-(4-trifluoromethyl-phenyl)-pyrrolidin-2-one) is a CB₁-selective inverse agonist. [¹¹C]MePPEP has high and stable brain uptake *in vivo*. Despite its moderately high lipophilicity (measured LogD_{7.4}=4.8)(Yasuno et al., 2008), its specific binding is relatively high; >85% in monkey brain and 65% determined using CB₁ knockout mouse brain (Yasuno et al., 2008, Terry et al., 2008, Terry et al., 2010b).

One reproducibility study with [¹¹C]MePPEP has been performed in humans so far, involving eight test-retest scans (Terry et al., 2009) after injection of high doses of [¹¹C]MePPEP (~650

MBq). Only standardised uptake values and distribution volumes derived from compartmental modelling were calculated.

1.3.2.2 Cannabinoid receptor 1 (CB₁) PET in epilepsy

Converging evidence from pre-clinical studies suggest activation of CB₁ receptors has an anticonvulsant effect. For example, the research teams of Jones and Marsicano established that CB₁-receptor-knockout mice had a lower threshold to kainic acid (KA)-induced seizures. These authors also provided evidence for increased anandamide release from hippocampal neurons in response to KA administration (Jones et al., 1994, Marsicano et al., 2002)

Using the rat pilocarpine model of epilepsy, Wallace et al. demonstrated that administration of the marijuana extract Δ^9 -tetrahydrocannabinol (THC) or the cannabimimetic R(+)-WIN55,212 abolished spontaneous epileptic seizures, whereas application of the CB₁ receptor antagonist SR141716A significantly increased both seizure duration and frequency (Wallace et al., 2003). Their study also provided evidence for increased release of the endogenous CB₁ ligand 2-arachidonylglycerol from the hippocampus during pilocarpine-induced seizures. The application of CB₁ receptor antagonists during febrile seizures in rats was also shown to prevent the emergence of long-term limbic hyperexcitability (Chen et al., 2007).

The endocannabinoid system has been implicated in the process of epileptogenesis in the pilocarpine rat model of mTLE (Goffin et al., 2009). An *in vitro* study of participants with mTLE in association with HS identified a decrease in CB₁ receptor immunostaining and CB₁ messenger ribonucleic acid (RNA) in the sclerotic hippocampus (Hammers et al., 2005).

More recently, Goffin's research team used [¹⁸F]MK-9470 PET to study CB₁ receptor availability in 12 participants with refractory mTLE-HS. Voxel-wise group-wise analysis of

modified SUV (mSUV) images revealed a significant increase in [^{18}F]MK-9470 uptake in the ipsilateral inferotemporal cortex, with significant decreases observed in the insular cortex (more significantly in the ipsilateral than contralateral insula). The ipsilateral inferotemporal increases were evident for six of the 12 participants on individual comparison with (50 participants); including all six of the participants who had been scanned within two days of a seizure. The localisation of the increases were concordant with SISCOM; all six had either Engel class I or II post-surgical outcome (Goffin et al., 2011).

A significant positive correlation between [^{18}F]MK-9470 uptake in the ipsilateral temporal lobe and the number of seizures in the month before scanning ($p = 0.04$, $r = 0.60$) was observed. Accordingly, a negative correlation with the latency since last seizure before scanning ($p = 0.04$, $r = -0.60$) was also observed. There were no significant correlations between insular [^{18}F]MK-9470 uptake and seizure frequency or latency. These data suggest region-specific changes in CB_1 receptor availability in response to spontaneous epileptic seizures arising from the temporal lobe, and a potential role for CB_1 receptor PET in the pre-surgical evaluation of participants with mTLE-HS (Goffin et al., 2011).

The endocannabinoid anandamide was significantly decreased in the cerebrospinal fluid (CSF) of nine participants with TLE, relative to controls (Romigi et al., 2010).

In humans, the effect of CB_1 receptor agonists in epilepsy is unclear. A recent Cochrane review concluded that the limited data available was of low quality and did not allow reliable conclusions to be drawn (Gloss and Vickrey, 2012).

In a survey of 28 cannabis users at a tertiary epilepsy service, 68% were found to associate cannabis use with decreased seizure severity, and 54% with decreased seizure frequency

(Hamerle et al., 2013). In contrast, a recent survey 63 cannabis users at a tertiary epilepsy service found no evidence of an effect on seizures (Cohen et al., 2012).

Patient accounts of improvement of seizures with cannabinoid ingestion are frequent (Gordon and Devinsky, 2001). Moreover, Savic's research team reported two patients in whom cessation of marijuana cigarettes was associated with a marked increase in seizure frequency (Savic et al., 1988). Reduced seizure frequency was also observed in a case report of two of three children with symptomatic epilepsy in whom treatment with THC (Lorenz, 2004).

Case reports which associate seizures with cannabinoid ingestion are available in equal measure. For example, there has been a case report of a patient who had two witnessed generalised convulsions shortly after smoking synthetic cannabinoids (Schneir and Baumbacher, 2012). Intoxication with synthetic cannabinoids was associated with seizures in two of six patients in one case series (Harris and Brown, 2013). In contrast, seizures were not reported in a case series of two young women (Gross et al., 2004) or in a case series of three adolescents (Gordon and Devinsky, 2001).

Table 1.3. PET and SPECT studies of the GABA_A receptor to date in human epilepsy (*in vivo*). TLE – temporal lobe epilepsy; m – mesial, ETLTLE – extra-temporal lobe epilepsy; IFE – idiopathic focal epilepsy; FLE – frontal lobe epilepsy; nMRI – MRI-negative; MCD - Malformation(s) of cortical development; HC – hippocampus; HS – hippocampal sclerosis; u – unilateral; b - bilateral; f – focal seizures; WM – white matter; GM – grey matter ; TL – Temporal lobe; FL – frontal lobe; V_T – Volume-of-distribution; B_{max}- receptor density; BP-SRTM – Binding potential on specified reference tissue model; EEG-fMRI – electroencephalography with functional magnetic resonance imaging; K_D - dissociation constant ; rCMr_{glu} - regional cerebral metabolic rate of glucose; ADD - summed radioactivity-weighted images ; SUV - standardized uptake value.

Authors	Year	Radiotracer	Participants	Outcome measure(s)	Conclusion(s)
Savic I, et al.	1988	[¹¹ C]FMZ	10 x nMRI IFE (6 mTLE) 5 x healthy controls	K _D ; B _{max}	Decreased [¹¹ C]FMZ binding in epileptogenic focus relative to contralateral homologue
Henry TR et al.	1993	[¹¹ C]FMZ; [¹⁸ F]FDG	10 x mTLE; healthy controls	B _{max} ; rCMr _{glu}	Decreased [¹¹ C]FMZ binding in anterior mTL; [¹⁸ F]FDG hypometabolism more extensive
Savic I et al.	1993	[¹¹ C]FMZ; [¹⁸ F]FDG	8 x IFE (6 x TLE) 7 x healthy controls	B _{max} ; rCMr _{glu}	Decreased [¹¹ C]FMZ B _{max} superior to decreased [¹⁸ F]FDG rCMr _{glu} in epileptogenic focus
Savic I et al.	1995	[¹¹ C]FMZ; [¹⁸ F]FDG	6 x FLE 7 x healthy controls	B _{max} ; rCMr _{glu}	Decreased [¹¹ C]FMZ B _{max} superior to decreased [¹⁸ F]FDG rCMr _{glu} in epileptogenic focus
Richardson MP, et al.	1996	[¹¹ C]FMZ	12 x MCD 26 x healthy controls	V _T	10/12 V _T abnormalities; decreases and increases
Szelies B, et al.	1996	[¹¹ C]FMZ; [¹⁸ F]FDG	10 x nMRI TLE	B _{max}	Decreased [¹¹ C]FMZ B _{max} more circumscribed than [¹⁸ F]FDG hypometabolism
Koepp MJ, et al.	1997	[¹¹ C]FMZ	9 x mTLE-uHS; 6 x mTLE-bHS; 15 x healthy controls	V _T	Decreased V _T in HC; bilateral in one-third of patients with uHS or bHS or asymmetric.

Richardson MP, et al.	1997	[¹¹ C]FMZ	10 x MCD 16 x healthy controls	V _T	6/10 V _T abnormalities; some localised to regions of abnormal GM volume
Richardson MP, et al.	1998	[¹¹ C]FMZ	6 x fETLE; 18 x nMRI ETLE; 24 x healthy controls	V _T	6/6 sETLE with single focus had decreased V _T ; 6/18 nMRI ETLE had decreased V _T ; 10/18 nMRI had increased V _T ;
Ryvlin P, et al.	1998	[¹¹ C]FMZ; [¹⁸ F]FDG	100 x IFE (52 x TLE); 12 x healthy controls	Late uptake	94% of participants with TLE had [¹¹ C]FMZ binding abnormality. Decreased [¹¹ C]FMZ binding often more circumscribed than [¹⁸ F]FDG Pet hypometabolism; [¹¹ C]FMZ PET not superior to [¹⁸ F]FDG PET in localising epileptogenic focus
Koepp MJ, et al.	2000	[¹¹ C]FMZ	10 x nMRI TLE	V _T	V _T abnormalities in 5/10 participants; increased V _T in 2/10 participants, decreased V _T in 3/10 participants; not consistently helpful in localising epileptogenic focus
Muzik O, et al.	2000	[¹¹ C]FMZ [¹⁸ F]FDG	10 x ETLE	[Nil]	[¹¹ C]FMZ coincided with EEG seizure onset zone in 10/10 participants; [¹⁸ F]FDG PET coincided with EEG seizure onset zone in 8/10 participants
Hammers A, et al.	2001	[¹¹ C]FMZ	15 x mTLE-HS	V _T	14/15 Decreased V _T in ipsilateral HC. Correlation between [¹¹ C]FMZ and WM neuron number
Hammers A, et al.	2001	[¹¹ C]FMZ	10 x fMCD	V _T	9/10 Multiple foci of increased/decreased V _T in/around MCDS
Juhász C, et al.	2001	[¹¹ C]FMZ [¹⁸ F]FDG	15 x IFES		[¹¹ C]FMZ PET abnormality size positively correlated with worsening outcome
Hammers A, et al.	2002	[¹¹ C]FMZ	18 x nMRI TLE; 21 x healthy controls	V _T	Decreased V _T in hippocampi; increased V _T in ipsi- and contralateral TL WM; increased V _T in ipsi- and contralateral FL WM.
Hammers A, et al.	2003	[¹¹ C]FMZ	44 x nMRI neocortical epilepsy	V _T	8/44 Decreased V _T , 16/44 increased V _T , 9/44 decreased and increased V _T ; Periventricular increases in 7/44.
Hammers A, et al.	2005	[¹¹ C]FMZ	15 x mTLE-HS	V _T	Increased V _T around posterior horns of ventricles in participants with poor post-surgical outcome

Bouvard S, et al.	2005	[¹¹ C]FMZ	6 x mTLE; 4 x ETLE; 10 x healthy control	B _{max}	10/10 epilepsy subjects showed one or several focal decreases of B _{max} . 3/3 nMRI mTLE ipsilateral B _{max} decrease.
Hammers A, et al.	2008	[¹¹ C]FMZ	15 x mTLE-uHS; 13 x healthy control	ADD BP-SRTM V _T	V _T : 15/15 decreased ipsilateral seizure onset; 3/15 bilateral decreased. 13/15 ipsilateral decreased uptake on ADD; 6/13 bilateral decreased ADD 10'-20'; 5/13 bilateral decrease ADD 20'-40'. BP-SRTM: 12/15 decreased ipsilateral and 3/15 bilateral decreases.
Juhász C, et al.	2009	[¹¹ C]FMZ	20 x nMRI neocortical epilepsy		17/20 decreased ipsilateral to seizure onset. 11/17 multiple areas of decrease.
Laufs H, et al.	2011	[¹¹ C]FMZ	18 x nMRI focal epilepsy; 19 x focal epilepsy on EEG-fMRI; 24 x healthy controls	V _T	18/18 V _T increase in ipsilateral putamen and no decreases compared to controls; decreased V _T compared with EEG-fMRI in frontal piriform area.
Hirvonen J, et al.	2012	[¹¹ C]PBR28 [¹¹ C]dLop	9 x TLE uHS; 6 x TLE nMRI; 30 x healthy controls	SUV	13% increase in uptake on ipsilateral hippocampus in TLE uHS with [¹¹ C]PBR28. 17/17 increase in uptake on ipsilateral HC, parahippocampal gyrus, amygdala, fusiform gyrus, and choroid plexus compared to [¹¹ C]dLop
Vivash L, et al.	2013	[¹⁸ F]FMZ [¹⁸ F]FDG	12 x mTLE HS; 19 x mTLE nMRI; 17 x [¹⁸ F]FMZ healthy controls; 20 x [¹⁸ F]FDG healthy controls.		Decreased ipsilateral uptake in epileptogenic TL 14/31 [¹⁸ F]FMZ and 18/29 [¹⁸ F]FDG.

Table 1.4 PET and SPECT studies of CB₁ to date in human epilepsy (*in vivo*). mTLE – mesial temporal lobe epilepsy; TL – temporal lobe; mSUV – modified standardized uptake value.

Authors	Year	Radiotracer	Participants	Outcome measure(s)	Conclusion(s)
Goffin K, et al.	2011	[¹⁸ F]MK-9470	12 x mTLE-HS; 50 x healthy controls	mSUV	Increased CB ₁ availability in ipsilateral TL. Decreased CB ₁ superior insular cortex.

CHAPTER 2

Common Materials and Methods

Methods common to both the [^{11}C]Ro15 4513 and [^{11}C]MePPEP studies are described below. Methods specific to each study are following chapters.

2.1 Approvals

Approval of the studies in this thesis were provided by Imperial College Healthcare NHS Trust, University College London Hospitals NHS Foundation Trust, Research Ethics Committee and the Administration of Radioactive Substances Advisory Committee (ARSAC) of the United Kingdom (UK). All participants provided written informed consent according to the Declaration of Helsinki prior to participation in the study.

2.2 Subject identification and recruitment

Participants with TLE were recruited from the epilepsy clinics of the National Hospital for Neurology and Neurosurgery, Queen Square, London, UK and the Epilepsy Society, Chalfont St. Peter, Buckinghamshire, UK. Their diagnoses were based on semiology, interictal and ictal (where available) EEG studies, MRI studies, and any other available relevant investigation. The seizure frequency and time of most recent seizure prior to scanning were obtained directly from participants.

Healthy controls were recruited through local advertising, newspaper advertising and word of mouth. I attempted to match the participant groups, by terms of age and gender. This however was only partially successful as it was difficult to recruit a control group that could

match the TLE cohort identically in all features other than illness status. Recruitment difficulties included: time constraints of the studies, limited budget, limited operational time for PET centre and the scheduling demands of busy PET and MRI scanning centres.

All control participants denied a history of neurological or psychiatric conditions, the regular use of medication, in particularly benzodiazepines, as well as habitual or recent use of illegal substances. General practitioners (GP) were contacted through post with consent of all participants. GP were informed and encouraged to contact the investigators if they believed the participant under their care did not fulfil the inclusion criteria.

All subjects underwent a urine drug screen cassette test for 11-nor- Δ^9 -THC, morphine, amphetamine, benzoylecgonine (the main metabolite of cocaine), methamphetamine and oxazepam (Monitect[®]; BMC, California, U.S.A.) prior to PET scanning. All female patients of childbearing age underwent a urine pregnancy test. All participants with positive urine drug screen were excluded, other than participants with epilepsy who tested positive for prescribed benzodiazepines. All female participants that tested positive in the urine pregnancy test were excluded.

The exclusion criteria were: inability to provide informed consent, claustrophobia, any contraindication for undergoing MR, positive urine drug test, positive urine pregnancy test, general practitioner's (family doctor's) advice against participation, use of cannabis within the previous three months or on more than five occasions over the subject's lifetime, pathological modified Allen's test for patency of the ulnar artery (Allen, 1929, Slogoff et al., 1983, Cable et al., 1999), participants taking part in other trials involving pharmacological products over the previous three months or in another procedure involving ionising radiation in the previous year, presence of relevant medical conditions (particularly neurological) in controls only, regular or recent medication intake in controls only.

2.3 Radiotracer synthesis and administration

The radioisotopes were synthesised on site by Hammersmith Imanet and then incorporated in ^{11}C -Ro15 4513 ((ethyl 8-azido-5,6-dihydro-5-methyl-6-oxo-4H-imidazo[1,5-a][1,4]benzodiazepine-3-carboxylate; (Osman, 2006)) and MePPEP (following a procedure described previously (Yasuno et al., 2008)), respectively, for injection. Each preparation was analysed by high-performance liquid chromatography (HPLC) to determine the specific activity of the radiotracer and the radiochemical purity. The presence of pathogens was excluded.

Radiotracers were injected intravenously using a 22-gauge cannula into an antecubital fossa vein prior to the emission scan. The radiotracer was injected as a smooth bolus thirty seconds after the start of the dynamic scan over the subsequent 15 seconds, followed by a flushing over a further 15 seconds.

2.4 PET data acquisition

PET scans were acquired on a Siemens/CTI ECAT EXACT HR+ 962 camera (Knoxville, TN, USA) in 3D mode. This camera has a 15.5 centimetre (cm) axial and 58.0 cm transaxial field of view (FOV), a spatial resolution of 4.8 ± 0.2 mm, and sensitivity of 69 cps/Bq/ml (Brix et al., 1997).

Ten-minute transmission scans for attenuation correction were obtained prior to dynamic emission scans using a rotating ^{137}Cs point source. Each dynamic acquisition was 90 minutes long and consisted of 24 studies frames for (^{11}C)-Ro15 4513 study) and 35 studies frames for (^{11}C)-MePPEP of increasing length. 30 seconds after the scan start, (^{11}C)-Ro15 4513 or (^{11}C)-MePPEP was injected as an intravenous bolus injection. Subjects were scanned on two separate days.

The images were acquired in a reduced-stimuli-room without background noise. Participants were placed in the centre of the scanner, as the resolution of the scanners decreases towards the periphery of the field of view. Subjects were positioned supine in a comparable way with their transaxial planes parallel to the line intersecting anterior and posterior commissure (AC–PC). Participants' heads were secured with foam head moulds and straps. Marks were drawn on the participants' skins over the midline forehead and axially on the maxillary bones; these marks were aligned with the scanner's projected laser lines. The alignments of these points were monitored continuously via a camera and monitor and intermittently with the scanner's positioning laser. Correct positioning was verified with a two-minute transmission scan, followed by a ten-minute transmission scan using a ^{137}Cs point source to allow emission scans to be corrected for attenuation. If movement was noticed, subjects were repositioned and underwent a second transmission scan at the end of the dynamic scan. To compensate for head movement during dynamic scans, a *post hoc* frame-by-frame realignment method was used, as described below (section "PET data processing"). Data were reconstructed using FORE (Defrise et al., 1997) and 2D FBP (ramp filter, kernel 2.0 mm FWHM). Voxel sizes of reconstructed images were: $2.092 \times 2.092 \times 2.42$ mm.

2.5 Input Function (IF) Derivation

Continuous and intermittent blood samples were collected to allow the subsequent generation of metabolite-corrected arterial plasma time-activity curves (TAC) and were subsequently used to generate plasma:whole blood activity ratios and determine metabolite levels (Hammers et al., 2008). During the first 15 minutes blood was withdrawn continuously at a rate of 300ml/hr and radioactivity measured in a bismuth germanium oxide (BGO) detection system (Ranicar et al., 1991, Jones et al., 1994). To quantify plasma and whole blood radioactivity, as well as to allow quantification of the parent fraction of the radiotracer,

intermittent discrete (10 ml) samples were taken with heparinised syringes before the scan (baseline) and at the following time points after scan start: 3, 5, 10, 15, 20, 30, and 50 minutes. At 75 minutes, a larger 17 ml sample was taken to allow quantification despite radioactivity decay. Parent fraction quantification was not possible at 90 minutes; hence at this time point only three millilitres were withdrawn for plasma and whole blood radioactivity measurement.

For each sample the plasma radioactivity concentration was acquired from an aliquot that had been centrifuged before measurement in a NaI(Tl) well counter. The plasma radioactivity concentration in whole blood was separately measured. HPLC was used to quantify the radiolabelled metabolites in plasma.

Continuous decay-corrected, and metabolite-corrected parent plasma input functions (IF) were derived for all participants using CLICKFIT versions 1.6 and 1.7 (in-house software, Cunningham et al., 2006) on MATLAB© 6.5 (MathWorks, 2006). In any interval of missing data due to regular flushing with saline solution to maintain arterial line patent, an interpolation of the continuous on-line time course of radioactivity concentration in whole blood was done.

The plasma:whole blood ratio was calculated from the discrete blood samples and fitted to a sigmoid function. Continuous plasma IF were derived by cross-calibration (Luthra et al., 1993) and combination of the continuous and discrete data, multiplication with the fitted plasma-over-blood ratio, and correction for parent radiotracer fraction, as described in detail in previous studies (Hammers et al., 2007b, Jones et al., 1994).

2.6 MRI data acquisition, analysis and generation of ROIs

All participants had 3D T1 weighted MRI scans with approximately millimetric voxel sizes on a Phillips Intera 3 Tesla (3T) MRI scanner (Best, The Netherlands) at the Robert Steiner MRI Unit, Hammersmith Hospital, for co-registration and regions of interest (ROIs) definition. Coronal T2 and FLAIR were also acquired for each participant.

T1-weighted images were segmented into tissue classes using the statistical parametrical mapping software SPM8[®] (Statistical Parametric Mapping, Wellcome Trust Centre for Neuroimaging, UCL, London, www.fil.ion.ucl.ac.uk/spm) under MATLAB[®] 7.4 (MathWorks, 2008).

The T1-weighted images were also anatomically segmented using MAPER (multi-atlas propagation with enhanced registration; (Heckemann et al., 2010)). Using high-dimensional image registration, 30 MRI data sets, each associated with manually determined labels of 83 regions (Hammers et al., 2003a, Gousias et al., 2008), were propagated to the target brain. Label fusion was used to obtain 83 ROIs in target space (Figure 2.1 (a)) (Heckemann et al., 2006).

The T1-weighted images and corresponding MAPER-derived individual segmentations as well as individual grey matter (GM) probability images (Figure 2.1 (b)) were co-registered (Figure 2.1 (c)) with each subject's corresponding processed PET summation image for test and retest scans separately, using the normalised mutual information optimisation algorithm in SPM8[®]. For the cortical ROIs, the individual atlases in PET space were then multiplied with the grey matter probability maps thresholded at 0.5 using Analyze[®] 8.1 biomedical imaging software (Mayo Clinic, 2002). These regions of interest were then used to sample the dynamic or parametric images (Figure 2.1 (d)).

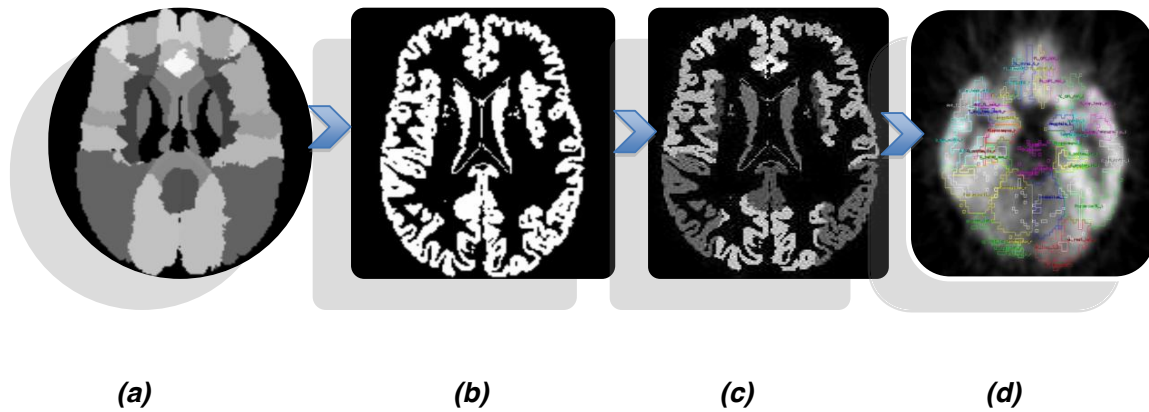


Figure 2.1. Example of generation of regions of interest (ROI). (a) multi-atlas propagation with enhanced registration (MAPER) outcome, (b) Grey matter (GM) tissue-class segmentation outcome, (c) co-registered MAPER and GM outcomes, (d) ROI after transfer on dynamic image.

2.7 PET data quantification

Attenuation and scatter-corrected dynamic PET images were de-noised and corrected for movements frame-by-frame using wavelets in Piwave 8.0 (Studholme et al., 1996, Studholme et al., 1997, Turkheimer et al., 1999). The frame starting at 4 minutes (frame 10) was used as reference due to its high signal-to-noise ratio and likelihood of subjects staying still during the first minutes of the scan. The first 93 seconds (frames 1 to 6) were not motion corrected due to their low signal-to-noise ratio. The remaining frames (7 to 24 in [^{11}C]Ro15 4513 and 7 to 35 for [^{11}C]MePPEP) were automatically re-sliced and re-concatenated into a new dynamic image (Hammers et al., 2007b) using a mutual information-based method (mpr; (Studholme et al., 1997)).

Binary contiguous masks encompassing each entire brain and extending approximately 10mm beyond the outer cortical boundaries were created semi-automatically using Analyze[®] 8.1 and applied to their respective dynamic and summed radioactivity-weighted (ADD) images (KBq/ml). The latter ADD images were generated for frames 0 – 24 in [^{11}C]Ro15

4513 and 0 – 35 in [^{11}C]MePPEP, using receptor parametric mapping (RPM; internal software, Cunningham & Gunn, 1997; (Cunningham and Jones, 1993a)) as described by Gunn's research team (Gunn et al., 1997, Aston et al., 2001)). The binary contiguous masks were applied during spectral analyses (SA) (Cunningham and Jones, 1993b). This was also the case for rank-shaping regularisation of spectral analyses (RS-SA; (Turkheimer et al., 2003)).

RS-SA was developed from the exponential SA estimation method that was optimised for datasets with noise by including a singular value decomposition (Golub and Reinsch, 1970) of the exponential bases. RS-SA outperformed traditional SA and Logan's graphical analysis method (Logan et al., 1990) at noise levels of 10% to 20%, in terms of bias, standard deviation of V_T , and mean squared error.

The quantitative acquired data was extracted from the ROIs within attenuation-corrected, scatter-corrected, and motion-corrected dynamic images, ADD images, and V_T images using the 'ROI' - 'sample images' tool in Analyze[®] 8.1 software.

TACs for each ROI were generated with CLICKFIT using the scanner information file and a weights file for each ROI within the sampled dynamic PET data.

2.8 Compartmental models, requiring arterial IFs

2.8.1 Reversible two-compartment (one tissue compartment) model with two rate constants and a variable blood volume (2kbv)

In this model, three microparameters are derived: K_1 is the clearance of the ligand from the plasma to the tissue compartment containing free, non-specifically bound, and specifically bound ligand, k_2 is the efflux constant from the ROI back to plasma (Huang et al., 1980,

Cunningham et al., 1991), and bv is a variable blood volume term. V_T is then calculated according to the compartmental model equation (Watabe et al., 2006, Gunn et al., 2002):

$$V_T = K_1/k_2 \quad (1)$$

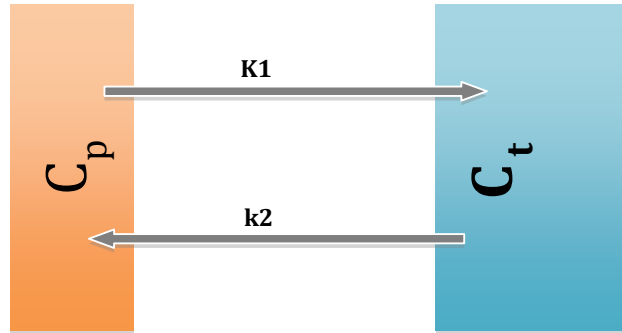


Figure 2.2. One brain compartment, two rate constants (reversible binding; 1c2kbv) model. Where C_t – the radioactivity concentration attributed to non-specific- and specific binding of the radiotracer in brain tissue. Adapted from (Watabe et al., 2006).

2.8.2 Reversible three-compartment (two tissue compartment) model with four rate constants and a variable blood volume (4kbv)

K_1 and k_2 were calculated as for the 2kbv compartmental model described above; in addition, two additional rate constants were estimated to describe transfer relating to the third compartment: k_3 , which describes the transfer from the free and non-specifically bound compartment to the specifically bound (third) compartment; and k_4 , which describes the opposite transfer (Huang et al., 1980, Cunningham et al., 1991). Again, a variable blood volume term was also computed according to compartmental model equations (Watabe et al., 2006, Gunn et al., 2002):

$$V_T = K_1/k_2 (1+k_3/k_4) \quad (2)$$

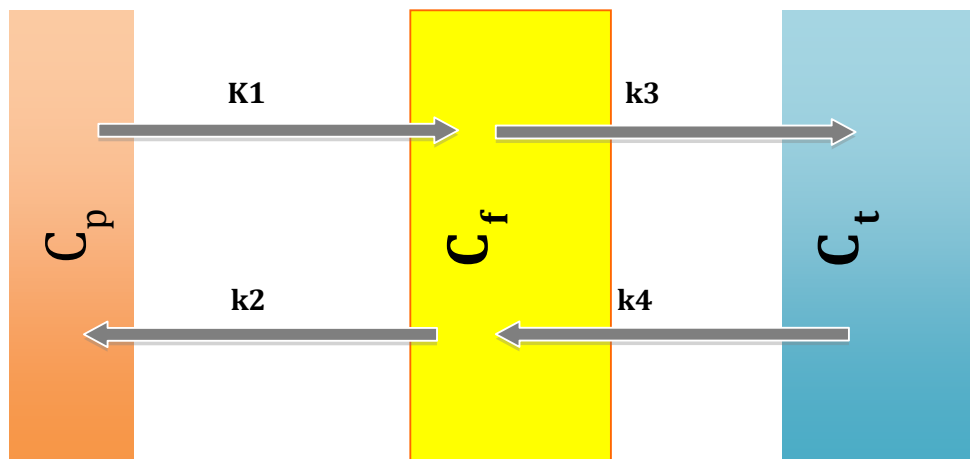


Figure 2.3 Two brain compartments, four rate constants (reversible binding; 2c4kbv) model. Where C_f - radioactivity concentration attributed to free (unbound) radiotracer in brain tissue; C_t – the radioactivity concentration attributed to non-specific- and specific binding of the radiotracer in brain tissue. Adapted from (Watabe et al., 2006).

2.9 Model-free analyses, requiring arterial IFs

2.9.1 Generation of parametric V_T images

2.9.1.1 “Classic” (non-regularised) SA

V_T s for each ROI were obtained from the dynamic images and the metabolite-corrected IFs using SA (Cunningham and Jones, 1993a, Turkheimer et al., 1994).

The principal advantage of SA is that the model structure, including the number of compartments, does not need to be known beforehand. However, in the presence of noise in the data, erroneous estimates of the coefficients can result (Turkheimer et al., 1998).

For the generation of V_T s, the fast frequency boundary was kept at the default value of 0.1 s^{-1} . The theoretical slow frequency boundary is given by the decay constant of ^{11}C ($t_{1/2} \approx 20$ minutes, decay constant 0.0005663 s^{-1} ; $\log_{10} = -3.25$). Based on previous work with another

tracer with relatively slow kinetics (Hammers et al., 2007b), this was changed to 0.00063 s^{-1} ($\log_{10} = -3.20$) in order to reduce noise.

2.9.1.2 Sampling parametric V_T images obtained voxel-by-voxel using spectral analysis (“Classic” SA)

Parametric images of V_T s were obtained from the dynamic images and the metabolite-corrected IFs using SA (Cunningham and Jones, 1993a, Turkheimer et al., 1994) and RPM with the same slow frequency boundaries as above. The resulting parametric maps of V_T values were then sampled in the eight selected ROIs.

2.9.1.3 Directly obtaining V_T values from ROI data with SA and rank shaping regularisation (RS)

V_T values were generated directly from dynamic data sampled using ROIs with RS-SA (orthogonalized-functional-base) regularization of SA (Turkheimer et al., 2003) using CLICKFIT software.

As previously described by Turkheimer et al. (2003), the metabolite-corrected IF were used, as well as a logarithmically spaced basis, an exponential range of bases extending to -3.2, and the regional tissue TAC. Furthermore, TACs were weighted according to (Gunn et al., 1998):

$$W_i = L_i/T_i \text{ (for frame } i = 1, 2, 3 \dots 24 \text{ for } [^{11}\text{C}]\text{Ro15 4513 and 35 for } [^{11}\text{C}]\text{MePPEP)} \quad (3)$$

Where w_i – weight for frame i ; L_i – length of frame i (in seconds); T_i – rate of true coincidences (per second).

The noise fraction was specified as 0.15; this is a conservative estimate of signal-to-noise based on exploratory analyses and the original guidance (Turkheimer et al., 2003)

The V_T for each ROI was then obtained as the plateau of the $V_T(R)$, where R is the expected signal-to noise ratio that is used as the regularization parameter (R) in RS-SA (Turkheimer et al., 2003).

2.10 Methods not requiring an arterial IF

2.10.1 Simplified reference tissue model (SRTM) using brainstem, cerebellum or as a pseudo-reference tissue

GABA_A α 5 subunit and CB₁ receptors are both widespread in the brain, and a true reference region devoid of specific binding does not exist for the latter. A recent attempt to obviate the invasive procedure of arterial cannulation has been to use the ROI with the lowest receptor concentration as a pseudo-reference region (Turkheimer et al., 2012). Two of the structures with the lowest concentration of GABA_A α 5 subunits are the brainstem and cerebellum (Pirker et al., 2000). One of the structures with a low concentration of CB₁ receptors is the pons (Yasuno et al., 2008). These regions were therefore used as pseudo-reference regions in the simplified reference tissue model (SRTM) for the [¹¹C]Ro15 4513 and [¹¹C]MePPEP studies.

2.11 Model-free analyses, requiring arterial IFs

2.11.1 Modified standard uptake values (mSUV)

Modified standard uptake values (mSUV; (Innis et al., 2007)) for frames 15 – 21 for [¹¹C]Ro15 4513 (corresponding to $t = 30'5'' - 50'5''$) and for frames 25 - 31 for [¹¹C]MePPEP, (corresponding to $t = 29'50' - 58'50''$) after injection of the radiotracers, were also derived for the ROIs according to (Goffin et al., 2011):

$$(\text{activity} \times [\text{weight (kg)} + 70 \text{ kg}]/2)/\text{injected dose} \quad (4)$$

2.11.2 Global intensities (GI)

Global intensities (GI) were calculated with an in-house script derived from SPM8[®] (Hammers et al., 2007b), where the GI is defined as the mean voxel value within a mask defined as all voxels exceeding 1/8 of the mean value of all voxels in the image matrix.

2.12 Statistical analyses

Power was determined using PS software (Dupont and Plummer, 1990).

For statistical testing SPSS[®] for Windows version 16 software (IBM 2008, New York, U.S.A.) and Microsoft Office Excel (Microsoft, 2007) were used.

Injectate data were compared between test and retest sessions using the non-parametric Wilcoxon signed-rank test.

Individual specific activities (SpAct) were calculated according to (Satyamurth, 2004):

$$\text{Specific Activity} = (\text{Injected Radiation}/(\text{Injected Stables/RMM})) \quad (5)$$

Where RMM - relative molecular mass/weight.

The percentage test – retest difference of parameters obtained with the various methods were calculated for all subjects in ROIs according to (Hammers et al., 2007b):

$$100((2(\text{retest value} - \text{test value}))/(\text{test value} + \text{retest value})) \quad (6)$$

The spread of binding parameters between subjects (inter-subjects variability) were estimated by the coefficient of variation (CV) as follows (Hammers et al., 2007b):

$$CV = (\text{Standard Deviation}/\text{Mean}) \times 100 \quad (7)$$

While reliability was calculated using intraclass correlation coefficients (Tai and Piccini) was computed in SPSS®, using the “one-way random” model and reporting the “single measures” ICC (Shrout and Fleiss, 1979, MacLennan, 1993):

$$ICC = (MSBS - MSWS) / (MSBS + (df_{WS} \times MSWS)) \quad (8)$$

Where MS = mean sum of squares, BS = between-subjects, WS = within-subjects, and df = degrees of freedom.

ICC values ≥ 0.75 were considered indicators of good reliability (Portney and Watkins, 2009).

CHAPTER 3

Test-retest reproducibility of quantitative binding measures of [^{11}C]Ro15 4513, a PET ligand for GABA_A receptors containing alpha 5 subunits

[^{11}C]Ro15 4513 is a novel PET radiotracer that selectively binds to the $\alpha 5$ subunit of GABA_A receptors (see Chapter 1 for further details). In this Chapter, the first-in-man evaluation of [^{11}C]Ro15 4513 PET is described.

3.1 Objectives

The objectives of the study were as follows:

1. Quantify [^{11}C]Ro15 4513 binding/distribution *in vivo* in the normal human brain using a variety of approaches,
2. Quantify the within-subject variability in [^{11}C]Ro15 4513 binding parameters in terms of percentage test-retest variability; the between-subject variability in terms of between-subject coefficient of variation; and overall reliability as their relationship, i.e. the intraclass correlation coefficient; ICC,
3. Determine the ability of the various methods to reflect binding heterogeneity, assessed as the ratio of the parameter estimated in a high binding region (hippocampus) over a low binding region (cerebellum).

3.2 Hypothesis

The primary hypothesis was:

1. In healthy controls [^{11}C]Ro15 4513 can be reliably quantified *in vivo* as demonstrated by high ICCs in test-retest studies.

3.3 Materials and methods

This study was approved by the Riverside NHS Research Ethics Committee, and the previously described regulatory authorities (see Chapter 2).

3.3.1 Study population

The inclusion criteria were as follows:

- Age between 35 and 65 years on the day of the PET scan
- Male

The exclusion criteria were listed in Chapter 2.

Seven healthy male subjects were recruited and gave written informed consent; from this sample two were excluded. One subject withdrew from study before the second scan and the arterial line could not be kept patent for the entire study in another subject. Hence, a total of five healthy male subjects (median age 40 years, range 38 to 49 years) with a median weight of 86 kg (range 64 – 119 kg) were scanned twice on two different days (median scan interval 12 days, range 7 – 59 days). They had no history of either somatic or psychiatric conditions or substance abuse. Demographic data are detailed in Table 3.1.

Subject No.	Age	Gender	BMI	Interval (days)	Dose (MBq)	Radiochemistry purity (%)	Co-injected mass (μ g)	Specific Activity (MBq/nmol)
1	40	M	24.6	12	431 435	99 98	2.0 2.4	72 59
2	38	M	26.5	30	430 437	99 98	4-4 2.2	32 66
3	39	M	19.9	11	447 441	99 99	3.0 5.1	49 28
4	49	M	34.4	59	440 442	97 98	2.6 3.5	55 42
5	44	M	23.0	7	452 444	96 98	3.0 0.4	26 72
Median	40		24.6	12	441	98	2.8	52
Interquartile range (25th-75th)	39 - 44		23-26.5	11 - 30	435-443	98-99	2.2 - 3.4	34- 64
Min	38		19.9	7	430	96	0.4	26
Max	49		34.4	59	452	99	5.1	72

Table 3.1. Subjects' demographic and injectate details. BMI - Body Mass Index; Min - minimum; Max - maximum; μ g – micrograms; μ mol – micromoles; M – male; MBq – Megabequerels.

3.3.2 Radiochemistry

Production and injection of the tracer was as described in Chapter 2. The target dose was 444MBq for each PET scan (leading to about 0.7 mSv/scan); 444MBq (minimum 333MBq) was injected, with a radiochemical purity of 95% and pH 4.5 to 7.5 (Osman, 2006). Specific radioactivities at the time of injection were calculated in relation to the relative molecular weight of Ro15 4513 (326 mol/g) (Osman, 2006). Details of the injectate are presented in Table 3.1.

3.3.3 PET data acquisition

PET image acquisition was as described in Chapter 2.

3.3.4 MRI data acquisition

MRI data acquisition was as described in Chapter 2.

3.3.5 Metabolism of [^{11} C]Ro15 4513

The plasma-over-blood ratios and metabolite model curves were calculated as described in Chapter 2.

The analyses did not account for potential penetration of radiolabelled metabolites across the blood-brain barrier.

3.3.6 Input Function (IF) Derivation

The IF was derived as described in Chapter 2.

3.3.7 PET image pre-processing

Motion correction and denoising was as described in Chapter 2.

3.3.8 Global uptake

Global V_T was calculated as described in Chapter 2.

3.3.9 Extraction of PET data from regions-of-interest

Data was extracted from ROIs as described in Chapter 2.

3.3.10 SUVs

SUVs were created from ADD images as described in Chapter 2.

3.3.11 ROI definition

I evaluated the test-retest reliability of the quantification methods (see “PET data quantification” section below) in a selection of five bilateral ROIs in total. Furthermore I chose representative regions with high GABA_A $\alpha 5$ subunit receptor concentrations; the grey matter masked cortical structures – hippocampus, anterior cingulate gyrus, inferior frontal gyrus, occipitotemporal (fusiform) gyrus. In addition, a region with low concentration of

GABA_A α 5 subunit was evaluated: the cerebellum. The data from left and right homologues were averaged prior to quantification.

3.3.12 Quantification of [¹¹C]Ro15 4513 V_T

I evaluated the ability of [¹¹C]Ro15 4513 to quantify α 5 GABA_A receptors in the control's brains by generating quantitative parametric V_Ts values using compartmental modelling and receptor parametric mapping.

3.3.13 Compartmental modelling of [¹¹C]Ro15 4513 cerebral tissue kinetics

ROIs' V_T were calculated using standard two- (2k) and four- (4k) compartments models, as described in Chapter 2.

3.3.14 Generation of parametric V_T images

Parametric V_T images were calculated by SA and RS-SA, as described in Chapter 2.

3.4 Results

3.4.1 Adverse or serious events

[¹¹C]Ro15 4513 was well-tolerated by all participants. No serious events were reported.

3.4.2 Injectate

Details are given in Table 3.1, there were no significant differences between test and retest studies in terms of the amount of injected radioactivity (median (i.q.r)): test 362 (358 – 368) MBq; retest 366 (360 – 372) MBq; co-injected mass of stable ligand: test: 3.2 (2.4 – 4.1) μ g, retest 3.3 (2.7 – 4.7) μ g; specific activity at the time of injection: test 50 (49 – 61) MBq/ η mol, retest: 51 (37 – 61) MBq/ η mol.

The amount of injected radioactivity was not significantly different between test and retest scans (MBq median, i.q.r.: test: 440, 443 - 445; retest: 441, 438 - 442; Wilcoxon signed-ranks test $p = 0.9$). The median specific radioactivity at the time of injection was 52 MBq/ η mol (i.q.r. 34 - 64 MBq/ η mol, range 26 - 72 MBq/ η mol); there was no difference between test and retest scans (MBq/ η mol median, i.q.r.: test: 49, 36 - 54; retest: 59, 46 - 64, $p = 0.7$). This was also the case for co-injected stable mass (μ g median, i.q.r.: test: 3.0, 2.7 - 3.0; retest: 2.4, 2.2 - 3.2; $p = 0.7$) and global radioactivity concentration in the images (kBq/ml median, i.q.r.: test: 1.2, 0.9 - 1.2; retest: 1.0, 0.9 - 1.1; $p = 0.14$).

3.4.3 Quantification results

The following sections describe the regional estimates for the parameters derived with the seven quantification methods. To assess test-retest variation, for each ROI the median percent difference between test and retest study as well as their signed range is given in the tables. The CV quantifies the between-subject variability of the measure. The ICC assesses the reliability of the measure as a function of both within-subject and between-subject variability; the closer to the value of 1, the more reliable the method, i.e. the smaller the intra-subject variability of the measure compared with natural between-subject variability. Finally, the ratio of the generally highest binding region (pallidum) over the lowest binding region (pons) assesses a method's ability to reflect known binding heterogeneity.

3.4.4 Compartmental models, requiring arterial IFs

3.4.4.1 Reversible two-compartment (one tissue compartment) model with variable blood volume term (2kbv)

The method yielded inconsistent data, with high test-retest variability. The region with the highest V_T was the hippocampus (5.8; Table 3.2). Regional heterogeneity of V_T values was

relatively low, with a ratio of hippocampus/cerebellum of 2.7. ICCs ranged between -4.19 and -0.52, with a median (i.q.r.) of -0.73 (-0.81 – -0.62).

ROI	Median V_T	Interquartile range	Min	Max	Median % diff.	% diff range	Mean %BS-CV	ICC
Hippocampus	5.80	5.31 – 7.31	5.15	7.48	28	8 – 37	17	-0.62
ACG	5.51	5.18 – 6.45	4.80	6.96	28	8 – 29	14	-0.81
Cerebellum	2.13	2.05 – 2.29	2.00	2.68	13	6 – 26	9	-4.19
Fusiform gyrus	5.11	4.85 – 6.40	4.62	6.79	30	8 – 32	16	-0.73
Inferior frontal gyrus	4.26	4.13 – 5.12	4.01	5.56	22	6 – 31	13	-0.52
							Median (iqr):	-0.73 (-0.81 – -0.62)

Table 3.2. Subjects' V_T (2kbv.) BS = between-subjects, CV = coefficient of variation, diff. = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, ACG = anterior cingulate gyrus, IFG = Inferior frontal gyrus, iqr = interquartile range.

3.4.4.2 Reversible three-compartment (two tissue compartment) model with variable blood volume term (4kbv)

The method yielded inconsistent data for several ROIs. The region with the highest V_T was the hippocampus (7.69; Table 3.3). Regional heterogeneity of V_T values was moderate, with a ratio of hippocampus/cerebellum of 3.9. ICCs ranged between -0.32 and 0.86, with a median (i.q.r.) of 0.34 (-0.12 – 0.59). Only hippocampus was relatively reliably estimated with the method, with an ICC of 0.60.

ROI	Median V_T	Interquartile range	Min	Max	Median % diff.	% diff range	Mean %BS-CV	ICC
Hippocampus	7.69	6.98 – 7.81	5.80	8.00	5	2 - 11	11	0.86
ACG	6.66	5.97 – 6.85	5.54	7.37	9	5 - 14	10	0.59
Cerebellum	1.99	1.95 – 2.37	1.94	3.13	31	15 - 46	16	-0.32
Fusiform gyrus	6.04	5.62 – 6.53	1.64	7.27	15	13 - 107	29	0.34
Inferior frontal gyrus	4.78	4.29 – 5.51	2.44	5.73	12	4 - 78	24	-0.12
							Median (iqr):	0.34 (-0.12 – 0.59)

Table 3.3. Subjects' V_T (4kbv). BS = between-subjects, CV = coefficient of variation, diff. = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, ACG = anterior cingulate gyrus, IFG = Inferior frontal gyrus, iqr = interquartile range.

3.4.5 Model-free analyses, requiring arterial IFs

3.4.5.1 “Classic” SA, applied to ROI data

The method yielded inconsistent data. The region with the highest V_T was the hippocampus (7.83; Table 3.4). Regional heterogeneity of V_T values was moderate, with a ratio of hippocampus/cerebellum of 3.24. ICCs ranged between -0.39 and 0.31, with a median (i.q.r.) of -0.22 (-0.32 – 0.09).

The region with the highest V_T was the hippocampus (7.83; Table 3.4). Regional heterogeneity of V_T values was low – moderate, with a ratio of hippocampus:cerebellum of 3.24. ICCs ranged between -0.39 and 0.31, with a median (i.q.r.) of -0.22 (-0.32 – 0.09).

ROI	Median VT	Interquartile range	Min	Max	Median % diff.	% diff range	Mean %BS-CV	ICC
Hippocampus	7.83	6.72 – 8.02	4.37	10.14	11	0 – 77	23	-0.22
ACG	6.40	5.68 – 6.92	2.65	7.61	14	8 – 90	21	-0.32
Cerebellum	2.42	2.15 – 2.77	1.99	2.95	18	-3 – 36	15	0.09
Fusiform gyrus	6.39	5.78 – 6.78	4.29	8.27	16	-13 – 43	17	-0.39
Inferior frontal gyrus	5.51	4.62 – 6.30	3.65	6.94	7	0 – 56	20	0.31
							Median (iqr):	-0.22 (-0.32 – 0.09)

Table 3.4. Subjects' V_T ("classic" SA applied to ROI data). BS = between-subjects, CV = coefficient of variation, diff. = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, ACG = anterior cingulate gyrus, IFG = Inferior frontal gyrus, iqr = interquartile range.

3.4.5.2 "Classic" SA applied on a voxel-by-voxel basis

The region with the highest V_T was the hippocampus (9.27; Table 3.5). Regional heterogeneity of V_T values was very high, with a ratio of hippocampus/cerebellum of 6.1. ICCs ranged between 0.35 and 0.83, with a median (i.q.r.) of 0.72 (0.61 – 0.73).

ROI	Median V_T	Interquartile range	Min	Max	Median % diff.	% diff range	Mean %BS-CV	ICC
Hippocampus	9.27	8.41 – 10.16	7.58	10.45	6	2 - 11	12	0.83
ACG	5.12	4.41 – 5.69	4.08	7.11	9	2 - 24	17	0.61
Cerebellum	1.53	1.40 – 1.69	1.34	1.85	12	1 – 19	10	0.35
Fusiform gyrus	4.97	3.97 – 5.33	3.76	6.42	6	0 – 21	19	0.73
Inferior frontal gyrus	4.06	3.35 – 4.54	3.13	4.96	10	1 – 22	17	0.72
							Median (iqr):	0.72 (0.61 – 0.73)

Table 3.5. Subjects' V_T (voxel-wise “classic” SA). BS = between-subjects, CV = coefficient of variation, diff. = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, ACG = anterior cingulate gyrus, IFG = Inferior frontal gyrus, iqr = interquartile range.

This method yields parametric maps of V_T . An example of such data is shown in Figure 3.1.

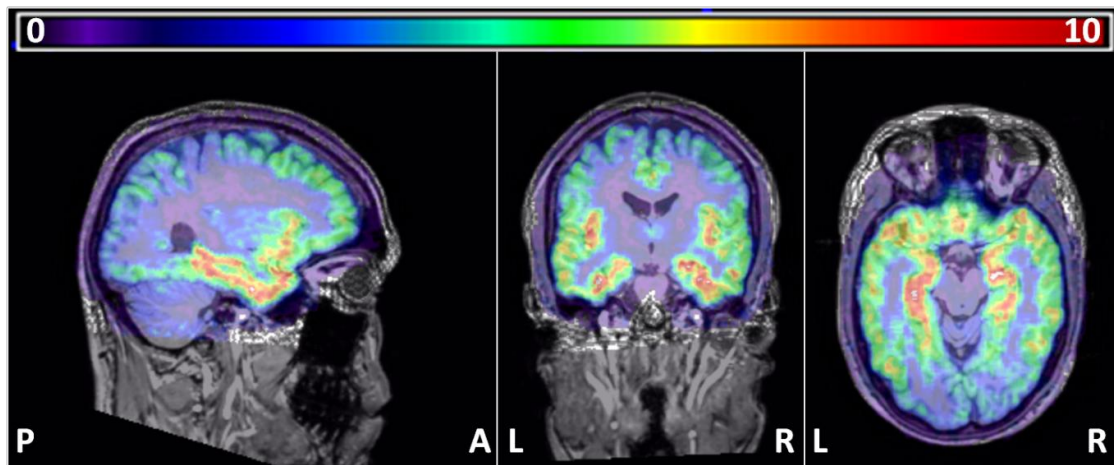


Figure 3.1. V_T image for participant 1, co-registered to the corresponding T1 - weighted MRI image. The image was produced by voxel-wise SA. Note the high uptake in the limbic regions. A = anterior, L = left, P = posterior, R = right; colour bar: V_T .

3.4.5.3 Rank shaping regularisation of spectral analysis (RS-SA), applied to ROI data

The method yielded inconsistent data. The region with the highest V_T was the hippocampus (8.21; Table 3.6). Regional heterogeneity of V_T values was moderate, with a ratio of hippocampus/cerebellum of 3.4. ICCs ranged between -0.28 and 0.49, with a median (i.q.r.) of 0.14 (-0.06 – 0.46).

ROI	Median V_T	Interquartile range	Min	Max	Median % diff.	% diff range	Mean %BS-CV	ICC
Hippocampus	8.21	7.84 – 8.79	6.44	10.30	10	6 - 23	14	0.46
ACG	6.85	5.45 – 7.33	5.10	8.42	15	7 - 36	19	0.49
Cerebellum	2.45	2.12 – 2.77	0.93	3.02	16	12 - 106	27	-0.28
Fusiform gyrus	6.48	5.64 – 7.30	4.93	8.65	10	4 - 49	18	0.14
Inferior frontal gyrus	4.87	4.27 – 5.29	3.92	6.12	16	8 - 39	14	-0.06
							Median (iqr):	0.14 (-0.06 -0.46)

Table 3.6. Subjects' V_T (regional rank-shaping). BS = between-subjects, CV = coefficient of variation, diff. = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, ACG = anterior cingulate gyrus, IFG = Inferior frontal gyrus, iqr = interquartile range.

3.4.6 Methods not requiring an arterial IF

3.4.6.1 SRTM with brainstem as a reference tissue

The region with the highest values was the hippocampus (4.50; Table 3.7). Regional heterogeneity of values, estimated as the ratio of hippocampus/cerebellum, was high at 5.0. ICCs ranged from 0.37 to 0.80, with a median (i.q.r.) of 0.65 (0.39 – 0.76). The regions with low ICCs were the anterior cingulate gyrus and the cerebellum.

ROI	Median BP _{ND}	Interquartile range	Min	Max	Median % diff.	% diff range	Mean %BS-CV	ICC
Hippocampus	4.50	4.26 – 4.70	3.80	5.01	5	4 – 9	8	0.76
ACG	4.17	4.01 – 4.34	3.70	4.59	9	7 – 10	6	0.37
Cerebellum	0.90	0.88 – 0.94	0.85	1.11	3	3 – 15	7	0.39
Fusiform gyrus	3.86	3.71 – 4.07	3.17	4.33	8	5 – 10	8	0.65
IFG	3.17	2.90 – 3.26	2.60	3.49	6	3 – 7	9	0.80
							Median (iqr):	0.65 (0.39 – 0.76)

Table 3.7. Subjects' BP_{ND} (SRTM; brainstem). BS = between-subjects, CV = coefficient of variation, diff. = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, ACG = anterior cingulate gyrus, IFG = Inferior frontal gyrus, iqr = interquartile range.

3.4.6.2 SRTM with cerebellum as a reference tissue

The region with the highest values was the hippocampus (1.91; Table 3.8). Regional heterogeneity of values could not be estimated, as the BP_{ND} of cerebellum as the reference region is ~0. ICCs ranged from 0.56 to 0.89, with a median (i.q.r.) of 0.80 (0.68 – 0.88).

ROI	Median BP _{ND}	Interquartile range	Min	Max	Median % diff.	% diff range	Mean %BS-CV	ICC
Hippocampus	1.91	1.84 – 2.07	1.67	2.33	4	1 – 11	11	0.89
ACG	1.71	1.59 – 1.78	1.54	1.98	9	0 – 10	8	0.56
Cerebellum	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Fusiform gyrus	1.62	1.45 – 1.69	1.27	1.79	6	1 – 13	11	0.72
Inferior frontal gyrus	1.11	1.04 – 1.23	0.93	1.37	8	1 – 10	13	0.88
							Median (iqr):	0.80 (0.68 – 0.88)

Table 3.8. Subjects' BP_{ND} (SRTM; cerebellum). BS = between-subjects, CV = coefficient of variation, diff. = difference, ICC = intraclass correlation coefficient, Min = minimum, Max =

maximum, ROI = region-of-interest, ACG = anterior cingulate gyrus, IFG = Inferior frontal gyrus, iqr = interquartile range.

3.4.6.3 Modified standard uptake values (mSUV)

The region with the highest values was the hippocampus (3.91; Table 3.9). Regional heterogeneity of values, estimated as the ratio of hippocampus/cerebellum, was moderate at 3.3. ICCs ranged from 0.41 to 0.58, with a median (i.q.r.) of 0.48 (0.42 – 0.55).

ROI	Median	Interquartile range	Min	Max	Median % diff.	% diff range	Mean %BSCV	ICC
Hippocampus	3.91	3.74 – 4.19	3.44	4.59	7	1 - 15	9	0.57
ACG	3.78	3.61 – 4.10	3.37	4.54	10	2 – 17	9	0.42
Cerebellum	1.19	1.05 – 1.26	1.01	1.37	13	0 – 17	10	0.41
Fusiform gyrus	3.54	3.25 – 3.90	3.09	4.37	6	0 - 19	12	0.54
Inferior frontal gyrus	3.10	2.77 – 3.20	2.45	3.59	9	1 - 21	12	0.58
							Median (iqr):	0.48 (0.42–0.55)

Table 3.9. Subjects' modified standard uptake values (mSUV). All data is decay-corrected. BS = between-subjects, CV = coefficient of variation, diff. = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, ACG = anterior cingulate gyrus, IFG = Inferior frontal gyrus, iqr = interquartile range.

3.4.7 Comparison between analysis methods.

Table 3.10 provides an overview of the median test-retest differences (%) for the different methods. The 2kbv method was very variable for most regions. The other methods had median differences of 6 – 15%, which were similar across regions as indicated by low spreads (interquartile ranges).

Method	mSUV	SRTM (brainstem)	SRTM (cerebellum)	2kbv	4kbv	SA- ROIs	SA-maps	RS-SA
Parameter	kBq/ml	BP _{ND}	BP _{ND}	V _T	V _T	V _T	V _T	V _T
Sampling	ROI on summed images	ROI on dynamic images	ROI on dynamic images	ROI on dynamic images	ROI on dynamic images	ROI on dynamic images	ROI on parametric maps	ROI on dynamic images
Hippocampus	7	5	4	28	5	11	6	10
ACG	10	9	9	28	9	14	9	15
Cerebellum	13	3	n/a	13	31	18	12	16
Fusiform gyrus	9	8	6	30	15	16	6	10
IFG	6	6	8	22	12	7	10	16
Median (iqr)	9 (7 – 10)	6 (5 – 8)	7 (5.5 – 8.3)	28 (22 – 28)	12 (9 – 15)	14 (11 – 16)	9 (6 – 10)	15 (10 – 16)

Table 3.10. Median test – re-test differences (%) for subjects' parameter estimates

(mSUV / BP_{ND} / V_T) obtained with the different methods. mSUV = modified standard uptake value, SRTM = simplified reference tissue model, 2/4kbv: 2/4 rate constants compartmental model with variable blood volume, SA = spectral analysis, RS-SA = rank shaping of spectral analysis, ROI = region-of-interest, ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, iqr = interquartile range.

Median between-subject coefficients of variation (BSCV; %) for the different methods are listed in Table 3.11. The median between-subject CVs were lowest using the SRTMs (8 – 11%). The other methods had similar median between-subject variability, 11 – 18%, with similar variability for the various regions.

Method	mSUV	SRTM (brainstem)	SRTM (cerebellum)	2kbv	4kbv	SA- ROIs	SA-maps	RS-SA
Parameter	kBq/ml	BP _{ND}	BP _{ND}	V _T	V _T	V _T	V _T	V _T
Hippocampus	12	8	11	17	11	23	12	14
ACG	15	6	8	14	10	21	17	19
Cerebellum	19	7	n/a	9	16	15	10	27
Fusiform gyrus	14	8	11	16	29	17	19	18
IFG	17	9	13	13	24	20	17	14
Median (iqr)	15 (14 – 17)	8 (7 – 8)	11 (10 – 12)	14 (13 – 16)	16 (11 – 24)	20 (17 – 21)	17 (12 – 17)	18 (14 – 19)

Table 3.11. Mean between-subject coefficients of variation (BSCV; %) for subjects' parameter estimates (V_T / BP_{ND} / mSUV) obtained with the different methods. mSUV = modified standard uptake value, SRTM = simplified reference tissue model, 2/4kbv: 2/4 rate constants compartmental model with variable blood volume, SA = spectral analysis, RS-SA = rank shaping of spectral analysis, ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, iqr = interquartile range.

ICCs for the different methods are listed in Table 3.12. The 2kbv model, regional SA, and voxel-wise RS-SA yielded non-reproducible results, as reflected by median ICCs of -0.73, -0.22 and -0.14, respectively, whereas mSUV and the 4kbv model yielded slightly higher ICCs (medians 0.48 and 0.34, respectively). The remaining methods had good to excellent reproducibility, ranging from 0.65 (0.39 – 0.76) for SRTM using the brainstem to 0.80 (0.68 – 0.88) for SRTM using the cerebellum. Low between-region spread of the ICC was seen with mSUV, SRTM using the cerebellum, and voxel-wise “classic” SA parametric maps, indicating reproducibility was good throughout the brain regions sampled.

Table 3.12 also shows the ratio between a high-binding region (hippocampus) and a low-binding region (cerebellum), indicating a method's ability to reflect the known between-region heterogeneity. SA applied to parametric maps yielded the highest differential.

Method	mSUVs	SRTM (brainstem)	SRTM (cerebellum)	2kbv	4kbv	SA- ROIs	SA- maps	RS-SA
Parameter	kBq/ml	BP _{ND}	BP _{ND}	V _T	V _T	V _T	V _T	V _T
Hippocampus	0.57	0.76	0.89	-0.62	0.86	-0.22	0.83	0.46
ACG	0.42	0.37	0.56	-0.81	0.59	-0.32	0.61	0.49
Cerebellum	0.41	0.39	n/a	-4.19	-0.32	0.09	0.35	-0.28
Fusiform gyrus	0.54	0.65	0.72	-0.73	0.34	-0.39	0.73	0.14
IFG	0.58	0.80	0.88	- 0.524	-0.12	0.31	0.72	-0.06
Median (iqr)	0.48 (0.42 – 0.55)	0.65 (0.39 – 0.76)	0.80 (0.68 – 0.88)	-0.73 (-0.81 – -0.62)	0.34 (-0.12 – 0.59)	-0.22 (-0.32 – -0.09)	0.72 (0.61 – 0.73)	0.14 (-0.06 – 0.46)
Ratio hippocampus/cerebellum	3.3	5.0	n/a	2.7	3.9	3.2	6.1	3.4

Table 3.12. Intraclass Correlation Coefficients (ICCs) for subjects' parameter estimates

(V_T / BP_{ND} / mSUV) obtained with the different methods. mSUV = modified standard uptake value, SRTM = simplified reference tissue model, 2/4kbv: 2/4 rate constants compartmental model with variable blood volume, SA = spectral analysis, RS-SA = rank shaping of spectral analysis, ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, n/a = not applicable, iqr = interquartile range

3.5 Discussion

I tested a wide range of methods for quantifying reversible binding of a radiotracer with relatively fast kinetics. Out of the eight methods tested, only three produced reliable values – the SRTMs with the pseudo-reference regions brainstem and cerebellum, and SA applied at the voxel level (SA-maps). For these methods, test-retest variation was low, below 10% (Table 3.10). Between-subject variation, expressed as the coefficient of variation, was at expected levels for SRTM (cerebellum) (11%) and SA-maps (17%), but rather low for SRTM (brainstem) (8%). Further studies should determine whether this is sufficient for capturing between-subject variation of interest.

The ICCs resulting from the combined assessment of the relationship between within-subject (test-retest) variation and between-subject variation, indicated good reliability (Portney and

Watkins, 2009) for SRTM (cerebellum; ICC 0.80) and SA-maps (ICC 0.72), with less good reliability for SRTM (brainstem; ICC 0.65). However, the actual BP_{ND} values were much lower for the SRTM using cerebellum as the pseudo-reference; likely due to some binding present in the cerebellum (V_T using SA-maps 1.53; see also illustration in Figure 3.1).

There was a marked difference in reliability between spectral analysis applied at the voxel-level (SA-maps) and the two methods applying it at the regional level. This phenomenon has been noted in less marked manner before (Hammers et al., 2007c) and is likely due to the analysis being able to accommodate differences in blood volume, tissue class partial volume and receptor concentration when applied at the voxel level, whereas averaged time-activity curves across all voxels in a ROI cannot be fit as well. The voxel-based method has the added advantage of allowing whole-brain surveys in diseases where the exact localisation of pathology is not known, e.g. refractory focal epilepsy.

Of the compartmental models, the 2kbv model did not produce reliable values; the 4kbv model was reliable only in the highest binding region, namely the hippocampus. This is in line with previous findings on model fits (Asai et al., 2009). While [^{11}C]Ro15 4513 has highest affinity for GABA_A receptors containing $\alpha 5$ subunits (Lingford-Hughes et al., 2002), it also binds to GABA_A receptors containing $\alpha 1$ subunits (Myers et al., 2012, Stokes et al., 2013a). Even if the affinity for $\alpha 1$ subunit containing GABA_A receptors is lower, they are overall more abundant. Given the overlapping regional distribution of α subunits, signal from brain regions will contain a mixture of both, with different kinetics (Myers et al., 2012); hence a two-tissue compartment, single binding site model will not be adequate except in the regions with highest $\alpha 5$ subunit concentration like the hippocampus.

For a radiotracer to be use widely, quantification without an arterial input function is desirable. Besides the SRTMs, mSUVs offer this possibility and may be reliable in other

contexts (e.g. (Riaño Barros et al., 2013) for [^{11}C]Ro15 4513, values were not reliable. Of note, standard SUVs - which correct less stringently for a subject's body weight - achieved somewhat higher ICCs, reflecting the large heterogeneity (between-subject variability) in weight in my sample, coupled with very little variation in the dose injected (Table 3.1; data not shown).

3.6 Conclusion

Quantification of [^{11}C]Ro15 4513 binding shows good-to-excellent reproducibility with regional SRTMs and voxel-wise SA. [^{11}C]Ro15 4513 PET is well placed as a tool to investigate binding to $\alpha 5$ subunit containing GABA_A receptors in health and neuropsychiatric disease.

CHAPTER 4

Test-retest reproducibility of cannabinoid-receptor type 1 availability quantified with the PET ligand [^{11}C]MePPEP

[^{11}C]MePPEP is a novel PET radiotracer that selectively binds to CB₁ (see Chapter 1 for further details). In this Chapter, I describe the evaluation of [^{11}C]MePPEP PET in 15 healthy control adult subjects.

4.1 Objectives

The objectives of the study were as follows:

1. Evaluate the kinetic behaviour of [^{11}C]MePPEP *in vivo* in the healthy human brain.
2. Quantify [^{11}C]MePPEP binding/distribution *in vivo* in the healthy human brain (in terms of BP or V_T).

4.2 Hypothesis

The primary hypothesis was:

1. The two brain-compartments, four-rate constants (reversible binding; 2c4kbv) model would best-describe the kinetic behaviour of [^{11}C]MePPEP *in vivo* in the healthy human brain. [This was based on evaluation of (Terry et al., 2009)].

4.3 Materials and methods

Ethical approval was obtained from the London – Surrey Borders Research Ethics Committee, and the previously described regulatory authorities (see Section 2.1).

4.3.1 Study population

The inclusion criterion was as follows:

- Age between 25 and 80 years on the day of the first PET scan

The exclusion criteria were as listed in Chapter 2.

Seventeen healthy subjects were recruited (as described in Chapter 2) and gave written informed consent. From this sample two were excluded: one subject with pathological modified Allen's test; another withdrew consent for the retest scan. Hence, a total of fifteen healthy subjects (8 females; median age 32 years, range 25 to 65 years), without history of either somatic or psychiatric conditions or substance abuse were scanned twice. Demographic data are detailed in Table 4.1.

Subject No.	Age	Gender	BMI	Scan Interval (days)	Dose (MBq)	Radiochemistry purity (%)	Co-injected mass (µg)	Specific Activity (MBq/nmol)
1	28	M	23	24	365 364	98 98	4.2 6.2	39 27
2	44	M	28	23	375 376	96 99	3.0 4.3	56 40
3	42	M	25	122	356 373	96 100	2.3 2.3	70 72
4	26	M	24	1	361 366	98 98	3.4 2.7	48 61
5	31	M	24	19	353 355	98 99	4.2 2.7	38 60
6	27	F	21	309	367 363	99 97	4.6 1.7	37 99
7	32	F	36	37	351 316	100 100	3.2 8.3	50 17
8	65	F	22	23	355 371	97 96	2.3 4.0	69 42
9	27	M	22	67	369 366	98 98	3.9 3.1	43 54
10	29	F	23	4	360 364	97 100	9.7 5.8	17 29
11	57	M	30	30	373 399	97 96	2.9 5.0	60 37
12	61	F	36	93	361 349	96 96	1.8 1.6	92 97
13	63	F	29	12	384 380	97 98	3.5 3.3	50 52
14	25	F	31	156	364 357	97 100	2.4 4.4	69 37
15	62	F	24	10	362 366	97 97	1.4 3.3	122 51
Median	32		24	24	364	98	3.3	98
Interquartile range (25th-75th)	27 - 61		23 - 30	12 - 93	356 - 372	97 - 99	2.4 - 4.3	38 - 69
Min	25		21	1	316	96	1.4	17
Max	65		36	309	399	100	9.7	122

Table 4.1. Subjects' demographic and injectate details. BMI = Body Mass Index; Min = minimum; Max = maximum; M = male; F = female.

4.3.2 Radiochemistry

Production and injection of [¹¹C]MePPEP was as described in Chapter 2. Details of the injectate are listed in Table 4.1.

4.3.3 PET data acquisition

PET image acquisition was as described in Chapter 2. Each dynamic acquisition consisted of 35 frames of increasing length ($1 \times 30''$, $6 \times 10''$, $3 \times 20''$, $3 \times 30''$, $3 \times 60''$, $6 \times 120''$, $8 \times 300''$ and $3 \times 600''$). [^{11}C]MePPEP was injected as an intravenous bolus injection of $\sim 370\text{MBq}$ (median 364 MBq , range $316 - 399\text{ MBq}$; Table 4.1). Subjects were scanned on two separate days with a median interval of 24 days (range 1 to 309; Table 4.1).

4.3.4 Input Function (IF) Derivation

The input function was derived as described in Chapter 2.

4.3.5 MRI data acquisition, analysis and generation of ROIs

The MRI data was acquired and analysed, and the ROIs generated, as described in Chapter 2.

4.3.6 Manual delineation of the pons

Because the pons is not included in the 83 regions obtained via MAPER, I delineated it manually using Analyze 8.1. The pons was first delineated on sagittal views, followed by coronal and axial views, with the following limits illustrated by the numbers in brackets on Figure 4.1:

Anterior/ventral: cisterna interpeduncularis and basilar artery (1).

Posterior/dorsal: floor of the fourth ventricle (2).

Superior: A line was drawn from the floor of the fourth ventricle below the superior cerebellar peduncle (3) along the lower limit of the cerebral peduncle, to the indentation between the pons and the midbrain (4).

Inferior: A line was drawn from the floor of the fourth ventricle above the inferior peduncle (6) to the upper limits of the olive and pyramid of the medulla oblongata (7), i.e. to the indentation between the pons and medulla oblongata (8).

On coronal view: Following anatomical boundaries of pons, which are clearly visible.

On axial view: Following delineation in both sagittal and coronal views, the pons is now clearly delineated, and the axial view is used for verification.

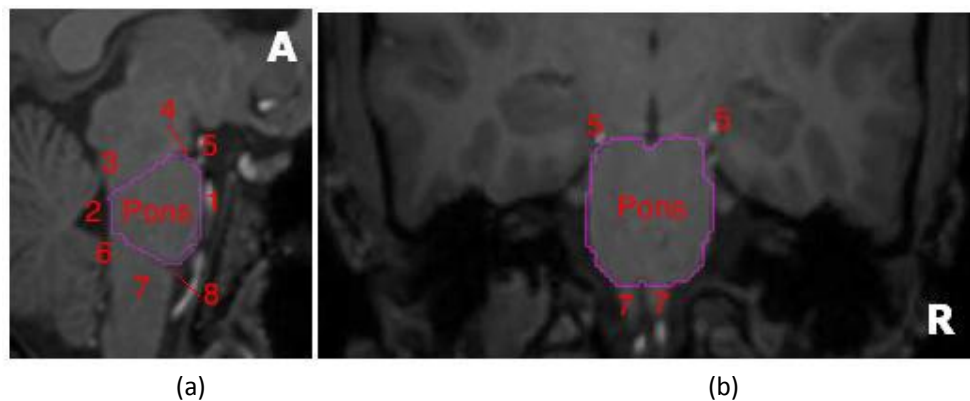


Figure 4.1. Manual delineation of the pons on MRI. (a) sagittal view, (b) coronal view, A

– Anterior, R – right. The pons was first delineated on sagittal views, followed by coronal and axial views, with the following limits; Anterior/ventral: cisterna interpeduncularis and basilar artery (1); Posterior/dorsal: floor of the fourth ventricle (2); Superior: A line was drawn from the floor of the fourth ventricle below the superior cerebellar peduncle (3) along the lower limit of the cerebral peduncle, to the indentation between the pons and the midbrain (4); Inferior: A line was drawn from the floor of the fourth ventricle above the inferior peduncle (6) to the upper limits of the olive and pyramid of the medulla oblongata (7), i.e. to the indentation between the pons and medulla oblongata (8); On coronal view: Following anatomical boundaries of pons, which are clearly visible; On axial view: Following delineation in both sagittal and coronal views, the pons is now clearly delineated, and the axial view is used for verification. [5 – posterior cerebral artery].

4.3.7 ROI definition

I evaluated the test-retest reliability of the quantification methods (see “PET data quantification” section below) in a selection of eight bilateral ROIs in total. I chose representative regions with high CB₁ receptor concentrations; the grey matter masked

cortical structures – hippocampus, anterior cingulate gyrus, inferior frontal gyrus; and the subcortical structures in their entirety, i.e. not grey matter masked (Heckemann et al., 2011) – caudate nucleus, globus pallidus, nucleus accumbens. In addition, two regions with low concentration of CB₁ receptors were evaluated: the thalamus and the manually defined (entire) pons. The data from left and right homologues were averaged prior to quantification.

4.3.8 Metabolism of [¹¹C]MePPEP

The metabolite model was fitted as described in Chapter 2.

4.3.9 PET data quantification

Attenuation and scatter-corrected dynamic PET images were de-noised and corrected for movements as described in Chapter 2.

ADD images were created as described in Chapter 2.

Regional quantification of distribution/binding/uptake was then performed. In all following analyses, assessment was based on the same ROIs. Binding parameters were calculated directly for the ROI TAC data, except in the “classic” SA, where the additional aim was the assessment of the quality of parametric maps for use in voxel-by-voxel analyses, and the parametric map itself was sampled using the same ROIs:

1. Compartmental models, requiring arterial IFs
 - Reversible 2kbv model
 - Reversible 4kbv model
2. Model-free analyses, requiring arterial IFs:
 - “Classic” (non-regularised) SA, applied to ROI time-activity data
 - “Classic” SA, applied on a voxel-by-voxel basis to create parametric maps of V_T , which were then sampled in the same ROIs as for the other methods

- RS-SA

3. Methods not requiring arterial IFs

- SRTM using pons as a pseudo-reference tissue
- Regional mSUV

Each of the above methods was described in Chapter 2.

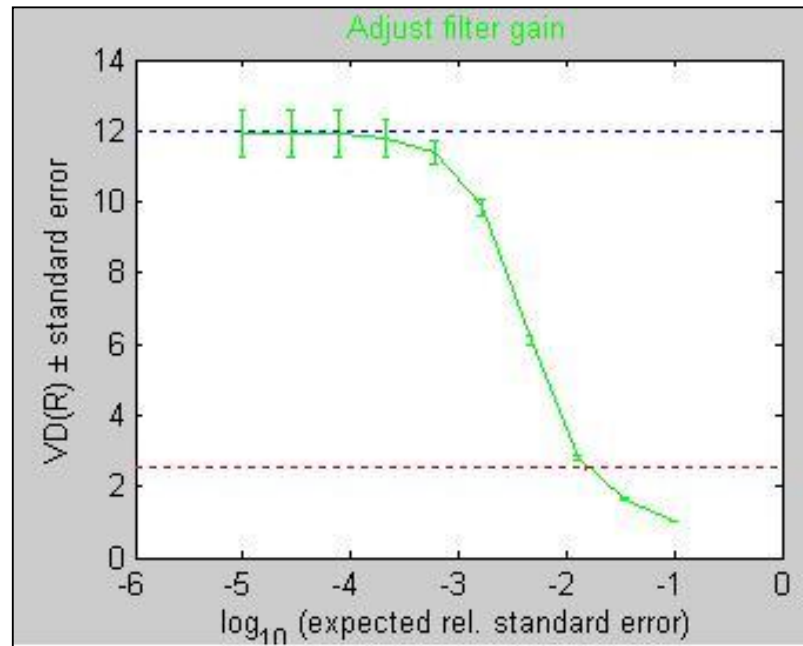


Figure 4.2. Example plot showing a V_T of 12 obtained for the hippocampus from the $V_T(R)$ plateau via rank-shaping regularisation of spectral analysis.

4.3.10 Global intensities (GI)

GIs were calculated as described in Chapter 2.

4.3.11 Statistical analyses

Statistical analyses were as described in Chapter 2.

4.4 Results

4.4.1 Adverse or serious events

[¹¹C]MePPEP was well-tolerated by all participants. One participant reported self-limiting abdominal discomfort approximately 24 hours after injection. No serious events were reported.

4.4.2 Injectate

Details are given in Table 4.1. There were no significant differences between test and retest studies in terms of the amount of injected radioactivity (median (i.q.r)): test 362 (358 – 368) MBq; retest 366 (360 – 372) MBq; co-injected mass of stable ligand: test: 3.2 (2.4 – 4.1) μg , retest 3.3 (2.7 – 4.7) μg ; SpAct at the time of injection: test 50 (49 – 61) MBq/ ηmol , retest: 51 (37 – 61) MBq/ ηmol .

4.4.3 Image data

GIs of ADD images did not differ between test and retest studies (median, interquartile 25th – 75th: test: 1.2, 1.0 – 1.6; retest: 1.3, 1.1 – 1.5; $p>0.6$). Figure 4.3 shows examples of TACs.

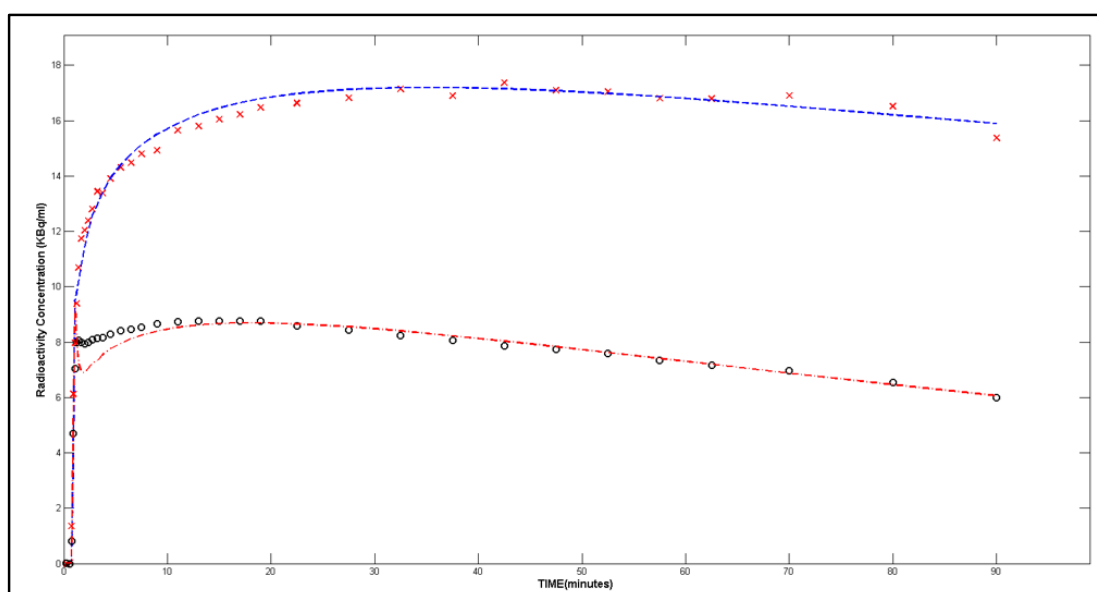


Figure 4.3. Time activity curve in the pallidum (Panayiotopoulos et al.) and pons (bottom) showing the average uptake as a function of time in minutes, fitted with a two-compartment (2kbv) model.

4.4.4 Quantification results

The following sections describe the regional estimates for the parameters derived with the seven quantification methods. To assess test-retest variation, for each ROI the median percent difference between test and retest study as well as their signed range is given in the tables. The CV quantifies the between-subject variability of the measure. The ICC assesses the reliability of the measure as a function of both within-subject and between-subject variability; the closer to the value of 1, the more reliable the method, i.e. the smaller the intra-subject variability of the measure compared with natural between-subject variability. Finally, the ratio of the generally highest binding region (pallidum) over the lowest binding region (pons) assesses a method's ability to reflect known binding heterogeneity.

A comparison of analysis methods and a synthetic overview of the various measures will be provided in section 3.4.

4.4.5 Compartmental models, requiring arterial IFs

4.4.5.1 Reversible two-compartment (one tissue compartment) model with variable blood volume (2kbv)

The region with the highest V_T was globus pallidus (10.8; Table 4.2). Regional heterogeneity of V_T values was high, with a ratio of pallidum:pons of 3.6. ICCs ranged between 0.69 and 0.95, with a mean $\pm$ SD of 0.80 ± 0.08 .

ROI	Median	Interquartile range	Min	Max	Median % diff	Median % diff range	Mean BS % CV	ICC
Hippocampus	8.0	6.2 – 10.8	4.1	24.8	14.8	-54.8 – 54.2	50.4	0.88
ACG	10.3	7.5 – 14.0	1.0	26.4	27.8	-154.4 – 64.2	47.4	0.76
IFG	8.8	7.3 – 12.3	4.1	22.4	18.1	-48.3 – 31.9	42.1	0.83
Caudate	7.4	6.0 – 12.2	3.9	22.9	19.7	-60.9 – 33.5	48.0	0.85
Pallidum	10.8	8.5 – 14.2	1.1	32.6	20.6	-158.7 – 44.1	48.1	0.70
Accumbens	8.4	7.2 – 13.2	1.2	31.7	35.8	-150.5 – 51.5	53.7	0.69
Thalamus	5.1	4.4 – 7.3	2.9	10.9	12.6	-35.5 – 28.5	35.9	0.89
Pons	3.0	2.8 – 3.9	2.0	6.2	8.3	-10.7 – 20.2	30.2	0.95
				Mean (SD)	19.7 (8.7)		44.5 (7.9)	0.82 (0.09)

Table 4.2. Subjects' volume of distribution (V_T) obtained with two-compartment model (2kbv) method. ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, caudate = caudate nucleus, pallidum = globus pallidus, accumbens = Nucleus accumbens. BS = between-subjects, CV = coefficient of variation, diff = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, SD = standard deviation.

4.4.5.2 Reversible three-compartment (two tissue compartment) model with variable blood volume (4kbv)

The method yielded highly variable data. Unlike for the other methods, the regions with the highest V_T values were nucleus accumbens (13.6; Table 4.3) and hippocampus (13.4). The ratio of pallidum / pons was 2.0. ICCs ranged between -0.13 and 0.50, with a mean $\pm$ SD of 0.08 ± 0.22 .

ROI	Median	Interquartile range	Min	Max	Median % diff	Median diff % range	Mean BS % CV	ICC
Hippocampus	13.4	7.5 – 19.6	-0.7	102.2	72.8	-219.9 – 175.6	81.1	-0.05
ACG	9.0	5.8 – 13.6	1.4	65.6	53.7	-136.2 – 126	81.4	0.23
IFG	8.9	4.7 – 11.2	0.8	18.6	61.1	-159 – 116.4	51.9	0.13
Caudate	6.2	4.2 – 8.5	0.0	31.5	68.5	-153.4 – 200.0	69.9	-0.13
Pallidum	10.6	6.7 – 18.3	3.4	79.8	69.7	-170.7 – 126.1	111.3	-0.03
Accumbens	13.6	8.7 – 39.5	0.0	163.5	122.1	-72.3 – 200.0	131.7	-0.06
Thalamus	6.1	4.5 – 8.4	1.2	30.5	32.7	-133.8 – 102.5	87.9	0.49
Pons	5.3	3.9 – 7.2	2.4	23.0	40.3	-23.4 – 146.5	79.0	0.50
				Mean (SD)	65,1 (27.1)		86.8 (24.6)	0.14 (0.25)

Table 4.3. Subjects' V_T obtained with three-compartment model (4kbv). ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, caudate = caudate nucleus, pallidum = globus pallidus, accumbens = Nucleus accumbens. BS = between-subjects, CV = coefficient of variation, diff = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, SD = standard deviation.

4.4.6 Model-free analyses, requiring arterial IFs

4.4.6.1 Directly obtaining V_T values from ROI data with “classic” spectral analysis (SA)

The region with the highest V_T values was globus pallidus (15.7; Table 4.4). Regional heterogeneity of V_T values, estimated as the ratio of pallidum over pons, was 2.6. ICCs ranged between 0.67 and 0.87, with a mean $\pm$ SD of 0.77 ± 0.07 .

ROI	Median	Interquartile range	Min	Max	Median % diff	Median % diff range	Mean BS % CV	ICC
Hippocampus	12.4	9.4 – 17.2	6.6	23.4	10.9	-39.3 – 29.6	34.1	0.87
ACG	14.4	11.9 – 19.9	7.3	27.1	12.4	-64.3 – 39.3	35.0	0.74
IFG	13.9	10.1 – 19.3	5.4	26.3	13.3	-76.3 – 47.3	39.0	0.71
Caudate	13.0	9.3 – 19.4	5.9	28.1	13.6	-80.8 – 26.3	44.2	0.74
Pallidum	15.7	12 – 21.7	8.2	28.9	9.4	-53.8 – 33.5	35.5	0.85
Accumbens	13.9	11.3 – 18.9	6.9	26.7	15.1	-63.6 – 47.1	35.2	0.80
Thalamus	9.6	6.9 – 12.2	4.8	20.3	24.7	-60.7 – 49.2	39.4	0.70
Pons	6.0	4.6 – 7.1	2.9	11.8	24.8	-62.0 – 39.9	35.5	0.67
				Mean (SD)	15.5 (6.0)		37.2 (3.4)	0.76 (0.07)

Table 4.4. Subjects' volume of distribution (V_T) obtained with Spectral Analysis (SA)

based on ROI data. ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, caudate = caudate nucleus, pallidum = globus pallidus, accumbens = Nucleus accumbens. BS = between-subjects, CV = coefficient of variation, diff = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, SD = standard deviation.

4.4.6.2 Sampling parametric V_T images obtained with “classic” SA

The regions with the highest V_T were pallidum (15.8; Table 4.5) and globus pallidus (15.8). Regional heterogeneity of V_T values was the highest of all methods tested, with a ratio of pallidum over pons of 3.6. ICCs were fairly homogenous between regions and ranged between 0.76 and 0.87, with a mean $\pm$ SD of 0.84 ± 0.04 . Figure 4.4 shows an example of a parametric map.

ROI	Median	Interquartile range	Min	Max	Median % diff	Median % diff range	Mean BS % CV	ICC
Hippocampus	11.5	9 – 16.2	7.0	22.6	9.6	-40.1 – 20.6	34.2	0.87
ACG	14.8	11.1 – 20.5	8.1	28.8	12.5	-44.0 – 30.1	34.7	0.85
IFG	14.8	10.4 – 20.3	7.6	27.6	12.0	-49.7 – 27.0	37.0	0.85
Caudate	12.9	10.2 – 19.3	6.5	26.9	12.3	-76.6 – 24.3	40.0	0.81
Pallidum	15.8	11.6 – 21.1	8.6	30.4	12.6	-53.0 – 23.5	35.4	0.83
Accumbens	9.5	7.8 – 13.9	5.3	18.7	12.3	-44.6 – 31.4	36.0	0.83
Thalamus	13.2	10.4 – 17.3	5.2	26.5	16.8	-78.9 – 50.8	37.1	0.76
Pons	4.4	3.5 – 5.3	1.7	9.6	20.2	-33.1 – 43.3	39.0	0.81
				Mean (SD)	13.5 (3.3)		36.7 (2.0)	0.83 (0.03)

Table 4.5. Subjects' volume of distribution (V_T) obtained with “classic” spectral analysis (SA) on parametric maps. ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, caudate = caudate nucleus, pallidum = globus pallidus, accumbens = Nucleus accumbens. BS = between-subjects, CV = coefficient of variation, diff = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, SD = standard deviation.

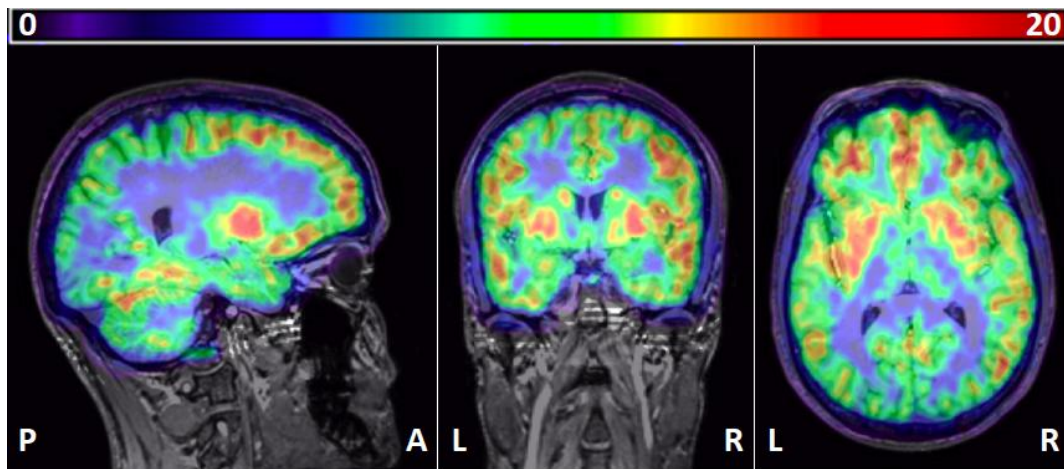


Figure 4.4. Example of a parametric map, co-registered onto the subject's MRI scan.

Left, sagittal; middle, coronal; right, transverse. Note high binding in the putamen and pallidum and intermediate binding in the hippocampus and thalamus. Colour bar: V_T values. P, posterior; A, anterior; R, right; L, left.

4.4.6.3 Spectral analysis with rank shaping regularisation (SA-RS)

The region with the highest V_T values was globus pallidus (10.3; Table 4.6). Regional heterogeneity of V_T values was lower than with the preceding methods, with a ratio of pallidum over pons of 2.4. ICCs ranged between 0.73 and 0.90, with a mean $\pm$ SD of 0.82 ± 0.05 .

ROI	Median	Interquartile range	Min	Max	Median % diff	Median % diff range	Mean BS % CV	ICC
Hippocampus	8.2	5.8 – 10.4	4.2	15.2	14.4	-48.5 – 34.9	35.9	0.81
ACG	9.9	7.6 – 13.7	5.5	18.8	14.6	-29.7- 32.4	34.0	0.86
IFG	9.9	7.2 – 13.8	5.3	18.2	15.6	-39.5- 29.0	34.8	0.85
Caudate	8.1	6.1 – 12.6	4.4	18.1	11.6	-88.7- 27.0	42.2	0.82
Pallidum	10.3	7.0 – 13.0	4.5	20.7	23.3	-64.1- 42.3	39.7	0.77
Accumbens	8.6	6.1 – 10.5	3.2	15.9	18.5	-77.8- 58.4	36.5	0.73
Thalamus	6.5	5.5 – 9.4	3.5	12.7	14.9	-43.9- 31.0	34.7	0.85
Pons	4.3	3.3 – 5.3	2.5	8.0	7.4	-30.6- 24.1	32.3	0.90
				Mean (SD)	15.0 (4.7)		36.3 (3.2)	0.82 (0.05)

Table 4.6. Subjects' Volume of distribution (V_T) obtained with Rank Shaping (RS)

regularization of Spectral Analysis (SA) method. ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, caudate = caudate nucleus, pallidum = globus pallidus, accumbens = Nucleus accumbens. BS = between-subjects, CV = coefficient of variation, diff = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, SD = standard deviation.

4.4.7 Methods not requiring an arterial IF

4.4.7.1 SRTM with pons as a pseudo-reference tissue

The method yielded inconsistent data. The regions with the highest values were the globus pallidus and the anterior cingulate gyri (both $BP_{ND} = 1.1$; Table 4.7). The ratio of pallidum over pons could not be calculated, as the BP_{ND} of pons as the reference region is ~ 0 . ICCs ranged between -0.29 and 0.50, with a mean $\pm$ SD of 0.08 ± 0.25 .

ROI	Median BP _{ND}	Interquartile range	Min	Max	Median % diff.	Median % diff range	Mean %BS- CV	ICC
Hippocampus	1.0	0.8-1.7	0.4	12.8	54.6	-155.5 – 176.6	125.2	- 0.06
ACG	1.1	0.9-1.2	0.7	3.4	40.2	-142.9 – 71.7	-574.1	0.50
IFG	0.9	0.9-1.0	0.0	1.7	16.0	-66.6 – 207.6	29.4	- 0.29
Caudate	0.9	0.6-1.0	0.2	2.7	30.5	-137.5 – 45.4	48.8	0.06
Pallidum	1.1	1.0-1.3	0.1	1.6	9.4	-37.9 – 158.4	25.1	0.27
Accumbens	1.1	0.7-1.6	-0.2	18.2	53.0	-213.9 – 180.6	157.4	- 0.15
Thalamus	1.0	0.5-6.2	0.4	10.4	42.3	-169.8 – 181.2	108.2	0.19
				Mean (SD)	35.1 (17.4)			0.07 0.27

Table 4.7. Subjects' binding potential (BP_{ND}) obtained with the SRTM and pons as a pseudo-reference region. ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, caudate = caudate nucleus, pallidum = globus pallidus, accumbens = Nucleus accumbens. BS = between-subjects, CV = coefficient of variation, diff = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, SD = standard deviation.

4.4.7.2 mSUV

The region with the highest values was globus pallidus (0.80; Table 4.8). Regional heterogeneity of values, estimated as the ratio of pallidum over pons, was 4.2. ICCs ranged from 0.79 to 0.86, with a mean $\pm$ SD of 0.83 ± 0.03 .

ROI	Median	Interquartile range	Min	Max	Median % diff.	Median % diff range	Mean %BS-CV	ICC
Hippocampus	2,7	2.1 – 3.2	1.8	4.2	8.3	-22.6 – 34.4	27.4	0.84
ACG	3,2	2.6 – 3.8	2.0	5.2	8.8	-24.1 – 32.7	27.2	0.86
IFG	3,2	2.6 – 3.7	2.1	5.3	8.9	-22.4 – 35.1	27.3	0.86
Caudate	2,9	2.5 – 3.9	1.9	5.1	7.6	-58.1 – 30.3	30.1	0.79
Pallidum	3,6	2.7 – 4.1	2.2	5.5	11.1	-28.6 – 30.9	27.1	0.84
Accumbens	3,0	2.4 – 3.6	1.5	4.7	6.7	-65.7 – 33.7	27.9	0.80
Thalamus	2,7	2.2 – 3.2	1.7	4.3	8.1	-16.7 – 38.1	25.8	0.83
Pons	0,9	0.6 – 1.3	0.3	3.4	36.7	7.3 – 108.2	62.6	0.47
				Mean (SD)	12.0 (10.1)		31.9 (12.5)	0.79 (0.13)

Table 4.8. Subjects' modified standard uptake values (mSUV). ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, caudate = caudate nucleus, pallidum = globus pallidus, accumbens = Nucleus accumbens. BS = between-subjects, CV = coefficient of variation, diff = difference, ICC = intraclass correlation coefficient, Min = minimum, Max = maximum, ROI = region-of-interest, SD = standard deviation.

4.4.8 Comparison between analysis methods.

I used a Bland-Altman plot to compare methods and assess bias. Relative to the 2kbv model, a bias towards overestimation of medium high hippocampal V_T of [^{11}C]MePPEP was seen for both analyses using “classic” SA (Figure 4.5). RS-SA did not show this bias but restricted the range of V_T estimates, with an underestimation of the highest V_T s. The 4kbv model was not assessed due to its lack of reliability.

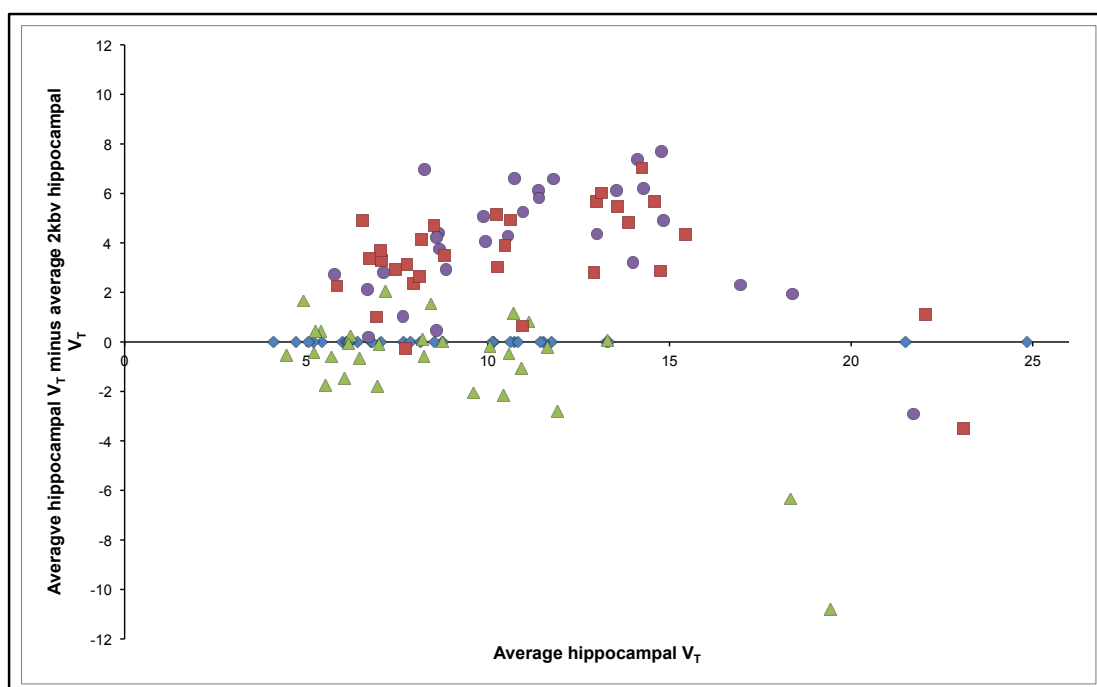


Figure 4.5. Bland-Altman plot for the different methods to obtain V_T s. Data for the bilateral hippocampi is shown as an example, relative to the one compartment, two-rate constants model (blue diamonds, 2kbv); green triangles: rank-shaping regularisation of spectral analysis; red squares: “classic” voxel-wise SA; purple circles: “classic” SA on ROI data.

Table 4.9 provides an overview of the median test–retest differences (%) for the different methods. 2kbv and the methods using SA had median differences between 13 and 20%, similar across regions as indicated by low spreads (SDs), while mSUVs varied even less on average, but with more between-region variation due to the pons showing high test–retest differences (37%). 4kbv and SRTM were very variable for most regions.

Method	2kbv	4kbv	SA-ROIs	SA-maps	RS-SA	SRTM	mSUV
Parameter	V_T	V_T	V_T	V_T	V_T	BP_{ND}	kBq/ml
Sampling	ROI on dynamic images	ROI on dynamic images	ROI on dynamic images	ROI on parametric maps	ROI on dynamic images	ROI on dynamic images	ROI on summed images
Hippocampus	14.8	72.8	10.9	9.6	14.4	54.6	8.3
ACC	27.8	53.7	12.4	12.5	14.6	40.2	8.8
IFG	18.1	61.1	13.3	12.0	15.6	16.0	8.9
Caudate	19.7	68.5	13.6	12.3	11.6	30.5	7.6
Pallidum	20.6	69.7	9.4	12.6	23.3	9.4	11.1
Accumbens	35.8	122.1	15.1	12.3	18.5	53.0	6.7
Thalamus	12.6	32.7	24.7	16.8	14.9	42.3	8.1
Pons	8.3	40.3	24.8	20.2	7.4	NA	36.7
Mean $\pm$ SD	19.7 $\pm$ 8.7	65.1 $\pm$ 27.1	15.5 $\pm$ 5.9	13.5 $\pm$ 3.3	15.0 $\pm$ 4.6	35.1 $\pm$ 17.4	12.0 $\pm$ 10.1

Table 4.9. Median test-retest differences (%) for subjects' parameter estimates (V_T / BP_{ND} / mSUV) obtained with the different methods. ACC = anterior cingulate gyrus, IFG = inferior frontal gyrus, caudate = caudate nucleus, pallidum = globus pallidus, accumbens = Nucleus accumbens. ROI = region-of-interest, SD = standard deviation.

Mean between-subject coefficients of variation (BSCV; %) for the different methods are listed in Table 4.10. The between-subject variability based on mSUVs (i.e. tissue data only) was approximately 32 %; however the BSCV for pons was 63 %. The three methods based on SA had similar between-subject variation, around 36 %, with similar variability for the various regions. Between-subject variability was higher for the 2kbv compartmental model, at 45%. SRTM and the 4kbv model yielded implausible values.

Method	2kbv	4kbv	SA-ROIs	SA-maps	RS-SA	SRTM	mSUV
Parameter	V_T	V_T	V_T	V_T	V_T	BP_{ND}	kBq/ml
Sampling	ROI on dynamic images	ROI on dynamic images	ROI on dynamic images	ROI on parametric maps	ROI on dynamic images	ROI on dynamic images	ROI on summed images
Hippocampus	50.4	81.1	34.1	34.2	35.9	125.2	27.4
ACG	47.4	81.4	35.0	34.7	34.0	-574.1	27.2
IFG	42.1	51.9	39.0	37.0	34.8	29.4	27.3
Caudate	48.0	69.9	44.2	40.0	42.2	48.8	30.1
Globus Pallidum	48.1	111.3	35.5	35.4	39.7	25.1	27.1
Accumbens	53.7	131.7	35.2	36.0	36.5	157.4	27.9
Thalamus	35.9	87.9	39.4	37.1	34.7	108.2	25.8
Pons	30.2	79.0	35.5	39.0	32.3	NA	62.6
Mean $\pm$ SD	44.5 $\pm$ 7.9	86.8 $\pm$ 24.6	37.2 $\pm$ 3.4	36.7 $\pm$ 2.0	36.3 $\pm$ 3.2	-11.4 $\pm$ 253.2	31.9 $\pm$ 12.5

Table 4.10. Mean between-subject coefficients of variation (BSCV; %) for subjects'

V_T / BP_{ND} /mSUV parameters obtained with the different methods. ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, caudate = caudate nucleus, pallidum = globus pallidus, accumbens = Nucleus accumbens. ROI = region-of-interest, SD = standard deviation, NA: not applicable.

ICCs for all methods are listed in Table 4.11. As expected from the high test-retest variability (Table 4.9) and unrealistically high between-subject variability (Table 4.10), the 4kbv model and SRTM yielded non-reproducible results, as reflected by an ICC around zero. All the other methods had good to very good reproducibility, ranging from 0.77 ± 0.07 for classic SA calculated on ROI data to 0.84 ± 0.04 for classic SA applied to parametric maps. Note the low between-region spread of the ICC for the five methods with good or very good reproducibility, meaning that reproducibility was good throughout the brain regions sampled.

Table 4.11 also shows the ratio between a high-binding region (pallidum) and a low-binding region (pons), indicating a method's ability to reflect the known between-region

heterogeneity. The 2kbv model and SA applied to parametric maps had the highest differential.

Method	2kbv	4kbv	SA-ROIs	SA-maps	RS-SA	SRTM	mSUV
Parameter	V_T	V_T	V_T	V_T	V_T	BP_{ND}	kBq/ml
Sampling	ROI on dynamic images	ROI on dynamic images	ROI on dynamic images	ROI on parametric maps	ROI on dynamic images	ROI on dynamic images	ROI on summed images
Hippocampus	0.88	-0.05	0.87	0.87	0.81	-0.06	0.84
ACG	0.76	0.23	0.74	0.85	0.86	0.50	0.86
IFG	0.83	0.13	0.71	0.85	0.85	-0.29	0.86
Caudate	0.85	-0.13	0.74	0.81	0.82	0.06	0.79
Pallidum	0.70	-0.03	0.85	0.83	0.77	0.27	0.84
Accumbens	0.69	-0.06	0.80	0.83	0.73	-0.15	0.80
Thalamus	0.89	0.49	0.70	0.76	0.85	0.19	0.83
Pons	0.95	0.50	0.67	0.81	0.90	NA	0.47
Mean $\pm$ SD	0.82 $\pm$ 0.09	0.14 $\pm$ 0.25	0.76 $\pm$ 0.07	0.83 $\pm$ 0.03	0.82 $\pm$ 0.05	0.07 $\pm$ 0.27	0.79 $\pm$ 0.13
Ratio pallidum / pons	3.6	2.0	2.6	3.6	2.4	NA	4.2

Table 4.11. Intraclass Correlation Coefficients (ICCs) for subjects' volume of distribution (V_T) and/or binding potential (BP) obtained with the different methods.

The ratio V_T /BP of a high binding region (pallidum) over that of a low-binding region (pons) is also given. ACG = anterior cingulate gyrus, IFG = inferior frontal gyrus, caudate = caudate nucleus, pallidum = globus pallidus, accumbens = Nucleus accumbens. ROI = region-of-interest, SD = standard deviation, NA = not applicable.

4.5 Discussion

I describe the test–retest reproducibility of quantification for CB₁-receptor availability, as assessed by [¹¹C]MePPEP PET, in 15 healthy human subjects. My major finding is that good-to-excellent reproducibility of estimates of availability is achievable using either the one tissue compartment, two rate-constant kinetic model with a variable blood volume term; model-free analyses using spectral analysis variants; or simple scaled measures of radioactivity (mSUV).

The performance of the various methods was consistent between measures – those having low percentage test-retest variability also had high ICCs, reflecting that among the well-performing methods, between-subject variability was comparable.

The 2kbv compartmental model was among the best performing methods for test–retest variability and reliability, and also had one of the highest ratios of pallidum over pons. This indicates low bias (i.e. a large range of concentrations between regions of known high and low receptor availability). This ratio was lower for the spectral analysis variants applied to ROI data, reflecting their known bias towards lower V_T estimates in high binding regions (Hammers et al., 2007b). In contrast, voxel-wise SA had the same high pallidum/pons ratio as the 2kbv model. This may be due to SA's ability to fit voxel-wise time courses – voxels with varying partial volume contributions of white matter or vasculature can be individually fitted, which is not the case for methods using the averaged ROI TAC.

Previous *in vivo* human studies with [^{18}F]MK-9470 (Burns et al., 2007), [^{11}C]MePPEP (Terry et al., 2009) and [^{18}F]FMPEP- d_2 (Terry et al., 2010b) involved 120 to 300 minutes scan times (Terry et al., 2009, Terry et al., 2010b). This requirement limits the usefulness of these authors' approaches, as patients with debilitating conditions and even healthy volunteers are unlikely to tolerate PET scans of 2 hours' duration or more. Here I present data that indicates that [^{11}C]MePPEP can be used to reliably quantify CB $_1$ receptor availability with just 90 minutes of data acquisition. In a previous study, it has been shown that 90 minutes of acquisition are sufficient for obtaining stable V_T estimates (Terry et al., 2009). In addition, the injected doses used in previous studies were generally approximately twice as high as the doses used in my study, up to 750 MBq of [^{11}C]MePPEP (Terry et al., 2009). I achieved reliable receptor availability estimation using only 370 MBq, entailing an effective dose of just ~1.7 mSv per scan (Terry et al., 2010a). I had previously observed that both image quality and the reliability of blood data

measurements were lower when injected doses of a radiotracer with similarly slow kinetics were lowered to ~180 MBq (Hammers et al., 2007b). In my hands, mSUVs – using only tissue data – yielded excellent test–retest properties and differentiation between regions, with the highest pallidum/pons ratio of all methods. The fact that excellent reliability and differentiation between regions with high and low receptor concentrations (Herkenham et al., 1990) could be achieved with methods using metabolite-corrected arterial plasma input functions indicates the reliability of the blood measurements.

Because arterial cannulations require skilled personnel and involve discomfort and small risk to volunteers and patients, non-invasive PET studies are usually preferred in research studies, and even more so in a clinical environment. Methods using a reference region devoid of the studied receptor are needed for full quantification in the absence of an input function. However, CB₁ receptors are present throughout the brain, and a true reference region does not exist. Here I used the pons as a pseudo-reference region. It has low CB₁ receptor concentration (Herkenham et al., 1990, Irving et al., 2002, Yasuno et al., 2008, Terry et al., 2009), motivating this attempt despite some specific binding (Yasuno et al., 2008, Terry et al., 2009). I was unable to achieve reliable data. The application of more sophisticated pseudo-reference region approaches as described in recent studies (Turkheimer et al., 2012) might improve on these results. However, I note that the pons tissue data (i.e. mSUV) measurements were far less reliable than measurements elsewhere. Even small variations in the amount of specific binding between individuals may thus have a large influence on the radioactivity concentration in this region, with resulting low reliability for the SRTM.

This is the first study to apply model-free analyses (SA with or without RS) to quantify cannabinoid receptor availability using [¹¹C]MePPEP PET. These have the advantage of being ‘data-driven’ rather than requiring an a priori model selection. I additionally describe

the first voxel-wise quantification of [^{11}C]MePPEP, yielding parametric VT images with high corresponding regional ICCs.

A major difference relative to the previous test-retest study (Terry et al., 2009) is the lack of reliability of V_T estimates obtained with the two-tissue compartment model (4kbv) in my study, as well as good reliability for V_T estimates obtained using the one-tissue compartment model 2kbv, whereas this had yielded poor fits for Terry et al. (2009). This might relate to longer scanning time and nearly twice the injected dose in the former study. An additional major difference in the models is that I estimated the blood volume contribution, whereas this had been set to 5% in the previous study. Of note, for similar time interval my SUVs are comparable to those of Terry et al. (2009).

4.6 Conclusion

In conclusion, quantification of CB_1 receptor availability showed good-to-excellent reproducibility with selected kinetic and model-free analyses, whether applied on a region-of-interest or voxel-wise basis. [^{11}C]MePPEP PET is well-placed as a tool to investigate CB_1 receptor-mediated neurotransmission in health and neuropsychiatric disease

CHAPTER 5

Cannabinoid Receptor Type 1 Availability Following Spontaneous Temporal Lobe Seizure

Activation of the CB₁ has been associated with both anti- and pro-convulsant effects (see Chapter 1 for further details). A post-ictal increase in the availability of CB₁ receptors was recently demonstrated in humans with TLE associated with HS, *in vivo* (Goffin et al., 2011). In this Chapter, I quantified activated CB₁ receptor availability in subjects with TLE, including nMRI subjects, using [¹¹C]MePPEP PET.

5.1 Objective

The objective of the study was as follows:

1. Demonstrate increased [¹¹C]MePPEP V_T in subjects with TLE, *in vivo*, relative to healthy control subjects.

5.2 Hypotheses

The primary hypothesis was:

1. Post-ictal increases in [¹¹C]MePPEP V_T would be identified in the ipsilateral temporal lobe for subjects with refractory TLE, relative to healthy control participants.

The secondary hypothesis was:

2. The focal increases in [¹¹C]MePPEP V_T identified for subjects with refractory TLE would be negatively correlated with post-ictal interval.

5.3 Materials and methods

Ethical approval was obtained from the London – Surrey Borders Research Ethics Committee, and the previously described regulatory authorities (see Section 2.1).

5.3.1 Epilepsy and control populations

The inclusion criteria for the TLE group were as follows:

- Age between 18 and 80 years on the day of the first PET scan.
- History of refractory TLE

The inclusion criterion for the healthy controls group was as follows:

- Age between 20 and 80 years on the day of the first PET scan.

Exclusion criteria were as listed in Chapter 2.

Seventeen healthy subjects were recruited and gave written informed consent, of whom 15 were scanned twice (see Chapter 4 for details). A further 5 healthy participants that were recruited and scanned by a collaborator were added to the cohort. Hence, the total controls group consisted of 20 healthy subjects (8 females; median age 29 years, range 20 to 66 years), without history of either somatic or psychiatric conditions or substance abuse.

Ten subjects with refractory TLE were recruited and gave written informed consent. One subject was excluded from the analysis as he got out of the scanner during both scans; another withdrew after a single scan. Hence, the total controls group consisted of 8 subjects with refractory TLE (5 females; median age 42 years, range 30 to 60 years). Their diagnoses were based on history, seizure semiology, prolonged and repeated interictal and ictal (where available) video-EEG recordings, and MRI data. Interictal [^{18}F]FDG PET data was available for one of the eight participants. Discordance between clinical, EEG, and

imaging data was not an exclusion criterion. Demographic and clinical details are listed in Table 5.1.

5.3.2 Radiochemistry

Production and injection of [^{11}C]MePPEP was as described in Chapter 2. Details of the injectate are listed in Table 5.2.

5.3.3 PET data acquisition

PET image acquisition was as described in Chapters 2 and 4. [^{11}C]MePPEP was injected as an intravenous bolus injection of ~370MBq (refractory TLE group median 369 MBq, range 359 – 396MBq; controls group median 363, range 316 – 398; Table 5.2). Subjects were scanned on two separate days (refractory TLE group median 25 days, range 6 – 67 days; controls group median 23 days, range 1 – 309; Table 5.2). The participants were closely observed for evidence of seizures throughout the scan.

5.3.4 Simultaneous electroencephalographic monitoring

All participants with refractory TLE had simultaneous electroencephalography (Panayiotopoulos et al., 2004) during the PET scan using a TrackitTM 18/8 (Lifelines Limited, Hants, U.K.) ambulatory EEG recorder and an ECI E1 Cap (Electro-Cap International, Eaton, Ohio, U.S.A.) with 19 electrodes placed according to the “10-20” system of the International Federation of Societies for Electroencephalography and Clinical Neurophysiology (Jasper, 1958). An additional reference electrode (Fz) was sited just anterior to Fz. The O1 and O2 electrodes were removed from the cap for several patients who complained of discomfort whilst in the scanner. The EEG recordings were used to detect possible sub-clinical epileptiform activity during the PET scans. The participants with epilepsy (and controls) were additionally observed directly and via a monitor for evidence of seizures.

5.3.5 Input Function (IF) Derivation

The IF was derived as described in Chapter 2.

5.3.6 MRI data acquisition and analysis

The MRI data was acquired and analysed as described in Chapter 2.

5.3.7 Metabolism of [^{11}C]MePPEP

The metabolite model was fitted as described in Chapter 2.

5.3.8 PET data quantification

Attenuation and scatter-corrected dynamic PET images were de-noised and corrected for movements as described in Chapter 2.

ADD images were created as described in Chapter 2.

“Classic” SA was applied on a voxel-by-voxel basis to create parametric maps of V_T , as described in Chapter 2.

5.3.9 Global intensities (GI)

GIs were calculated as described in Chapter 2 from the transformed, smoothed V_T images.

5.3.10 Statistical analyses

Age, weight, injected dose, radiochemical purity, specific activity, co-injected mass, scan interval, and global intensities were compared between groups and scans (where applicable; post-ictal, interictal, controls 1, controls 2) using the non-parametric Wilcoxon signed-rank test (Wilcoxon, 1945). Gender balance was compared between groups using the non-parametric Mann – Whitney U test.

5.3.11 Normalisation

An in-house, approximately symmetrical template of [^{11}C]MePPEP 90-minutes ADD images was as follows:

1. Normalisation of each ADD image to Montreal Neurological Institute (MNI)/International Consortium for Brain Mapping (ICBM) 152 template in standard space using SPM8[®].
2. Average the normalised ADD images.
3. Right-left reversed averaged, normalised images and rigid-body co-register to average (non-reversed) image).
4. Average resultant and non-reversed images.
5. Repeat steps 3 and 4 twice.

Each subject's second PET scan was co-registered to their first, but not re-sliced, as described in Chapter 2, using the ADD images as source files and applying the parameters to the V_T images. The ADD images, and corresponding V_T images, were then normalised to the symmetrical [^{11}C]MePPEP template.

The normalised V_T images were smoothed by a 12mm FWHM (full-width at half-maximum) isotropic Gaussian kernel for an approximate final smoothness of 14 mm x 15 mm x 13 mm FWHM.

The extent of smoothing employed in PET pre-processing is variable and subject to debate. Traditionally, a minimum smoothness of at least twice the voxel size has been recommended, in order to meet the requirement that the data constitute a smooth Gaussian field (Worsley and Friston, 1995). Here, the 12mm isotropic Gaussian FWHM kernel, which constitutes a modest degree of smoothing in PET pre-processing, was selected as a compromise between this requirement and the desire to evidence small, but clinically-relevant foci of altered V_T .

5.3.12 Focal increases and decreases in [^{11}C]MePPEP V_T

[^{11}C]MePPEP V_T was compared on a voxel-by-voxel basis, in SPM8[®] analyses based on the transformed, smoothed V_T images. The V_T images were classified as follows

- Early post-ictal (acquired up to one hour after a seizure; one image);
- Post-ictal (acquired up to 48 hours after a seizure; four images);
- Interictal (acquired more than 48 hours after a seizure; seven images);
- Control (acquired from healthy subjects; 40 images).

None of the V_T images were flipped prior to comparison. All comparisons used an implicit mask, threshold masking with relative threshold equal to 0.8, and an explicit mask that consisted of the grey- and white-matter regions of the symmetrical template. All findings were inspected in conjunction with the normalised summation images, in order to exclude spurious results due to transformation error.

5.3.12.1 Post-ictal versus controls (group-wise)

Post-ictal V_T images (four in total, two from the first scan session, two from the second scan session) were compared against V_T images generated for the controls (selected at random; 10 from each scan session; one per subject) using an independent samples t-test, assuming equal variances, with grand mean scaling, and global activity and age taken into account via an ANCOVA. The contrasts (1 -1) and (-1 1) were used to identify focal increases and decreases, respectively, in V_T . Voxels were assessed at $p < 0.005$ (uncorrected), and clusters at $p < 0.05$ (uncorrected).

ID	Age/sex/ handed- ness	Probable lateralisation & localisation	Post- ictal interval (hours)	Treatment	Seizures	EEG (inter- /ctal)	MRI	[¹⁸ F]FDG-PET
TLE-1	60/F/R	L	36/336	LVT, ?TPM	SFS	L T/Un.	?L HS/NAD	--
TLE-2	34/F/R	L	50/144	CLB, LEV, LTG, OXC	SFS, ?CFS, ?SGS	L F-T/Un.	NAD	--
TLE-3	39/F/R	L	168/192	CBZ, CLB, LEV, PGB, citalopram, levotyroxine, naproxen	CFS	L T/Un.	L HS; Tiny WM lesion L T	--
TLE-4	44/M/L	L / ?Bil.	1/30	CBZ, CLB	SFS, CFS	L F-T, L & R T / L & R TL	NAD	NAD
TLE-5	60/M/--	L	192/216	CLB, LVT, LTG, OXC	CFS, SGS	Bi-T/L T	NAD	--
TLE-6	30/F/R	?Bil.	12/120	LEV, LTG, OXC	SFS, CFS, SGS		Bil. HS, L > R	--
TLR-7	38/F/R	L	26/120	CBZ, LAC, St. John's Wort	CFS	R C-T/	Bil. subependymal heterotopia	--
TLR-8	49/M/L	L	96/96	CBZ, CLB, LAC, LEV	CFS	L & R T/L T	Tiny WM lesion R F	--
Median	42; 38% M;		43/132					
Interquartile range (25 th -75 th)	37 - 52		23 - 114/114 - 198					
Min	30		1/30					
Max	60		192/336					

Table 5.1. Participants with refractory TLE – clinical details. (--- = unavailable; [¹⁸F]FDG-PET = [¹⁸F]fluorodeoxyglucose Positron

Emission Tomography; ant = anterior aspect; Bil. = Bilateral; C = Central region; CBZ = Carbamazepine; CFS = Complex Focal Seizures;

CLB = Clobazam; EEG = Electroencephalography; F = Female/Frontal lobe; HS = Hippocampal Sclerosis; ID = Identification; ins. = insula;

L = Left; LAC = Lacosamide; LEV = Levetiracetam; LTG = Lamotrigine; M = Male; NAD = No abnormality demonstrated i.e. no significant findings; OXC = Oxcarbazepine; P = Parietal lobe; PGB = Pregabalin; prn = pro re nata i.e. as required; R = Right; SFS = Simple Focal

Seizures; SGS = Secondary Generalised Seizures; T = Temporal lobe; TPM = Topiramate; Un = Unhelpful).

Table 5.2. Subjects' demographic and injectate details. -- = unavailable; F = Female; ID = Identification; kg = kilograms; M

= Male; Max = maximum; MBQ = Megabecquerels; Min = minimum; µg = micrograms; nmol = nanomols.

ID	Age	Gender	Weight (kg)	Interscan interval (days)	Dose (MBq)	Radiochemistry purity (%)	Co-injected mass (µg)	Specific Activity (MBq/nmol)
C-1	28	M	69	24	365 364	98 98	4.2 6.2	39 27
C-2	44	M	95	23	375 376	96 99	3.0 4.3	56 40
C-3	42	M	86	122	356 373	96 100	2.3 2.3	70 72
C-4	26	M	77	1	361 366	98 98	3.4 2.7	48 61
C-5	31	M	76	19	353 355	98 99	4.2 2.7	38 60
C-6	27	F	61	309	367 363	99 97	4.6 1.7	37 99
C-7	32	F	101	37	351 316	100 100	3.2 8.3	50 17
C-8	65	F	57	23	355 371	97 96	2.3 4.0	69 42
C-9	27	M	67	67	369 366	98 98	3.9 3.1	43 54
C-10	29	F	68	4	360 364	97 100	9.7 5.8	17 29
C-11	57	M	88	30	373 399	97 96	2.9 5.0	60 37
C-12	61	F	91	93	361 349	96 96	1.8 1.6	92 97
C-13	63	F	85	12	384 380	97 98	3.5 3.3	50 52
C-14	25	F	85	156	364 357	97 100	2.4 4.4	69 37
C-15	62	F	61	10	362 366	97 97	1.4 3.3	122 51
C-16	20	M	60	35	357 357	99 99	3.0 8.5	54 19
C-17	25	M	91	18	345 356	99 97	3.7 2.7	43 59

C-18	21	M	69	21	336 347	99 96	3.2 2.2	48 73
C-19	24	M	--	24	374 353	98	3.0	57
C-20	23	M	77	23	363 356	-- --	-- --	-- --
Median	29	(60% M)		24	363	98	3.2	51
Interquartile range (25 th -75 th)	25 – 47			19 – 45	355 – 367	97 – 99	2.7 – 4.2	39 – 61
Min	20			1	316	96	1.4	17
Max	65			309	399	100	9.7	122
TLE-1	60	F	51	12	363 366	97 98	5.0 2.6	33 63
TLE-2	34	F	73	67	372 365	95 95	2.6 1.5	64 103
TLE-3	39	F	94	47	375 373	95 95	3.1 3.7	54 45
TLE-4	44	M	92	49	378 375	97 95	3.1 7.8	56 22
TLE-5	60	M	76	35	396 365	97 97	2.5 4.7	73 35
TLE-6	30	F	44	14	364 359	96 96	5.3 1.7	31 95
TLR-7	38	F	61	7	374 366	98 96	1.6 5.5	106 31
TLR-8	49	M	92	6	388 366	96 97	2.8 3.4	62 49
Median	42	(38% M)	75	25	369	97	3.1	55
Interquartile range (25 th -75 th)	37 – 52		58 – 92	11 – 48	365 – 375	96 – 97	2.6 – 4.8	35 – 66
Min	30		44	6	359	95	1.5	22
Max	60		94	67	396	98	7.8	109

5.3.12.2 Interictal versus controls (group-wise)

Interictal V_T images (seven in total, three from the first scan session, four from the second) were compared against V_T images generated for the controls (selected at random; nine from the first scan session, 11 from the second scan session) using an independent samples t-test as described above. Where two interictal scans were acquired from the same participant, the V_T image that was derived from the scan with the longest post-ictal interval was used. For TLE-8, whose scans were approximately equal in terms of post-ictal interval, the second V_T image was used. The contrasts and thresholds used were as described above.

5.3.12.3 Paired t-test (refractory TLE group)

Five pairs of V_T images were using a paired samples t-test, assuming equal variances, with grand mean scaling, and global activity taken into account via an ANCOVA. Subjects TLE-3, TLE-5, and TLE-8 were excluded as a post-ictal V_T image was not captured. TLE-2 had a scan 50 hours after a seizure; although not strictly post-ictal, the subject was included in order to boost power. Similarly, the V_T image derived from TLE-4 40 minutes after a seizure was were classified as 'interictal', as decreases rather than increases in V_T would be expected (see e.g. discussion in). The contrasts and thresholds used were as described above.

5.3.12.4 Early post-ictal and post-ictal versus controls (single subject)

$[^{11}\text{C}]\text{MePPEP } V_T$ was compared for each early post-ictal and post-ictal V_T image against 20 control V_T images (1 versus 20). This was performed separately for scan sessions one and two. The images were compared using an independent samples t-test, assuming equal variances, with grand mean scaling, and global activity and age taken into account via an ANCOVA. The contrasts used were as described above. Voxels were assessed at $p < 0.023$ (uncorrected). This is equivalent to 2 standard

deviations from the mean and hence similar to how images derived from Subtraction Ictal SPECT Co-registered to MRI (SISCOM) would be analysed in the analogous clinical situation of determining a seizure focus. The cluster threshold was $p < 0.05$ (uncorrected).

5.3.12.5 Interictal versus controls (single subject)

[^{11}C]MePPEP V_T was compared for each interictal V_T image against 20 control V_T images (1 versus 20), as described above.

5.3.13 Correlations between V_T and post-ictal interval

Correlations between V_T and post-ictal interval were explored by log-transforming the post-ictal interval (in hours), due to their highly skewed distribution (Hammers et al., 2007a). I then used using the MarsBar toolbox (Brett M et al., 2002) to sample each of the refractory TLE group's smoothed, transformed V_T images, using a cluster derived from the post-ictal versus controls (group-wise) comparison. The V_T image derived from TLE-4 at 40 minutes after a seizure was again excluded as it was captured too early in the post-ictal period. The data were correlated using Spearman's rho correlation coefficient in SPSS.

5.4 Results

5.4.1 Adverse or serious events

[^{11}C]MePPEP PET procedure was well-tolerated by all participants. No serious events were reported.

5.4.2 Demographic data and injectate

The refractory TLE group was significantly older than the controls group ($p = 0.033$). There were no significant differences in terms of weight, injected dose, radiochemical

purity, specific activity, co-injected mass, interscan interval, or gender between the groups or across scans (all $p > 0.07$).

5.4.3 EEG

Technical and timing issues led to the acquisition of incomplete EEG data; these were not further analysed. No features suggestive of seizure were reported by the subjects or observed by myself or my co-workers.

5.4.4 Image data

Global V_T did not differ significantly between the groups (refractory TLE group median 8.7, range 6.3 – 11.2; control group median 8.2, range 4.1 – 14.1; $p = 1$) or across scan conditions ($p = 0.963$).

5.4.5 Focal increases and decreases in V_T

5.4.5.1 Post-ictal versus controls (group-wise)

Comparison of post-ictal V_T images versus those derived from controls revealed four focal increases, the largest of which was in the left (ipsilateral) temporal pole and lateral temporal neocortex (495 voxels; Table 5.3; Figure 5.1). Four focal decreases were seen, the largest of which was in the right (contralateral) parietal lobe (458 voxels; Table 5.3).

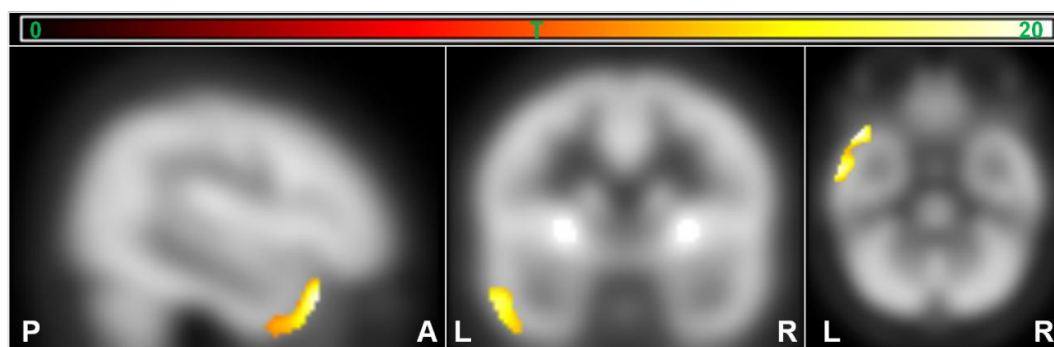


Figure 5.1. Post-ictal increases in $[^{11}\text{C}]\text{MePPEP } V_T$ (group-wise). Data is overlaid on the symmetrical $[^{11}\text{C}]\text{MePPEP}$ template. $p < 0.005$ uncorrected.

5.4.5.2 Interictal versus controls (group-wise)

Comparison of interictal V_T images versus those derived from controls did not reveal a focal increase. Two focal decreases were seen, the largest of which was in the left (ipsilateral) parietal lobe (978 voxels; Table 5.3).

Scan Condition	Increases /Decreases	Localisation	Cluster size (voxels)	Peak voxel coordinates (x, y, z; mm)	Z_{max} peak
Post-ictal	Increases	L T	495	-48 20 -28	3.95
		R F	403	20 62 02	3.82
		L F	310	-06 54 -12	3.61
		R T	279	52 06 -20	3.34
	Decreases	R P	458	16 -42 68	3.49
		L O	396	-32 -80 -12	3.67
		R O	384	32 -78 -04	3.59
		L P	338	-34 -62 50	3.79
Interictal	Decreases	L P	978	-34 -64 52	3.85
		R F	374	30 00 62	3.41

Table 5.3. Focal increases and decreases in $[^{11}\text{C}]\text{MePPEP } V_T$ (group-wise). F =

Frontal lobe; L = Left; mm = millimetres; P = Parietal lobe. R = Right; T = Temporal lobe.

5.4.5.3 Paired t-test (refractory TLE group)

Pair-wise comparison of post-ictal and interictal V_T images did not reveal any focal increases or decreases.

5.4.5.4 Early post-ictal and post-ictal versus controls (single subject)

Post-ictal increases in V_T were seen in the ipsilateral (left) temporal lobe for three of four participants with suitable scans (Table 5.4). However, the increases were not restricted to this region; in two of the three, the increases were mostly right-lateralised.

5.4.5.4 Interictal versus controls (single subject)

Interictal decreases in V_T were seen in the ipsilateral (left) temporal lobe each of 11 interictal scans. The decreases were not restricted to this region; in five of the interictal scans, the decreases were mostly right-lateralised.

5.4.7 Correlation between V_T and post-ictal interval

A negative correlation between V_T and $\log_{10}(\text{post-ictal interval})$ was observed in the left basolateral temporal neocortex ($p < 0.005$ uncorrected; Figure 5.2). A smaller cluster was observed in the left frontal white matter.

A very small focus in the right cerebellum showed a positive correlation between V_T and $\log_{10}(\text{post-ictal interval})$.

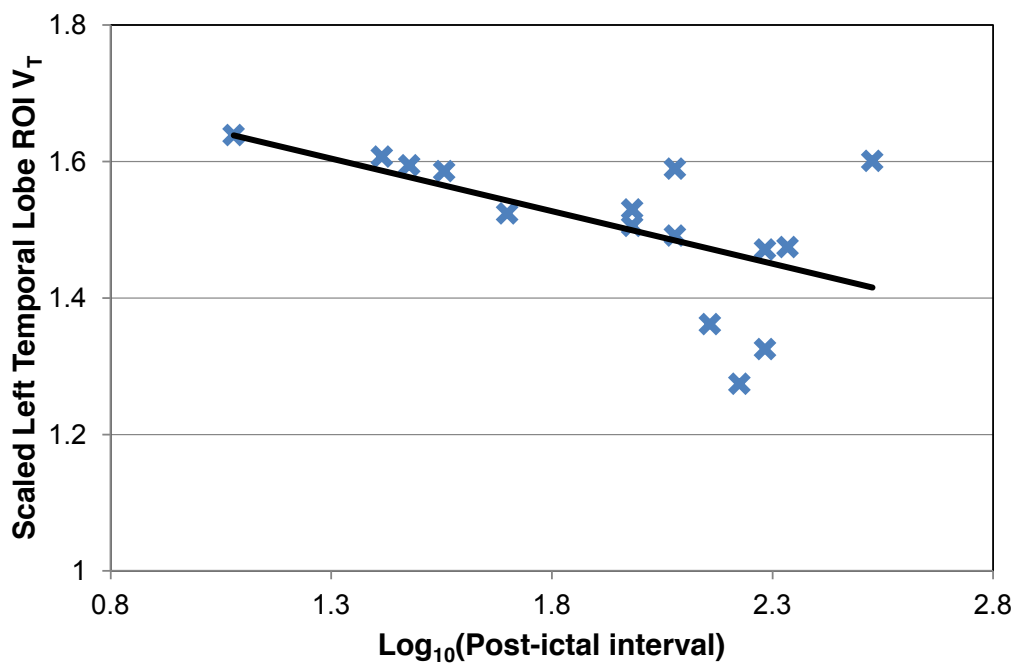


Figure 5.2. Negative correlation between scaled [¹¹C]MePPEP V_T and log₁₀(post-ictal interval) in the left (ipsilateral) basolateral temporal neocortex. (Spearman's rho = -0.584; p = 0.018).

ID	Scan condition	Increases/Decreases	Localisation	Predominant Hemisphere
TLE-1	Post-ictal (36h)	Increases	R F, L F, R T	R
		Decreases	L O, R P	L
	Interictal (336h)	Increases	L F-T, R T	L
		Decreases	R F-P, R O	R
TLE-2	Interictal 1 (50h)	Nil	N/a	N/a
	Interictal 2 (144h)	Increases	R T, L cerebellum, L P,	R
		Decreases	L P, R T	L
TLE-3	Interictal 1 (168h)	Increases	L O, L T, L F-P	L
		Decreases	R F, L T	R
	Interictal 2 (192h)	Nil	N/a	N/a
TLE-4	Early post-ictal	Nil	N/a	N/a
	Post-ictal (30h)	Increases	L cerebellum	L
		Decreases	L P	N.L.
TLE-5	Interictal 1 (192h)	Nil	N/a	N/a
	Interictal 2 (216h)	Increases	L O, R O	N.L.
TLE-6	Post-ictal (12h)	Increases	L T	L
	Interictal (120h)	Increases	L F-T	L
		Decreases	R P-O, L P, R T-O	R
TLE-7	Post-ictal (26h)	Decreases	L F	N.L.
	Interictal (120h)	Increases	R cerebellum	N.L.
		Decreases	R O, L T, R T	N.L.
TLE-8	Interictal 1 (96h)	Increases	R/midline F	N.L.
		Decreases	R P, R T-O, L F-P	R
	Interictal 2 (96h)	Nil	N/a	N/a

Table 5.4. [^{11}C]MePPEP V_T increases and decreases in all individual refractory

TLE scans. The scans are labelled as interictal or post-ictal as a function of the interval since last seizure (see 'Materials and Methods'). Regions are listed in descending order of cluster size. F = frontal lobe, L = left, P = Parietal lobe, R = Right, T = Temporal lobe, O = Occipital, NL = Non-lateralising.

5.5 Discussion

I describe the CB₁-receptor availability, as assessed by [¹¹C]MePPEP PET, in eight participants with refractory TLE. My major finding is the expected increase, in the ipsilateral epileptogenic temporal lobe, of [¹¹C]MePPEP V_T in the post-ictal scans. This increase was negatively correlated with the log₁₀ of post-ictal interval, i.e. the increased availability of CB₁ receptors returned towards baseline over time.

Such behaviour of G-protein coupled receptors has previously been observed in humans, *in vivo*, for opioid receptors (Hammers et al., 2007a). Similar behaviour has also been observed with GABA_A receptors, ligand-gated ion channels, in one study (Bouvard et al., 2005) but not others (Hammers et al., 2002). My findings replicate those of Goffin et al. (2011), who found a post-ictal increase in CB₁-selective [¹⁸F]MK-9470 in the ipsilateral temporal lobe of subjects with refractory TLE and hippocampal sclerosis, which was negatively correlated with post-ictal interval. I newly extend this finding to subjects with refractory TLE and normal MRI. The post-ictal increases in [¹¹C]MePPEP V_T are unlikely to reflect changes in cerebral blood flow as individual parent plasma input functions were used for spectral analyses, and V_T should be independent of blood flow (Holthoff et al., 1991)

The post-ictal increase is consistent with increased CB₁ staining in the hippocampus in the ‘chronic’ phase of the pilocarpine model of refractory TLE (Karlocai et al., 2011). It is not possible from my data to determine whether the post-ictal increase exerts an anti- or proconvulsant effect, in part because [¹¹C]MePPEP does not distinguish between glutamatergic (excitatory) or GABAergic (inhibitory) neurons. Preclinical data is controversial in this regard (e.g (Wallace et al., 2003, Chen et al., 2007, Echegoyen et al., 2009).

Here, I had hypothesised that due to the about tenfold higher concentration of CB₁ receptors compared to opioid receptors, post-ictal changes would be more marked and possibly visible for longer than the 24hr period investigated in the previous paper from our group (Hammers et al., 2007a). This would potentially allow the determination of the seizure onset zone on single individual post-ictal PET scans, complementing or replacing the logistically challenging acquisition of ictal SPECT scans for SISCOM (Van Paesschen, 2004). However, in my analyses so far, the individual comparisons of post-ictal scans with the control group did not allow the detection of plausible and unequivocal focal abnormalities in the ipsilateral temporal lobe (see Table 5.4). Similarly, the pair-wise comparison of post-ictal and interictal scans did not reveal the expected post-ictal increases; this may suggest that the ipsilateral temporal lobe V_T declines slowly over the first 12 days of the interictal period.

My finding suggests that [¹¹C]MePPEP V_T scans cannot be used for individual determination of the seizure onset. However, several limitations have to be considered. First, the sample size is small. Goffin et al. (2011) benefitted from a population of 50 healthy controls, whereas due to financial and time constraints my control group was restricted to 15. Post-ictal scans are challenging to acquire, particularly in the absence of an on-site video-telemetry unit, and I only succeeded in obtaining eight pairs of refractory TLE scans, despite major recruitment efforts over two years. Of these, only four truly post-ictal scans and three truly post-ictal–interictal scan paired datasets were obtained. This restricts the usefulness of some of the data. It is possible that the results derived from my small cohorts are not representative for the majority of patients with refractory TLE.

Secondly, the interictal scans were obtained within 14 days of the last seizure. Ipsilateral temporal lobe CB₁ receptor availability was normal or low-normal in Goffin et al.'s (2011) interictal mSUV data, however, only one of these five scans was acquired

within 20 days from the last seizure. It is possible that my pair-wise, SISCOM-like comparisons were uninformative because the rate of V_T decline was slower than anticipated.

Thirdly, my group was less homogenous than that of Goffin et al. (2011), who had recruited refractory TLE patients with hippocampal sclerosis only. Hippocampal sclerosis visible on MRI is a strong predictor of the seizure onset zone, but my study is the first to include the clinically more important and difficult group of patients with normal MRI (Keihaninejad et al., 2012). CB_1 receptor PET might be expected to have a lower yield in MRI-normal participants, similar to other investigations. The cohort assembled by Goffin and co-workers was extremely well-localised, whereas my cohort included subjects in whom ictal EEG, [^{18}F]FDG PET, and SISCOM was unavailable or normal. It is therefore possible that misidentification of the epileptogenic zone confounded the interpretation of the findings. A larger, well-defined cohort is required to evaluate the localising capacity of [^{11}C]MePPEP in MRI-normal subjects *in vivo* on an individual basis. The findings could be corroborated with post-surgical outcome.

Finally, my preliminary analysis can be further refined. For example, specific masks could be used for refining the statistical assessment of the ipsilateral temporal lobe to that of the epileptogenic zone (e.g.(Hammers et al., 2003b)). The negative correlation of [^{11}C]MePPEP V_T with $\log_{10}$ of post-ictal interval was found using a simple correlation; as two scans per patient were used, a regression using a mixed model would be more appropriate. These additional analyses will be performed before this work is submitted for publication.

5.6 Conclusion

In conclusion, [^{11}C]MePPEP PET showed that CB_1 receptor availability is increased in the ipsilateral temporal lobe during the post-ictal period at the group level, in subjects

with refractory TLE (including those with normal MRI). Moreover, CB₁ receptor availability in this region was negatively correlated with the length of post-ictal interval. Individual analyses were uninformative; further study in a larger, better-defined cohorts is required to assess the clinical potential of [¹¹C]MePPEP PET.

CHAPTER 6

General Discussion

In this thesis, I performed the first evaluation of the test – re-test reliability of [^{11}C]Ro15 4513 PET in healthy human control subjects. In addition, I performed the most comprehensive evaluation of the test – re-test reliability of [^{11}C]MePPEP PET data to date. I subsequently used [^{11}C]MePPEP to study the variation in CB₁ receptor availability following spontaneous temporal lobe seizures. The major emphasis of the work has been the study of neurotransmission in TLE, through the evaluation of novel PET tools.

6.1 Summary of major findings

Building on a thorough review of the relevant literature, the main objectives of the thesis, (defined in the Preface). were met. The major findings were:

1. Quantification of the concentration of GABA_A receptors containing $\alpha 5$ subunits using [^{11}C]Ro15 4513 shows good-to-excellent reproducibility with regional SRTM and voxel-wise SA.
2. Quantification of CB₁ receptor availability showed good-to-excellent reproducibility with selected kinetic and model-free analyses, whether applied on a region-of-interest or voxel-wise basis, or via semi-quantification with mSUVs.
3. At the group level, CB₁ availability in TLE was higher in the ipsilateral temporal lobe in post-ictal scans than in controls, as hypothesised. In addition, CB₁ availability was negatively correlated with time since last seizure. However, in individual patients, focal increases were inconsistently found in the

epileptogenic temporal lobe, and it is unlikely that the method will be widely applicable.

6.2 Implications

This thesis has characterised the reproducibility of quantitative measures derived using two PET radiotracers and applied one to the study of an important clinical problem, namely the localisation of the seizure onset zone, in the most common adult focal epilepsy type, TLE.

6.2.1 [^{11}C]Ro15 4513

The findings from the [^{11}C]Ro15 4513 study suggest that this radiotracer permits quantification of the concentration of GABA_A receptors containing an $\alpha 5$ subunit, *in vivo*, provided one of a relatively small number of reproducible methods of quantification is used, such as voxel-wise SA.

Importantly, this study thus allows the use of parametric maps for surveying the entire brain. This is useful for finding differences in the concentration of GABA_A receptors containing $\alpha 5$ subunits between patients and controls or between brain states in the same subject, or for the correlation of behavioural or neuropsychological parameters with such measures.

As the regional SRTM models performed well, the radiotracer is not limited by the need for arterial cannulation; however, I did not assess parametric maps constructed using the SRTM. If no arterial input functions can be acquired, this might be an avenue for further research.

One issue with [^{11}C]Ro15 4513 is that its selectivity for GABA_A receptors containing $\alpha 5$ subunits is not high enough to ensure the signal exclusively comes from such

receptors. In the case of structures with high concentration of $\alpha 5$ subunits, like the hippocampus, a standard two-tissue compartment model performs well (see chapter 3); in other structures with stronger contribution of $\alpha 1$ subunits, a rigid model structure leads to insufficient reliability. It is interesting to note that prior studies investigating model fits exclusively in hippocampus and insula (Asai et al., 2009) did not allow making this observation.

6.2.2 [^{11}C]MePPEP

The findings from my evaluation of [^{11}C]MePPEP test-retest variability highlight the capacity to derive reproducible estimates of CB_1 receptor availability using this tracer. Parametric V_T images can be used with confidence to perform brain-wide comparisons between populations. Moreover, prolonged scan times beyond 90 minutes' duration are not necessary. Importantly, for this tracer, mSUV maps were also reproducible. Therefore, imaging centres with a more clinical focus may be able to use the tracer without an arterial input function. However, differences in mSUV are more difficult to interpret than differences in V_T or BP_{ND} , and may be due to factors of no interest, e.g. differences in radiotracer metabolism between healthy controls without medication and epilepsy patients on common antiseizure drugs which induce hepatic enzymes, as for example carbamazepine.

6.2.3 General considerations regarding test-retest studies

The test-retest studies in this thesis have revealed features of ligand behaviour that had not previously been noted, e.g. the dependence of the appropriate model on the balance of $\alpha 1$ and $\alpha 5$ subunits in a given region.

On a more general note, then, it is important to perform test-retest studies of novel radiotracers, ideally ahead of widespread use in clinical research, and ideally investigating a large selection of regions with high, intermediate and low expected

concentrations of the radiotracer's target. Our group's expertise in automatic multi-region atlasng has helped achieving this goal.

In addition, it is important to choose a large enough array of models. Compartmental models, applied to ROI data, have remained the standard assessment method for new radiotracers since the 1980s. However, in both test-retest studies in this thesis, as well as in our previous work (Hammers et al., 2007b), models applied at the voxel-level performed better. This may be due to better accounting for tissue composition partial volume effects, different content in blood volume, etc. Given the much improved sensitivity and resolution of PET scanners, it may be time to routinely expect better performance of such methods applied at the voxel level. This has the added advantage of allowing whole-brain assessments in clinical research.

6.2.4 TLE and post-ictal CB₁ availability

After the first *in vivo* demonstration of an increase in availability of a G-protein coupled receptor following a spontaneous seizure (Hammers et al., 2007a) at the group but not individual level, I had hypothesised that this phenomenon might be better detectable using the much more concentrated cannabinoid system. During my thesis, another group has already succeeded in replicating the phenomenon using another PET tracer for CB₁ (Goffin et al., 2011).

The lack of clear-cut results at the individual level in my study is disappointing, but may be due to a number of factors as explained in chapter 5. Further analyses are possible and may yield more clinically useful results.

The pathophysiological result – a demonstration of a role of CB₁ receptors in seizures - remains interesting and links in well with current interest in possible therapeutic uses of the CB system for suppressing seizures (Gloss and Vickrey, 2012, Robson, 2013)

6.3 Limitations

The work presented in this thesis is limited by the small sample cohorts that were available for study, a common limitation of *in vivo* clinical epilepsy research. In the case of the [^{11}C]MePPEP studies, I intended to collate a larger dataset. Unfortunately, the PET centre, Hammersmith Imanet Limited, closed unexpectedly during the second year of this PhD research program before data acquisition could be completed. Data acquisition was also limited by the challenges of identification and recruitment of participants with low – moderate seizure frequency.

The heterogeneity in the TLE group in terms of seizure frequency, and extent of localising clinical data available, are additional limitations of the [^{11}C]MePPEP refractory TLE study. As a consequence of the challenging cohort assembled, however, remains the difficulty in assigning clinical significance to the findings.

Recruitment of well-localised refractory TLE cases without is challenging as such individuals have been investigated extensively as part of their clinical workup, are often invited to participate in multiple research studies, and where refractory, rapidly progress to resective surgery. Co-morbid illnesses such as anxiety or depressive disorders are also extremely common.

The refractory TLE group was also limited to a lesser extent by a being a mixed cohort of subjects with and without hippocampal sclerosis. Therefore, the population presumably consists of a mixture of mesio-basal and neocortical epilepsies. These do not necessarily share a common pattern of [^{11}C]MePPEP distribution and alterations in response to seizures.

CHAPTER 7

Future Research Directions

7.1 Future studies now possible with data already acquired

With the help of our research team, I intend to apply the SFS-RR (Structural and Functional Synergistic - Resolution Recovery) partial volume effect correction method (Shidahara et al., 2009, Shidahara et al., 2012) to the [^{11}C]MePPEP and [^{11}C]Ro15 4513 PET data reported in this thesis. This will improve the accuracy of quantification in smaller ROIs, thus facilitating the quantification of test – re-test variability and potentially identifying additional alterations in [^{11}C]MePPEP V_T in the subjects with refractory TLE. The evaluation could be facilitated by the incorporation of further sub-regions of interest into the Hammer^{smith} atlas (Hammers et al., 2003a) prior to sampling.

During this thesis, I also applied acquired [^{11}C]Ro15 4513 data from 12 participants with nMRI (MRI-normal) neocortical TLE. Each participant in this [^{11}C]Ro15 4513 study also completed a battery of memory tests. At the same time, I collaborated with the PET psychiatry group who acquired healthy control data using the same acquisition protocols as me, in order to pool resources. Together with the five controls scanned twice described in chapter 3, there are 17 controls available. I intend to build on preliminary analyses performed during the thesis (Barros et al., 2010) and compare the [^{11}C]Ro15 4513 TLE dataset with the pooled controls to examine any differences in GABA_A $\alpha 5$ subunit containing receptors and controls, and also the association between GABA_A $\alpha 5$ subunits and memory deficits in TLE.

As part of the [^{11}C]MePPEP study, diffusion-weighted images acquired in 64 different directions were acquired in all participants. Each participant also completed a battery of psychiatric and psychology assessments. I will use this dataset to investigate the relationships between CB₁ receptor availability, the diffusion of water in white matter, and affective, cognitive and personality traits. In those patients with focal abnormalities of [^{11}C]MePPEP, it will also be possible to use tractography to examine the connectivity of the abnormalities to other areas, and possible correlations with neuropsychiatric performance.

In addition to the subjects with refractory TLE, paired [^{11}C]MePPEP scans were acquired from a further four participants with refractory frontal lobe epilepsy, with the assistance of a collaborator. I intend to analyse these data as described in this thesis for refractory TLE group.

7.2 Potential prospective studies

My test-retest studies have already enabled the choice of appropriate methods for multiple clinical studies undertaken or underway at the MRC-CSC (Myers et al., 2012, Stokes et al., 2013a). The data has also been used for assessing the test-retest reliability of novel information obtained through spectral analysis (Stokes et al., 2013b). It is to be hoped that they will underpin more studies to come.

7.2.1 [^{11}C]Ro15 4513

[^{11}C]Ro15 4513 PET could be further used as an *in vivo* marker of GABA_A receptors containing $\alpha 5$ subunits in clinical settings. Pre-clinical studies would also be possible, as memory is a function that can be assessed eg. in rodents. Larger test – retest studies in healthy controls and with more regions of interest would allow confirmation and extension of the findings, and possibly optimisation of the scanning protocol (e.g. perhaps shorter scans would suffice). The evaluation could be facilitated by the

incorporation of further sub-regions of interest into the Hammer^{smith} atlas (Hammers et al., 2003a) prior to regional quantification.

Evaluation of the localising capacity of [¹¹C]Ro15 4513 PET in a large, pre-surgical cohort with well-localised, refractory TLE would offer the potential to corroborate V_T estimates with subunit autoradiography in resected brain tissue.

Further evidence of a specific role of GABA_A $\alpha 5$ subunit binding could be sought by thorough demonstration of a change in hippocampal [¹¹C]Ro15 4513 V_T with performance of a hippocampal-dependent, episodic memory paradigm.

Longitudinal studies could be performed in subjects with new-onset temporal lobe epilepsies, to assess the prognostic value of [¹¹C]Ro15 4513 V_T .

7.2.2 [¹¹C]MePPEP

[¹¹C]MePPEP PET could be further used as an *in vivo* marker CB1 receptors in pre-clinical and clinical settings, including the epilepsies and neuropsychiatric disease. I have demonstrated that scans of more than 90 minutes are not necessary, but further study will allow optimisation of the scan protocol. For example, is quantification at 60 minutes reproducible? If so, this will facilitate the incorporation of [¹¹C]MEPPEP PET into clinical practice.

Although my hypotheses were confirmed, the [¹¹C]MePPEP refractory TLE study would benefit from further evaluation in a larger cohort of paired post-ictal – interictal datasets. The study should target well-localised cases and should be supported by post-surgical outcome data. As there is currently a discrepancy between my largely negative results and the Leuven group's results indicating localising value in about half of participants (and all scanned within 48 hours) (Goffin et al., 2011), a comparison of

[¹¹C]MePPEP and [¹⁸F]MK-9470 would be interesting. Another study could compare ipsilateral temporal lobe CB₁ receptor availability between mTLE-HS and nMRI.

Another consideration that should be taken is that at present, PET is increasingly moving towards hybrid PET/MRI systems (Sauter et al., 2010, Zaidi and Del Guerra, 2011). More sophisticated MRI analysis (e.g. voxel-based morphometry or FLAIR image assessment (Huppertz et al., 2011), grey matter-white matter junction analysis (Antel et al., 2002), automated MAPER-based assessment of TLE cases (Keihaninejad et al., 2012) could be directly combined with the assessment of the PET data, e.g. focussing the analysis on certain regions.

CHAPTER 8

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