DOPAMINERGIC MECHANISMS UNDERLYING PSYCHOSIS

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Declaration of Originality

I declare that all the work in this thesis is my own except where otherwise stated.

Copyright Declaration

Dedication

I would like to dedicate this thesis to my family.

Acknowledgements

For all the help and support I have received, I would like to thank all my colleagues within the Psychiatric Imaging Group, all the participants in these studies, the radiographers and staff at Imanet and Imanova, my collaborators at the Institute of Psychiatry and UCL, the MRC, and my friends and family.

Above all, I would like to thank my supervisor, Dr Oliver Howes.

Abstract

Schizophrenia is a potentially devastating mental illness with a complex aetiology, in which the odds ratios for environmental risk factors for the disorder are greater than the odds ratios of any single gene hitherto identified. Within schizophrenia, striatal dopamine dysfunction has been proposed to underlie the development of psychosis. The Aberrant Salience hypothesis provides an explanatory model based on empirical findings to explain how psychotic symptoms may arise from striatal hyperdopaminergia, whereby multiple risk factors converge to elevate striatal dopamine synthesis capacity as the Final Common Pathway to psychosis.

Two important epidemiological risk factors for the disorder are chronic cannabis use and long-term psychosocial stress, both of which have evidence supporting effects on the dopamine system. Environmental risk factors are by their very nature modifiable, and so this thesis examined whether these environmental risk factors were associated with the same dopaminergic abnormalities that have been observed in schizophrenia with 3,4-dihydroxy-6-[^{18}F]-fluoro-*l*-phenylalanine Positron Emission Tomography. This thesis also examined whether cannabis users exhibit aberrant salience processing using a behavioural task, the Salience Attribution Task.

This thesis found that long-term cannabis use was associated with reduced dopamine synthesis capacity and no relationship was found between striatal dopamine synthesis capacity and cannabis-induced psychotic-like symptoms. Whilst cannabis use was not associated with increased aberrant salience processing, there was a relationship between cannabis-induced psychotic-like symptoms and aberrant salience processing. This thesis found that long-term

psychosocial stress is associated with reduced dopamine synthesis capacity, although this finding may be due confounding factors. However, a positive relationship was observed between childhood and recent adult stressors and dopamine synthesis capacity.

These findings call into question the hypothesis that cannabis increases the risk of psychosis by inducing the same changes observed in schizophrenia, although there some evidence to support the hypothesis that psychosocial stressors do increase risk via this mechanism.

Table of Contents

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Table of Figures

Table of Tables

Glossary of Abbreviations and Symbols

[^{11}C]2b-CFT	[^{11}C]2b-carbomethoxy-3-b-(4-fluorophenyl)tropane
[^{11}C]-PHNO	[11C]-(+)-4-Propyl-3, 4, 4a, 5, 6, 10b-hexahydro-2H-naphtho[1, 2-b][1, 4]oxazin-9-ol
[^{123}I]-IBZM	[^{123}I]-Iodobenzamide
[^{18}F]-DOPA	3,4-dihydroxy-6-[^{18}F]-fluoro-*l*-phenylalanine
^{1}H-MRS	Single proton magnetic resonance spectroscopy
$5HT_{2A}$	Serotonin (5-hydroxytryptamine) type 2A (receptor)
A	Living alone
A	*n*–1 vector of the amount of model solute in each exchange compartment
AB	Asian British
ACTH	Adrenocorticotrophic hormone
AES	Apathy Evaluation Scale
AESOP	Aetiology and Ethnicity of Schizophrenia and Other Psychoses (Study)
AES-S	Apathy Evaluation Scale (self-rated)
AM	Amygdala
A_m	Total amount of material in the tissue samples

AMBRA1	Autophagy/Beclin-1 Regulator 1 gene
α-MPT	Alpha-methyl-p-tyrosine
ANCOVA	Analysis of co-variance
ANK3	Ankyrin 3, Node Of Ranvier (Ankyrin G) gene
ANOVA	Analysis of variance
ARSAC	Administration of Radioactive Substances Advisory Committee
AS	Aberrant Salience
ASI	Aberrant Salience Inventory
AST	Associative functional subdivision of the striatum
AWC	Living Alone with children
AX-CPT	'AX' Continuous Performance Task
b	bound
BAI	Beck Anxiety Inventory
BB	Black British
BC	black Caribbean
BDI	Beck Depression Inventory
BIS11	Barratt Impulsiveness Scale
B_{max}	maximum number of binding sites
BOLD	Blood oxygen level dependent (signal)

BP	Binding Potential
Bq	Becquerel
BRP44	Brain protein 44, also known as mitochondrial pyruvate carrier 2 (MPC2), gene
CAARMS	Comprehensive Assessment of At-Risk Mental State
CACNA1C	Calcium Channel, Voltage-Dependent, L Type, Alpha 1C Subunit gene
CB_1	Endocannabinoid type 1 (receptor)
CBF	Cerebral blood flow
CCDC68	Coiled-Coil Domain Containing 68 gene
CECA	Childhood Experiences of Care and Abuse Questionnaire
CEQ	Cannabis Experience Questionnaire
CHP	Living with co-habiting partner
CHR	clinical high risk
CI	Confidence interval
CN	Caudate nucleus
CNNM2	Cyclin M2 gene
CNVs	Copy number variants
COMT	Catechol-*0*-methyltransferase
Cp(t)	plasma tracer concentration

CPSS	Combined psychosocial stress score
CRH	Corticotrophin-releasing hormone
CS	Conditioned or cue stimuli
CSF	Cerebrospinal fluid
CSMD1	CUB And Sushi Multiple Domains 1 gene
CT	Computed Tomography
CTQ	Childhood trauma questionnaire
d	Day
d	Cohen's d effect size
D_2	dopamine type 2 (receptor)
$D_{2/3}$	Dopamine types 2 and 3 (receptors)
D_3	Dopamine type 3 (receptor)
DA	Dopamine
DAO	D-amino acid oxidase (gene)
DAT	Dopamine transporter
del	Deletion
df	Degrees of freedom
DISC1	Disrupted in schizophrenia -1 gene
dlPFC	Dorsolateral pre-frontal cortex

DMN	Default Mode Network
DOPA	Dihydroxyphenylalanine
DOPAC	3,4-Dihydroxyphenylacetic acid
DRD1	Dopamine D_1 receptor gene
DRD2	Dopamine D_2 receptor gene
DSM-III-R	Diagnostic and Statistical Manual of Mental Disorders 3rd Edition Revised
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders 4th Edition
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders 4th Edition Text Revision
DSM-5	Diagnostic and Statistical Manual of Mental Disorders 5th Edition
DTI	Diffusion tensor imaging
DTNBP1	Dysbindin 1 gene
Dup	Duplication
DZ	Dizygotic
ECG	Electrocardiogram
EPQ	Eysenck's Personality Questionnaire
ERBB4	Erb-b2 receptor tyrosine kinase 4 (gene)
EU	European Union

EU-GEI	European Union Gene-Environment Interactions Study
eV	Electron-volt
Ex del	exonic deletion
F	Living with friends
FDG	[18F]deoxy-glucose
FGM	First generation migrant
FIGS	Family Interview for Genetic Studies
fMRI	Functional magnetic resonance imaging
g	gram
GE	General Electric corporation
GR	Glucocorticoid receptor
GRIA1	Glutamate Receptor, Ionotropic, AMPA 1 gene
GRIN2A	Glutamate Receptor, Ionotropic, N-Methyl D-Aspartate 2A gene
GRM3	Glutamate Receptor, Metabotropic 3 gene
GWAS	Genome-wide association study
H	Hydrogen
HAM-A	Hamilton Rating Scale for Anxiety
HAM-D	Hamilton Rating Scale for Depression

HCL	Hydrogen chloride
HIST1H2BJ	Histone Cluster 1, H2bj gene
HPA	hypothalamo-pituitary-adrenal (axis)
HPSS	High Psychosocial Stress Group
HVA	Homovanillic Acid
IBM	International Business Machines Corporation
IC_{50}	Half maximal inhibitory concentration
ICC	Intraclass correlation coefficient
ICC	Inner Capital City
ICD-10	International Classification of Disease 10th Edition
IES-6	Brief Impact of Events Scale
IMP	Investigational Medicinal Product
IRR	Incidence rate ratio
ITIH3	Inter-Alpha-Trypsin Inhibitor Heavy Chain 3 gene
ITIH4	Inter-Alpha-Trypsin Inhibitor Heavy Chain Family, Member 4 gene
K	(n x n) matrix of the K_{ij} rate constants
K_{bp}	Rate constant for the direct movement of material from the plasma to the trap

K_D	Receptor affinity
K_e	Efflux rate constant
K_i	Influx rate constant
K_i^{cer}	Influx rate constant relative to the cerebellum
L	Litre
L-DOPA	L-3,4-dihydroxyphenylalanine
LPSS	Low Psychosocial Stress Group
LSD	Lysergic acid diethylamide
LSM1	U6 Small Nuclear RNA Associated Sm-like protein LSm1 gene
LST	Limbic functional subdivision of the striatum
M	Mole
μA	Micro-ampere
MAO-A	Monoamine oxidase A
MAO-B	Monoamine oxidase B
Mat dup	Maternally-derived duplication
MCL	Married, in a civil partnership or living with long-term partner
MDMA	3,4-methylenedioxy-N-methylamphetamine (“Ecstasy”)
MDMQ	Multidimensional Mood State Questionnaire

ME	Mixed ethnicity
mEPSCs	Miniature excitatory postsynaptic currents
MFB	medial forebrain bundle
mg	milligram
MHRA	Medicines and Healthcare products Regulatory Agency
min	Minute
MIR137	MicroRNA 137 gene
mm	Millimetre
MMP16	Matrix Metallopeptidase 16 (Membrane-Inserted) gene
MNI	Montreal Neurologic Insitute
mol	Molar
MPFC	Medial prefrontal cortex
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MR	Mineralocorticoid receptor
MRC	Medical Research Council
MRI	Magnetic Resonance Imaging
mRNA	Messenger Ribonucleic acid
MRS	Magnetic resonance spectroscopy
ms	Millisecond
MTG	Medial temporal gyrus

MTHFR	Methylenetetrahydrofolate reductase gene
MZ	Monozygotic
N	Native
NA	Nucleus accumbens
NAA	*N*-acetylaspartic acid
NART	National Adult Reading Test
NCS	Non-cohabiting stable relationship
NEO-FFI	New Five Factor Inventory
NICE	National Institute for Health and Care Excellence (United Kingdom)
NIMH	National Institute of Mental Health (United States of America)
NKAPL	Nuclear factor kappa-light-chain-enhancer of activated B cells Activating Protein-Like gene
NMDA	N-methyl-D-aspartate (receptor)
NRES	National Research Ethics Service
NRG1	Neuregulin 1 (gene)
NRGN	Neurogranin (Protein Kinase C Substrate, RC3) gene
NRXN1	Neurexin 1 gene
NT5C2	5'-Nucleotidase, Cytosolic II gene
NY	New York

O	Oxygen
OB	Olfactory bulb
OCC	Outer Capital City
OE	Other Ethnicity
OF	Living with other family members
OLA	Other living arrangement
O-LIFE	Oxford-Liverpool Inventory of Life Experiences
ONS	Office of National Statistics
OR	Odds ratio
$_{p}$	plasma
P	Parents
p	Probability
PANSS	Positive And Negative Syndrome Scale
PC	Provincial City
PCGEM1	Prostate-Specific Transcript 1 (Non-Protein Coding) gene
PCP	phencyclidine
PET	Positron Emission Tomography
PFC	Pre-frontal cortex
PHNO	(+)-4-propyl-9-hydroxynaphthoxazine

PRSS16	Protease, Serine, 16 (Thymus) gene
PSI	Psychotomimetic States Inventory
PSS	Psycho-social Stress
PVN	Paraventricular nucleus of the hypothalamus
Q	*n* vector of rate constants from plasma to the exchangeable compartments (K_{ip})
r	Pearson's correlation coefficient
r	Reversible
R	Rural
rCBF	regional cerebral blood flow
RNA	Ribonucleic acid
ROI	Region of Interest
RR	Relative Risk
RT	Reaction Time

S	Single
SAE	Serious Adverse Event
SAT	Salience Attribution Test
SCID	Structured Clinical Interview for DSM-IV
SCZ	Schizophrenia
SD	Standard deviation
SDF	Standard deviation of the fastest half of the trials
SEAT	Social Environment Assessment Tool
SEP	Septum
SGM	Second generation migrant
SMST	Sensorimotor functional subdivision of the striatum
SN	Substantia nigra
SNPs	Single nucleotide polymorphisms
SPECT	Single photon emission computed tomography
SPM	Statistical Parametric Mapping
SPM5	Statistical Parametric Mapping version 5
SPM8	Statistical Parametric Mapping version 8
SPQ	Schizotypal personality questionnaire
SPSDS	Striatal Presynaptic Dopamine Synthesis Capacity
SRR	Serine Racemase gene

Std	Standardised
STR	Striatum
STT3A	3A Subunit Of The Oligosaccharyltransferase Complex (Catalytic) gene
Sv	Sievert
t	Time
TACs	Time activity curves
TCF4	Transcription Factor 4 gene
THC	Δ9-tetrahydrocannabinol
TPH1	Tryptophan hydroxylase 1 gene
TPPS	Transient Positive Psychotic Symptoms
TRIM26	Tripartite Motif Containing 26 gene
USA	United States of America
VAS	Visual analogue scale
VOI	Voxel of Interest
VTA	Ventral tegmental area of the midbrain

WAIS	Wechsler Adult Intelligence Scale
WB	White British
WHO	World Health Organization
ZNF804A	Zinc Finger Protein 804A gene

List of papers published in peer reviewed journals arising from this thesis

Bloomfield MAP, Morgan CJA, Egerton A, Kapur S, Curran HV, Howes OD (2014) Dopaminergic function in cannabis users and its relationship to cannabis-induced psychotic symptoms. *Biol Psychiatry.* 75(6):470-8.

Bloomfield MAP, Morgan CJA, Egerton A, Kapur S, Curran HV, Howes OD (2014) The link between dopamine function and apathy in cannabis users: an [18F]-DOPA PET imaging study. *Psychopharmacology (Berl).* 231(11):2251-9.

Bloomfield MAP, Pepper F, Egerton A, Demjaha A, Tomasi G, Mouchlianitis E, Maximen L, Veronese M, Turkheimer F, Selvaraj S, Howes OD (2014) Dopamine Function in Cigarette Smokers: An [^{18}F]-DOPA PET Study. *Neuropsychopharmacology* 39: 2397–2404.

Chapter 1: Background

1.1 Schizophrenia and Psychosis: Historical Context

The word "psychosis" is defined by the Oxford English Dictionary (2014) as "*a severe mental disorder in which thought and emotions are so impaired that contact is lost with external reality"* and first began to be used in the mid-Nineteenth century. It was around this time that the modern medical study of mental disorders began. Bénédict Morel (1809-1873) coined the term *démence precoce*, literally a "precocious dementia", to describe a cognitive disorder he had observed in young people. Later it was Aloysius Alzheimer's (1864-1915) supervisor, Emil Kraepelin (1856-1926), who adapted the term and applied it to patients who developed a significant cognitive and social impairment at a young age, calling this *dementia praecox*, in comparison to the senile dementia affecting older adults. Post-mortem neuropathological examination of the brains of patients with late onset dementia revealed the now cardinal lesions of the disorder i.e. neurofibrillary tangles and senile plaques. Kraepelin called this disorder Alzheimer's disease to differentiate it from dementia praecox. At the time he believed it would be possible to identify the neuropathology typical of dementia praecox (Kraepelin *et al.* 1919), although the technology with which to achieve this remained out of his grasp. Writing on dementia praecox, what we would now call schizophrenia, Kraeplin said:

> *"If it should be confirmed that the disease attacks by preference the frontal areas of the brain, the central convolutions and the temporal lobes, this distribution would in a certain measure agree with our present views about the site of the psychic mechanisms which are principally injured by the disease… it is easy to believe that the frontal cortex, which is specially well developed in man, stands in closer relation to his higher intellectual abilities, and these are the faculties which in our patients invariably suffer profound loss… On the other hand, the peculiar speech disorder*

resembling sensory aphasia and the auditory hallucinations, which play such a large part, probably point to the temporal lobe being involved."

Eugen Bleuler (1857–1939) later modified the concept of dementia praecox to include symptoms that were not based on external reality and were therefore, by the contemporary definition given above, psychotic. These were hallucinations (seemingly real perceptions in the absence of apparent sensory stimuli) and delusions (abnormal beliefs that are maintained despite contradiction from evidence in reality or rational argument). Bleuler called this disorder *Schizophrenia* (skhiz- 'to split' + phrēn- 'mind') to reflect the fragmenting of mental functions which were then seen as underlying the internal psychological mechanisms of the disorder (Bleuler 1911).

Around the same time, the British Neurologist Sir John Reynolds (1828-1896) who wrote extensively on the clinical diagnosis of brain disorders, coined the terms "positive symptoms" and "negative symptoms" to describe symptoms that were *added* by a disorder or lesion and those that were *taken away* by a disorder or lesion, respectively (Berrios 1985). Reynold's contemporary John Hughlings-Jackson (1835 – 1911), at the time based at the National Hospital for Paralysis and Epilepsy in London's Queen Square (now the National Hospital for Neurology and Neurosurgery), developed an evolutionary hierarchical organization of the nervous system which he used to explain why some patients have particular constellations of positive and/or negative neurological symptoms (Berrios 1991). Hughlings-Jackson's theories were later highly influential for leading continental psychiatrists of the age (Berrios 1977) resulting in their application to schizophrenia. Examples of positive and negative symptoms of schizophrenia as used in contemporary Psychiatry are given in Box .11.

This thesis is primarily concerned with the neurobiology underlying positive symptoms and will use the term "psychotic symptoms" to mean positive symptoms throughout, unless otherwise stated.

Box 1.1 Examples of positive and negative symptoms of schizophrenia from the Positive and Negative Syndrome Scale (PANSS) for schizophrenia (Kay *et al.* 1997)	
Positive symptoms:	Delusions Conceptual disorganization Hallucinations Excitement Grandiosity Suspiciousness (Paranoia)
Negative symptoms:	Blunted affect Emotional withdrawal Poor Rapport Passive-apathetic social withdrawal Difficulty in abstract thinking Lack of spontaneity and flow of conversation Stereotyped thinking

1.2 Introduction: Schizophrenia, Psychosis & Dopamine

Schizophrenia, a leading global cause of disability (WHO, 2008), is a clinical syndrome characterised by episodic psychosis composed of hallucinations, delusions and disordered thinking. It is associated with increased risk of early death from suicide (Palmer *et al.* 2005). The commonest feature of acute schizophrenic psychosis is loss of insight. The constellation of signs and symptoms that can give rise to a diagnosis of schizophrenia were standardised over the course of the Twentieth century into the World Health Organization's International Classification of Disease 10th Edition (ICD-10) (1992) and the American Psychiatric Association's Diagnostic and Statistical Manual 4th Edition Text Revision (DSM-IV-TR) (2000). Whilst the ICD-10 is widely used internationally and is the standard used in clinical practice in the United Kingdom, for historical reasons the DSM-IV-TR is more commonly used in the research literature. The American Psychiatric Association has since published a 5th Edition of their Diagnostic and Statistical Manual (DSM-5) (2013). However, the DSM-5 has received substantial criticism over doubts about the scientific validity and reliability of its diagnostic constructs and the *"medicalisation of... natural and normal responses to... experiences"* (British Psychological Society, 2011). Therefore, DSM-IV-TR diagnoses will be used throughout this thesis unless otherwise stated. The DSM-IV-TR diagnostic criteria for schizophrenia are reported in Box 1.2.

Box 1.2: DSM-IV(TR) Diagnostic Criteria for Schizophrenia (American Psychiatric Association 2000)

A: *Characteristic Symptoms:* Two or more of the following, each present for a significant portion of time during a one month period (or less if successfully treated):

(1) Delusions
(2) Hallucinations
(3) Disorganised speech (e.g. frequent derailment or incoherence)
(4) Grossly disorganised or catatonic behaviour
(5) Negative symptoms i.e. affective flattening, alogia (poverty of speech) or avolition

Note: Only one Criterion A symptom is required if delusions are bizarre or hallucinations consist of a voice keeping up a running commentary on the person's behaviour or thoughts, or two or more voices conversing with each other.

B: *Social/occupational dysfunction:* For a significant portion of the time since the onset of the disturbance, one or more major areas of functioning such as work, interpersonal relations, or self-care are markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, failure to achieve expected level of interpersonal, academic, or occupational achievement).

C: *Duration*: Continuous signs of the disturbance persist for at least 6 months. This 6 month period must include at least 1 month of symptoms (or less if successfully treated) that meet criterion A (i.e. active-phase symptoms) and may include periods of prodromal or residual symptoms/ During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or two or more symptoms listed in Criterion A present in an attenuated form (e.g. odd beliefs, unusual perceptual experiences).

D: *Schizoaffective and Mood Disorder Exclusion:* Schizoaffective Disorder and Mood Disorder with Psychotic Features have been ruled out because either (1) no Major Depressive, Manic, or Mixed Episodes have occurred concurrently with the active-phase symptoms; or (2) if mood episodes have occurred during active-phase symptoms, their total duration has been brief relative to the duration of the active and residual periods.

E: *Substance/general medical condition exclusion:* The disturbance is not due to the direct physiological effects of a substance (e.g. drug of abuse, a medication) or a general medical condition.

F: *Relationship to a Pervasive Developmental Disorder*: If there is a history of Autistic Disorder or another Pervasive Developmental Disorder, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations are also present for at least a month (or less if successfully treated).

1.3 Aetiology

Schizophrenia has a complex aetiology in which genetic-environment interactions are likely to be central. In terms of epidemiology, the twentieth century saw an initially divergent approach to schizophrenia with American studies focussing on socio-ecological risk factors whilst European studies focussed on genetic risks and population distributions (Jablensky 2012). Arguably the first epidemiological study was a Swiss case-control genealogical inquiry from records of psychiatric units by Koller (1895) who reported that *'the hereditary loading of healthy subjects is much higher than generally assumed'*, that *'the strongest loading is that of psychosis'*, and that *'the loading in distant relatives is quite low, unless a person at risk is exposed to multiple factors'*. It was this ground-breaking study that first highlighted the interactions between multiple risk factors, namely hereditary (i.e. genetic) and then unknown environmental factors. Much epidemiological work has been conducted since and the results will be summarised below. Based on large systematic reviews, the point prevalence of schizophrenia has been estimated at 4.6 per 1,000 people and the lifetime prevalence is 7.2 per 1,000 (Saha *et al.* 2005), whilst the annual incidence has been estimated to be 0.24 per 1000 (McGrath *et al.* 2003). Schizophrenia can occur throughout the lifespan, although the majority of onsets occur in the 15 to 54 year age range, with peak onset between the ages of 20 and 24 years (Jablensky 2012), followed by a second peak in later life which is most pronounced in women (Hafner *et al.* 1998). There is also evidence of a male preponderance for the illness (Kirkbride *et al.* 2006), see Figure 1.1.

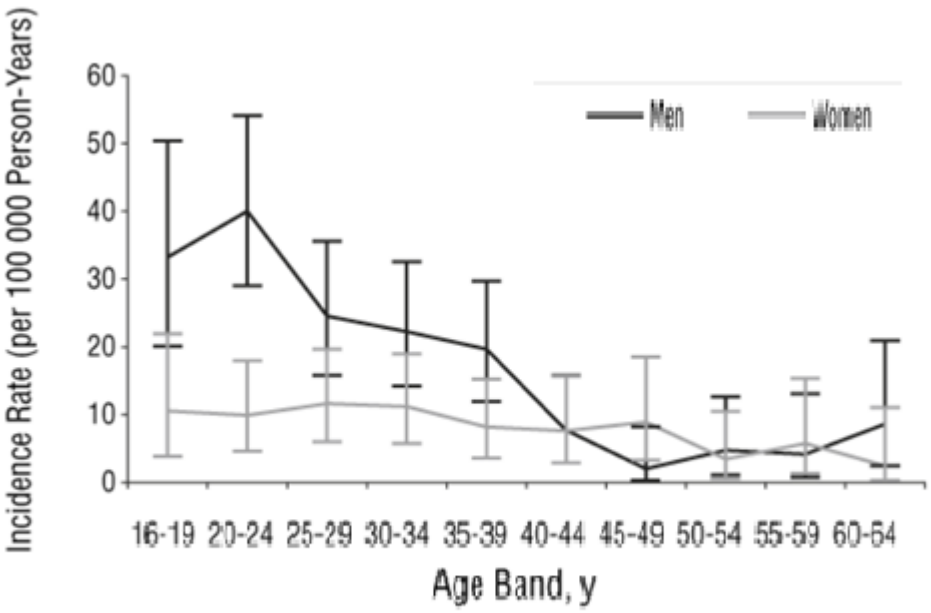

Figure 1.1 Age specific incidence rates of schizophrenia by sex from the 3-Center AESOP study (Kirkbride *et al.* 2006)

1.4 Risk Factors for Schizophrenia

1.4.1.1 Genetic Risk Factors

At the individual level, having a relative with schizophrenia is the most important risk factor for the illness, with lifetime risk of schizophrenia increasing with increasing genetic similarity, such that risk is most elevated amongst monozygotic (MZ) twins and in those with two parents with the disorder (Gottesman 1991), see figure 1.2. The heritability of a disorder can be defined as the proportion of phenotypic variance attributable to genetic variance i.e. the extent to which genetic individual differences contribute to phenotypic individual differences.

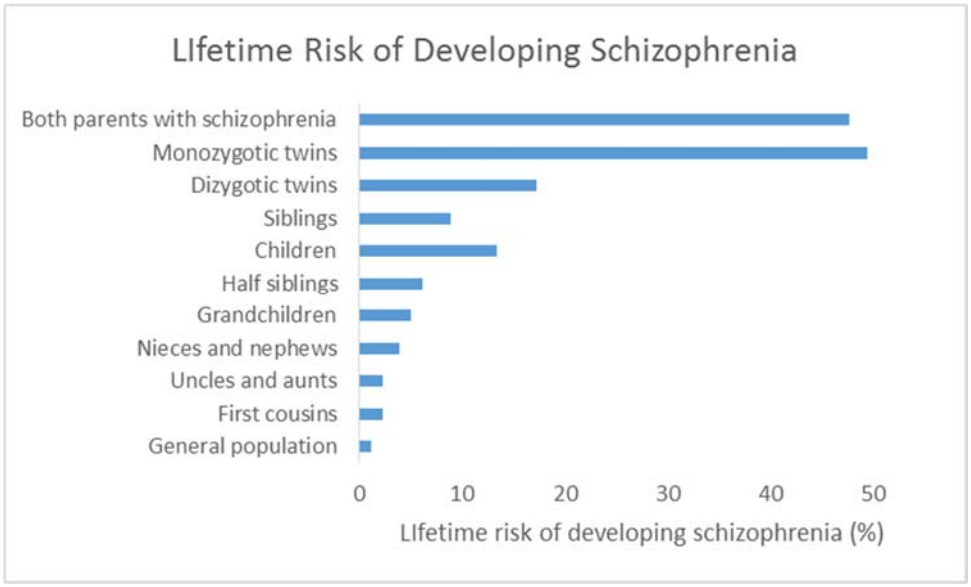

Figure 1.2 Lifetime risk of developing schizophrenia in relatives of individuals (Gottesman 1991)

High familial aggregation has been widely replicated in family studies (e.g. Kendler *et al.* 1993). Twin studies (Cardno & Gottesman 2000) have estimated high heritability of the disorder, approximately 80 to 85%, with probandwise concordance rates (i.e. the proportion of twins who have the illness given an affected twin) in MZ twin pairs of 41 to 65 % and up to 28 % in dizygotic (DZ) pairs. Other studies have estimated the heritability for the disorder at been between 65% and 80% (Lichtenstien *et al.* 2009; Sullivan *et al.* 2003). The heritability of schizophrenia has been confirmed by adoption studies which are able to separate environmental from genetic effects. Kety *et al.* (1994) reported that 23.5 % of adoptees who had a biological first-degree relative with schizophrenia later developed the illness, compared to 4.7 % of control adoptees with no biological parental history of schizophrenia. Furthermore, a reverse adoption study (Wender *et al.* 1974) found that the grown-up children of individuals without schizophrenia who had been adopted by a parent who later developed schizophrenia were not at increased risk of the disorder. Taken together, these findings confirm a high heritability for the disorder, suggesting that genetics contribute to altered biology giving rise to the disorder.

Obstacles remain in the genetic investigation of schizophrenia due to non-Mendelian inheritance and a likely complex genetic architecture. The most parsimonious genetic account for schizophrenia has been proposed to be a polygenic model in which a number of genes of small effect contribute to elevated risk (Iyegbe *et al.* 2014). Evidence in favour of this comes from family studies in which the risk to an individual increases with the number of affected relatives (Gottesman 1991); the concordance rate being higher in MZ twins who have an earlier rather than a later onset of the illness (Cardno *et al.* 1999); and relatives of those with an earlier onset of the illness have a higher risk of schizophrenia than those with a later illness onset. Taken together these findings also lended support to the idea that susceptibility genes may be involved in the control of neurodevelopment (Jones & Murray 1991).

1.4.1.2 Pre Genome-Wide Association Study (GWAS) era

Early genetic work using karyotyping, linkage studies (assessing co-segregation of genetic loci with disease within families given that genes which are close together on the genome tend to be inherited together) and candidate gene association studies (the identification of commonly occurring variants of small effect) in velocardiofacial syndrome led to the findings that large deletions on chromosome 22q11.2 are associated with increased risk of schizophrenia (Bassett *et al.* 1998) and higher rates of the 22q11 deletion are found in schizophrenia compared to the general population (Karayiorgou *et al.* 1995). However, there does not appear to be any significant clinical variation in schizophrenia with or without 22q11.2 deletion (Bassett *et al.* 2003) affecting the COMT and TBX1 genes

Until recently, although dozens of linkage analyses have been performed in schizophrenia, with some reporting genome-wide significant findings (Paunio *et al.* 2001; Maziade *et al.* 2005; Holliday *et al.* 2009; Holmans *et al.* 2009), no locus had been consistently replicated across studies, and meta-analyses had failed to identify any locus that surpasses genome-wide significance (Ng *et al.* 2009). This could be explained by multiple genes of small effect. More than one thousand candidate gene association studies have been performed to date. However, the results from earlier studies had been inconsistent and they were limited by low power. Meta-analyses of these studies had identified four out of sixteen candidate genes that had *"strong"* epidemiological credibility (Allen *et al.* 2008): DRD1 (dopamine D1 receptor) on chromosome 5q35.1, DTNBP1 (Dysbindin 1; dystrobrevin binding protein 1) on chromosome 6p22.3, MTHFR (methylenetetrahydrofolate reductase) on chromosome 1p 36.3 and TPH1 (tryptophan hydroxylase 1) on chromosome 11p15.

1.4.1.3 Current thinking of the genetic architecture of schizophrenia

Since these earlier studies, more definitive evidence that genetic risk for schizophrenia arises from variation in DNA sequences has come from genome-wide association studies (GWAS) studies of single nucleotide polymorphisms (SNPs) and copy number variants (CNVs). A significant strength of GWAS is that it provides a hypothesis-free method. GWAS SNPs in the genome and data from GWAS arrays can also be used to identify rare sub-microscopic chromosomal deletions and duplications i.e. CNVs. These studies were recently reviewed by Harrison (2015) and their findings are summarised below. For illustrative purposes, selected loci and genes that confer risk of schizophrenia identified through studying SNPs with brief notes on what is known of their neurobiology are given in Table 1.1.

Table 1.1 Selected loci and genes showing association to schizophrenia: nomenclature and notes (adapted from Harrison 2015)

Locus	Implicated gene	Name of gene or product	Notes
12p13.33	*CACNA1C*	L-type calcium a subunit, type 1c	Important in neuronal function. Mutations cause Timothy syndrome and Brugada's syndrome (Bhat *et al.* 2012)
12q24.11	*DAO*	D-amino acid oxidase	Enzyme which degrades the NMDA receptor co-agonist D-serine. Expression and activity increased in schizophrenia. (Verrall *et al.* 2010)
1q42.2	*DISC1*	Disrupted in schizophrenia 1	Identified in a large Scottish pedigree with a chromosome 1:11 translocation. A multifunctional scaffolding protein. (Brandon & Sawa 2011)
11q23.2	*DRD2*	Dopamine D_2 receptor	Long known to be the key target of antipsychotic drugs, GWAS data now indicate that the DRD2 gene may play a role in schizophrenia (Beaulieu & Gainetdinov 2011)
2q33-34	*ERBB4*	Receptor tyrosine inase erbB4	Receptor for neuregulin 1 and some other ligands. Mutations can cause cancers (Mei & Xiong 2008)
5q33.2	*GRIA1*	AMPA receptor subunit 1 (GluA1; GluR1)	The subunit influences properties of the AMPA receptor, and affects synaptic plasticity and behaviour (Barkus et al. 2014)
16p13.2	*GRIN2A*	NMDA receptor subunit 2A (GluN2A; NR2A)	The subunit influences properties of the NMDA receptor, including synaptic localisation and channel conductance (Paoletti et al. 2013)
7q21.11-12	*GRM3*	Metabotropic glutamate receptor 3 (mGlu3)	Group II metabotropic glutamate receptor (along with mGlu2), acting primarily as inhibitory autoreceptors (Harrison et al. 2008)
1p21.3	*MIR137*	MicroRNA 137	Non-protein-coding gene. A micro RNA, which regulates other genes by binding to the 3'untranslated region of their transcripts (Pasquinelli 2012)
8p12	*NRG1*	Neuroregulin 1	Growth factor, involved in many aspects of nervous system development and plasticity (Mei & Nave 2014)
17p13.3	*SRR*	Serine racemase	Enzyme which synthesises D-serine from L-serine (Balu et al. 2013)
18q21.2	*TCF4*	Transcription factor 4	Basic helix-loop-helix transcription factor. Haploinsufficiency causes Pitt-Hopkins syndrome (Forrest et al. 2014)
2q32.1	*ZNF804A*	Zinc finger protein 804A	Putative transcription factor (Hess & Glatt 2013)

1.4.1.4 Single Nucleotide Polymorphisms

As genome-wide association studies (GWAS) have grown in sample size, there is increasing evidence that common SNPs confer increased risk of schizophrenia (Table 1.2). A recent large meta-analysis by the international Psychiatric Genetics Consortium (2014), identified over 100 genetic loci that contain SNPs significant for association to schizophrenia at the genome-wide level. Each locus contains one or more genes and one or more variant within the gene or genes that contribute to the risk of schizophrenia. That study, in almost 37,000 cases and 113,000 controls implicates approximately 600 genes.

Table 1.2 Multi-stage GWAS reporting genome-wide significant ($<5 \times 10^{-8}$) findings in schizophrenia, or schizophrenia combined with bipolar disorder (adapted from Mowry & Gratten 2013)

Lead author	***Locus***	***SNP***	***Odds ratio***	**P-*value***	***Closest gene (abbreviation)***
Schizophrenia					
Purcell *et al.* 2009	6p22.1	rs13194053	.82	9.54×10^{-9}	*HIST1H2BJ*
Shi *et al.* 2009	6p22.1	rs13194053	.88	9.54×10^{-9}	*HIST1H2BJ*
Stefansson *et al*. 2009	6p21.3-22.1	rs6932590	1.16	1.4×10^{-12}	*PRSS16*
	11q24.2	rs12807809	1.15	2.4×10^{-9}	*NRGN*
	18q21.2	rs9960767	1.23	4.1×10^{-9}	*TCF4*
Rietschel *et al.* 2012	11p11.2	rs11819869	1.25	3.89×10^{-9}	*AMBRA1*
Ripke *et al.* 2011	1p21.3	rs1625579	1.12	1.59×10^{-11}	*MIR137*
	2q32.3	rs17662626	1.20	4.65×10^{-8}	*PCGEM1*
	6p21.3-22.1	rs2021722	1.15	2.18×10^{-12}	*TRIM26*
	8p23.2	rs10503253	1.11	4.14×10^{-8}	*CSMD1*
	8q21.3	rs7004633	1.16	1.45×10^{-8}	*MMP16*
	10q24.32	rs7914558	1.10	1.82×10^{-9}	*CNNM2*
	10q24.33	rs11191580	1.15	1.11×10^{-8}	*NT5C2*
	11q24.2	rs548181	1.20	2.91×10^{-8}	*STT3A*
	18q21.2	rs12966547	1.09	2.60×10^{-10}	*CCDC68*
	18q21.2	rs17512836	1.40	2.35×10^{-8}	*TCF4*
Shi *et al.* 2011	1q24.2	rs10489202	1.19	9.50×10^{-9}	*BRP44*
	8p12	rs16887244	.83	1.27×10^{-10}	*LSM1*
	8p12	rs1488935	.87	5.06×10^{-9}	*WHSC1L1*
Steinberg *et al.* 2011	2p15.1	rs2312147	1.09	1.9×10^{-9}	*VRK2*
	18q21.2	rs4309482	1.09	7.8×10^{-9}	*TCF4*
Yue *et al.* 2011	6p21-22.1	rs1635	.78	6.91×10^{-12}	*NKAPL*
	11p11.2	rs11038167	1.29	1.09×10^{-11}	*TSPAN18*
Schizophrenia and bipolar disorder					
O'Donovan *et al.* 2008	2q32.1	rs1344706	1.12	9.96×10^{-9}	*ZNF804A*
Ripke *et al.* 2011	3p21.1	rs2239547	1.12	7.83×10^{-9}	*ITIH3/4*
	10q21.2	rs10994359	1.22	2.5×10^{-8}	*ANK3*
	12p13.3	rs4765905	1.11	7.0×10^{-9}	*CACNA1C*

To date, many of the schizophrenia-associated SNPs are in non-coding intronic DNA regions or are synonymous exonic polymorphisms. As there is often no evidence of functional difference between the risks and non-risk alleles, identifying the biological basis for the genetic

association has been problematic. A further difficulty has that the identified SNPs are unlikely to be themselves the causative SNP but instead are tagging a length of DNA where the causative variants lie (Kircher *et al.* 2014). One possible explanation for these findings is that the risk SNP may alter gene expression.

Schizophrenia risk SNPs typically have odds ratios in the order of 1.1 meaning that each has a very small effect on disease risk. Furthermore, there are a number of SNPs that associate with broader phenotypes including bipolar affective disorder, depressive disorder and autism (Cross Disorder Group of the Psychiatric Genomics Consortium, 2013; Table 1.2). This emergent genetic pleiotropy suggests that clinical commonality amongst these disorders may, to a degree, result from shared genetic predisposition.

There was some initial controversy following the GWAS studies as those studies were not able to replicate findings from earlier candidate gene approaches because the first GWAS studies initially suggested these genes were not associated with commonly occurring disease-related genetic variants. The debate centred around whether genome-wide statistical significance was essential in deciding whether or not a particular gene should be considered to be a risk factor or if other biological evidence should also be taken into account (Abbott 2008), as was the case with scientific arguments around DISC1 (e.g. Porteous 2014). However, since these earlier studies, much progress has been made by combining samples into single systematic analyses.

However, the most recent meta-analysis (Psychiatric Genetics Consortium, 2014) implicates several candidate genes with genome-wide association for several candidate genes with high biomechanistic plausibility including the D_2 receptor gene and several glutamate receptor genes including GRIN2A, GRIA1 and GRM3.

1.4.1.5 Copy Number Variants (CNVs)

In addition to SNPs, genetic risk for schizophrenia is also mediated via CNVs. These are lengths of DNA which are duplicated or deleted, yet were too small to be observed using older methods such as karyotyping. CNVs are a normal feature of the genome, but microarray technology has revealed that certain CNVs in particular genomic regions are associated with increased risk of schizophrenia.

There is evidence for a small increase in the rate of CNVs in people with schizophrenia compared to controls (Walsh *et al.* 2008; Stone *et al.* 2008; Levinson *et al.* 2011) and *de novo* CNVs are associated with a higher risk of schizophrenia (Malhotra *et al.* 2011; Xu *et al.* 2008; Kirov *et al.* 2012). A number of CNVs have been identified that confer an increased risk of schizophrenia and these are reported in Table 1.3. These occur at low frequency in the general population and whilst they have higher odds ratios than the SNPs that have been identified to date (CNVs with odds ratios over 8) none have yet been identified that are necessary and sufficient to cause disease.

Table 1.3 Replicated CNVs in schizophrenia (adapted from Mowry & Gratten 2013)

Locus	*CNV*	*Odds ratio*	P-*value*	*Genes*	*Other Disorders*
1q21.1	Del	8.3	2.2×10^{-8}	11	Autistic Spectrum Disorder, Mental Retardation
2p16.3	Ex del	8.2	5.5×10^{-9}	1 (NRXN1)	Autistic Spectrum Disorder, Mental Retardation
3q29	Del	17.0	9.7×10^{-3}	19	Autistic Spectrum Disorder, Mental Retardation
3q29	Dup	∞	1.0×10^{-2}	2	
7q36.3	Ex dup	4.0	2.0×10^{-3}	1 (VIPR2)	
15q11.2	Del	2.7	6.0×10^{-4}	4	Autistic Spectrum Disorder, Prada-Willi & Angelman syndromes
15q13.3	Del	9.9	2.0×10^{-9}	8	Autistic Spectrum Disorder, Mental Retardation, Epilepsy
15q11.2-13.1	Mat dup	7.3	1.0×10^{-2}	13–24	
16p11.2	Dup	11.6	1.5×10^{-12}	26	Autistic Spectrum Disorder, Mental Retardation, Developmental Delay
16p13.1	Dup	3.3	7.1×10^{-3}	11	Autistic Spectrum Disorder, Mental Retardation
17p12	Del	9.9	5.0×10^{-5}	15	Hereditary neuropathy with liability to pressure palsies
22q11.2	Del	∞	$<1.0 \times 10^{-16}$	29–43	Velo-cardio-facial syndrome

Abbreviations: Del, deletion; Dup, duplication; Ex del, exonic deletion; Mat dup, maternally-derived duplication

CNV regions hitherto identified include those with multiple and single genes. Each CNV is penetrant (Kirov *et al.* 2014), although with the exception of deletion at 22q11 giving rise to velocardiofacial syndrome (Schneider *et al.* 2014) each CNV is very rare such that it is estimated that CNVs may have a causal significance in approximately 5 per cent of cases (Costain *et al.* 2013), although the odds ratios associated with CNVs for these cases are likely to be much larger than any other identifiable factors.

In addition to SNPs and CNVs, recent research has also given rise to an association between schizophrenia and insertion or deletions affecting a few nucleotides and also single nucleotide coding variants (Purcell *et al.* 2014). It was estimated that these mutations may account for a proportion of risk to similar to CNVs, although CNVs and these newly identified mutations appear to contribute approximately 10 per cent of the heritability as common SNPs. It is interesting to note this early evidence suggests some shared genetic aetiology with a number of neurodevelopmental disorders including autistic spectrum disorder (Malhotra & Sebat 2012). Lately, it has been suggested that these mutations are associated with cognitive deficits and are likely to represent *de novo* changes in the genome (Fromer *et al.* 2014).

1.4.1.6 Linking genes to neurobiology

There is growing evidence that from genes associated with increased risk of schizophrenia converge upon a number of neurobiological pathways that may shed light on the underlying pathophysiology of the disorder. Of particular relevance to this thesis, examples of these relevant to the current major aetiological hypotheses of schizophrenia include the dopamine D2 receptor (Schizophrenia Working Group 2014), NMDA receptor signalling (e.g. Purcell *et al.* 2014), immune function (Corvin & Morris 2014), calcium signalling (Ripke *et al.* 2013), the NRG1-ERBB4-PI3K-AKT1 pathway (Emamian *et al.* 2004; Harrison & Law, 2006; Hatzimanolis *et al.* 2013; Law *et al.* 2012; Nicodemus *et al.* 2010; Norton *et al.* 2006) which is likely involved in plasticity. The AKT1 pathway is of particular interest given its resultant inactivation of glycogen synthase kinase 3 (GSK-3), a downstream mediator of the effects of D_2 receptor activation. Interestingly, using a combined GWAS across three psychiatric disorders, the Network and Pathway Analysis Subgroup for the Psychiatric Genomics Consortium (2015) found using a pathway approach that there were significant genetic associations across epigenetic, immune and neuronal signalling pathways.

Whilst the findings summarised above advance the field they account for only a minority of the heritability of schizophrenia (Harrison 2015). For example, the common SNPs identified in schizophrenia exceeding genome-wide significance explain little of the variance in susceptibility to the illness, either individually (<0.1%) or collectively (~2-3%) (Visscher *et al.* 2011). Yet, attempts to overcome this using polygenic risk scores, which are capable of explaining more of the variance, appear to be promising (Iyegbe *et al.* 2014). One interpretation of the current lack of a robust understanding of the genetics of schizophrenia is that the heritability lies in epigenetic factors: *"the failure of the search for psychosis genes by linkage and association therefore reveals the trans-generational reality of the epigenetic phenotype"* (Crow 2008). Indeed it is plausible that epigenetic factors including histone modification and DNA methylation contribute to gene-environment interactions (Daxinger & Whitelaw 2012; Dempster *et al.* 2013). Likewise, it may well be the case that instead of a cumulative effect of independent genes, genetic risk for schizophrenia is moderated by epistasis i.e. gene-gene interactions (Phillips 2008; Mackay 2014). Another possibility, as mentioned briefly earlier, is that disease prevalence is maintained by *de novo* mutation across many genes in spite of negative selection. There is some evidence in favour of the latter possibility, as increased exonic *de novo* mutations have been found in people with schizophrenia compared to controls (Girard *et al.* 2011). Further whole exome sequencing studies, together with GWAS and epigenetic studies involving very large sample sizes, will therefore be needed in the future to dissect the possible molecular pathways that can lead to this disorder and in particular, how these interact with the environmental risk factors described below to result in the phenotypic expression of schizophrenia.

1.4.1.7 Gene–Environment Interactions

The first studies of gene x environment interactions in schizophrenia have begun to yield interesting results. These shall be outlined before environmental risk factors are considered in more detail below.

Whilst initially a COMT polymorphism was proposed to contribute to the risk of schizophrenia in cannabis users (Zammitt et al. 2011), this now seems less pronounced than initially thought. Yet, there is much current interest in AKT1 polymorphisms in cannabis users (e.g. Di Forti *et al.* 2012). Amongst cannabis users AKT1 c/c carries appear to have a seven-fold increased risk of schizophrenia compared to t/t carriers. Here, the increased risk may be occurring via downstream effects in the dopamine signalling cascade. A Val66Met polymorphism in the BDNF gene has been associated with increased risk in positive psychotic symptoms in those who have experienced child abuse (Alemany *et al.* 2011). This is interesting given that the BDNF polymorphism is proposed to impair plasticity, and so may impair how the brain processes psychological trauma. The COMT gene has also been investigated in the context of stress reactivity in psychosis (e.g. Peerboms *et al.* 2012) where the Val158Met polymorphism may interact with MTHFR genotype, providing a tentative gene-gene-environment interaction. Given that there are likely many genes of small of small effect, future research on polygenic risk scores may well benefit from specificity to the environments in which these genes are expressed.

1.4.2.1 Environmental Risk Factors

> *"Developments in molecular genetics need to go hand in hand with progress in the study of environmental factors that interact with genes. It is easier (and ethically preferable) to change the environment than it is to change one's genes… Even if this were possible, it would not be ethically justifiable, especially in Psychiatry"*
>
> *Khong 1997*

There is consistent epidemiological evidence for significant associations between a number of environmental factors and increased risk of schizophrenia. Whilst a history of schizophrenia in a first degree relative is associated with the highest relative risk of having the illness at the individual level, environmental risk factors account for many more cases on a population basis (Mortensen *et al.* 1999). Furthermore, environmental factors appear necessary for the manifestation of frank illness in the majority of cases (Van Os & Marcelis, 1998). There is now renewed interest in further understanding the biological mechanisms underlying environmental risks for schizophrenia, which are outlined below.

1.4.2.2 Obstetric Factors

Individuals with schizophrenia are more likely to have a history of prenatal or perinatal obstetric complications than healthy subjects from the general population and their own healthy siblings (Geddes *et al.* 1999). Evidence in favour of this association comes from studies that have collected obstetric data from the time of birth (Hultman *et al.* 1999) and meta-analyses of epidemiological studies (Cannon *et al.* 2002). The obstetric events that are most associated with an increased risk of schizophrenia include low birth weight, prematurity, resuscitation at

birth, retarded foetal growth and rhesus incompatibility (Geddes *et al.* 1999). One feature that most of these adverse events have in common is that of being associated with increased risk of hypoxia, which could interfere with foetal and/or neonatal brain development. An alternative possibility is that the increased rate of obstetric complication in schizophrenia may be caused by an unknown pre-existing abnormality since women with schizophrenia are more likely to have obstetric complications e.g. caesarean sections or instrument-assisted deliveries than mothers without schizophrenia (Bennedsen *et al.* 2001).

Being born in the winter and early spring is associated with an increased risk of schizophrenia (Mortensen *et al.* 1999) (Figure1.3), an effect which remains evident both the Northern and Southern hemispheres. This effect does not interact with familial risk of schizophrenia (Hettema *et al.* 1996). One explanation for this is that an excess of maternal infection in the winter months, such as influenza underlies this association. Controlling for seasonality, it was found that rates of maternal influenza, particularly in the sixth month of gestation, were associated with rates of birth of people with schizophrenia (Barr *et al.* 1990), although one study reported an association between schizophrenia and the presence of anti-influenza antibodies in the first trimester only (Brown *et al.* 2004).

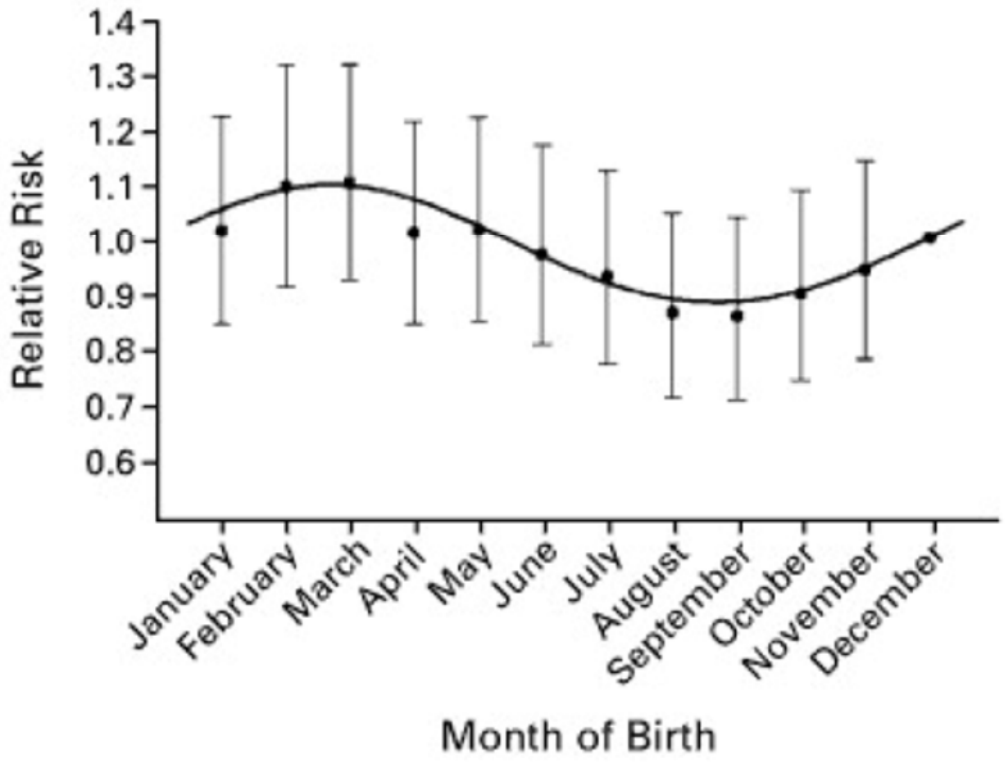

Figure 1.3 The relative risk of schizophrenia according to month of birth (Mortensen *et al.* 1999). The data points and vertical bars show the relative risks and 95 percent confidence intervals, respectively, with the month of birth analysed as a categorical variable, and the curve shows the relative risk as a fitted sine function of the month of birth. The reference category is December.

There is also evidence that severe prenatal malnutrition, as would occur in a famine for example, increases the risk of schizophrenia. This association has been following in the Nazi-engendered Dutch Hunger Winter during the Second World War (Susser & Lin 1992) and the Chinese Famine (1951-1961) in the wake of the Chinese Communist Party's *"Great Leap Forward"* resulting in flawed agricultural policies (St Clair *et al.* 2005).

1.4.2.3 Childhood Risk Factors

There is a two-fold increase in the risk of schizophrenia among people with unknown fathers compared with people with known fathers, which may be related to the lower socioeconomic status of the mothers of these offspring and/or by difficulties in growing up in a family without a father (Mortensen *et al.* 1999). Individuals who have experienced the death of or long-term separation from a parent before the age of 16 years have been shown to have an increased risk of psychotic disorder, with the odds ratio in the most robust studies (e.g. Morgan *et al.* 2007, Agid *et al.* 1999) being in the order of two to three. The largest case-control evidence comes from the AESOP study, which found the level of risk to be over two fold higher in those with a history of separation from or death of a parent before the age of 16, after adjusting for potential confounds (Morgan *et al.* 2007).

Studies have also found associations between childhood abuse/neglect and psychotic risk, although this evidence is less consistent and less methodologically robust (Morgan & Fisher 2007). Despite methodological challenges, these studies suggest traumatic events may increase the likelihood of experiencing psychotic symptoms (reviewed in van Os *et al.* 2010) and it has been suggested there may be specific associations between different types of trauma and specific psychotic phenomena (Bentall & Fernyhough, 2008), with ongoing interest in the tentative association between childhood sexual abuse and hallucinosis (e.g. Read *et al.* 2003; Varese *et al.* 2012).

1.4.2.4 Urbanicity

Schizophrenia is associated with a non-random geographical distribution. That is, urban dwelling, or "urbanicity", is associated with schizophrenia (Faris & Dunham 1939) and there is increased risk of psychosis in people who are born or brought up in inner cities compared to rural areas (Figure 1.4). In a study of Swedish conscripts, individuals who developed schizophrenia were 1.65 times more likely to have been brought up in urban vs. rural areas (Lewis *et al.* 1992). Likewise, Marcelis *et al.* (1998) found that urban birth carried twice the risk of later schizophrenia than rural birth. There is also evidence of a dose-response relationship between degree of urbanicity and risk of schizophrenia (Pedersen & Mortensen, 2001).

A systematic review by Kelly *et al.* (2010) found that all but one of 18 register-based studies examining rates of psychosis according to urbanicity found a positive association. The relative risk was between 1.4 and 4.3, and in most cases was approximately 2.

A number of possible aetiological mechanisms underlying the link between urbanicity and schizophrenia have been suggested, including obstetric complications (e.g. Torrey *et al.* 2000), perinatal infection, (e.g. McDonald *et al.* 2001) and perinatal vitamin D deficiency (based on McGrath's hypothesis, 1999). However research into these specific factors is either lacking or has shown that urbanicity continues to make an independent contribution to risk (Kelly *et al.* 2010).

A recent population-based study of over 20,000 individuals (Zammit *et al.* 2010) concluded that the association between urbanicity and psychosis appears to be a reflection of greater social fragmentation present within cities. Other commentators (e.g. Van Os *et al.* 2010) have identified individual social factors (e.g. being single, living alone), area-level factors such as social fragmentation, inequality (see Boydell *et al.* 2004) and the relationship between individual and area-level factors as candidate contributing factors.

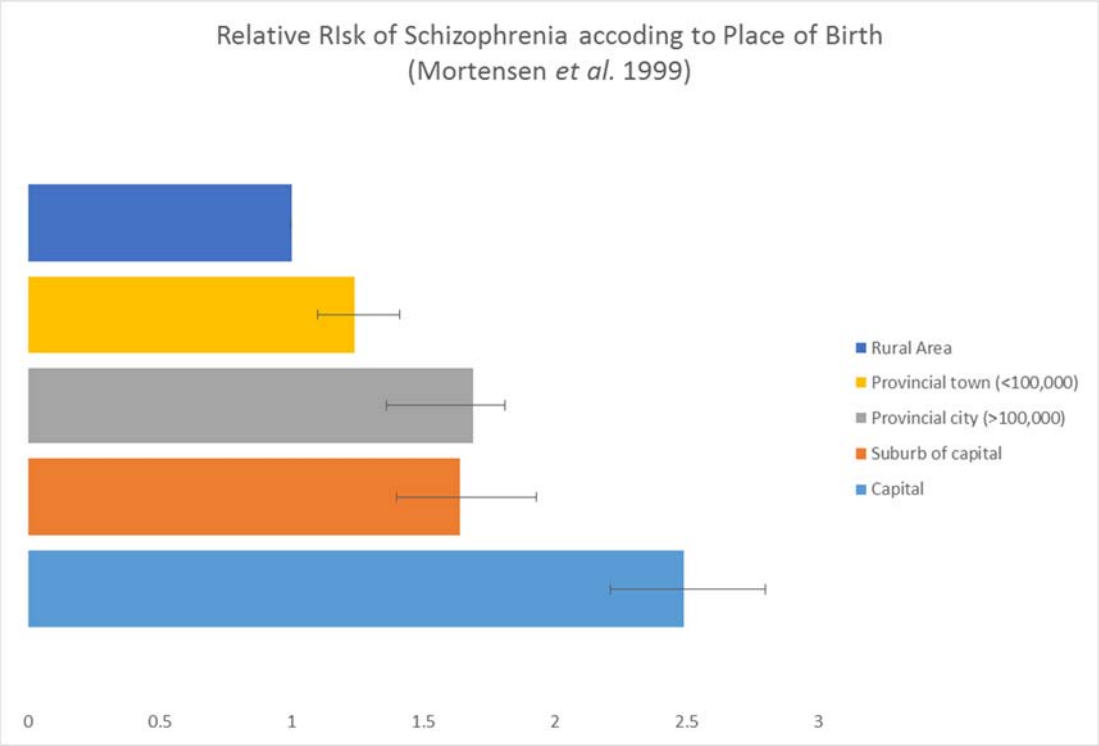

Figure 1.4 Relative risk of schizophrenia in Denmark according to pace of birth. In this figure the reference category is the rural area (from Mortensen *et al.* 1999).

1.4.2.4 Migration & Ethnicity

Since Ødegaard (1932) described high rates of Norwegians undergoing a schizophrenic breakdown after migration to Minnesota, many studies have replicated the finding that migrants are at increased risk of schizophrenia compared to those in the country of origin. Whilst Ødegaard hypothesised that individuals predisposed to schizophrenia are more likely to migrate, this has since been refuted (Selten *et al.* 2002). Associated with migration are the findings that individuals from minority ethnic groups are also at increased risk of schizophrenia, and that this showed a dose-dependency, with greatest risk in those who were most in the minority (Boydell *et al.* 2001).

In a meta-analysis (Cantor-Craee & Selton, 2005) the relative risk (RR) of schizophrenia was higher in migrants from less economically developed countries and particularly for black migrants moving to a white-majority country. The authors concluded this provides evidence for a role of social adversity in schizophrenic aetiology.

The multicentre AESOP study (Fearon *et al.* 2006) demonstrated a nine-fold and six-fold increase in the incidence of schizophrenia among African-Caribbeans and Africans respectively in three English cities. This appears very unlikely to be predominantly genetically determined as, if this were the case, rates of schizophrenia would be significantly raised in the origin countries compared to the native population of the destination, which they are not (e.g. Sugarman & Craufurd, 1994). In a British household survey, Brugha *et al.* (2004) found that African-Caribbeans and Black Africans were more likely than other ethnic minority groups to experience indicators of social disadvantage including low socioeconomic class,

unemployment, lone parent status, low perceived social support and poverty. Adjusting for these factors modestly reduced the risk of psychosis in these groups.

These findings have also been extended to migrants of other ethnicities to other countries (e.g. Selten & Sijben, 1994 and Cantor-Graae *et al.* 2003). A more recent meta-analysis (Bourque *et al.* 2011) has confirmed an increased risk of psychosis in both first and second generation migrants with incidence rate ratios (IRR) of 2.3 and 2.1 respectively, with no significant difference between the two generations. Possible explanations for heterogeneity between ethnic groups include perceived discrimination (Veling *et al.* 2008a) and a replicated finding that incidence of psychosis in migrants increases as they form a decreasing proportion of the population (e.g. Boydell *et al.* 2001 & Veling *et al.* 2008b). In summary, these studies strongly suggest a potential aetiological role for being a member of an 'outsider' social grouping, either as a migrant, descendant of a migrant or minority. Other factors such as urbanicity (above) do not appear to mediate the risk associated with this (Bourque *et al.* 2011).

1.4.2.5 Cannabis

Cannabis is the most widely used illicit drug in the world (United Nations 2010). 10% of cannabis users will at some point become dependent on the drug and the lifetime prevalence of cannabis dependence or abuse (as defined in DSM-III-R, American Psychiatric Association, 1987) in United States adults was estimated at 4.4% (Anthony *et al.* 1994). Until as recently as a decade ago many psychiatrists would not have regarded cannabis as dangerous to mental health, however this changed following a series of large scale epidemiological studies (Morrison & Murray 2007) and wide public debate on the issue. There is consistent epidemiological evidence that the drug is a risk factor for schizophreniform psychotic disorders (Moore *et al.* 2007), exhibiting dose-dependence (Moore *et al.* 2007) and dose-duration effects (Di Forti *et al.* 2009). In cannabis users who do not have schizophrenia, there is also evidence that use of the drug is associated with increased paranoid ideation (Freeman *et al.* 2013, Freeman *et al.* 2014), a key symptom of the illness.

The main psychoactive substance in cannabis is Δ9-tetrahydrocannabinol (THC) (Wachtel *et al.* 2002). THC was originally described as an agonist of endocannabinoid CB_1 receptors (Felder *et al.* 1992), however there is growing evidence of partial agonist properties from both *in vitro* (Sim *et al.* 1996; Petitet *et al.* 1998; Shen & Thayer 1999; Breivogel & Childers 2000; Govaerts et al. 2004; Kelley and Thayer, 2004) and *in vivo* (Paronis 2012*)* studies. THC was first isolated from hashish in 1964 by Gaoni & Mechloulam. Subsequently, the effects of THC on dopaminergic function have been widely studied and shall be described here. As THC has a number of double bond and stereo isomers, this discussion will focus on the main THC isomer found in cannabis, i.e. (−)-*trans*-Δ^9-tetrahydrocannabinol, which is also

referred to in some older studies by its alternative name Δ^1-tetrahydrocannabinol and as a pharmaceutical preparation using the International Non-Proprietary Name dronabinol.

Cannabis and THC can induce transient positive psychotic symptoms in healthy individuals (Moreau 1845, D'Souza *et al.* 2004, Morrison *et al.* 2009; Bhattacharyya *et al.* 2010; Morrison *et al.* 2011), and sensitivity to the acute psychotogenic effects of cannabis has been shown to be a predictor of subsequent psychotic disorders (Arendt *et al.* 2005). There is also evidence that THC can elicit schizophrenia-like negative symptoms which are distinct from sedation (Morrison & Stone 2011). It has been proposed that cannabis can precipitate psychosis in susceptible individuals (Murray *et al.* 2007), and therefore represents a potentially modifiable risk factor for psychotic disorders.

1.4.2.6 Acute and Chronic Stressors in Adulthood

Sudden changes in an individual's life (e.g. bereavement, unemployment or moving house) are termed *life events* which have been classified according to the stress they cause an individual (Holmes & Rahe, 1967). In 1968 Brown and Birley reported acute life events to be associated with relapse of schizophrenia and Bebbington *et al.* (1993) found a significant relationship between life events and onset or relapse of schizophrenia. More recently, Myin-Germeys & van Os (2007) have extended this to show increased stress-reactivity (i.e. sensitivity to the small stressors of everyday life) in people with and those at genetic risk of schizophrenia. In a case-control study of the effects of cumulative social disadvantage and first-episode psychosis Morgan *et al.* (2008) found a relationship between social disadvantage and odds of psychosis (see table 1.4). However, there is an intrinsic difficulty in determining

causality with this study design and it is well established schizophrenia is accompanied by a marked decline in social functioning (Hafner et al. 1999). Taken together with the evidence above there is therefore evidence of causal bidirectionality. Nonetheless, given the impact of similar stressors in childhood on later adult risk of psychosis, it would be reasonable to conclude that adult social stressors indeed are contributing to increased schizophrenia risk to a degree.

Table 1.4 Indicators of social disadvantage and isolation by case-control status (adapted from Morgan *et al.* 2008). Across all the domains considered, cases were more likely to be socially disadvantaged and isolated than were controls. Data presented below are from a restricted sample which yields more conservative adjusted odds ratios.

		Odds Ratio	95 % CI
Current Employment	Unemployed	3.61	2.19-5.96
	Employed	1	-
Long-term Employment	Unemployed > 1yr	2.44	1.4-4.25
	Employed	1	-
Housing Status	Self-owned	1	-
	Family owned	3.15	1.41-7.06
	Rented	1.93	1.13-3.28
Housing Stability	Moved in past 6 months	2.03	1.02-4.05
	Not moved in past 6 months	1	-
Current Living Arrangements	Live alone	2.66	1.62-4.37
	Live with relatives	5.2	2.63-10.25
	Live with others	1	-
Long-term living Arrangements	Lived alone for > 1 year	2.19	1.26-3.82
	Live with others in past year	1	-
Relationship	Single	3.36	2.14-5.27
	In a stable relationship	1	-
Long-term relationship	Never	3.81	2.21-6.59
	At least 1	1	-
Networks - Friends	Daily	1	-
	Weekly	3.4	2.01-5.77
	Less than weekly	5.02	2.73-9.22
Confidants	No	7.74	3.78-15.86
	Yes	1	-

Odds ratios presented are adjusted odds ratios, adjusted for age, gender, ethnicity, study centre, parental social class, special needs education before the age of 16, and pre-morbid IQ. CI, confidence interval.

1.4.3 Summary of Risks

In summary, at the individual level the most important risk factor for schizophrenia is having an affected relative which likely represents the inheritance of multiple genes of small effect which together will contribute to a genetic loading for the illness. This then interacts with a number of environmental risk factors occurring through the life-span from conception through birth, childhood, adolescence and adulthood. These are summarised in figure 1.5.

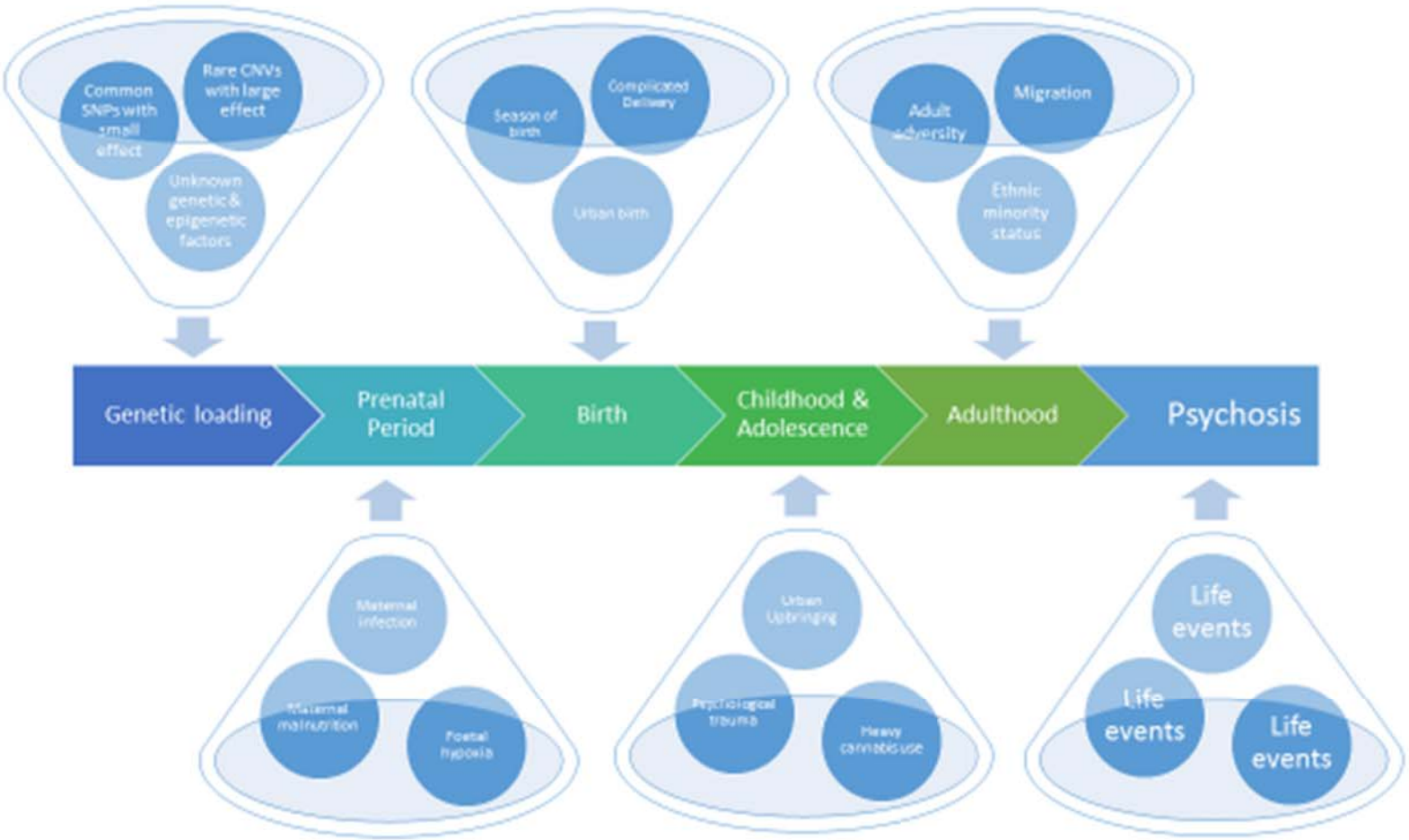

Figure 1.5 The developmental risk factor model of schizophrenia to illustrate causality over the life course.

These risk factors occur throughout the lifespan and have complex interactions with each other.

Whilst epidemiological risks are likely crude proxies for more complicated underlying variables and interactions, it is possible to conceptualise these across broad categories: hereditary and perinatal factors, psychosocial stressors, and cannabis use. Our current understanding of hereditary and perinatal factors has been discussed above, as have the psychosocial stressors including childhood and adult psycho-socio-economic adversity. This thesis is concerned with investigating the neurobiology of how psychosocial stressors and cannabis use increase psychosis risk, as these represent two of the largest modifiable risk factors for the illness.

1.5 The Neurobiology of Schizophrenia

1.5.1 Introduction

Much has been learnt regarding the neurobiological basis of schizophrenia with progress being catalysed by advances in human *in vivo* brain imaging over recent decades. The main findings will be summarised below including studies of macroscopic and microscopic brain structure, studies of brain function and the major neurochemical theories including current conceptualisation of the dopamine hypothesis of schizophrenia.

1.5.2 Macroscopic Brain Structure

The first major study of brain structure in schizophrenia using modern imaging technology utilised computed tomography (CT) to find that patients with schizophrenia had enlargement of the lateral ventricles (Johnstone *et al.* 1976). This finding was replicated in further imaging studies (e.g. Andreasen *et al.* 1986) and subsequent meta-analyses (e.g. Wright *et al.* 2000), with evidence suggesting that the degree of ventricular enlargement is related to poorer prognosis (Kolakowska *et al.* 1985). Together with the increase in ventricular size there have been findings of reduced whole brain weight (Harrison *et al.* 2003) and reduced grey matter volume (Wright *et al.* 2000). Subsequent work has identified a number of brain regions which show greater volume reductions compared to others including a) the left superior temporal gyrus (Honea *et al.* 2005), which is notable in the context of auditory hallucinations which are common in the illness, b) the medial temporal lobe and hippocampus (Nelson *et al.* 1998; Honea *et al.* 2005), and c) the thalamus (Konick &

Friedman 2001). Other findings include the reversal of the normal asymmetry of planum temporale (Petty *et al.* 1995) with further subsequent evidence of reduced cerebral asymmetry (Sommer *et al.* 2001). Findings of reduced asymmetry have been hypothesised to be related to language and the speciation of *Homo sapiens* (Crow 2004), although these findings may be epiphenomena of abnormal developmental processes which interfere with normal brain lateralisation (Harrison 2012). There is evidence that the major brain changes are present in individuals before they develop frank psychosis (Pantelis *et al.* 2003), that there are some volume changes which develop when people who are at high risk of psychosis proceed to develop psychosis (Velakoulis *et al.* 2006), and in patients with a first episode psychosis (Steen *et al.* 2006). Volume deficits have been observed in unaffected relatives of patients with psychosis (Boos *et al.* 2007), yet there is some evidence of progressive deficits with illness duration (Woods *et al.* 2005), which would suggest that these volumetric changes represent a mixture of both state (psychosis) and trait (psychosis-proneness) related effects. However, the interpretation of longitudinal studies is complicated by a current incapability to adequately tease out the effects of illness *vs*. antipsychotic treatment on the brain (Lieberman *et al.* 2005). It should be emphasised here that on an individual basis, these findings remain of limited clinical utility, for the time being, as they do not alter clinical management in the majority of cases, and are therefore not currently recommended by the United Kingdom's National Institute for Health and Care Excellence (NICE) in the routine management of first-episode psychosis (NICE 2008).

In addition to volumetric studies, advances in MRI have enabled the measurement of white matter connectivity via diffusion tensor imaging (DTI). A meta-analysis of DTI studies in schizophrenia (Ellison-Wright & Bullmore 2009) found significant reductions in connectivity in the left frontal deep white matter tracts, interconnecting the frontal lobe,

thalamus and cingulate gyrus, and in the left temporal deep white matter tracts, interconnecting the frontal lobe, insula, hippocampus, amygdala, temporal and occipital lobes. These findings of structural dysconnectivity are considered with those relating to functional dysconnectivity below.

1.5.3 Microscopic Brain Structure

Post-mortem cell counting and microscopic analysis suggested regions of cell loss and cortical volumetric reduction associated with schizophrenia (Benes 1993; Bogerts 1993; Shapiro 1993; Heckers 1997; Dwork 1997). Following these initial studies, morphometric findings in the prefrontal cortex gave rise to the hypothesis that impoverished neuronal connectivity was associated with cognitive dysfunction in schizophrenia (Selemon *et al.* 1995; Selemon *et al.* 1998; Rajkowska *et al.* 1998). This was conceived by some as the Reduced Neuropil Hypothesis (Selemon *et al.* 1995), whereby atrophy of neuronal processes occurs without local neuronal loss, and it was speculated that this was associated with cell loss elsewhere in the cortex (Selemon & Goldman-Rakic 2005). Yet, reductions in the neuropil may be due to antipsychotics (e.g. Vernon *et al.* 2014) or environmental impoverishment (Cannon *et al.* 2003).

Since post-mortem histological studies have yielded a number of controversial findings, with some authors suggesting that genetic neuropathology studies hold the key to bridging gaps in our current understanding (Kleinman *et al.* 2011). There is some evidence to suggest that altered synaptic proteins may be associated with reduced plasticity which could manifest itself

via decreased numbers of synapses (Eastwood 2003). There are a number of important negative histological findings in schizophrenia suggesting that classically described pathological process of neurodegeneration are not in operation. Specifically, these are the absence of neurofibrillary tangles, amyloid plaques, or Lewy bodies (Arnold *et al.* 1998). Whilst it had been suggested that inflammatory processes were unlikely to underlie the illness (Roberts & Harrison 2000), there is now renewed interest in inflammatory processes in the neuropathology of schizophrenia (Schnieder & Dwork 2011).

1.5.4 Functional Brain Imaging

One of the earliest findings in functional neuroimaging of schizophrenia was that patients with schizophrenia had reduced regional cerebral blood flow (rCBF) compared to controls in frontal areas, as measured with the xenon-133 technique (Ingvar & Franzen 1974). This gave rise to the concept of "hypofrontality" in schizophrenia, which was widely replicated using positron emission tomography (PET) (Weinberger & Berman 1996). However, using functional magnetic resonance imaging (fMRI) there have been inconsistencies in results with some studies (e.g. Callicott *et al.* 2003) including reports of increases in blood-oxygen level-dependent (BOLD) signal associated with lower accuracy on working memory tasks, which was interpreted as less efficient recruitment of cortical networks for the task in patients compared to controls. There has been some recent interest in cerebellar involvement in schizophrenia, where the illness was hypothesised to be caused by a state of "cognitive dysmetria" (Andreasen *et al.* 1999). Whilst there is evidence to support a role of the cerebellum in the disorder, findings from studies have been heterogeneous (Picard *et al.* 2008). As per the DTI literature, using resting state fMRI scans there is further evidence of dysconnectivity in

the illness, with hyperconnectivity of the default mode network and dysconnectivity between the cortex and striatum (Karbasforoushan & Woodward 2012).

1.5.5 The Glutamate System

The glutamatergic system has been implicated in the pathophysiology of the illness (Goff & Coyle 2001; Coyle *et al.* 2003) although more recently attempts have been made to combine the glutamate and dopamine models of the disorder (Stone *et al.* 2007). One of the tenets of the glutamate hypothesis of schizophrenia is that uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonists such as ketamine and phencyclidine (PCP) produce psychotic-like states in humans. However, it should be emphasised that these states are characterised primarily by subjective disembodiment, in addition to dissociation and visual hallucinations (Vollenweider *et al.* 1997), but lacking the auditory hallucinations commonly seen in schizophreniform psychosis. In support of glutamatergic involvement in the illness, reductions in both NMDA receptor binding (Pilowsky et al 2006) and levels of NMDA receptor mRNA (Law & Deakin 2001) in the hippocampus have been reported in schizophrenia. More recently, using single proton magnetic resonance spectroscopy (MRS), individuals at risk of psychosis have been found to have elevated glutamine in the anterior cingulate and reduced thalamic glutamate, compared to controls (Stone *et al.* 1999). There is also some evidence that NMDA receptor manipulated animals give rise to a "schizophrenia-like phenotype" (Lipska & Weinberger 2000).

1.5.6 The Dopamine Hypothesis of Schizophrenia

1.5.6.1 Dopamine in the Brain

Dopamine is a monoamine neuromodulator, although it remains described in many texts as a neurotransmitter. It is most abundant in the striatum, limbic system, frontal cortex and hypothalamus (see figure1.7; figure 1.8). The dopamine synthesis pathway involves conversion of tyrosine to DOPA via tyrosine hydroxylase, followed by conversion of DOPA to dopamine via dopa decarboxylase (figure 1.6) Dopamine is stored in presynaptic vesicles, transported via vesicular monoamine transporter (VMAT) proteins, prior to release into the synapse.

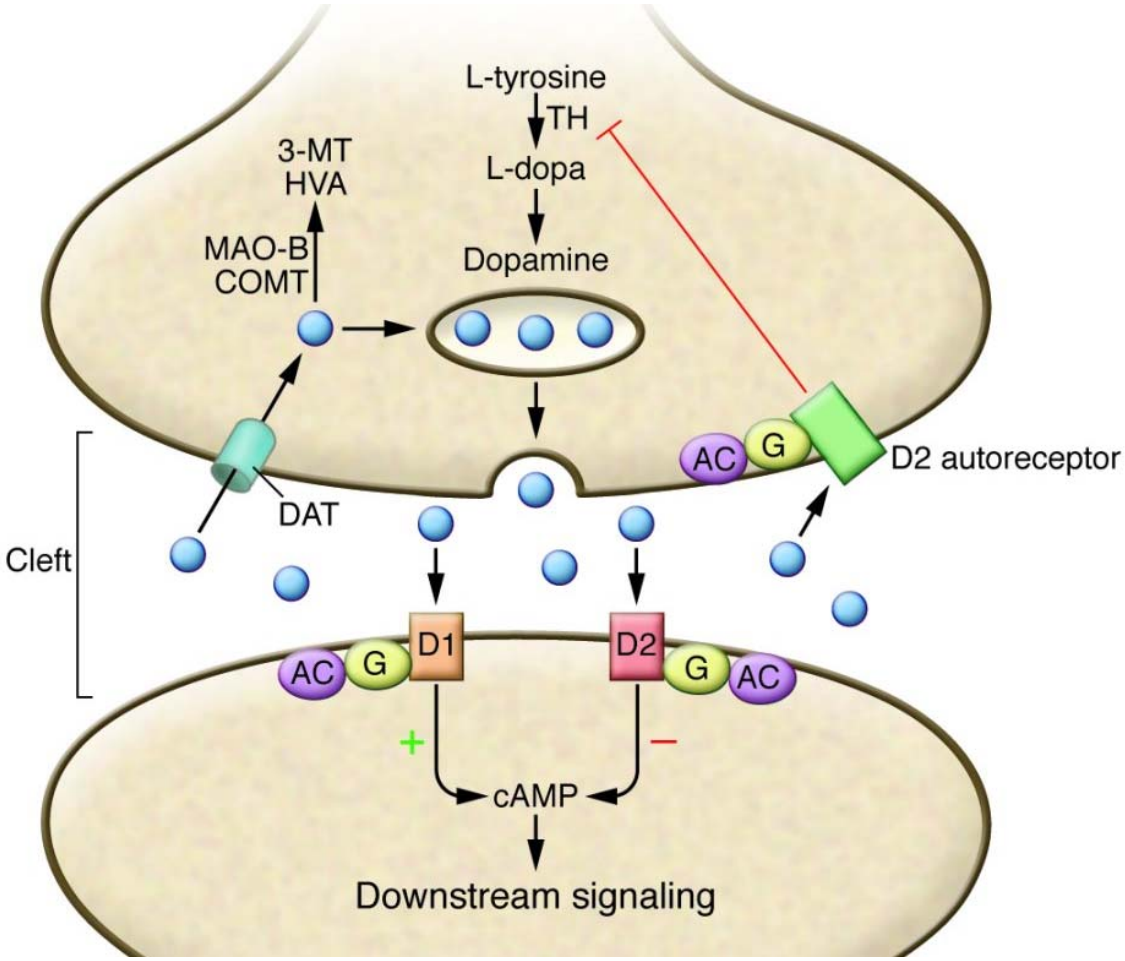

Figure 1.6 A simplified dopaminergic varicosity and synapse. Presynaptic and postsynaptic neuron membranes and the synaptic cleft are indicated. D_1 and D_2-class dopamine receptors are positively or negatively coupled to adenylate cyclase (AC) via G proteins (G). Coupling to other signalling pathways is not shown. Catechol O-methyltransferase (COMT) and monoamine oxidase B (MAO-B) are involved in the metabolism of dopamine (blue circles) to products such as homovanillic acid (HVA) and 3-methoxytyramine (3-MT). Dopamine in the cleft can bind presynaptically to D_2 autoreceptors or the DAT, or postsynaptically to D_1- and D_2-class receptors. Stimulation of D_2 autoreceptors inhibits phosphorylation-dependent activation of tyrosine hydroxylase (TH), which is rate limiting for the production of dopamine. Figure adapted from Blackstone (2009).

The striatum projects topographically to the pallidal complex, the ventral tegmental area and substantia nigra (Haber, 2012). The outputs from the globus pallidus pars interna and substantia nigra (GPi/SN) then projects back to the cortex via the thalamus, completing the basic cortico-basal ganglia circuit. This is known as the direct pathway. The side loop, from the striatum via the globus pallidus pars externa (GPe) passes through the subthalamic nucleus (STN) to the GPi, and is referred to as the indirect pathway (Figure 1.8). In addition, there are other projections of the striatum including those to the brainstem (Haber 2012).

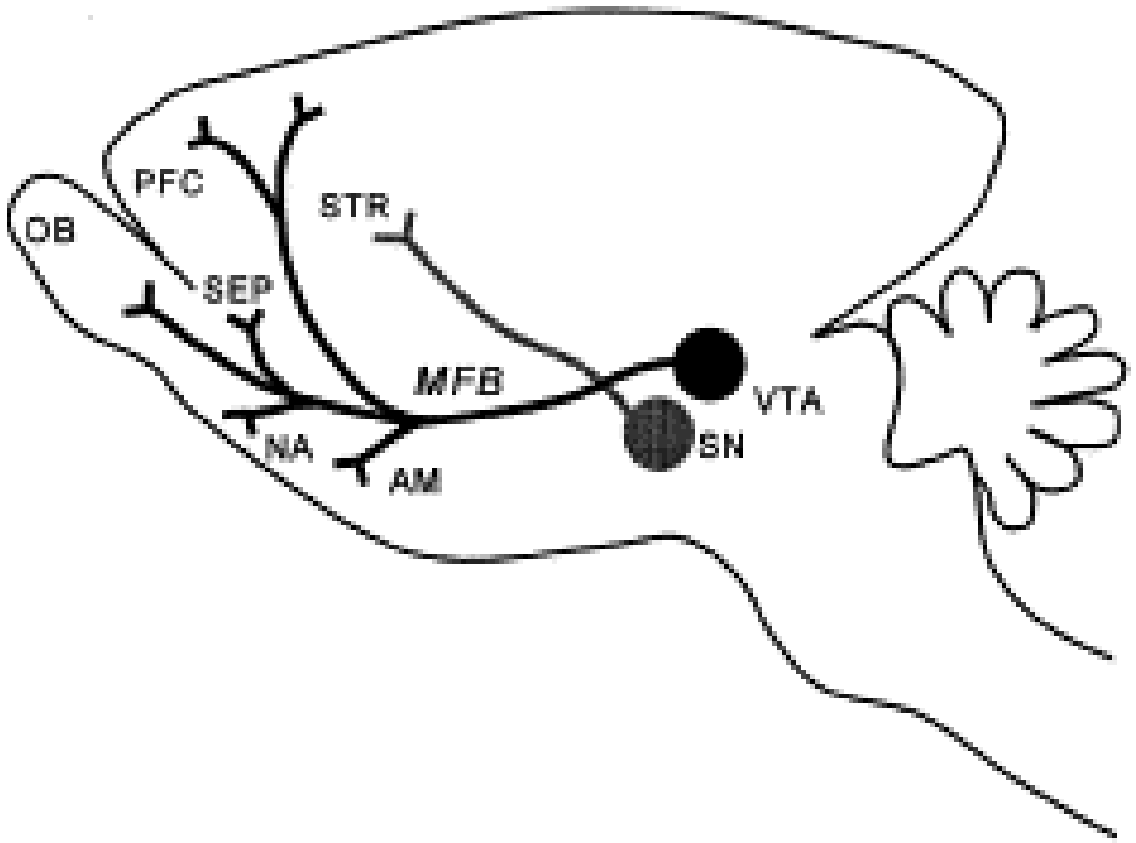

Figure 1.7 Simplified schematic of dopaminergic neurons and their projections in the rat brain. The nigrostriatal fibres project from the substantia nigra to the striatum. The ventral tegmental area and the nucleus accumbens represent key structures of the dopaminergic mesolimbic reward system. AM, amygdala; MFB, medial forebrain bundle; NA, nucleus accumbens; OB, olfactory bulb; PFC, prefrontal cortex; SEP, septum; SN, substantia nigra; STR striatum; VTA, ventral tegmental area. (from Aremi 1999).

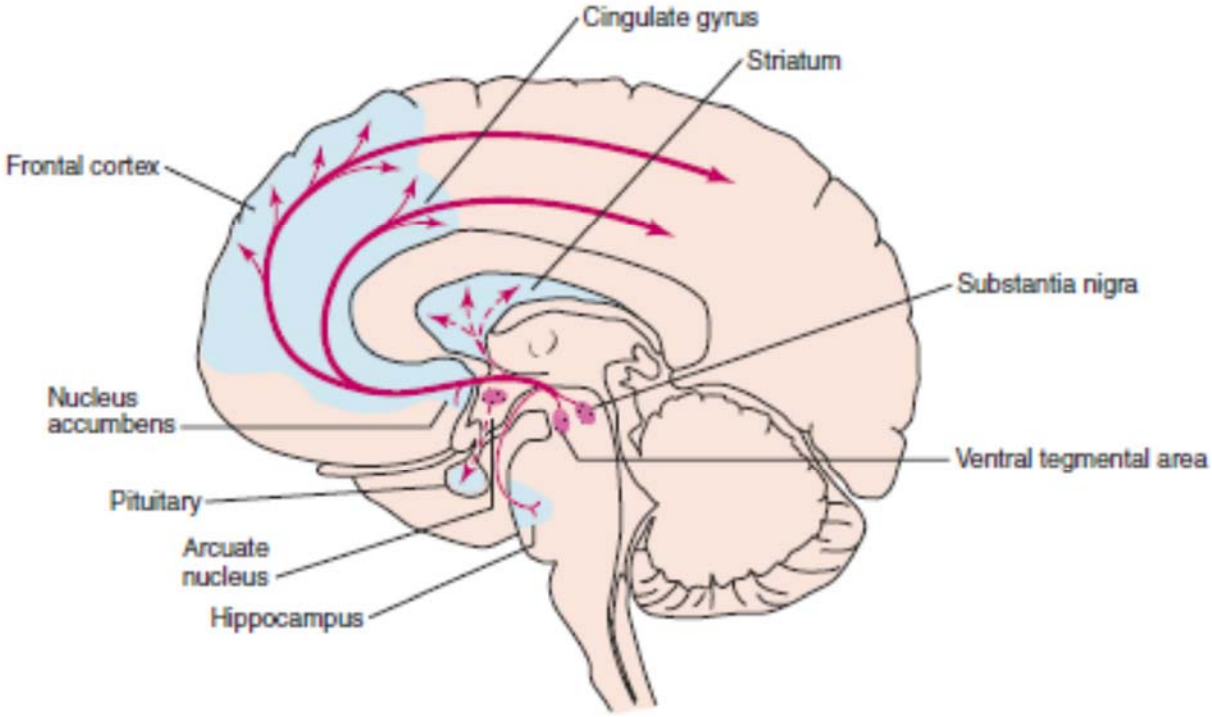

Figure 1.8 Illustration of the major dopaminergic projections in the human CNS: the nigrostriatal pathway (projecting from the substantia nigra to the striatum), the mesolimbic pathway (projecting from the ventral tegmental area [VTA] to the nucleus accumbens), the mesocortical pathway (projecting from the VTA to the frontal cortex). Figure from Malenka *et al.* (2009).

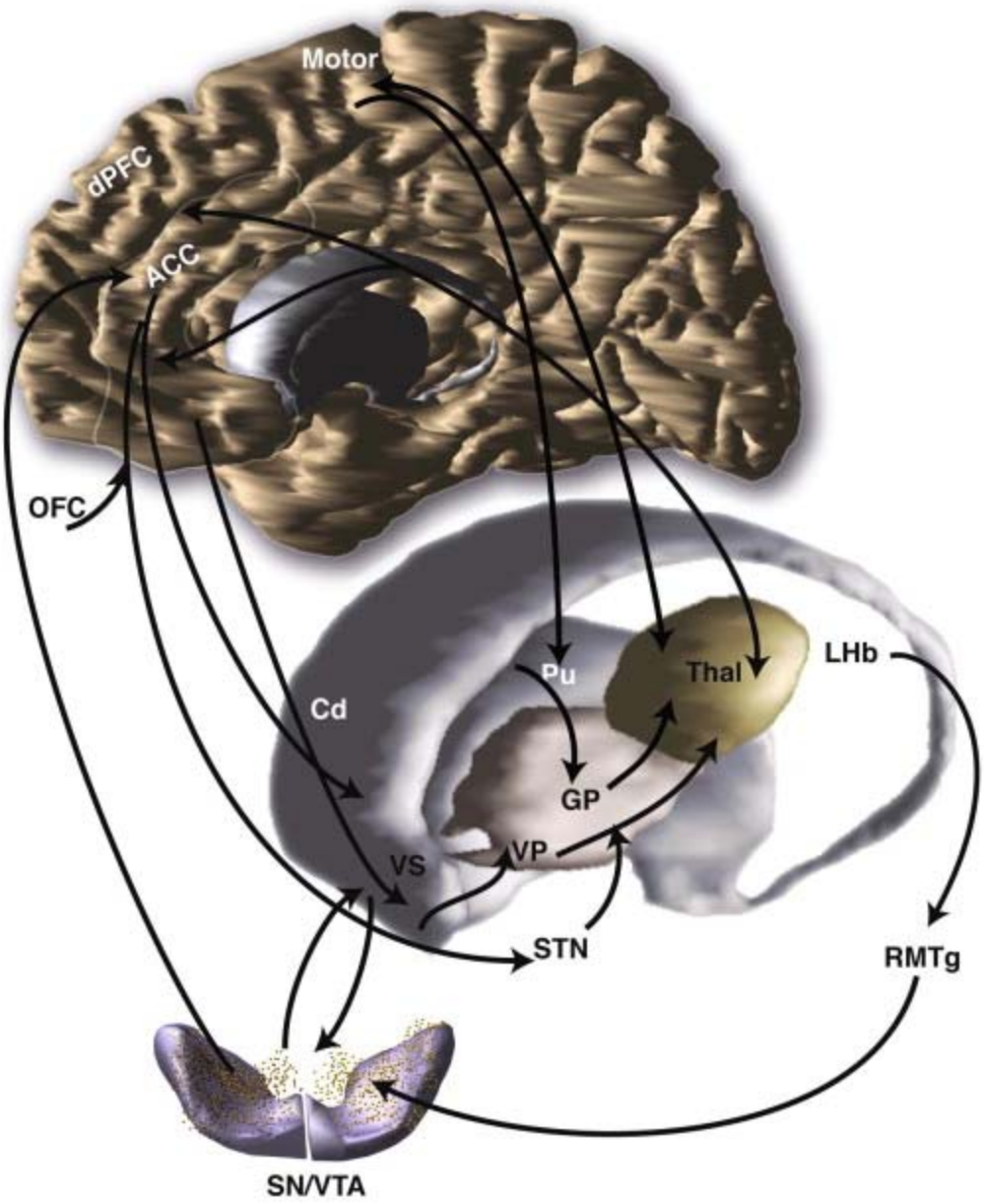

Figure 1.9 Schematic illustrating key structures and pathways of the basal ganglia. Note: brainstem motor connections are not illustrated to simplify the figure. Cd, caudate nucleus; ACC, anterior cingulate cortex; dPFC, dorsal prefrontal cortex; GP, globus pallidus; LHb, lateral habenula; OFC, orbital frontal cortex; Pu, putamen; RMTg, rostromedial tegmental nucleus; SN, substantia nigra; STN, subthalamic n; Thal, thalamus; VP, ventral pallidum; VS, ventral striatum; VTA, ventral tegmental area (from Haber 2014).

There are four major dopaminergic projections in the human brain: the nigrostriatal pathway (projecting from the substantia nigra to the striatum), the mesolimbic pathway (projecting from the ventral tegmental area [VTA] to nucleus accumbens and amygdaloid nucleus via the medial forebrain bundle), the mesocortical pathway (projecting from the VTA to the frontal cortex via the median forebrain bundle), and the tuberoinfundibular pathway (projecting from the hypothalamus to the pituitary gland and median eminence) See figure 1.7.

1.5.6.2 The Role of Dopamine in the Brain: motivation and reward

Initially it had been thought that the brain's dopamine system was primarily involved in motor function via the extrapyramidal system and basal ganglia, based in part on the clinical syndrome and nigral dopaminergic lesions observed in Parkinson's disease. However, this view was changed by research which found that administration of dopamine receptor antagonists reduced an animal's instrumental response to rewards including food, water and sex, as well as intracranial stimulation and drugs (Dews & Morse 1961). Likewise, a key development in our knowledge of the role of dopamine in the brain came with the finding that selective damage to dopaminergic fibres gave rise to feeding and drinking deficits (Ungerstedt 1971).

Dopaminergic anatomical projections were found to extend beyond the classical motor regions of the brain to the limbic system (Ungerstedt 1971) and the prefrontal cortex (e.g. Thierry *et al.* 1973). These mesolimbic regions included brain areas that were previously and serendipitously found to be associated with positive reinforcement upon direct electrical stimulation during (Olds & Milner 1954). Lesions to mesolimbic projections were found to reduce the forward locomotion required for reward-seeking behaviour (Smith 1976; Schneirla

1959), whilst nigrostriatal lesions disrupt appetitive/preparatory behaviour (Ervin *et al.* 1977). Likewise, dopamine antagonism was found to reduce the rewarding effects of food (Wise *et al.* 1978), water (Gerber *et al.* 1981), cocaine (de Wit & Wise 1977), amphetamine (Yokel & Wise 1975) and lateral hypothalamic electrical stimulation (Fouriezos & Wise 1976). Whereas dopamine antagonist treated rodents were able to perform operant conditioning tasks (Wise 1982), they displayed attenuated acquisition for reward-related lever pressing (Wise & Schwartz 1981). Taken together these findings give rise to a number of competing and complementary theories of dopaminergic function in behaviour relating to hedonia (Wise *et al.* 1978); reward (Wise *et al.* 1978); reinforcement (Fibiger 1978); and motivational salience (Berridge & Robinson 1998). This section will outline some of the important findings in this field of research.

1.5.6.3 Anatomy

Based on connective anatomy and histochemistry, the striatum and related anatomy (referred to in some papers as the striatal complex), has been distinguished into three functional subdivisions: a limbic subdivision comprising ventral structures including the nucleus accumbens (NAc) core and shell (Heimer & Wilson 1975); an intermediate associative subdivision and a sensorimotor subdivision comprising dorsolateral structures (Joel & Weiner 2000; Riedel *et al.* 2002).

Studies have found that the nucleus accumbens is a critical site in dopaminergic motivation and reward processing. The accumbens has been divided into three sub-territories: the shell, core and rostral pole (Zahm & Brog 1992; Zahm & Heimer 1993). Some of the key anatomical sites related to motivational processing for feeding behaviour provide an example of how the functions of these circuits can be related to the underlying anatomy (Kelley 2004).

The nucleus accumbens receives taste and visceral information from the brainstem via direct inputs from the nucleus of the solitary tract to the shell, and indirect inputs from the cortex to the shell and core via parabrachial-thalamic projections (Ricardo & Koh 1978; Saper 1982). Additional taste information is relayed to the accumbens from the amygdala which integrates taste information with multimodal sensory input (McDonald & Jackson 1987). The amygdala also sends information to dopaminergic cells in the ventral tegmental area. There are also projections from the amygdala to other forebrain nuclei which, with the mesolimbic and mesocortical dopamine neurons, comprise the forebrain arousal system (Robbins & Everitt 1987).

The accumbens also has efferent projections to the ventral globus pallidus and lateral hypothalamus (Hiemer *et al.* 1991). The accumbens shell receives information on the internal physiological state of the organism from the hypothalamus (Maldonado-Irizarry & Kelley 1995). The accumbens shell integrates sensory, spatial, visceral and memory information related to food reward with aminergic modulatory input, to drive hypothalamic and brainstem motor centres independently from a motor cortical relay. The accumbens core integrates amygdala inputs related to the affective value of sensory information with frontal inputs in a striatocortical loop (Alexander *et al.* 1990). Taken together, differences in anatomical connectivity suggest the accumbens shell is involved in activational and incentive aspects of processing, whilst the accumbens core is involved in directional aspects of motivation (Corbit *et al.* 2001).

The accumbens shell is greatly interconnected with the medial extended amygdala complex (Aheid & Heimer 1988; Heimer *et al.* 1991) comprising the central amygdala and bed nucleus of stria terminalis, which is innervated by dopaminergic projections with a striato-pallidal organisation (Cassell *et al.* 1999). The terminal dopaminergic areas of the extended amygdala have been grouped functionally into a parastriatal complex which is distinct from the striatum and accumbens shell, on the basis of comparative distribution of calcium binding proteins (Riedel *et al.* 2002).

1.5.6.4 Terminology

There are inconsistencies in the literature in the precise meanings attributed to the constructs used to describe behaviour. These shall be described as per Di Chiara (2005).

An underlying principle in the study of behaviour is that life has the goal of self-perpetuating and organisms reproduce for the survival of their species. The concept of motivation is closely linked to this principle. Aspects of motivation are thought to be hard-wired into organisms by evolution such that organisms have an innate ability to encode the intrinsic biological value of stimuli and respond accordingly (Glickman & Schiff 1967). Therefore, certain stimuli, such as the taste of food or the sound of a predator will result in behaviours that will either approach or avoid the stimulus, depending on its motivational valence. These responses are unconditioned primary responses which means that are neither based on learnt experiences nor based on imitating the behaviours of others.

Motivation refers to the thought processes and behaviours that are triggered within an organism in response to stimuli that are relevant for the survival of the organism, and are therefore termed "motivational stimuli". In addition to the unconditioned primary responses, an organism must learn the predictive relationships, termed "contingencies", between stimuli that are "salient", i.e. relevant and biologically meaningful in terms of survival, the organisms own response to these stimuli, and the consequences of the organism's response, termed an "outcome". The process of an organism learning these contingencies so that it can change its behaviour to increase or decrease the occurrence of biologically valuable events is termed "instrumental action".

Motivational stimuli will have a "motivational valence", which determines the direction of the organism's response in relation to the stimulus whereby a positive motivational valence elicits an approach to the stimulus and a negative motivational valence elicits an aversion to the stimulus. The motivational valence can either be learnt by the association of a motivational stimulus with a particular outcome, which is referred to as "conditioned". This is in contrast to valences which are not learnt and are therefore "unconditioned".

Behaviour which is motivated (i.e. directed) in response to environmental stimuli can be divided into two phases: a preparatory phase, termed "appetitive", and a "consummatory phase". These phases can be thought of in terms of patterns of behaviour to two different types of motivational stimuli (Woodworth 1918; Konorski 1967): "incentives" and "rewards". Incentive stimuli operate during the appetitive phase, whilst rewarding stimuli operate in the consummatory phase of the motivated behaviour. Incentive stimuli are experienced via sensory modalities that can act at a distance, such as smell, sound and vision, and therefore do not require direct contact between the organism and the stimulus. Thus, incentive stimuli can allow the organism to achieve it's a goal, but are not necessarily themselves for the goal in question. Rewarding stimuli are experiences via senses requiring direct contact with the stimulus, e.g. taste and touch, and unconditionally predict the biological outcome providing the final goal of the motivated behaviour. It must be pointed out that incentives and rewards can co-exist within the same object. For example, the smell of a particular food would be considered the incentive stimulus, which motivates the organism to eat it and result in taste, the rewarding stimulus. A stimulus which can strengthen or weaken the responses elicited by it is termed a positive or negative reinforcer, respectively. Rewards, therefore, can act as positive reinforcers.

Incentive stimuli can have two properties: a directional property related to its valence, resulting in attraction or repulsion, and an activational property which is associated with a particular level of arousal within the organism termed "incentive arousal". The incentive arousal of a stimulus serves to increase or decrease the incentive properties of other stimuli in the particular environment in which it is experienced. Again these different properties can be unconditioned or conditioned. The process of conditioning in this context is termed "incentive conditioning".

The learning of stimulus-reward contingencies is termed Pavlovian learning (Mackintosh 1983), which is also known as classical conditioning. In this process, new salient stimuli that predict the occurrence of an unconditioned stimulus (US) acquire conditioned responses (CR) in line with the valence of the US, thereby becoming conditioned stimuli (CS). CS can elicit both conditioned consummatory or conditioned preparatory/incentive responses (Konorski 1967). Preparatory/incentive behavioural responses to the CS are thought of as being due to the excitation of the motivational system common to different US (Konorski 1967). Therefore, the association with the US results in a representation of the CS which is associated with the motivational system through the process of Pavlovian incentive learning (Dickson & Balleine 1994) and able to induce preparatory/incentive responses (Konsorski 1967). Incentive act-outcome instrumental responding is gradually transformed into "habit responding" based on stimulus-response associations (Dickinson 1994), meaning that it becomes automatic and is *learnt*. When a previously conditioned stimulus no longer gives rise to a conditioned response, then an "extinction" of that response has occurred.

Early animal studies into the effects of dopamine receptor antagonism on instrumental behaviour gave rise to four main hypotheses, which are not mutually exclusive:

1. The anhedonia hypothesis, which infers a role for dopamine in hedonia, whereby dopamine antagonism results in blunting of the rewarding properties of primary reinforcers.
2. The revised anhedonia hypothesis whereby dopamine antagonists result in the loss of incentive-motivational and arousal properties of incentives and rewards.
3. Dopamine antagonism results in impaired performance and sensory-motor functions.
4. Impairment of Pavlovian incentive learning.

The roles of dopamine in hedonia, incentive motivation and learning will be described in more depth below. However, owing to discrepancies in experimental findings related to the role of dopamine in motivation, which may be related to methodological differences, inferences related to the role of dopamine in different anatomical regions regarding particular aspects of motivation should be made with caution (Di Chiara 2005). Furthermore, dopamine plays an important role in motor functions. Given the relationship between response-reinforcement and motor performance, this poses a challenge in the study of the role of dopamine in motivation and reward processing (Salamone 1992), although approaches to overcome this have included measures of the effects of dopamine antagonism on response rates during experimental tasks (e.g. Willner *et al.* 1990).

1.5.6.5 Dopamine and Hedonia

Following the work by Olds & Milner (1954) reporting that direct electric stimulation of mesolimbic brain regions was associated with positive reinforcement, further evidence that dopamine had a role in hedonia came from the finding that dopamine receptor antagonists disrupt instrumental responding in a manner which extends beyond motor impairment as they produce a within-session reduction in the rate of lever pressing continuous reinforcement schedules (Wise 1982). This finding was generalised to a number of rewards including water, food and psychostimulants (Wise *et al.* 1978; Wise 1982; Salamone 1987). Since dopamine receptor antagonists had similar effects to extinction, it was hypothesised that dopamine antagonists blunted the hedonic impact of rewards (Wise *et al.* 1978; Wise 1982).

However, Treit & Berridge (1990) found that administration of high dose haloperidol does not induce changes in hedonic or aversive taste reactions. Furthermore, 6-hydroxydopamine lesions did not alter hedonic taste reactivity (Berridge 1998; Berridge & Robinson 1998). Later, Pecina *et al.* (1997) concluded that dopamine receptor antagonism results in sensorimotor impairment rather than blunting of taste hedonia.

Nonetheless, there is evidence from human *in vivo* imaging that stimulant-induced euphoria is correlated with drug-induced dopamine release (Laruelle *et al.* 1995; Volkow *et al.* 1999; Drevets *et al.* 2001). Furthermore, a role for dopamine in euphoria is implicated in some hypotheses regarding normal and abnormal mood states including euthymia, dysthymia, depression and mania (Papp *et al.* 1991). Taken together, these studies suggest that there exist both dopamine-independent stimulus-hedonic responses and dopamine dependent state-hedonic responses. Yet, it must also be noted here that in addition to processing *positively*

rewarding events, dopamine is also involved in *negatively* rewarding events, i.e. aversive stimuli (Salamone *et al.* 1997; Salamone 1994).

1.5.6.7 Dopamine and aversive stimulation

Dopamine likely has a role in processing aversive stimuli, in addition to the examples of rewarding stimuli outlined above, suggesting a role for dopamine in the stress response. Increases in both dopaminergic neuron firing rates, as measured with electrophysiology, and extracellular dopamine in response to aversive stimuli and stress have been reported in a number (Horvitz *et al.* 1997; Horvitz 2000; Abercrombie *et al.* 1989; Imperato *et al.* 1989; McCullough *et al.* 1993; Kalivas & Duffy 1995; Bassareo *et al.* 1996) but not all (Mirenowicz & Schultz 1996) studies. Based on these results, it has been suggested that dopamine also plays a role in aversive motivation. Given that different parts of the dopamine system appear to respond differently to aversive stimuli, including the accumbens core and shell (Bassareo *et al.* 2002; Deutch & Cameron 1992), the responsiveness of dopamine transmission may also be dependent on additional properties of the stimulus including novelty and motivational valence.

1.5.6.8 Incentive-motivation hypothesis

The priming (drive-like) effects of an encounter with an otherwise neutral stimulus that has acquired motivational importance through prior association with a reward is termed incentive motivation. Gray & Wise (1980) provided a revision to the anhedonia hypothesis by hypothesising that dopamine mediates the incentive-motivational properties of rewards and conditioned reinforcers. This became known as the incentive-motivation hypothesis.

Whilst dopamine has been proposed to be central for incentive motivation (e.g. Stewart *et al.* 1984), it has been argued by some that the main role of dopamine in incentive motivation lies in the "stamping in" of the reward value of a stimulus (Wise 2004). This is based on the finding that dopamine blockade severely impedes Pavlovian conditioning (Spyraki *et al.* 1982), whilst established incentive motivational stimuli can result in conditioned behaviours under dopaminergic blockade (McFarland & Ettenberg 1998). Yet, the capability of incentive stimuli to elicit behaviour becomes extinguished over time as the incentive value of the stimulus is weakened over time since the pairing of the stimulus with the reinforcer becomes ineffective under such conditions over time (Franklin & McCoy 1979).

Studies by Pecina *et al.* (2003) using dopamine transporter (DAT) knock-down mice carrying reduced DAT expression, resulting in increased steady-state extracellular dopamine, demonstrated that knockdown mice show faster running for food and increased food intake compared to wild-type. This increased motivation for food was not secondary to increased reward from food as estimated from the hedonic reactions to intraoral sucrose infusion. These studies support the hypothesis that that dopamine plays a role in the incentive, rather than the rewarding properties of food *per se*.

A number of studies (Berridge 1996; Berridge & Robinson 1998; Robinson & Berridge 1993) report that dopamine is involved in a process called "incentive salience attribution". This process refers to stimuli conditioned to a reward by dopamine-independent Pavlovian learning being imbued with incentive properties due to conditioned dopamine release. In other words, it was proposed that an incentive stimulus obtains its ability to elicit a response as reward-predictive stimuli (Schultz 1998) trigger a burst of spikes in dopamine neurons and a consequent striatal phasic release of dopamine. In this model, the stimulus-bound release of dopamine was thought of as enabling response-eliciting properties of the stimulus that triggered it. This views the role of phasic dopamine in response expression in a series of events between the stimulus and the response i.e. having a stimulus-bound role.

Dopamine neurons have been found to fire with a delay of 100ms after the unpredicted presentation of a reward or a reward-conditioned stimulus (Shultz 1998). *In vivo* studies in rodents and monkeys have found that a behaviourally significant stimulus takes less than 150 ms to produce a response in the efferent basal ganglia neurons of the substantia nigra pars reticulata and the medial globus pallidus (Hikosaka & Wurst 1983). Thus, by the time a stimulus presentation results in activation of dopamine neurons and dopamine starts to elicit its post synaptic effects, responsive units along the efferent pathway of the basal ganglia would have already initiated their discharge sequences leading to the inhibition of output neurons in the substantia nigra and globus pallidus by fast globus pallidus γ-aminobutyric acid (GABA) receptors. It was therefore proposed that phasic dopamine release is a "teaching signal", strengthening future transmission through striatal synapses activated in coincidence with it. This built on earlier work by Beninger & Phillps (1980) who suggested that dopamine receptor antagonism impairs Pavlovian incentive learning. Therefore, reinforcement refers to the "stamping-in" of the association between a stimulus and a reward (Wise 1989) and dopamine

antagonism results in a progressive decline in reinforced behaviour (Wise 1982). Likewise, place preference for reward in Pavlovian conditioning paradigms is disrupted by dopaminergic blockade (Spyraki *et al.* 1982).

1.5.6.9 Dopamine and Arousal

Rewarding and reward-associated stimuli result in motivational arousal (Bindra 1968). The motivating effect of free reward obtained before a behaviour is known as *priming*, i.e. the precipitation of a learned response habit by administration of an unearned sample of the reward. In other words, priming refers to the phenomena by which exposure to a rewarding stimulus without (e.g. sugar) without working for it can increase future responses to conditions in which that reward (e.g. sugar) or indeed other rewards are obtained. There is evidence that priming is, to some extent, dopamine-dependent (Esposito *et al.* 1979; Wasserman *et al.* 1982) and dopaminergic potentiation can prime food and drug-seeking behaviour (Roitman *et al.* 2004; de Wit *et al.* 1981).

Similarly, dopamine plays a role in the facilitation of responding associated with states of behavioural arousal induced by reinforcers. This process is called "incentive arousal" and it is thought to facilitate instrumental responding for the reward to witch the incentive has been conditioned, in addition to other rewards. This property acts as a transfer from classical conditioning (i.e. Pavlovian) state to an instrumental one, termed "PIT". Consistent with this role, amphetamine facilitates PIT (Wyvell & Berridge 2000).

As Wise proposed in his paper (1982):

"Dopaminergic impairment disrupts first and most strongly the motivational arousal function of external rather than internal stimuli…. Reinforcers and their associated environmental cues lose their sensory impact in terms of arousal function but not in terms of cue function."

According to this hypothesis, dopamine is a substrate of an arousal state, termed "incentive arousal", which non-specifically increases the ability of incentives to facilitate instrumental responding. This notion is similar to the "incentive state" described by earlier theorists (Cofer 1972; Killeen 1975), corresponding to the earlier notion of behavioural arousal as being distinct from directional effects of reinforcers.

However, dopaminergic mechanisms are not necessary for pre-reward motivation, but rather they amplify this process (Wyvell & Berridge 2000). In other words, there is "enhancement of reward '*wanting'* without enhanced '*liking"*", as wanting and liking have been proposed to represent two separate dimensions of reward function (Berridge & Robinson 1995).

1.5.6.10 Dopaminergic transmission

Dopaminergic neuron activity and dopamine transmission is activated in relation to the presentation of motivational stimuli or the expression of specific phases of motivated behaviour. Studies using electrophysiology have shown that dopaminergic neurons respond by a burst of spikes to the unpredicted occurrence of primary food stimuli and associated conditioned stimuli (Schultz *et al.* 1993; Schultz 1998). On the other hand, the unpredicted omission of reward leads to the inhibition of dopaminergic activity. Once a reward occurrence or omission becomes predictable, there is a reduction in the activation or inhibition of dopaminergic neuron activity. It was therefore argued that dopamine neurons encode reward prediction error signals (Hollerman & Schultz 1998). Initially unpredictable rewards elicit dopamine neuron activations and as the reward becomes predicted by the conditioned stimulus with continued experience, the activations elicited by the reward decrease whilst the conditioned (reward-predicting) stimulus can induce dopamine neuron activation. If the predicted reward does not occur (e.g. because a test animal makes an incorrect response) then a depression in dopamine neuron activity occurs at the same time as an increase in activity would have occurred had the reward been obtained (See figure 1.10). This property is thought to be a neural correlate of associative learning. It is thought that phasic dopamine serves a teaching signal in learning process related to the ability to predict reward occurrence in the context of motivated behaviour (Schultz 1998) as mentioned previously. Likewise, studies using microdialysis to directly measure extracellular dopamine concentrations, have been used to measure the effect of feeding and food-related stimuli in specific brain regions which vary depending on a number of experimental conditions (e.g. Gratton & Wise 1994). The results of those studies suggest that dopamine plays different roles in behaviour depending on where in the brain it is acting.

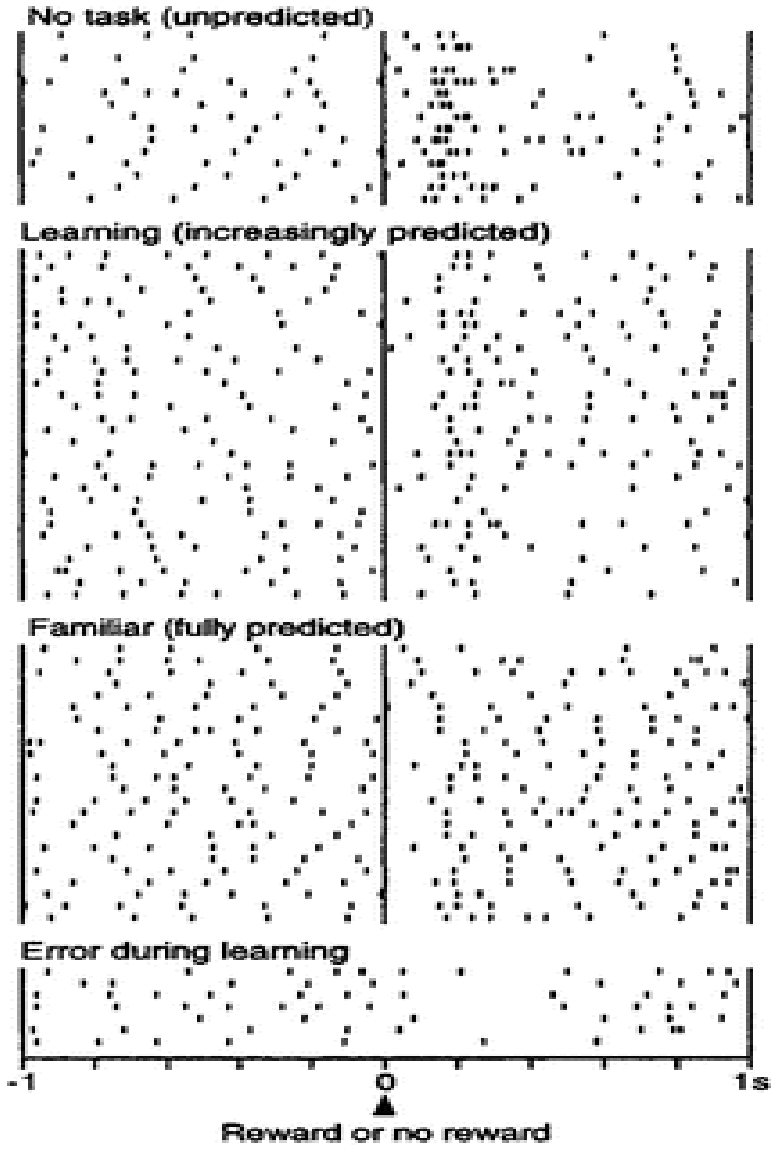

Figure 1.10 Coding of reward-prediction error during learning by a single dopamine neuron. (*Dots*) Neuronal impulses, each line showing one trial, with the chronological sequence in each panel being from *top* to *bottom.* Rewards were small quantities of apple juice delivered to the mouth of a monkey. No task: The temporally unpredicted occurrence of reward outside of any task induces reliable neuronal activation. Learning: The presentation of a novel picture pair in a two-picture discrimination task leads to uncertain behavioural performance with unpredictable occurrence of reward and dopamine response. (*Top* to *bottom*) Response decreases with increasing picture acquisition (only correct trials shown). Familiar: Presentation of known pictures in same task leads to predictable occurrence of reward and no dopamine response. Error during learning: Error performance with novel pictures leads to omission of reward. (From Hollerman & Schultz 1998.)

1.5.6.11 The Dopamine Hypothesis of Schizophrenia

The dopamine hypothesis of schizophrenia gained popularity based on the finding that the clinical potency of antipsychotics being directly related to their affinity for dopamine receptors (e.g. Seeman *et al*. 1976) (Figure 1.11). It had first been proposed in an early form by van Rossum (1966) who hypothesised that *"dopamine receptor blockade is an important factor in the mode of action of neuroleptic drugs"*. Van Rossum (1966) subsequently wrote that overstimulation of dopamine receptors could be part of the pathophysiology of schizophrenia.

Elevated levels of brain dopamine in patients with schizophrenia were initially reported in post-mortem studies, which at first reported increases in limbic regions of the forebrain (Bird *et al.* 1979). Subsequently, increased brain dopamine and dopamine receptors were reported in a post-mortem study comparing patients with schizophrenia to controls (Mackay *et al.* 1982), and it was found that increased dopamine in the nucleus accumbens and caudate nucleus were unrelated to antipsychotic medication, whereas the reported increase in dopamine receptor binding sites was only present for patients in whom antipsychotic medication had been continued until death. The authors interpreted this as reflecting an iatrogenic increase in dopamine receptor availability. However, an earlier post-mortem study (Lee & Seeman 1980), which likewise reported increased dopamine receptor availability, also found increased availability in antipsychotic-naïve patients in contrast to the results of Mackay *et al.* (1982).

The dopamine hypothesis was later modified by Davis (1991), who attempted to incorporate the emergent hypofrontality literature, outlined above. Davis proposed that schizophrenia was caused by subcortical hyperdopaminergia, giving rise to positive symptoms, and frontal hypodopaminergia, giving rise to negative symptoms. Other earlier versions of the dopamine hypothesis incorporated the other neurotransmitters, such as the dopamine-serotonin hypothesis proposed by Huttunen (1995).

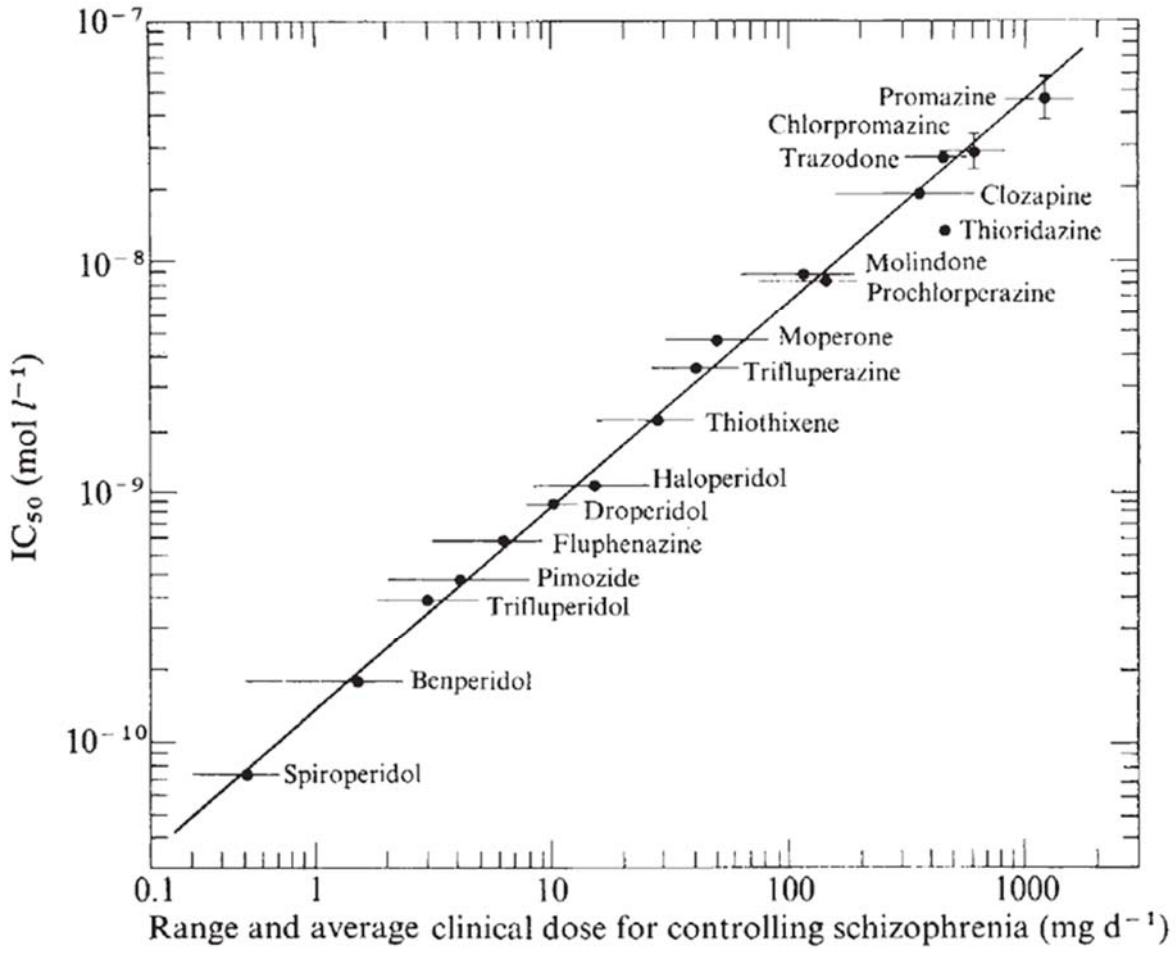

Figure 1.11 The relationship between dopamine receptor half maximal inhibitory concentration and the clinical dose of antipsychotics required for treating schizophrenia (reproduced from Seeman *et al.* 1976)

The dopamine hypothesis of schizophrenia found further support from studies reporting that drugs which increase dopamine release, such as amphetamine, induce transient positive psychotic symptoms in healthy volunteers and worsen psychosis in patients with schizophrenia (Wallis *et al.* 1949, Lieberman *et al.* 1987, Laruelle *et al.* 2000). Positron Emission Tomography (PET) has led to evidence that elevated striatal pre-synaptic dopamine synthesis leads to the psychotic symptoms of schizophrenia (Howes *et al.* 2009). Using [^{18}F]-DOPA uptake to index dopamine synthesis capacity, elevated striatal dopamine synthesis capacity has been found in patients with schizophrenia taking antipsychotics and those that were medication-naïve (Reith *et al.* 1994; Hietala *et al.* 1999; Meyer-Lindenberg *et al.* 2002; McGowan *et al.* 2004; Howes *et al.* 2007), people at risk of psychosis (Howes *et al.* 2009) and first-degree relatives of patients with schizophrenia (Huttunen *et al.* 2008). However, the findings of increased dopamine synthesis in patients with schizophrenia were not replicated in all studies (e.g. Dao-Castellano *et al.* 1997; Elkashef *et al.* 2000). Yet, dopamine elevation is positively correlated with severity of prodromal psychotic symptoms (Howes *et al.* 2009). Furthermore, striatal [^{18}F]-DOPA uptake is a reliable predictor of striatal dopamine release upon amphetamine challenge in non-human primates (Doudet & Holden 2003), but this relationship has not been tested in humans. Subsequently, two separate meta-analyses (Howes *et al.* 2012; Fusar-Poli & Meyer-Lindenberg 2013) have reported that, compared to healthy controls, individuals with schizophrenia have increased striatal dopamine synthesis capacity, associated with a large effect size of d=.79. In addition to studies of presynaptic dopamine synthesis, other aspects of dopaminergic function have also been investigated. In a study using the D_2 receptor SPECT radiotracer [^{123}I]-IBZM following dopamine depletion via α-MPT administration, patients with schizophrenia exhibited increased D_2 receptor availability compared to control subjects. A meta-analysis (Howes et al. 2012) of studies comparing baseline D2 receptor availability reported a small increase in patients with schizophrenia

compared to controls with an effect size *d*=.26. A meta-analysis of studies of dopamine transporter availability (Howes *et al.* 2012) found no significant difference between patients and controls. Elevated dopamine synthesis capacity is therefore the most widely replicated *in vivo* neurochemical finding in psychosis (Howes *et al.* 2012) and has been found to predict the subsequent development of psychosis in at risk individuals (Howes *et al.* 2009) (Figure1.12). However, a meta-analysis of studies of dopamine synthesis capacity using only medication-naïve patients with schizophrenia has yet to be conducted. Therefore, the possibility that these effects are somehow related to medication effects, possibly via effects on D_2 auto-receptors which modulate the dopamine synthesis pathway, cannot be completely excluded.

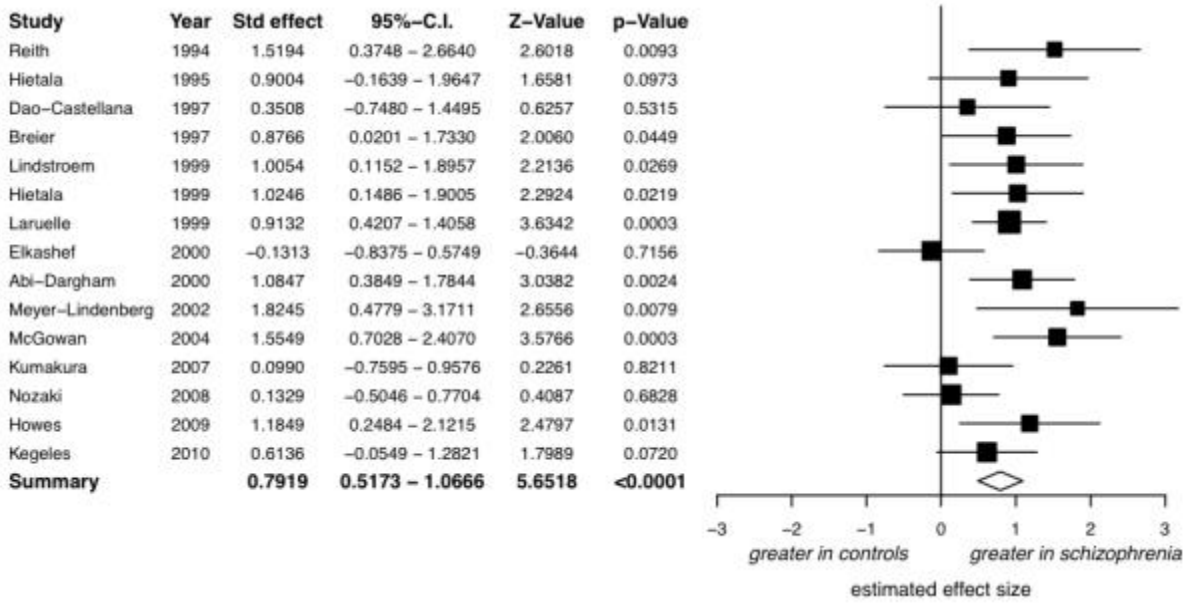

Figure 1.12 Studies of presynaptic dopaminergic function: Forrest plot showing the effect size and 95% confidence intervals of the difference between patients with schizophrenia and controls by study. There was evidence of a significant elevation in schizophrenia with a summary effect size of *d* = .79. Reproduced from Howes *et al.* (2012).

1.5.6.12 Limitations of the Dopamine Hypothesis

The dopamine hypothesis of schizophrenia is not without criticism (e.g. Moncrieff 2008). The main tenets of this relate to cause and effect in terms of dopamine and psychosis, and the mode of action of antipsychotics. Moncrieff argues that the question of whether altered dopamine synthesis necessarily *causes* psychosis still needs to be addressed. In particular, she argues that if patients are more aroused, as would occur in psychosis, then this may account for findings of elevated dopamine synthesis capacity. Moncrieff states that *"abnormalities of neurotransmitters may be better understood as correlations of psychological states than as causes of them. For example, the surge of adrenalin that accompanies a frightening experience does not in itself produce fear… It may be difficult to clarify experimentally whether biochemical states qualify as causes of mental experiences or as symptoms. To establish causality, longitudinal studies would be needed".* Indeed, such studies have begun to yield results supporting the dopamine hypothesis. For example, Howes *et al.* (2009) found that elevated dopamine synthesis is found in the prodromal phase of psychosis, and longitudinal studies are underway.

Rather than targeting a pathophysiological process, Moncrieff *et al.* (2005) proposed that antipsychotics achieve their desired effects via the inducing a characteristic neurological state. For example, in healthy volunteers antipsychotics produce a state of physical and mental slowing, and emotional flattening or detachment (Belmaker & Wald 1977; Healy & Farquhar 1998; McClelland *et al.* 1990). It is argued that these subjective effects may account for the ability of antipsychotics to reduce the impact of psychotic phenomena such as hallucinations or delusions.

Along similar lines, it has also been proposed that there are differences in the clinical phenomenology of amphetamine-induced psychosis compared to schizophreniform psychosis (Snyder *et al.* 1972), such as reduced thought disorder and delusions of control. However, others have argued that the two states are indistinguishable (Batki & Harris 2004; Harris & Batki 2000). Amphetamine-induced psychosis results in abnormal stereotyped movements and posturing in animals (van Rossum & Hurkmans 1964), and this is likewise observed in untreated schizophrenia, albeit without the external motor hyperactivity (Batki & Harris 2004; Owens *et al.* 1982; McCreadie *et al.* 2002).

Furthermore, studies of dopamine metabolite CSF levels have produced conflicting results which may reflect complicated interactions between antipsychotics and dopamine metabolite levels (Widerlov 1988). However, a likely confounding factor in the interpretation of these results is that CSF levels provide a marker of whole brain dopamine metabolism and therefore lack the spatial specificity of chemical imaging studies.

A further complicating factor in the dopamine hypothesis is that, clozapine, which is the treatment of choice for refractory schizophrenia (Taylor *et al.* 2009), has relatively weak affinity for the D_2 receptor (Seeman 2002) and it has been hypothesised that its therapeutic superiority to the other antipsychotics is due to antagonism of the $5HT_{2A}$ receptor (Huttunen 1995). This is particularly interesting given that $5HT_{2A}$ agonism results in marked hallucinations, albeit predominantly visual, as per the mode of action of the "classic" psychedelics such as LSD, psilocybin and mescaline (reviewed in Nichols 2004).

Not all patients with schizophrenia appear to exhibit increases in striatal dopamine synthesis capacity. Demjaha *et al.* (2012) found that patients who do not respond to standard antipsychotic treatment have no significant elevation in dopamine synthesis capacity, compared to controls, whilst patients who do respond to antipsychotic do exhibit increased dopamine synthesis capacity. The authors concluded that this could be either because of differential underlying pathophysiology or differential responsiveness of the dopaminergic system to antipsychotic treatment. The idea that patients who remain symptomatic despite antidopaminergic treatment may not have a primary elevation in dopamine synthesis capacity gave rise to the hypothesis that schizophrenia as a syndrome may arise from multiple neurochemical abnormalities. This argument was later put forward in a paper by Howes & Kapur (2014), which suggested classifying schizophrenia according to the presence or absence of hyperdopaminergia (type A and type B schizophrenia, respectively).

1.5.6.13 Linking Dopamine Dysfunction to Symptoms: The Aberrant Salience Hypothesis

In light of the findings linking dopaminergic dysfunction to schizophrenia it was suggested that dopamine is "the wind of the psychotic fire" (Laruelle & Abi-Dargham 1999). However, a coherent account of how dopaminergic dysfunction gave rise to psychotic symptoms was lacking. Animal models support the view that striatal dopamine release underlies the attribution of motivational salience to stimuli (Berridge & Robinson 1998). Based on this and evidence in humans, a mechanism to explain how elevated striatal dopamine could lead to psychotic symptoms was proposed (Kapur 2003), where dopamine dysregulation leads to the aberrant assignment of salience to internal and external stimuli, and psychotic symptoms are secondary to cognitive rationalisation of these experiences. Kapur's thesis will now be outlined in relation to the role of dopamine in the brain, which was discussed earlier in this chapter.

Kapur limits the extent to which reward prediction error electrophysiological findings (Schultz *et al.* 1997; Schultz 1997) account for dopaminergic function, as findings had neither been related to aversive stimuli nor the longer-term modulatory role of dopamine (Berridge & Robinson 1998; Schultz *et al.* 1997; Redgrave *et al.* 1999). Kapur instead favoured the incentive motivational hypothesis of dopaminergic function proposed by Berridge & Robinson (1999) and others of a similar theoretical persuasion (Horvitz 2000; Martin-Soelch *et al.* 2001; Di Chiara 1998). In line with these theories, the mesolimbic dopamine theory attributes salience to stimuli which Kapur explained as:

"A process whereby events and thoughts come to grab attention, drive action, and influence goal-directed behaviour because of their association with reward or punishment (Berridge & Robinson, 1998; Berridge 1999). This role of dopamine in the attribution of motivational salience does not exclude the roles suggested by previous theorists; instead it provides an interface whereby the hedonic subjective pleasure, the ability to predict reward, and the learning mechanisms allow the organism to focus its efforts on what it deems valuable and allows for the seamless conversion of motivation into action (Berridge & Robinson, 1998; Berridge 1999)."

Kapur emphasised some of the phenomenological aspects of delusion formation, including the fact that the development of schizophrenic psychosis is slow (Yung & McGorry 1996) and follows a series of stages in which a period of heightened awareness and anxiety is followed by a *drive* to make sense of the situation and then followed by a sense of relief which occurs when a "new awareness" is obtained through the crystallisation of the delusion (Yung & McGorry 1996; Bowes & Freedman 1966; Bowers 1968; Roberts 1992). In parallel, dopamine-releasing drugs do not produce psychosis upon single doses in most humans (Yui *et al.* 1999), but do so after chronic administration (Harris & Batki 2000). This is likely due to sensitising effects of these drugs on the dopamine system, whereby initially repeated doses result in greater dopamine release. Furthermore, delusions, Kapur argues, are generally highly improbable, *vs.* impossible beliefs, and are therefore disorders of inferential logic (Roberts 1992), whilst hallucinations are aberrantly recognised internal percepts (David 1999; Bentall 1990; Grossberg 2000), i.e. stimuli that are aberrantly recognised as being external when they are internal in origin, and/or aberrantly produced internal stimuli.

Kapur proposed that under a hyperdopaminergic state, a stimulus-independent release of dopamine could give rise to psychotic symptoms via the "aberrant assignment of salience to external objects and internal representations" such that in a psychotic state the dopamine system creates salience from otherwise neutral stimuli. In Kapur's model, delusions are a top-down cognitive explanation of aberrantly salient stimuli. In other words, an individual tries to make sense of their experiences by using the explanations that will be influenced by their past experiences and culture. He also explains the mechanism of antipsychotic action in the treatment of schizophrenia by dampening salience (Courvoisier 1956), which Kapur argues would include aberrant salience, giving rise to the gradual symptomatic relief which is observed clinically (Miller 1987).

Using L-DOPA and haloperidol Pessiglione *et al.* (2006) found that dopaminergic agents modulated reward processing so that L-dopa resulted in increased reward-learning relative to subjects treated with haloperidol. Subsequently, tasks have been developed to measure aberrant salience including the Salience Attribution Task (SAT) and these have shown that psychotic patients exhibit elevated Aberrant Salience (Roiser *et al.* 2009). In contrast to most other reward-learning tasks, the SAT includes a task-irrelevant stimulus dimension as well as a task-relevant stimulus dimension. The subject's reaction time and subjective rating of reinforcement probabilities for relevant and irrelevant stimuli provide measures of the salience attached to these dimensions. Salience attached to the irrelevant dimension is considered aberrant.

Aberrant salience is directly related to the presence of delusions in medicated patients with schizophrenia (Roiser *et al.* 2009). In a study using fMRI, Murray *et al*. (2008) found a disruption in reward prediction error among first-episode psychosis patients, as well as abnormal activations in dopamine brain regions. A further fMRI study involving healthy volunteers (Roiser *et al.* 2010) and employing the SAT found that participants who showed greater aberrant learning exhibited greater dorsolateral pre-frontal cortex (dlPFC) responses, an area targeted by dopamine projections. Whilst Kapur's model has heuristic value, its predictions are based on animal studies using simple conditioning paradigms and require continued investigation in humans.

Using an fMRI version of the SAT in un-medicated individuals at ultra-high risk of psychosis compared to healthy controls, Roiser *et al.* (2012) found that individuals at ultra-high risk of psychosis were more likely to attribute motivational salience to irrelevant stimulus features and this was related to the severity of their delusion-like symptoms. In addition, it was reported that ventral striatal BOLD responses to irrelevant stimulus features were also correlated with delusion-like symptoms in the ultra-high risk group. The authors concluded that these findings were consistent with and provided support for the Aberrant Salience hypothesis.

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1.5.6.13 Dopamine Dysregulation as a Potential Mechanism Linking Risk Factors to Psychotic Symptoms

In summary, within schizophrenia, striatal dopamine dysfunction has been proposed to underlie the development of psychosis via a process involving 'Aberrant Salience' (Kapur 2003, Kapur 2004, Kapur & Mizrahi 2005, Howes & Kapur 2009). Two major modifiable environmental risk factors for psychosis are cannabis use (Moore *et al.* 2007) and psychosocial stress (Cantor-Graae & Selton, 2005). This thesis aims to determine the relationships between the striatal dopamine system, Aberrant Salience and these environmental risk factors in order to further our understanding of the neurobiology of psychosis.

1.6 Cannabis & Dopamine

1.6.1.1 Animal Evidence

Several lines of evidence indicate that dopamine dysfunction may mediate the link between cannabis exposure and psychosis as has been proposed to occur in schizophrenia. Several early animal studies described the interactions of amphetamine, which increases striatal dopamine release (Costa *et al.* 1972), with THC (Garriott *et al.* 1967; Howes 1973a; Howes 1973b; Kubena & Barry 1970; Pirch *et al.* 1973; Zitko *et al.* 1972), reporting that the behavioural effects of amphetamine may be potentiated or antagonised depending on the dose of administered THC. As Howes & Osgood (1974) suggested, *"dopamine appears to be a prime candidate for consideration in the mode of action of Δ^9-tetrahydrocannabinol"*.

1.6.1.2 THC & Dopamine Synthesis

Synaptosomes have been used as an *in vitro* tool to investigate neurotransmitter release, uptake and synthesis (DeBelleroche & Bradford 1973). The use of rodent synaptosomes has enabled the measurement of dopamine synthesis from tyrosine (Kuczenski & Segal 1974; Thierry *et al.* 1973; Patrick & Barchas 1974). Maitre *et al.* (1970) reported increased [^{3}H]-dopamine synthesis from [^{3}H]-tyrosine in the brains of rats treated with THC, although this may have been related to changes in blood [^{3}H]-tyrosine. Likewise, Bloom *et al.* (1978) reported THC increased dopamine synthesis. It was later found that THC caused a concentration-dependent decrease in the uptake of [^{14}C]-dopamine into striatal synaptosomal preparations from Charles-River mice, along with inducing a small but significant release of [^{14}C]-dopamine from pre-incubated synaptosomes (Howes & Osgood 1974). However, Hershkowitz and Szechtman (1979) reported that THC caused an increase in dopamine uptake in both cortical and striatal synaptosomes, with greater dopamine uptake in striatal *vs*. cortical preparations. Bloom (1982) found that in mice synaptosomes, THC produced increases in the conversion of tyrosine to dopamine, but high doses of THC resulted in decreased dopamine synthesis in both whole brain and striatal synaptosomes (see figure 1.13), in line with previous findings (Poddar & Dewey, 1980). THC also inhibited the active uptake of tyrosine into all synaptosome preparations studied. It was suggested that the decrease in precursor uptake may be the cause of the decreased dopamine synthesis observed at the higher concentrations of THC (Bloom 1982). This was also seen in previous research (Hershkowitz *et al.* 1977). There are however differences in the concentrations of dopamine in synaptosomal subfractions which preclude direct comparisons between studies (Bloom 1982). The dose-related THC increase in the accumulation of newly synthesised dopamine without producing significant change in

the endogenous levels of dopamine was found to be under opioid modulation since pre-treatment with the μ-opioid receptor antagonist naloxone reduced dopamine accumulation by 66% (Bloom & Davey 1978). This ties in with earlier behavioural experiments with Charles-River rats that had shown interactions between THC and morphine via dopaminergic mechanisms (Hine *et al.* 1975). Later microdialysis studies also reported blockade of THC-induced dopamine efflux via μ-opioid receptor antagonism (Chen *et al.* 1990a).

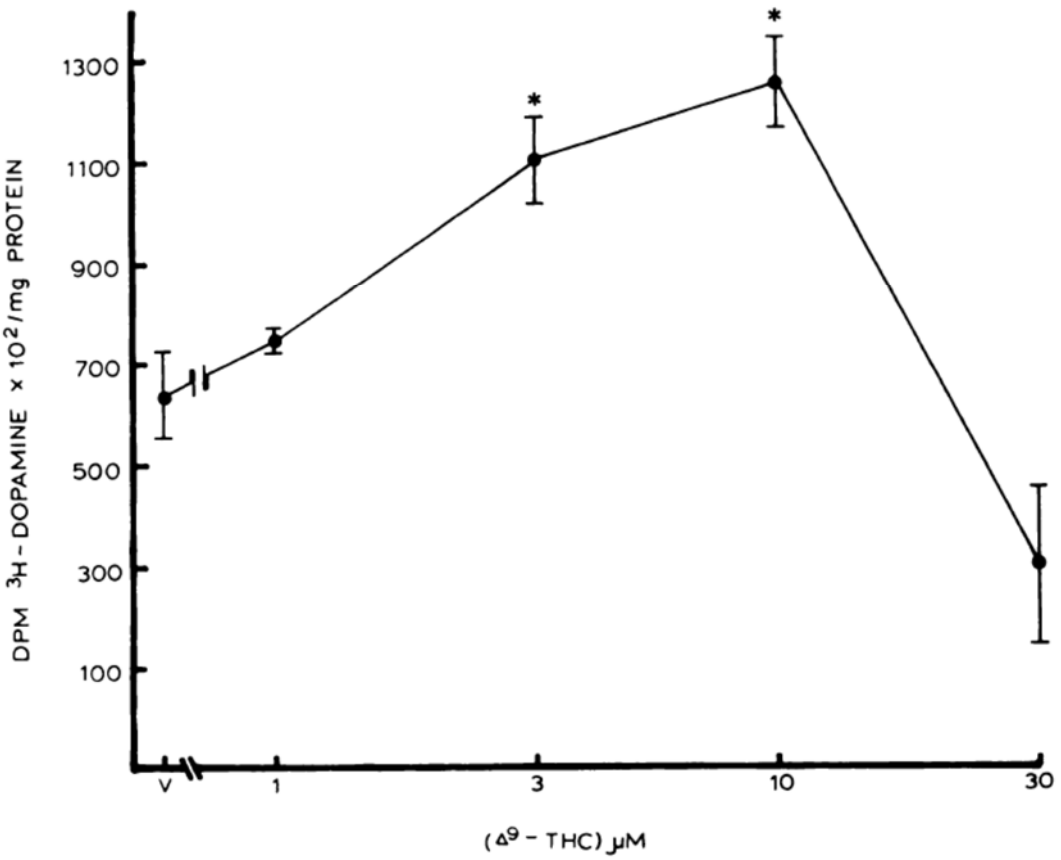


Figure 1.13 The effects of THC on the synthesis of [^{3}H]-dopamine in synaptosomes prepared from mouse striata. Values shown are the mean and error bars indicate standard error of the mean. Samples were incubated with 5 x 10^{-7} M [^{3}H]-tyrosine for 15 minutes. Each sample consisted of the tissue from the striatum of approximately one brain. Dopamine synthesis was significantly increased by the 3 and 10 μM concentrations of THC. The effect was maximal at 10 μM with that concentration producing a 97% increase. Dopamine synthesis was decreased by the 30 μM concentration. * $p < 0.05$ when compared to vehicle controls. (Figure from Bloom [1982])

Since THC induces hypothermia, which in turn can affect dopamine synthesis, the increased synaptosomal dopamine synthesis elicited by THC was shown not to be related to drug-induced hypothermia (Bloom & Kiernan 1980). Whilst some studies found no change in THC-induced dopamine synthesis (Bracs *et al.* 1975) or reductions in synthesis (Fenessy & Taylor 1977), this may have been related to the reported biphasic dopamine response to THC. Interestingly, sleep deprivation was found to result in decreased dopamine turnover in response to THC, whilst normal sleep led to no THC-induced dopamine turnover effects (Carlini *et al.* 1993).

Agrawal *et al.* (1985) compared single *vs*. repeated, over 14 consecutive days of 25, 50, or 100 mg/kg, oral dosing of cannabis extract in mice. Single dose cannabis extract resulted in increased in [^{3}H]-spiroperidol binding, indicative of increased dopamine receptor availability. Multiple doses at 50 and 100mg/kg, resulted in decreased [^{3}H]-spiroperidol binding. Scatchard analysis indicated this was due to a change in receptor affinity (K_D) rather than maximum number of binding sites (B_{max}) implying a functional change in dopamine receptors associated with repeated cannabis exposure.

A study of brain homogenates did not find a significant effect of THC on whole brain dopamine (Taylor & Fennessy 1977). Whilst a later study found that THC did result in increased dopamine turnover rates in the cortex and brainstem (including the striatum) (Bensemana & Gascon 1978). However, following a 90 day dosing of THC in Sprague-Dawley rats, no significant change in dopamine receptor or dopamine levels were observed in the caudate nucleus, hypothalamus, septum or hippocampus (Ali *et al.* 1989).

Bhattacharyya *et al.* (1980) reported a triphasic dopaminergic response to THC in which the drug led to an initial decrease in caudate nucleus and midbrain dopamine, followed by an increase in dopamine and then a return to baseline. Aulakh *et al.* (1980) found a complicated temporal relationship between THC and dopamine. A single dose of THC led to a decrease in dopamine levels in the caudate nucleus (CN) and midbrain immediately post administration. However, repeated daily THC administration led to a decrease in dopamine levels on day 5, and then gradually returning to normal on day 15 in brains that were studied one hour after THC dosing. When the brains were studied two hours after dosing there was an increase in brain dopamine that peaked to a maximum between day 8 and 10 of the regimen before reducing. One potential explanation for the complex temporal course of dopaminergic effects is the finding that THC alters monoamine-oxidase activity. THC has been found to inhibit (Schurr & Livne 1976) and increase (Banerjee *et al.* 1975) the activity of monoamine oxidase.

As described earlier, THC is a partial agonist of endocannabinoid CB1 receptors. More recently, CB1 receptor agonism has been found to selectively increase the expression of tyrosine hydroxylase, a key enzyme in the dopamine synthesis pathway (Bosier *et al.* 2012).

1.6.1.3 THC & Dopamine Release

THC has been found to increase the release of striatal dopamine (Ng Cheon Ton *et al.* 1998) although some findings have been contradictory (Cheer *et al.* 2004). One study found an increase in THC-induced forebrain dopamine efflux (Chen *et al.* 1990b) although the same research group later reported differences in microdialysis results associated with the strain of experimental rat (Chen *et al.* 1991). Microdialysis with Sprague-Dawley rats showed that pre-exposure to THC resulting in behavioural sensitization was associated with reduced stimulation dopamine transmission in the nucleus accumbens shell and an increased stimulation in nucleus accumbens core in response to THC challenge (Cadoni *et al.* 2008). In Long-Evans rats THC had no effect on extracellular concentrations of dopamine, DOPAC or homovanilic acid, and no effect on amphetamine-induced dopamine release nor fluphenazine-induced transient increase in dopamine, DOPAC and homovanilic acid (Castaneda *et al.* 1991).

1.6.1.4 THC & Dopamine Electrophysiology

Electrophysiological studies show acute administration of THC increases ascending mesolimbic dopaminergic nerve firing rates via cannabinoid CB_1 receptor agonism (French 1997; Gessa *et al.* 1998). This finding was replicated by Diana *et al.* (1998a) who also showed that acute THC withdrawal elicited by both abrupt cessation of chronic THC treatment or administration of the cannabinoid CB1 receptor antagonist SR 141716A resulted in decreased dopamine nerve activity (Diana *et al.* 1998b). However, one study did not find an effect of THC on dopaminergic cell activity (Gifford *et al.* 1997). Other dopaminergic projections have also been studied with THC likewise resulting in increased firing (Melis et al. 2000; Moreno-Herreras 2008).

1.6.2 Human Evidence

Patients with cannabis-induced psychoses have elevated levels of peripheral dopamine metabolites (Bowers & Kantowitz 2007) and a case report of a drug-free schizophrenic patient who smoked cannabis between single photon emission tomography (SPECT) scans with the dopamine $D_{2/3}$ receptor antagonist radiotracer [^{123}I]iodobenzamide, found evidence of dopamine release and an exacerbation of symptoms after cannabis use (Voruganti *et al.* 2001). However, only 1 of 3 subsequent molecular imaging studies of the acute effects of THC in healthy volunteers has found evidence of dopamine release (Bossong *et al.* 2009, Barkus *et al.* 2011, Stokes *et al.* 2009), suggesting that the acute effects of THC on dopaminergic function may not be large or consistent in humans. These studies all administered 10mg or less of THC because older pharmacological studies indicated that the "standard joint" (defined by the US National Institute of Drug Abuse) delivered an approximate THC dose of 8mg-15mg (Lindgren et al. 1981, Smart & Adlaf 1986). Yet, the THC content of cannabis has increased significantly since these studies (Hardwick & King 2008), such that THC doses may be over 40mg per *spliff* (joint; cannabis cigarette) (Hunault et al. 2010), and so the THC doses used in those imaging studies may not be sufficient to elicit a consistent dopamine release. Furthermore, there is evidence of sensitisation of the dopamine response to THC in animals (Gorritti *et al.* 1999); repeated THC administration results in a greater post-synaptic response, indicating that dopaminergic effects are likely to be greater in regular cannabis users.

Recent studies in ex-cannabis users have found no significant difference in striatal dopamine release or post-synaptic dopamine D_2/D_3 receptor availability (Urban *et al.* 2012,

Stokes *et al.* 2012) compared to individuals with no history of chronic cannabis use. However, as these studies were in abstinent ex-cannabis users, it is not clear if this is due to the lack of dopaminergic effects of cannabis in humans or the normalisation of dopaminergic function with abstinence, as has been reported to be the case with dopamine transporter availability in abstinent cocaine users (Volkow *et al.* 1996) and alcohol users (Volkow *et al.* 1996). Therefore presynaptic dopaminergic function will be studied in active cannabis users who experienced cannabis-induced psychotic-like symptoms because these individuals are most at risk of psychosis (Arendt et al. 2005).

1.7 Psychosocial Stress & Dopamine

1.7.1 The Stress Response and Dopamine

The stress response can be described as the brain and body's response to a threat or potential threat or from internal cues such as memories (Mora *et al.* 2012). This requires the involvement of multiple brain regions including the prefrontal cortex, amygdala, hippocampus, nucleus accumbens, hypothalamus and dynamic feedback processes involving multiple neurotransmitters and neuromodulators including dopamine, noradrenaline, acetylcholine and GABA (Joels & Baram 2009; Ulrich-Lai & Herman 2009). The role of fast limbic inputs in cortical arousal will be briefly described below, followed by a description of the HPA axis.

Stressors produce a change in extracellular concentrations of multiple neurotransmitters resulting in the activation and modulation of behavioural processes to cope with the stressor (Robbins 1997; Robbins 2005) and indirect outputs to the hypothalamus modulating the final stress responses by increased sympathetic output and the activation of the HPA axis (Ulrich-Lai and Herman, 2009).

Under normal conditions, the prefrontal cortex (PFC) regulates behaviour, thought and emotion in a top-down fashion. The PFC has direct and indirect connections to monoamine cell bodies in the brainstem including the origins of noradrenergic projections in the locus coeruleus and dopaminergic projections in the substantia nigra (SN) and ventral tegmental area (VTA). The PFC can therefore regulate its own catecholamine levels. The ventromedial prefrontal cortex (VMPFC) has extensive connections with subcortical structures including the

amygdala, the nucleus accumbens and the hypothalamus that generate emotional responses (Price & Amaral 1981; Price *et al.* 1996; Ghashghaei & Barbas 2002). Under stress conditions, the amygdala activates stress pathways in the hypothalamus and brainstem, resulting in increased noradrenaline and dopamine release (see below). This increased catecholamine output during stress strengthens amygdala mediated fear conditioning (Debiec & LeDoux 2006). In parallel, acute stress impairs higher-order PFC functions such as working memory and attention regulation. Thus, attention regulation switches from 'top-down' control by the PFC (Goldman-Rakic 1987) to 'bottom-up' control, whereby salient stimuli capture attention (Gazzaley et al. 2007). In addition, the amygdala biases behaviour away from flexible spatial navigation towards habitual motor responding (Elliott & Packard 2008). Therefore, during stress, the brain's response patterns switches from slower, more thoughtful PFC regulation to the reflexive and rapid emotional responses of the amygdala and related subcortical structures.

Processed sensory information enters the limbic system via the amygdala (LeDoux 2000; McGaugh 2004) which receives dopaminergic, noradrenergicc, serotonergic, cholinergic inputs and afferents releasing glutamate from multiple brain regions (Arnsten 2009; Carlsen *et al.* 1985; Mo *et al.* 2008; Pérez de la Mora *et al.* 2010). Stress results in acute amygdalar increases in these neurochemicals, including dopamine (Galvez et al. 1996; Tanaka *et al.* 1991; Kawahara *et al.* 1993; Mo *et al*. 2008; Inglis & Moghaddam 1999; Stevenson *et al.* 2003; Reznikov *et al.* 2007; Singewald *et al.* 2000), with the exception of acetylcholine (Mark *et al.* 1996).

In terms of the dopamine system, the ventral tegmental area projects to and receives inputs from a number of regions that are involved in the stress response. These regions include the amygdala, prefrontal and cingulate cortices, hippocampus and nucleus accumbens. Other projections include those from the hypothalamus to the VTA such as those arising from the pre-optic area (Simerly & Swanson, 1988; Zahm *et al.* 2011) which are likely involved in the stressors associated with sexual behaviour (Hull & Dominguez 2007; Hull *et al.* 2007). Activation of glutamatergic afferents results in increased VTA activity (Figure 1.14; see also figure 1.15 for further circuitry). On the other hand, the VTA receives inhibitory inputs via the tail of the VTA (tVTA), also called the rostromedial tegmental nucleus (RMTg) which is rich in GABA neurons (Perotti et al. 2005; Bourdy & Barrott 2012; Jhou *et al*. 2009). Since wakefulness is also essential to the stress response, the VTA also receives cholinergic input from the reticular activating system via nicotinic receptors (Liu *et al*. 2012). It is thought that tobacco addiction involves this cholinergic-dopaminergic mechanism (Balfour *et al.* 2000; Pidoplichko *et al.* 1997), through the main addictive compound in tobacco, nicotine (Stolerman *et al.* 1995)

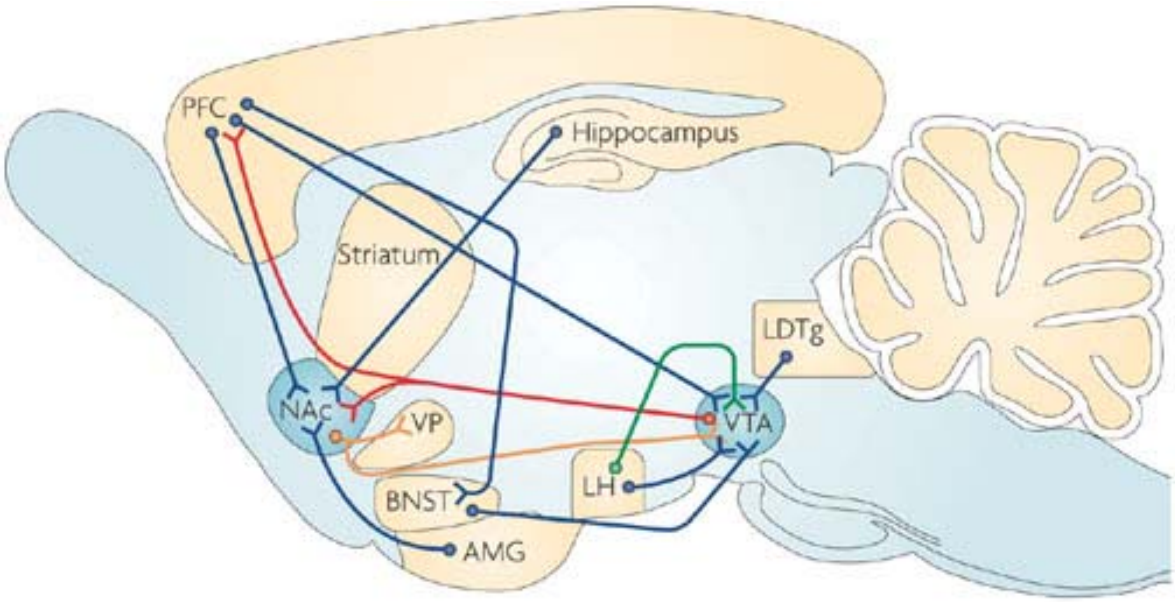


Figure 1.14 Mesolimbic dopamine system circuitry. Simplified schematic of the circuitry of the mesolimbic dopamine system in the rat brain highlighting the major inputs to the ventral tegmental area (VTA) (glutamatergic projections, blue; dopaminergic projections, red; GABAergic projections, orange; orexinergic projections, green). Glutamatergic synapses excite postsynaptic neurons and GABAergic synapses inhibit postsynaptic neurons. Dopamine release exerts more complex modulatory effects. AMG, amygdala; BNST, bed nucleus of the stria terminalis; LDTg, laterodorsal tegmental nucleus; LH, lateral hypothalamus; PFC, prefrontal cortex; VP, ventral pallidum. (Figure from Kauer & Malenka 2007).

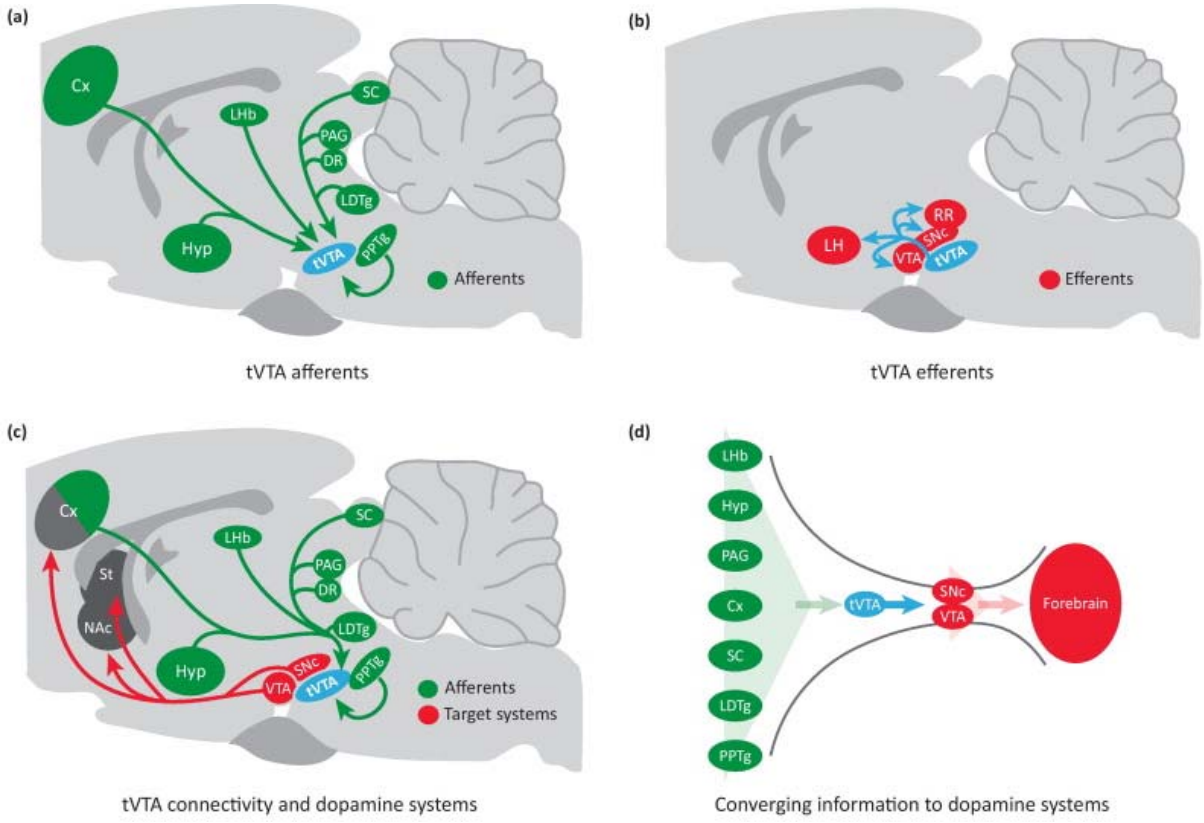


Figure 1.15 Schematic of tVTA main connectivity. (a) The tVTA receives afferents (in green) from a broad range of cerebral structures. The main afferents arise from the frontal cortex (Cx), lateral habenula (LHb), hypothalamus (Hyp), superior colliculus (SC), periaqueductal gray (PAG), dorsal raphe (DR), laterodorsal tegmentum (LDTg), and pedunculopontine tegmental nucleus (PPTg). (b) The tVTA efferents (in red) are more restricted and preferentially target midbrain dopamine nuclei: ventral tegmental area (VTA), substantia nigra pars compacta (SNc) and the retrorubral field (RR). The tVTA also heavily projects to the lateral hypothalamus (LH). (c) The tVTA is well placed to exert control of both mesocorticolimbic and nigrostriatal systems. (d) The tVTA may act as a hub, integrating converging multimodal signals from widespread structures and channelling them toward dopamine systems and their forebrain targets. It is important to note that these tVTA afferents (in green) also directly project to the VTA. Abbreviations: NAc, nucleus accumbens; St, dorsal striatum (Figure from Bourdy & Barrot 2012).

Early theoretical work (Zubin & Spring 1977) formulated a stress-diathesis model of schizophrenia suggesting endogenous vulnerability interacted with environmental stressors leading to the illness. Potential interactions between stressors and neurobiological mechanisms have been suggested including dopaminergic sensitization whereby exposure to sensitising stressors leads to increased neurochemical activation possibly via the hypothalamo-pituitary-adrenal (HPA) axis (e.g. Yuii *et al.* 2007).

1.7.2 Preclinical Evidence

1.7.2.1 The Hypothalamo-Pituitary-Adrenal (HPA) Axis

In addition to the fast neurotransmission described above, at the whole organism level, the acute stress response is mediated via the HPA axis (Armario *et al.* 2006), which regulates the release of corticosteroids. Perception of a potentially threatening situation, results in activation of the hypothalamic paraventricular nucleus (PVN), stimulating corticotrophin-releasing hormone (CRH) and vasopressin neurons. This leads to adrenocorticotrophic hormone (ACTH) release from the anterior pituitary gland, which in turn causes cortisol, a corticosteroid, to be released from the adrenal glands. A circadian pattern of adrenal corticosteroid release has been observed as a release of corticosteroids occurs in hourly pulses that are highest in amplitude during the active period (Young 2004). Background pulsatility has been proposed to maintain tissue-responsiveness to stress-induced peaks in corticosteroid release (Lightman & Conway-Campbell 2010).

1.7.2.2 Corticotrophin-Releasing Hormone Pharmacology

The corticotrophin-releasing factor (CRF) system is a major modulator of the stress response. At the hypothalamic–pituitary level, it is a potent stimulator of adrenocorticotrophic hormone (ACTH) (Vale *et al.* 1981). Furthermore, CRF is located in various corticomesolimbic structures including the amygdala, the bed nucleus of the stria terminalis (BNST), and the septum, where it is thought to be involved in the integration of emotional responses to stress (Chalmers *et al.* 1995; Lovenberg *et al.* 1995), as well as brainstem structures, where they regulate the autonomic stress response (Valentino *et al.* 1991). Two types of CRF receptors, type 1 (CRF1) and type 2 (CRF2), have been identified. CRF1 receptors are expressed primarily in the medial septum, pituitary, cortex, cerebellum, hindbrain, and olfactory bulbs, whereas CRF2 receptors are found in the lateral septum, ventromedial hypothalamus (VMH), medial amygdala, paraventricular nucleus (PVN), and choroid plexus (Chalmers *et al.* 1995; Van Pett *et al.* 2000).

1.7.2.3 Corticosteroid Neuropharmacology

Corticosteroids readily enter the brain and bind to the mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR) (Reul & de Kloet 1985). Activation of the receptors results in waves of gene regulation including transactivation and transrepression (Datson *et al.* 2008). The mineralocorticoid receptor and glucocorticoid receptor result in differential gene regulation (Datson *et al.* 2001) and have different localization patterns in the brain. The mineralocorticoid receptor is mainly expressed in limbic neurons i.e., the PFC, amygdala and hippocampus (Reul & de Kloet 1985). The glucocorticoid receptor is expressed throughout the brain on both neurons and glia, with highest expression in the PVN and hippocampus (Reul & de Kloet 1985). The mineralocorticoid receptor is established in maintaining homeostasis of limbic circuits (Joëls *et al.* 2008) whilst glucocorticoid receptor becomes activated only after exposure to stress or during the circadian peaks, reacting to the stress response (de Kloet & Reul 1987). In cognition, the mineralocorticoid receptor is involved in appraisal of novel situations, while activation of the glucocorticoid receptor facilitates the consolidation of stress-related information (de Kloet *et al.* 1999).

Adaptation to a stressful situation is a coordinated effort of the limbic system, consisting of the hippocampus, amygdala and PFC. Collectively, these limbic areas also facilitate the formation of a memory trace of the event. Processing of contextual aspects depends predominantly on hippocampal function. The hippocampus is a region with one of the highest concentrations of corticosteroid receptors in the mammalian brain (Kim & Diamond 2002). One function of the hippocampus is to participate in terminating the stress response

through glucocorticoid-mediated negative feedback that inhibits the hypothalamus–pituitary–adrenal (HPA) axis (Sapolsky et al. 1992; McEwen & Sapolsky 1995).

The primary physiological model of memory storage has been long-term potentiation (LTP) whereby a sustained enhancement of synaptic efficacy is produced by stimulation of excitatory afferent fibres in line with Hebb's theorem (1949). Activation of GRs impairs hippocampal LTP whereas activation of MRs results in the facilitation of LTP (Pavlides *et al.* 1995; Kim & Yoon 1998; Smigra *et al.* 1998; Pavlides & McEwen 1999). In addition to modulating LTP and therefore hippocampus dependent memory, corticosterone exerts strong genomic effects on the activity and plasticity of the hippocampus (McEwen 2001; Kim & Diamond 2002; Mirescu & Gould 2006; Joëls 2008). Low levels of corticosterone, through mineralocorticoid receptor activation, facilitate plasticity and hippocampus-dependent memory (Diamond *et al.* 1992). By contrast, absence or very high levels of corticosterone inhibits plasticity; the latter is mediated through the glucocorticoid receptor (Alfarez *et al.* 2002; Kim *et al.* 2004).

In recent years, corticosteroids have been found to have rapid, non-genomic effects (e.g. Groeneweg *et al.* 2011). Similar to neurons in the hypothalamus, hippocampal neurons spontaneously show miniature excitatory postsynaptic currents (mEPSCs). Corticosteroids have been found to rapidly increase the frequency of hippocampal mEPSCs in a manner not dependent on gene transcription (Karst *et al.* 2005). This mechanism likely interacts with other transmitters and hormones, including monoaminergic systems such as dopamine, in the limbic system and HPA axis as part of the stress response. It has been shown for example that the

administration of the potent dopamine transport blocker cocaine (benzoylmethylecgonine) and stress are both associated with increased strength at excitatory synapses on midbrain dopamine neurons, with the synaptic effects of stress but not cocaine being blocked by the glucocorticoid receptor antagonist RU486 (Saal *et al.* 2003).

1.7.2.3 The Stress Response & Dopamine

Preclinical studies indicate that both acute and chronic stress alter mesocortical dopaminergic function. Tidey (1996) found that social defeat stress selectively increased mesocorticolimbic dopamine release and Hall *et al.* (1999) found that maternal deprivation of neonatal rats produced enduring increases in dopamine release in the nucleus accumbens. Likewise, rats who had undergone neonatal isolation were found to have greater cocaine-induced increased in nucleus accumbens dopamine as measured with microdialysis (Kosten 2003). More recently social defeat has been found to result in increased phasic dopamine neuron firing in the VTA three weeks after a 10 day exposure to social defeat stress (Razzoli *et al.* 2011). However, in an experiment using Sprague−Dawley rats, unavoidable stress administered over one week and three weeks was associated with a decrease in nucleus accumbens dopamine output that remained evident two weeks after administration of stress had abated (Mangiavacchi *et al.* 2001). Likewise, Long-Evans rats confronted with 30 minutes of stress twice daily for 21 days exhibited a reduced cocaine-induced nucleus accumbens dopamine response (Shimamoto *et al.* 2011). These divergent results may underlie the nature of the stress paradigm deployed in different studies. For example, Miczek *et al.* (2011) found that Long-Evans rats under a ten day episodic defeat paradigm had a sensitised dopamine response in the nucleus accumbens, whilst when under a 5 week continuous subordination paradigm they exhibited a suppressed dopamine response.

There is evidence that altered dopaminergic function caused by early life persists into adulthood. For example, maternally separated and non-handled Long-Evans rats responded to

a mild stressor with greater increases in nucleus accumbens dopamine levels and maternally separated rats had reduced nucleus accumbens-core and striatal dopamine transporter sites and increased dopamine D_3 receptor binding and D_3 mRNA levels in the nucleus accumbens-shell (Brake *et al.* 2004). Likewise, Lister hooded rats that were reared in isolation for six to eight weeks post-weaning exhibited increased and prolonged extracellular dopamine responses to acute stress compared to group-reared rats (Fulford & Marsden 1998). In a study of the effects of maternal separation on Wistar rats, a transient decrease in substantia nigra pars reticulata and ventral tegmental neurons expressing tyrosine hydroxylase was observed at post-natal day 15 in males and females, followed by an increase at post-natal day 70 in the numbers of ventral tegmental tyrosine hydroxylase expressing neurons in females only (Chocyk *et al.* 2011). However, maternal separation in Sprague-Dawley rats was associated with decreased acute stress ventral tegmental tyrosine hydroxylase expression and reduced nucleus accumbens and midbrain levels, compared to normally housed animals (Jahng *et al.* 2010). Yet, repeated periods of maternal separation results in decreased dopamine transporter expression and increased dopamine response to acute stress, and behavioural responses to stress or cocaine (Meaney *et al.* 2002). Again, the discrepancy in findings may reflect divergent effects of differential stressors on distinct aspects of dopaminergic function, although species effects cannot be excluded. Nonetheless, there is consistent evidence that early life stress results in aberrant dopaminergic function later in life.

1.7.3 Human Evidence

Elevated urinary dopamine and other catecholamine metabolites has been reported in girls with a history of sexual abuse compared to those without (De Bellis *et al.* 1994). In an fMRI study, left basal ganglia reward pathway dysfunction was observed in adults who were abused as children (Dillon *et al.* 2009). In a further fMRI study, adolescents who had suffered severe early life deprivation exhibited ventral striatal hyporesponsivity during anticipation of monetary reward (Mehta *et al.* 2010). In an [^{11}C]-raclopride study by Pruessner *et al.* (2004) ventral striatal dopamine release was increased in response to psychosocial stress in humans who reported insufficient early life maternal care. Similarly, a further [^{11}C]-raclopride study Wand *et al.* (2007) found stress-induced cortisol levels were positively associated with amphetamine-induced dopamine release in the striatum. More recently, using the radioligand [^{11}C]-PHNO, a $D_{2/3}$ agonist, Mizrahi *et al.* (2011) found increased psychosocial stress-induced dopamine release in the associative and sensorimotor functional subdivisions of the striata of antipsychotic-naïve subjects with schizophrenia and those at clinical high-risk of the illness compared to matched healthy controls, possibly indicative of a sensitised dopaminergic stress response (see figure 1.16). Whilst these findings support acute dopamine release in response to stress in humans, it is unknown if long-term psychosocial stress has the same effect. A subsequent study by Oswald *et al.* (2014) reported positive associations between childhood adversity and amphetamine-induced dopamine release. Elevated dopamine synthesis capacity is associated with people at clinical risk of schizophrenia (Howes *et al.* 2009). People at clinical risk of schizophrenia, including those in that sample, report higher rates of long-term psychosocial stressors. However it is not known if this is associated with the altered dopamine synthesis capacity, or if they are unconnected and stress acts on cortical regions to cause cognitive impairments seen in schizophrenia and at risk individuals.

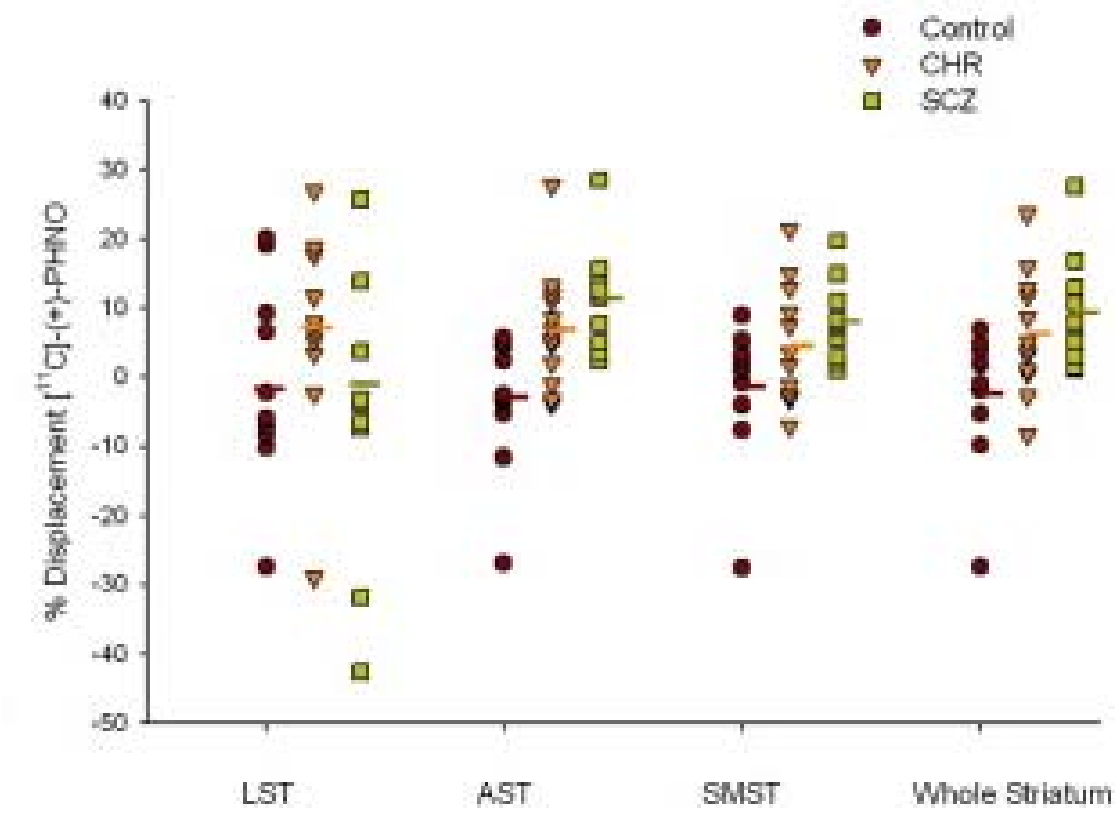


Figure 1.16 [^{11}C]-PHNO positron emission tomography response to stress in the corpus striatum and its functional subdivisions. AST, associative striatum; CHR, clinical high risk group; LST, limbic striatum; SCZ, schizophrenia group; SMST, sensorimotor striatum.

In summary, the dopaminergic system has complex reciprocal projections with many areas involved in the stress response, including afferents from multiple transmitter systems and there is evidence from preclinical and clinical research that stress alters dopaminergic function.

1.8 The Final Common Pathway Theory

As is evidenced above, the hypothesis that dopaminergic mechanisms are central to psychosis has become highly influential in our understanding of the neurobiology of schizophrenia. Howes & Kapur (2009) synthesised previous hypotheses, research evidence from the literature, and Kapur's Aberrant Salience hypothesis into a key paper in which various risk factors for schizophrenia converge on dopaminergic pathways to cause psychosis through creating a state of aberrant salience, which they termed the "Final Common Pathway" theory. The authors discuss the main developments of the dopamine hypothesis, namely the discovery of the dopaminergic action of antipsychotic drugs (Carlsson & Lindqvit 1963) and the theory proposed by Davis *et al.* (1991) relating positive symptoms to subcortical hyperdopaminergia and negative symptoms to frontal hypodopaminergia, which Howes and Kapur refer to as versions I and II of the dopamine hypothesis, respectively. Howes and Kapur conceptualise their Final Common Pathway theory as version III of the dopamine hypothesis. A summary of the rationale for the Final Common Pathway follows.

As described previously, a consistent finding in schizophrenia imaging research which has been subject to meta-analysis has been that patients with schizophrenia have elevated striatal dopamine synthesis capacity (Howes *et al.* 2012). Likewise, there is evidence of increased striatal dopamine release in schizophrenia (Abi-Dargham *et al.* 1998; Breier *et al.* 1997; Kestler *et al.* 2001; Laruelle *et al.* 1996; Laruelle *et al.* 1999) and a dopamine depletion study indicated that baseline occupancy of D_2 receptors is increased in schizophrenia (Abi-Dargham *et al.* 2000). Furthermore, in terms of treatment, all currently licensed antipsychotic drugs block D_2 receptors (as reviewed by Frankle & Laruelle 2002). The emergent genetic evidence is also in keeping with a role for dopaminergic dysfunction in schizophrenia. Whilst

other genes are also significant at the genome-wide level, a significant contributor to the polygenic risk of schizophrenia is likely to be the DRD2 gene, as identified by the Schizophrenia Working Group of the Psychiatric Genomics Consortium (2014) genome-wide association study.

A variety of environmental psychosocial risk factors, described previously, increase the risk of schizophrenia. Many of these share the common factor of being psychosocial stressors. These risk factors include migration, urban upbringing and abuse during childhood, and these relate to experiences of social isolation or subordination (van Winkel *et al.* 2008). Evidence from animal studies indicate that both social isolation and subordination are associated with hyperdopaminergia (Hall *et al.* 1998; Hall *et al.* 1999; Morgan *et al.* 2002; Tidey *et al.* 1996), as are models of maternal separation (Kehoe *et al.* 1996a; Kehoe *et al.* 1996b). Other risk factors including obstetric complications have also been linked in animal models mesostriatal hyperdopaminergia (Boksa & El-Khador 2003).

It is suggested that the multiple genetic and environmental risk factors for schizophrenia interact with each other in terms of their effects on the dopamine system, for example social isolation rearing potentiates the later effects of simulants (Howes *et al.* 2000; Jones 1992) or of stress (Fulford & Marsden 1998). Likewise in humans, striatal dopamine release in response to stress was elevated in people who reported low maternal care during their early childhood (Pruessner *et al.* 2004). A further area of the Final Common Pathway hypothesis is that research has also extended to the schizophrenic prodrome and the extended, or schizotypal, phenotype. As described above, these groups exhibit hyperdopaminergia (Abi-Dargham *et al.* 2004; Howes *et al*. 2011).

To encapsulate, in describing their Final Common Pathway (Figure 1.17), Howes and Kapur (2009) wrote:

> *"Firstly we hypothesise that multiple "hits" interact to result in dopamine dysregulation – the final common pathway to psychosis in schizophrenia… Second, the locus of dopamine dysregulation moves from being primarily at the D_2 receptor level to being at the presynaptic dopaminergic control level. Third, dopamine dysregulation is linked to "psychosis" rather than schizophrenia, and perhaps in the fullness of time it will be about "psychosis proneness". The exact diagnosis, however, reflects the nature of the hits coupled with sociocultural factors and not the dopamine dysfunction per se. And finally, the dopamine dysregulation is hypothesised to alter the appraisal of stimuli, perhaps through a process of aberrant salience."*

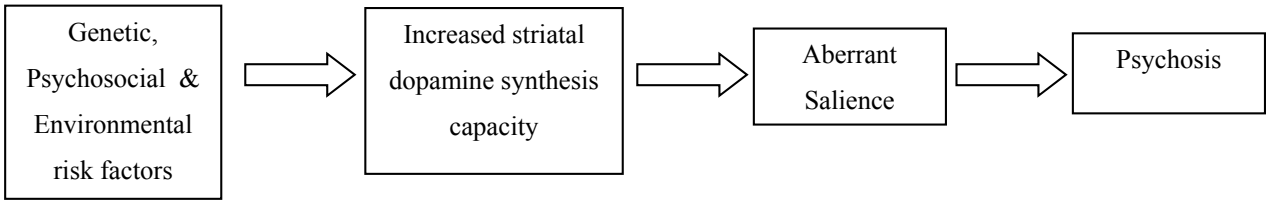


Figure 1.17 Multiple hits interact to result in striatal dopamine dysregulation to alter the appraisal of stimuli and resulting in psychosis, whilst current antipsychotic drugs act downstream of the primary dopamine dysregulation (adapted from Howes & Kapur 2009).

1.9 Summary

Schizophrenia is a potentially devastating mental illness with a complex aetiology. With the exception of 22q11 genetic deletion, the odd ratios for environmental risk factors for the disorder are greater than the odds ratios of any single gene hitherto identified. Within schizophrenia, striatal dopamine dysfunction has been proposed to underlie the development of psychosis. Two important epidemiological risk factors for the disorder are chronic cannabis use and long-term psychosocial stress, both of which have evidence supporting effects on the dopamine system and that the Salience Attribution hypothesis provides an explanatory model based on empirical findings to explain how psychotic symptoms may develop. Thus, elevated dopamine synthesis capacity may represent the final common neurobiological pathway (Howes & Kapur 2009). Environmental risk factors are by their very nature modifiable, and so this thesis will therefore examine whether these environmental risk factors are associated with the same dopaminergic abnormalities that have been observed in schizophrenia.

1.10 Hypotheses Relating to this Thesis

1. Regular cannabis users sensitive to cannabis' psychotogenic effects will exhibit elevated dopamine synthesis capacity compared with non-user healthy control participants.

2. There will be a direct relationship between dopamine synthesis capacity and cannabis-induced psychotic-like symptom severity.

3. Regular cannabis users sensitive to cannabis' psychotogenic effects will exhibit elevated aberrant salience compared with non-user healthy control participants.

4. There will be a direct relationship between aberrant salience attribution and cannabis-induced psychotic-like symptom severity.

5. Subjects with a high cumulative exposure to psychosocial risk factors for schizophrenia will exhibit elevated dopamine synthesis capacity compared to subjects with low cumulative exposure to the same environmental stressors.

6. Across subjects there will be a direct relationship between dose of psychosocial stress and dopamine synthesis capacity.

Chapter 2: Materials and Methods

Methods are described in this Chapter starting with those used in positron emission tomography and followed in a behavioural experiment of salience attribution.

2.1 Ethical Approval

The studies were granted favourable ethical opinions via the National Research Ethics Service. Permission to administer radioligand was provided by the Administration of Radioactive Substances Advisory Committee (ARSAC). The studies were conducted in accordance with the Helsinki Declaration (World Medical Association, 2008). All subjects provided informed written consent to participate after an oral and written explanation of the studies.

2.2.1 Participant Recruitment

Inclusion criteria for all participants were: minimum age 18 years, good physical health with no history of major medical condition and capacity to give written informed consent. Exclusion criteria for all subjects were current or past psychiatric illness (except Cannabis Use Disorders in the cannabis user group and Nicotine Use Disorder in all subjects) using the Structured Clinical Interview for DSM-IV (SCID) (First *et al.* 1996), current use of psychotropic medication, history of serious mental illness (including psychosis) in a first degree relative determined via the Family Interview for Genetic Studies (FIGS) (NIMH Genetics Initiative 1992), evidence of an At Risk Mental State for psychosis (Phillips *et al.* 2000), DSM-IV TR (American Psychiatric Association 2005) Substance Dependency or Abuse (other than cannabis in the cannabis user group and tobacco for all subjects), blood and needle

phobia, contra-indications to PET (including pregnancy, breast-feeding, severe obesity, previous clinical procedures involving exposure to significant ionizing radiation within the last year), contra-indications to MRI (including claustrophobia, the presence of ferromagnetic foreign bodies including aneurysm clips, neural stimulators, cardiac pacemakers or defibrillators, cochlear implants, ocular foreign bodies [e.g. metal shavings], other implanted medical devices [e.g. Swan-Ganz catheter], insulin pump, shrapnel or bullet injuries, patients with a history of surgery of uncertain type where the presence of metal clips or wires cannot be excluded).

Detailed drug histories were obtained from all subjects using the Cannabis Experience Questionnaire (CEQ) (Barkus *et al.* 2006), structured interview and timeline follow-back. Lifetime cannabis use was estimated as the total number of '*spliffs'* (cannabis cigarettes; '*joints'*) consumed. The time taken to smoke an '*eighth'* of cannabis ($^{1}/_{8}$ Ounce; approximately 3.5g, representing the standard unit of sale in Britain) was chosen as the primary index of cannabis use as this provides a measure of the amount of current drug consumption (shorter time indicating greater consumption). This is likely to be more accurate than subjective recall of the number of '*spliffs'* consumed because of variability in cannabis dose between '*spliffs'* and inconsistencies in self-reported cannabis use (Akinci *et al.* 2001).

2.2.2 Cannabis User Group

Cannabis user *cases* were recruited from an on-going cohort study in which over 400 cannabis users were tested when intoxicated with cannabis and when not intoxicated (Morgan *et al.* 2012). Subjects met the following criteria: current, at least weekly use of cannabis and the induction of psychotic-like symptoms in response to smoking cannabis, which was defined

as a positive change on the psychotic items score of the Psychotomimetic States Inventory (PSI) (Mason *et al.* 2008) measured 5 minutes after smoking their usual amount of cannabis (i.e. when acutely intoxicated) compared to when not intoxicated with the drug. Cannabis users consumed their own cannabis and testing occurred in the presence of a researcher in the environment where users habitually consumed cannabis in their usual drug-taking context (e.g. at home), as drug effects are typically larger in naturalistic as opposed to laboratory environments (Barkus *et al.* 2006). Cannabis-induced psychotic-like symptoms abated within two hours of consumption and no subject met the DSM-IV TR criteria for a diagnosis of a psychotic disorder. The psychotic items from the PSI covered 'Delusional Thinking', 'Perceptual Distortions', 'Cognitive Disorganization' (thought disorder) and 'Paranoia'. Each item is rated on a 4-point scale from "not at all" (score=0) to "strongly" (score=3). Examples of items include "People can put thoughts into your mind" and "You can sense an evil presence around you, even though you cannot see it". A sample of the cannabis that each participant smoked was taken on the day of testing and analysed for levels of THC (Forensic Science Service, UK).

2.2.3 Control Group (Cannabis studies)

Non-user control subjects were recruited from the same geographic area, i.e. London, by public advertisement in a newspaper. Control subjects were required to have no lifetime history of cannabis dependence or abuse (DSM-IV), no more than 10 total uses of cannabis in their lifetime, no report of the induction of psychotic symptoms by cannabis, and no history of cannabis use in the preceding three months. Community surveys indicate over 30% of young adults in England report trying cannabis in their lifetime (Home Office 2011). Control subjects were therefore permitted to have had a minimal exposure to cannabis to ensure the control

group was representative of the same general population from which the cannabis users were recruited.

2.2.4 High Psychosocial Stress Group ("HPSS")

Subjects with a history of high exposure to psychosocial stress were recruited by advertisement in a London-wide newspaper. HPSS *"cases"* were required to meet at least two of the following criteria:

A) Inner city upbringing (before age 16) or current dwelling according to the Office for National Statistics (ONS) definition of Inner London used by Eurostat i.e. residents of the City of London & the following London Boroughs: Camden, City of Westminster, Hackney, Hammersmith & Fulham, Haringey, Islington, Kensington & Chelsea, Lambeth, Lewisham, Newham, Southwark, Tower Hamlets, and Wandsworth.

AND/OR

B) History of Migration from outside the European Union (1st or 2nd generation)

AND/OR

C) History of childhood (before age 16 years) adversity and/or psychological trauma - at least one of: Parental separation/divorce OR death of a first-degree relative OR physical or sexual abuse OR neglect OR going into foster care/adoption OR major disaster OR war OR admission to hospital with life-threatening medical problem

AND/OR

D) Current adult adversity - At least one of: living with parents OR living alone for over a year OR lack of confidant OR unemployed OR not currently in a relationship.

2.2.5 Low Psychosocial Stress Group ("LPSS")

LPSS *"control"* subjects were recruited from the southern United Kingdom by public advertisement and were required to meet all of the following criteria:

A) No personal history of migration from outside the European Union.

AND

B) Current rural dwelling

AND

C) No history of inner city dwelling (as above) for longer than 6 months.

AND

D) No history of significant childhood difficulty: Both parents still together at subject's age 16 and no history of significant childhood psychological trauma (as above)

AND

E) No history of significant life-events in the 6 months prior to the study.

2.3 Positron Emission Tomography

2.3.1 Background to Positron Emission Tomography

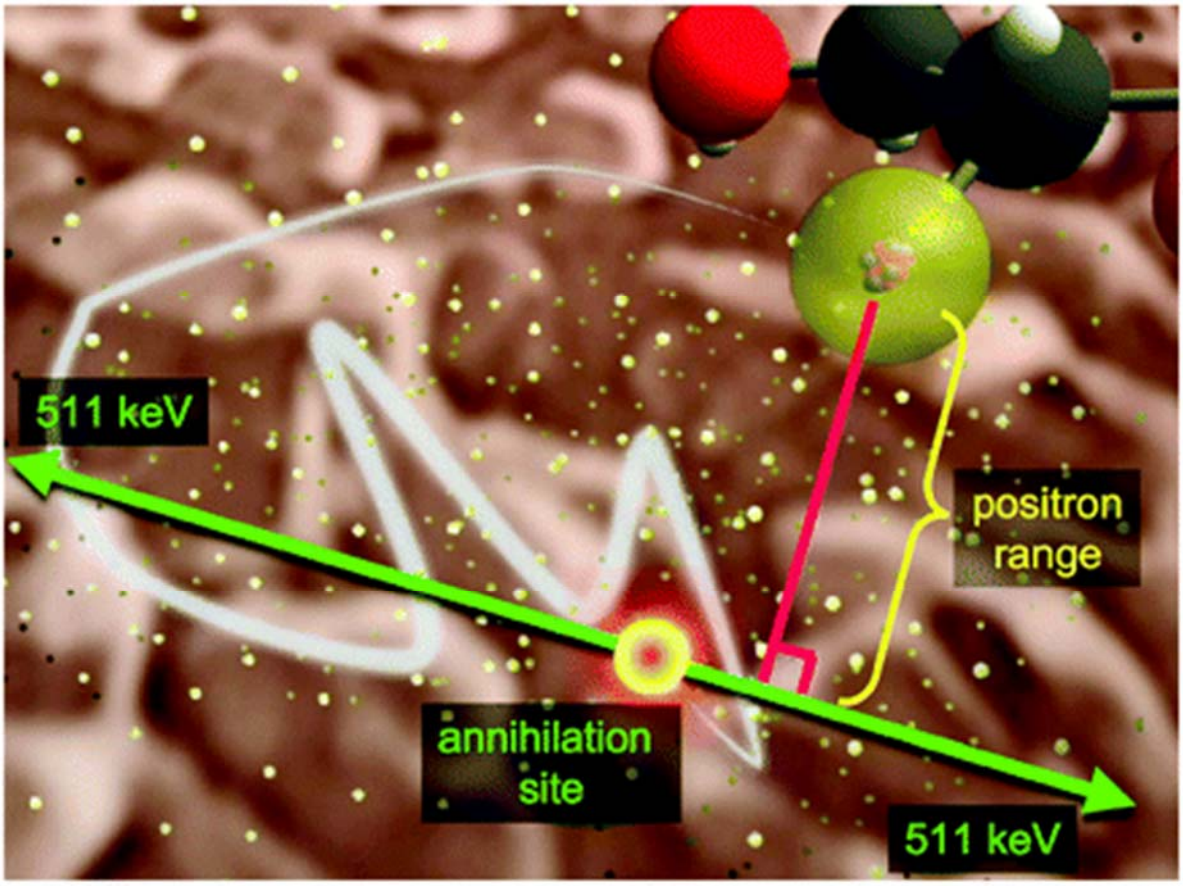


Figure 2.1 Basic physics of PET. The ^{18}F nucleus (light green) of a radioligand molecule decays, emitting a positron that scatters in tissue until it undergoes matter-antimatter annihilation with an electron (yellow circle), in which the mass of the positron and the electron are converted into two back-to-back 511keV γ-photons. (Figure reproduced from Cherry et al. 2006)

Positron Emission Tomography (PET) is a molecular imaging technology (Phelps *et al.* 1975; Phelphs *et al.* 1976; Hoffman *et al.* 1976; Cho *et al.* 1976; Derenzo *et al.* 1977) which utilises positron emitting radioisotopes labelled to compounds to image and compare biochemical processes *in vivo*. The commonly used radioisotopes include carbon (^{11}C), oxygen (^{15}O) and fluorine (^{18}F). Radiolabelled compounds, also referred to as radiotracers, are used to trace a restricted number of steps in the biochemical process of interest, enabling a kinetic analysis to be used to estimate biochemical, and therefore pharmacological, reaction rates. In a standard PET assay the radiotracer is injected intravenously and the PET scan provides a measure of the tissue concentration of the radiotracer over time. These measures are combined with a measure of the time course of the radiotracer in a different biological compartment, e.g. plasma or a different tissue pharmacological compartment, to model the kinetics of the radiotracer. An overview of PET is outlined below.

PET relies upon the principle that the positron emitted from a radionuclide will travel a short distance (less than 3mm) and then undergo matter-antimatter annihilation when it encounters an electron. The maximum resolution of PET is limited to 2-3mm because of the distance the positron travels before annihilation. The energy from this process is equal to the combined mass of the positron and electron, and is released in the form of two 511keV γ-photons, released at 180^{o} to each other. This phenomenon enables the positron decay to be detected by scintillation detectors arranged around in the ring of the tomograph. Scintillation detectors contain a crystal which emits photons in the visual spectrum upon gamma ray absorption, examples of the crystals include bismuth-germanium oxide. The light signal is amplified by a photomultiplier tube which enables its detection reliably. Two photons detected within an ultra-short time interval are assumed to have arisen from the same annihilation event, termed a coincidence event. As the coincidence event releases two photons at 180^{o} from each

other, the coincidence event is deemed to have occurred at a point along a straight line between the two detectors, termed a coincidence line. A normal tomograph will contain hundreds of detectors, and during the PET scan acquisition millions of coincidence lines are recorded. The coincidence lines from this process are stored as a two-dimensional matrix called a sinogram. To enable the formation of a three dimensional image, the sinogram must corrected for irregular absorption of photons by the body (attenuation correction) and detector non-uniformity. The images are then reconstructed using a filtered back-projected algorithm.

Post reconstruction data are then presented as a series of three dimensional images acquired over a sequence of time frames, known as frames. The duration of each frame will depend upon the decay properties of the radiotracer. Data collected from a particular region of the image enables the formation of time activity curves (TACs), which are then used to calculate kinetic variables.

Incorporation of the radionuclide into a pharmaceutical compound enables deductions to be made about the biodistribution of binding sites to the radiotracer from the positron emission data. A variety of PET radiotracers exist, thereby permitting the examination of an array of biochemical and pharmacological systems. As an example, much early work in PET was concerned with measures of brain metabolism using the radioligand [^{18}F]deoxy-glucose (FDG), which is incorporated into the glycolytic pathway after phosphorylation. Radiotracers have also been developed for different aspects of the dopamine system, examples of which include [^{18}F]-DOPA for dopamine synthesis, [^{11}C]-Raclopride for dopamine $D_{2/3}$ receptor densities and dopamine release, and [^{11}C]2β-carbomethoxy-3-β-(4-fluorophenyl)tropane

([^{11}C]2β-CFT) for the dopamine transporter. A description of the PET measurement of dopamine synthesis capacity is given in Box 2.1

Box 2.1 Indexing Dopamine Synthesis Capacity with PET (Adapted from supplementary material to Howes *et al.* 2012)

Dopamine synthesis capacity can be indexed with PET using two radiolabeled homologues of *l*-3,4-dihydroxy-phenylalanine (L-DOPA): [β-^{11}C]L-DOPA ([^{11}C]-DOPA) and 6-[^{18}F]fluoro-DOPA ([^{18}F]-DOPA) (Kumakura *et al.* 2009; Garnett *et al.* 1983). Brain metabolism of radiolabeled-DOPA parallels that of endogenous L-DOPA (Cumming *et al.* 1987). In dopamine neurons, these radiotracers are converted by aromatic L-amino acid decarboxylase (AADC) into [^{11}C]dopamine and 6-[^{18}F]fluoro-dopamine, respectively, and trapped in vesicles in the nerve terminals ready for release (see review by Kumakura *et al.* 2009). AADC is a regulated enzyme and its activity in dopamine neurons is relative to other aspects of dopamine metabolism (Cumming *et al.* 1995). AADC is present in other monoaminergic neurons in addition to dopamine neurons (Snow *et al.* 1993). Nevertheless, radiolabeled-DOPA uptake in the striatum is predominantly due to dopaminergic innervation, is highly correlated with striatal dopamine levels in post mortem brains, and responds to experimental manipulation of brain dopaminergic systems (Cuming *et al.* 1997; Snow *et al.* 1993; Vernaleken *et al.* 2006; Gjedde *et al.* 1991).

2.3.2 Theoretical Basis of K_i (Patlak *et al.* 1983)

Classical pharmacological studies of solute transfer across membranes have derived one of three transfer numbers: the extraction fraction, the influx constant (K_i) and the efflux constant (K_e). Early experimental work typically involved administering the solute of interest into the blood stream and taking samples from the two biological compartments e.g. from the blood to the brain across the blood-brain barrier. The calculation of K_i enables reliable quantification of solute transfer rates across the blood-brain barrier, except for rapidly transported solutes. K_i had historically been calculated by measurement of the unidirectional flux rate across amphibian epithelia from a source solution at constant concentration such that K_i would be defined as the steady state flux rate of the solute across the membrane divided by the test solute concentration in the source solution. Using the blood-brain barrier as an example, K_i would be defined as the steady state rate of solute flux across the blood-brain barrier from plasma at constant concentration divided by the solute plasma concentration. Patlak *et al.* (1983) proposed a method for the graphical evaluation of K_i from multiple time uptake data when the source solution concentration is not constant as would occur in a bolus intravenous administration of solute compared to constant infusion, for example. This model is thus well suited to measuring presynaptic dopamine synthesis capacity with [^{18}F]-DOPA because:

1) It enables the administration of a single bolus injection.
2) Radiolabelled L-DOPA accumulates in presynaptic terminals where it is converted into dopamine, therefore allowing the measurement of the activity of the enzymes aromatic acid decarboxylase and DOPA decarboxylase.

For the purposes of this calculation, the net transfer of solute across the blood-brain barrier is assumed to be unidirectional (i.e. irreversible) throughout the entire experimental period, such that this model best fits a solute that is held or bound within the brain compartment by a biochemical process. The equations derived from this model indicated that unidirectional influx could be examined by plotting uptake data from multiple measurements within the same subject in such a way as to determine whether the resultant curve has a linear phase. A linear phase would therefore indicate that the net transfer process is unidirectional over the time period in question, such that the K_i can be calculated (Bradbury & Kleeman 1967).

2.3.3 Assumptions of the Patlak Model

The Patlak model relies on the following assumptions:

1. There is a single source for the solute, i.e. plasma (denoted by "$_p$"), in the system.
2. The plasma solute concentration is permitted to vary with time.
3. Rapid exchange of the solute occurs between plasma and a tissue region comprised of *n* compartments. The solute can flow freely from plasma into any of these compartments and back to plasma. Therefore, solute transfer between plasma and this tissue region is exchangeable or reversible (denoted by "$_r$").
4. The solute can enter a further compartment, upon which it cannot leave. This region is the irreversible or bound region (denoted by "$_b$") that is treated mathematically as a single compartment.
5. The solute within the exchangeable region can leave the region only by entering the plasma or the irreversible region.
6. Solute transfer within the system obeys first-order kinetics, such that the rate constant for movement from the j^{th} to the i^{th} compartment denoted as "K_{ij}". The validity of this assumption being enhanced by the use of trace amounts of radioligand.
7. The solute does not alter the system.
8. System metabolism of the solute only occurs in the irreversible region and produces a metabolite that is trapped in the irreversible region and is measurable.
9. The solute is not initially present in the either the exchangeable or irreversible compartment.

2.3.4 Derivation of K_i

In addition to the terms defined above, the following will be used in the equations detailed below:

t = time

$A = n - 1$ vector of the amount of model solute in each exchange compartment.

K = (n x n) matrix of the K_{ij} rate constants

$Q = n$ vector of rate constants from plasma to the exchangeable compartments (K_{ip})

$C_p(t)$ = plasma tracer concentration.

$$\frac{dA}{dt} = KA + CQ_p(t)$$

Equation 1 Tracer accumulation in the reversible compartment.

If G = (n × n) diagonal matrix of rate constants from the exchangeable to the bound regions (K_{bi}), K_{bp} is the rate constant for the direct movement of material from the plasma to the trap and $U'_n = (1 \ldots . 1)$, $a(1 \times n)$ vector, then the equation for the amount of material T in the irreversible region is

$$\frac{dT}{dt} = U'GA + K_{bp}\, C_p$$

Equation 2

C_p is measurable from the experimental system, as is the total amount of material in the tissue samples (A_m). If V_p is the volume of the plasma in the tissue sampled then:

$$Am = U'A + T + V_p C_p$$

Equation 3

Equation 1 can be solved (Hearon, 1963):

$$A = e^{Kt} \int_0^t C_p e^{-Kt}\, d\tau Q$$

Equation 4

Substituting equation 4 into equation 2 and then solving the resultant equation yields:

$$T = U'_n G \int_o^t e^{K\tau} \int_0^\tau e^{K\theta} d\theta d\tau Q + K_{bp} \int_0^t C_p d\tau$$

Equation 5

Integrating equation 5, inserting equations 3 and 4, and the rearranging yields:

$$A_m = \left(-U'_n G K^{-1} + K_{bp}\right) \int_0^t C_p d\tau + U'_n (GK^{-1} + I)A + V_p C_p$$

Equation 6

If C_p is constant, A will approach a finite limit as $t \rightarrow \infty$, since the real parts of the eigenvalues of K are negative (Hearon, 1963). So, when $t \rightarrow \infty$:

$$A_m\left(C_p = \text{constant}\right) = -U'_n G K^{-1} Q + K_{bp}$$

Equation 7

As defined above, the rate of uptake for constant plasma level is given by $K_i C_p$. Therefore, equation 7 shows:

$$K_i = -U'_n G K^{-1} Q + K_{bp}$$

Equation 8

Therefore, equation 6 may written as:

$$A_m = K_i \int_0^t C_p d\tau + U'_n (K + G) K^{-1} A + V_p C_p$$

Equation 9

As detailed in Patlak *et al.* (1983), equation 9 can be worked through further such that:

$$A_{m_{t>t*}} = K_i \int_0^t C_p d\tau + (V_0 V_p)$$

Equation 10

Where t^* is a sufficient length of time after which the amounts in each of the components of the exchangeable region "follow" the plasma concentration and V_0 meets the following criteria:

$0 \leq V_0 \leq$ (steady-state space of A) $\leq$ (space of A)

The curve produced by plotting $\frac{A_m}{C_p}$ vs. $\int_0^t C_p d\tau / C_p$ starts at the origin and becomes a straight line with slope K_i by $t=t^*$.

2.4 Power Calculations

In a recent of test-retest reliability of [^{18}F]-DOPA PET (Egerton *et al.* 2010) striatal K_i^{cer} had an intra-class correlation coefficient of approximately 0.9 [mean (SD) K_i^{cer} = 0.01417(0.00127)min^{-1} (test) and 0.01381(0.00127)min^{-1} (re-test)]. Previous [^{18}F]-DOPA uptake work by Howes *et al.* found an effect size of 1.25 in patients with schizophrenia (which compares well with previous studies: 1.89 [Meyer-Lindenberg *et al.* 2002]; 1.57 [McGowan *et al.* 2004] and 0.75 in patients exhibiting prodromal symptoms of psychosis [Howes et al. 2009]. On this basis, it was reasonable to anticipate an effect size of 0.80 in both the Cannabis Group *vs.* Controls and the "HPSS" *vs.* "LPSS" comparisons (equivalent to 8% ±10 difference in [^{18}F]-DOPA uptake between the groups). Therefore, to achieve a power of 0.8, with an effect size of 0.8, p=0.05, using independent t-tests, 21 subjects in each group will be required.

2.5 Blinding

PET imaging was conducted on the same participant visit to the research institute to conduct psychiatric interviews and so it was not possible to conduct image acquisition blind to group status. However, image analysis was conducted blind to group status.

2.6 Pre-PET Scan Acquisition (all subjects)

Subjects were asked to fast and abstain from cannabis for 12 hours, and to refrain from smoking tobacco for 2 hours before imaging. The time period of 12 hours was chosen so that cannabis users would not be acutely intoxicated on the day of the PET scan whilst not significantly interfering with cannabis users normal pattern of drug use.

On the day of the PET scan, urine drug screen (Monitect HC12, Branan Medical Corporation, Irvine, California) confirmed no recent drug use (other than cannabis in the user group) and a negative urinary pregnancy test was required in all female subjects. Subjects received carbidopa 150 mg and entacapone 400 mg orally 1 hour before imaging (Sawle *et al.* 1994) to reduce the formation of radiolabelled [^{18}F]-DOPA metabolites (Cumming *et al.* 1993; Guttman *et al.* 1993).

2.7 [^{18}F]-DOPA Production

For the cannabis study, [^{18}F]-DOPA was produced by GE Imanet using a 17 MeV GE PET-trace cyclotron. The gas target was filled with $^{18}O_2$ and bombarded at 40 μA for 30 minutes followed by a passivation bombardment of 0.1% F_2 in argon at 20 μA for 20 minutes. This produced [^{18}F]-F by the ^{18}O(p,n) ^{18}F reaction. An electrophilic fluorination procedure was then used to synthesise 6-[^{18}F]fluoro-L-DOPA on a GE tracerlab synthesis platform. In brief, [^{18}F]F was bubbled through a solution of 6-Trimethylstannyl-L-DOPA (60mg) stirring in Deutero-chloroform (5ml) over 20mins at 5°C. 6M HCL (2ml) was added and the Chloroform evaporated at 70°C (Forsback *et al.* 2009). The resulting aqueous mixture was heated at reflux for 10 minutes and then allowed to cool. The cooled crude mixture was purified by semi-

preparative high pressure liquid chromatography using a polymer X column eluted with ammonium acetate buffer. The peak corresponding to [^{18}F]-L-DOPA eluted at 15 minutes was stabilised with 1mg ascorbic acid and sodium phosphate dibasic. Typical yields were 2.96–3.33GBq. For quality assurance, a sample was analysed by reverse phase high-pressure liquid chromatography to confirm identity and purity. To be able to proceed with the injection, a radiochemical purity of 95.0% or higher was required.

For the stress study, [^{18}F]-DOPA was produced at Imanova using an 11 MeV Siemens RDS Eclipse HP cyclotron. The gas target was filled with $^{18}O_2$ and bombarded at 40 μA for 40 minutes followed by a passivation bombardment of 0.8% F_2 in argon at 20 μA for 20 minutes. This produced [^{18}F]-F by the ^{18}O(p,n) ^{18}F reaction. An electrophilic fluorination procedure was then used to synthesise 6-[^{18}F]fluoro-L-DOPA on an Eckert & Ziegler Pharmtracer synthesis platform. In brief, [^{18}F]F_2 was bubbled through a solution of 6-Trimethylstannyl-L-DOPA (60mg) stirring in Deutero-chloroform (5ml) over 15mins at 5°C. 4M HCL (1ml) was added and the Chloroform evaporated at 70°C (Forsback *et al.* 2009). A further addition of 4M HCl (3ml) was heated at reflux for 15 minutes. The cooled crude mixture was purified by semi-preparative high pressure liquid chromatography using a polymer X column eluted with sodium dihydrogen phosphate buffer. The peak corresponding to [^{18}F]-L-DOPA eluted at 20 minutes was stabilised with 10mg ascorbic acid and sodium phosphate dibasic. Typical yields were 0.8–1.5GBq. For quality assurance, a sample was analysed by reverse phase high-pressure liquid chromatography to confirm identity and purity. To be able to proceed with the injection, a radiochemical purity of 95.0% or higher was required.

2.8 PET Scan Acquisition (Cannabis Study)

All subjects underwent an [^{18}F]-DOPA scan on an ECAT HR+ 962 PET scanner (CTI/Siemens) in 3D mode, with an axial field of view of 15.5cm, performed as previously reported (Lieberman *et al.* 1987). Psychotic symptoms were assessed using the PANSS at the time of scanning. Head position was marked and monitored via laser crosshairs and a camera, and minimized using a head-strap. A 10-minute transmission scan was performed before radiotracer injection for attenuation and scatter correction. Approximately 180 MBq of [^{18}F]-DOPA was administered by bolus intravenous injection 30 seconds after the start of PET imaging. Emission data were acquired in list mode for 95 minutes, re-binned into 26 time-frames (30-second background frame, four 60-second frames, three 120-second frames, three 180-second frames, and fifteen 300-second frames).

2.9 PET Scan Acquisition (Stress Study)

All subjects (stress study) underwent an [^{18}F]-DOPA PET scan, acquired on a Siemens Biograph HiRez XVI PET scanner (Siemens Healthcare, Erlangen, Germany). Head position was marked and monitored via laser crosshairs and a camera, and minimized using a head-strap. A low-dose computed tomography scan (effective dose=0.2 mSv) was acquired for attenuation and model-based scatter correction. Approximately 150 MBq of [^{18}F]-DOPA was administered by bolus intravenous injection at the start of PET imaging. Emission data were acquired in list mode for 95 minutes, re-binned into 34 time-frames (eight 15 second frames, three 60 second frames, five 120-second frames, and sixteen 300 second frames).

2.10 Method for Compensation of Movement Correction

2.10.1 Raw Data

No compensation for subject head movement was applied to the image.

2.10.2 Frame-by-frame realignment

To correct for head movement, non-attenuation-corrected dynamic images were de-noised using a level 2, order 64 Battle-Lemarie wavelet filter (Turkheimer *et al.* 1999), and individual frames were realigned to a single frame with high signal to noise ratio acquired 10 minutes after the [^{18}F]-DOPA injection using a mutual information algorithm (Studholme *et al.* 1996). Transformation parameters were then applied to the corresponding attenuation-corrected frames, and the realigned frames were combined to create a movement-corrected dynamic image (from 6 to 95 minutes following [^{18}F]-DOPA administration) for analysis.

2.11 Volume of Interest Definition

After movement correction standardised volumes-of-interest (VOIs) were defined bilaterally in the whole striatum, the limbic (ventral), associative (pre-commisural dorsal caudate, precommisural dorsal putamen and postcommisural caudate), and sensorimotor (postcommisural putamen) striatal functional subdivisions and the cerebellar reference region in Montreal Neurologic Institute space (Martinez *et al.* 2003; Egerton et al. 2010). An [^{18}F]-DOPA template was normalized with the VOI map to each individual PET summation (add) image using statistical parametric mapping software (SPM5, http://fil.ion.ucl.ac.uk/spm), allowing VOIs to be placed automatically on individual [^{18}F]-DOPA PET images without observer bias.

2.12 K_i^{cer} Calculation

[^{18}F]-DOPA uptake was calculated, relative to the cerebellum [K_i^{cer} (min^{-1})], for each VOI using the Patlak graphical analysis adapted for a reference tissue input function (Hartvig et al. 1991; Hartvig et al. 1997; Hoshi et al. 1993; Patlak *et al.* 1985). Good test-retest reliability has been demonstrated for striatal [^{18}F]-DOPA K_i^{cer} determined this way (Egerton *et al.* 2010).

2.13 Voxelwise Analysis

For the study in cannabis users, the VOI analysis was complemented by an independent voxelwise analysis using a wavelet-based Patlak method (Turkheimer et al. 2006) as previously described (Howes *et al.* 2009). The parametric image for each participant was normalised into standard space using the participants PET summation image and the [^{18}F]-DOPA template (Howes *et al.* 2009). Statistical Parametric Mapping was conducted using SPM5 and a striatal mask defined according to previously described criteria (Egerton *et al.* 2010) to compare groups. Results are presented corrected for multiple comparisons using random field theory as applied in SPM5 (p<0.05, corrected at the family-wise error rate).

2.14 Behavioural Task: The Salience Attribution Test (SAT)

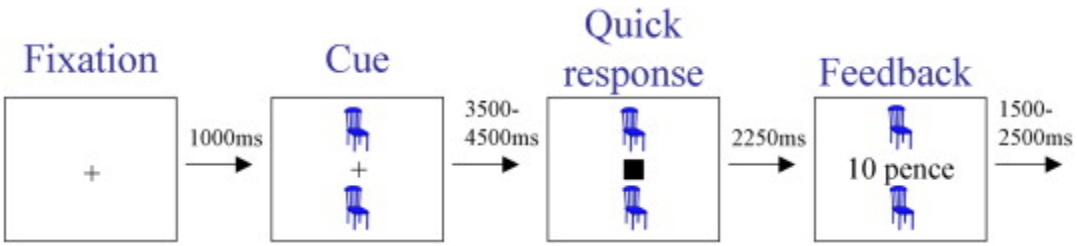


Figure 2.2 The Salience Attribution Test Subjects are presented with a fixation cross followed by a cue. They then have to respond to the solid square as quickly as possible. During 50% of trials, participants are rewarded with money for faster responses, with the probability of the reward signaled by the cue.

The SAT is a speeded-response game, with monetary reward, which measures responses to task-relevant and task-irrelevant cue features (Roiser *et al.* 2009; Roiser *et al.* 2010). These measures reflect incentive salience processing, described in Chapter 1, as the task-relevant cues are associated with a reward and task-irrelevant cues are not associated with reward. The "aberrant salience" measure from the SAT has displayed good face validity in previous results, with elevated scores both in high-schizotypy individuals, and in patients with schizophrenia suffering from delusions (Roiser et al. 2009). Furthermore, using a factor analysis Schmidt & Roiser (2009) demonstrated the psychometric construct validity of the SAT. In an fMRI study of the SAT, Roiser *et al.* (2010) found that cues associated with high relative to low reward probabilities elicited robust hemodynamic responses in brain networks implicated in motivational salience including the midbrain, in the vicinity of the ventral tegmental area, and regions targeted by its dopaminergic projections, i.e. medial dorsal thalamus, ventral striatum and prefrontal cortex. Adaptive salience was strongly correlated with responses in the medial dorsal thalamus and polar PFC, whilst participants who showed

greater aberrant salience exhibited greater dorsolateral PFC responses, and reduced medial temporal gyrus (MTG) responses. Data on the test-retest reliability of the measure are lacking.

As per the description of the task by Roiser *et al.*: During the task, participants respond to a probe (a black square) after seeing 1 of 4 categories of cues (blue animals, red animals, blue household objects, and red household objects), which vary along 2 dimensions (colour and form); see figure 2.2. Each cue set comprised 16 different pictures, each of which was presented once per block. Subjects receive monetary reward (£0.05 to £1.00) on 50% of trials, with more money available for faster responses. Feedback was given at the end of each trial. The probability of reward varies along one of the cue dimensions (such that if colour was the task-relevant dimension if blue stimuli were reward 87.5% of the time, red stimuli would be rewarded 12.5% of the time), but not for the other (such that if form was the task-irrelevant dimension both animals and household objects would be rewarded 50% of the time). On rewarded trials where participants either made no response or responded after the probe had disappeared, the message "Missed: 5 pence" was displayed. If participants responded prematurely (<100ms after the onset of the probe), the message displayed was "Too early: 5 pence". On rewarded trials where participants responded before the probe disappeared, but slower than their mean RT from the practice block, the message "Hit – good: 10 pence" was displayed. When participants responded more quickly than their mean practice RT, the message "Quick – very good: X pence" was displayed (for responses up to 1.5 standard deviations (SDs) faster than their practice mean RT) and "Very quick – excellent: X pence" (for responses over 1.5 SDs faster than their practice mean RT). The reward was scaled according to $X=10+90\times(\text{mean RT} - \text{trial RT})/(3\times\text{SDF})$, up to a maximum of £1.00. For example, a response 1 SDF faster than the mean was reinforced with 40 pence, a response 2

SDFs faster was reinforced with 70 pence, and any responses 3 SDFs or faster than the mean were reinforced with 100 pence. The money won on each trial was added to the participant's running total for that block, Y, which was displayed underneath the feedback: 'Total – £Y'. On reinforced trials, a 0.5 s tone sounded, frequency: (300+(10×X)) Hz. At the end of each block, participants indicated, using 10 mm visual analogue scales (VAS), their estimate of the reinforcement probabilities for each of the four different CSs.

On the 50% of trials that were not rewarded, the message "Sorry – no money available" was displayed, regardless of the speed of response. Participants were not informed of the contingencies between the different pictures and reward. Participants could earn a maximum of £20 on the test (minimum £5).

Category and reward probability contingencies are counterbalanced across participants and remained constant throughout the task. Two experimental sessions (64 trials each) were performed each session. The SAT provides measures of adaptive (relevant) and aberrant (irrelevant) motivational salience on the basis of visual analogue scale ratings (VAS; explicit salience) and reaction times (RTs; implicit salience).

Before the main task, participants performed a computerised tutorial, with neither rewards nor cues, on which they were required to respond as quickly as possible to the onset of the probe only. The tutorial featured example displays, written instructions and test trials before the main test. The tutorial was embedded with two practice sessions to familiarize

participants with the test and provide a measure of baseline response time (RT). During practice sessions, a fixation cross appeared at the beginning of each trial. Following a variable interval (minimum 0.5 s, maximum 1.5 s) the probe appeared, and participants responded by pressing a button as quickly as possible. Participants were instructed to try to respond as quickly as they were able to, and before the box disappeared. During the first practice session the probe was on the screen for randomized variable periods, with a maximum duration of 1.5 s, minimum duration 0.5 s and mean duration 1 s. Feedback was provided after 2 s as 'Good' if the participant responded before the box disappeared, 'Try to respond faster' if they responded after the box disappeared, 'Too early' if they responded before the box appeared, and 'No key pressed' if they made no response. On the second practice session, the mean probe duration was set to be the mean RT from the first, ensuring participants were responding as quickly as possible and to yoke task difficulty to individual performance, such that difficulty in the active task was calibrated on a participant-by-participant basis. The standard deviation (s.d.) of the fastest half of the trials (SDF) was also calculated, and was used to set the minimum and maximum probe durations for the second practice session (mean from first practice session±2×SDF). For the main test, the mean, minimum and maximum probe durations were calculated from the second practice session in the same way. No monetary reinforcement was provided during the practice sessions.

2.15 SAT Trial Structure

A fixation cross was presented at the beginning of each trial. After 1 s, while the fixation-cross remained on-screen, one of the four cues was displayed at the left and right of the screen and remained on-screen until the end of the trial. After a period of time that varied randomly across trials (between 3.5 and 4.5 s) the probe appeared, replacing the fixation-cross, and participants attempted to respond before it disappeared using the index finger of their right hand on a button-box. The onset of the probe was therefore unpredictable, ensuring that participants were unable to anticipate its appearance. The duration of the probe also varied randomly across trials, and was calibrated for each participant separately from their own practice session data. After 2.25 s feedback was presented for 1.5 s as described above. On rewarded trials, an auditory tone with a frequency proportional to the amount of money won on that trial sounded at feedback. After feedback, a blank screen of variable duration was inserted to result in a constant inter-trial interval of 9.25 s.

Four different versions of the SAT were used, each with a different stimulus feature (blue, red, animal or household object) reinforced with high probability. Each participant was administered the same version for both blocks of the SAT.

Two measures of motivational salience were calculated for each block. Adaptive salience was defined in two ways: behaviourally (implicit measure) and subjectively (explicit measure). RT adaptive salience (implicit) was defined as the speeding of responses on high-probability-reinforcement trials relative to low-probability-reinforcement trials (collapsing across the task-irrelevant stimulus dimension), and VAS adaptive salience (explicit) was defined as the increase in probability rating for high-probability-reinforcement trials relative to low-probability reinforcement trials (again, collapsing across the task-irrelevant stimulus

dimension). The aberrant salience measures were defined as follows: implicit aberrant salience is the absolute difference in reaction times between the task-irrelevant cues and task-relevant cues; explicit aberrant salience is the difference in visual analogue scale rating, i.e. the subjective probability ratings of reward, between the task-irrelevant stimulus dimension. These measures are averaged across the test for each participant. The aberrant salience measures are defined as any deviation from equal reaction time or subjective reinforcement probability rating of the task-irrelevant stimulus dimension away. Therefore, it does not make a difference to the measure whether the deviation is positive or negative. Therefore aberrant salience was always positive, whereas adaptive salience could be positive or negative. The number of premature responses and omissions were also recorded for each stimulus type on each block.

2.16 Statistical Analysis

Data were analysed using the Statistical Package for the Social Sciences, version 21 (IBM, Armonk, NY, USA). Statistical significance was defined as $p<0.05$ (two-tailed) throughout. Normality of distributions was assessed using the one-sample Kolmogorov-Smirnov test. Between-group comparisons were made with two-tailed independent *t*-tests for normally distributed data and Mann-Whitney *U* tests for non-normally distributed data. Relationships between K_i^{cer}, levels of cannabis use and cannabis-induced psychotic-like symptom severity were tested using Pearson's product-moment correlation coefficient. Potential confounders were explored using a single ANCOVA with subject group as the fixed factor; K_i^{cer} as the dependent variable and confounders as covariates. Pearson's product-moment correlation coefficient to determine if there was a relationship between K_i^{cer} and cannabis use and stressors. The primary PET outcome measure was K_i^{cer} in the whole striatum.

Exploratory analyses were conducted in the striatal sub-divisions (presented uncorrected for multiple comparisons).

For the SAT, SAT data were analyzed using repeated-measures analysis of variance. Block (1 / 2) was the within-subjects variable and Group (cannabis user / control) was the between-subjects variable. Normality of distributions was assessed using the one sample Kolmogorov-Smirnov test. Salience outcome measures were assessed for statistically significant skew. RT and VAS aberrant salience scores from the SAT were square root transformed prior to analysis to reduce skew, though untransformed values are presented in the text, figures and tables for clarity. Relationships between data were assessed using Pearson's *r* product–moment correlation coefficient for normally distributed data and Spearman's ρ rank correlation was used for non-normally distributed data. To determine whether subjects consistently assigned aberrant salience to any particular stimulus feature χ^2 tests were employed.

2.17 Summary

Dopamine synthesis capacity will be compared between cannabis users who experience a transient increase in psychotic-like symptoms and non-user controls, and between individuals with a high exposure to psychosocial stress and individuals with a low exposure to psychosocial stress. Dopamine synthesis capacity will be indexed as the influx rate constant K_i^{cer}, and measured with 3,4-dihydroxy-6-[^{18}F]-fluoro-*l*-phenylalanine ([^{18}F]-DOPA) positron emission tomography.

Adaptive and aberrant motivational salience will be compared between cannabis users and non-user controls using the Salience Attribution Test (SAT). The SAT provides behavioural measures of motivational salience during functional magnetic resonance imaging to assess neural haemodynamic responses to relevant and irrelevant task features.

Chapter 3:

Dopamine Synthesis Capacity

and its Relationship to Cannabis-Induced Psychotic Symptoms

3.1 Introduction

Cannabis is the most widely used illicit drug in the world and users are at increased risk of schizophrenia in a dose-dependent fashion (Moore *et al.* 2007). Dopamine dysfunction is thought to underlie the development of psychosis (Howes & Kapur 2009). A recent meta-analysis has found elevated striatal dopamine synthesis capacity in people with schizophrenia (Howes *et al.* 2012). It has been proposed, though never directly tested, that cannabis may increase the risk of schizophrenia by creating a hyperdopaminergic state in the striatum (Voruganti *et al.* 2001).

3.2 Hypotheses

1) Cannabis users who experience cannabis-induced psychotic-like symptoms have increased striatal dopamine synthesis capacity compared to non-user controls.

2) Within the cannabis user group: elevated dopamine synthesis capacity is directly related to a higher severity of transient psychotic phenomena.

3.3 Materials and Methods

3.3.1 Research Ethics

The study was conducted according to the research ethics that were described in Chapter 2.

3.3.2 Power Calculation

Baseline F-DOPA uptake is the primary measure and the study is powered for this variable. In a study of the test-retest reliability of F-DOPA PET (Egerton *et al.* 2010) striatal F-DOPA uptake had a within group intra-class correlation coefficient of approximately 0.9. A previous study of F-DOPA uptake in patients with schizophrenia by Howes *et al.* (2009) found an effect size of 1.25 in patients with schizophrenia (which compares well with effect sizes from other studies, e.g. 1.89 [Meyer-Lindenberg *et al.* 2002] and 1.57 [McGowan *et al.* 2004]) and 0.75 in patients exhibiting prodromal symptoms of psychosis (Howes *et al.* 2009). On this basis an effect size of 0.80 in the cannabis user group versus non-user controls was anticipated. To achieve a power of 0.8, with an effect size of 0.8 and statistical significance of p=0.05 (two-tailed) using an independent t-test, it was calculated that 21 subjects would be required in each group.

3.3.3 Study Population

The study population including inclusion and exclusion criteria were described in Chapter 2.

3.3.6 PET Scans

Subjects were asked to fast and abstain from cannabis for 12 hours and to refrain from smoking tobacco for 2 hours before imaging. On the day of the PET scan, urine drug screen (Monitect HC12, Branan Medical Corporation, Irvine, California) confirmed no recent drug use (other than cannabis in the user group), and a negative urinary pregnancy test was required in all female subjects. Psychotic symptoms were assessed using the Positive and Negative Syndrome Scale at the time of scanning. No subjects had psychotic symptoms at the time of scanning (mean [SD] Positive and Negative Syndrome Scale positive score cannabis users = 7.3 [0.5]; control subjects = 7.2 [0.4]). Subjects received carbidopa 150 mg and entacapone 400 mg orally 1 hour before imaging (Sawle *et al.* 1994) to reduce the formation of radiolabeled [18F]-DOPA metabolites (Cumming *et al.* 1993; Guttman *et al.* 1993). Head position was marked and monitored via laser crosshairs and a camera and minimized using a head-strap. A 10-minute transmission scan was performed before radiotracer injection for attenuation and scatter correction. Approximately 180 MBq of [^{18}F]-DOPA was administered by bolus intravenous injection 30 seconds after the start of PET imaging. Emission data were acquired in three-dimensional mode for 95 minutes, rebinned into 26 timeframes (30-second background frame, four 60-second frames, three 120-second frames, three 180-second frames, and fifteen 300-second frames).

3.4 Results

3.4.1 Subject Characteristics and Scan Parameters

Twenty cannabis users were recruited to the study. Owing to tomograph malfunction during one scan, complete data were available on nineteen users. All cannabis users consumed the drug as a spliff. The mean (SD) age of first cannabis use was 15.5 (1.6) years, and the mean (SD) duration of at least weekly use was 4.7 (3.1) years. The median (interquartile range) time taken to smoke an eighth and lifetime exposure to cannabis was 4.0 (13.5) days and 2340 (6240) spliffs, respectively. Within the user group, the median (interquartile range) time between the scan and the last cannabis exposure and self-reported cannabis-induced psychotic-like symptoms was 14.0 (23.8) hours. Ten users met DSM-IV criteria for cannabis dependence (n = 5) or abuse (n = 5). Mean (SD) time to smoke an eighth was 2.3 (2.2) days in users who met dependency/abuse criteria and 6.9 (4.7) days in users who did not meet criteria. Mean (SD) age of first cannabis consumption was 14.8 (1.6) years in users who met dependency/abuse criteria, and 16.2 (1.3) years in users who did not meet criteria. Nineteen control subjects were matched to the user group for age (±5 years) and sex. Subjects' characteristics are reported in Table 3.1. Urine drug screen was positive for THC and negative for all other substances (amphetamine, opiates, cocaine, methamphetamine, benzodiazepines) in every cannabis user and negative for all drugs (including cannabis) in every control subject. There was a significant group difference in current cannabis consumption, as expected, and also in tobacco and ecstasy use (Table 3.1).

Table 3.1 Sample characteristics and scan parameters

	Controls (*n*=19)	Cannabis Users (*n*=19)	*p*[a]
Sample characteristic			
Age (years) [mean(SD)]	22.3 (2.8)	20.8 (1.7)	0.07
Sex (*n*)	2 female, 17 male	2 female, 17 male	1.00
Handedness (*n*)	2 left, 17 right	4 left, 15 right	0.37
Ethnicity (*n*)	4AB, 3BB, 1ME, 11WB	4AB, 15WB	0.16[b]
Current Drug Use[c,d]			
Cannabis users (*n*)	0 users, 19 non-users	19 users, 0 non-users	1.00
Cannabis use (grams of cannabis/month) [median(IQR)]	0.0 (0.0)	26.3 (90.0)	0.00
THC content of cannabis (%) [mean(SD)]	-	8.7 (3.8)	-
Time since last cannabis exposure (hours) [median(IQR)	-	14.0 (23.8)	-
Time taken to smoke an '*eighth'* of cannabis (days) [median(IQR)]	-	4.0 (13.5)	-
Age of onset of regular cannabis use (years) [mean(SD)]	-	15.5 (1.6)	-
Tobacco cigarette smokers (*n*)	8 users, 11 non-users	15 users, 4 non-users	0.02
Tobacco use in whole sample (cigarettes/day) [median(IQR)]	0.0 (0.0)	4.0 (7.0)	0.01
Tobacco use in smokers (cigarettes/day) [median(IQR)] (tobacco users)	1.0 (9.0)	7.0 (8.0)	-
Alcohol use in last 3 months (*n*)	19 users, 0 non-users	19 users, 0 non-users	1.00
Alcohol[e] use (UK alcohol units/week) [median(IQR)]	9.0 (12.0)	12.0 (21.0)	0.34
MDMA/ecstasy use in last 3 months (*n*)	5 users, 14 non-users	11 users, 8 non-users	0.05
MDMA/ecstasy use in whole sample (grams of MDMA/month) [median(IQR)]	0.0 (0.0)	0.3 (1.0)	0.02
MDMA/ecstasy use in MDMA/ecstasy users (grams of MDMA/month) [median(IQR)]	0.3 (0.8)	1.0 (1.7)	-
Cocaine use in last 3 months (*n*)	3 users, 16 non-users	3 users, 16 non-users	1.00
Cocaine use in whole sample (grams of cocaine/month) [median(IQR)]	0.0 (0.0)	0.0 (0.0)	0.60
Cocaine use in cocaine users (grams of cocaine/month) [median(IQR)]	<0.1 (<0.1)	<0.1 (1.0)	-
Amphetamine use in last 3 months (*n*)	1 user, 18 non-users	4 users, 15 non-users	0.15
Amphetamine use in whole sample (grams of amphetamine/month) [median(IQR)]	0.0 (0.0)	0.0 (0.0)	0.27
Amphetamine use in amphetamine users (grams of amphetamine/month) [median(IQR)]	<0.1	0.5 (0.3)	-
Ketamine use in last 3 months (*n*)	1 user, 18 non-users	6 users, 13 non-users	0.04
Ketamine use in whole sample (grams of ketamine/month) [median(IQR)]	0.0 (0.0)	<0.1 (0.5)	0.10
Ketamine use in ketamine users (grams of ketamine/month) [median(IQR)]	<0.1	1.5 (2.9)	-

Psilocybin use in last 3 months (*n*)	1 user, 18 non-users		1 user, 18 non-users		1.00
Psilocybin use in whole sample (grams of "magic mushrooms"/month) [median(IQR)]	0.0	(0.0)	0.0	(0.0)	0.80
Psilocybin use in psilocybin users (grams of "magic mushrooms"/month)	<0.1	2.0	-		
Scan parameter					
Injected dose (MBq) [mean(SD)]	180.6	(7.2)	184.4	(5.2)	0.11
Specific activity (MBq/μmol) [mean(SD)]	31.1	(17.3)	30.5	(14.0)	0.92
Whole striatal volume (mm^3) [mean(SD)]	17,587.82	(1729.50)	17,942.90	(1286.73)	0.48
Associative striatal volume (mm^3) [mean(SD)]	10,801.19	(1134.46)	10,772.76	(1161.24)	0.94
Limbic striatal volume (mm^3) [mean(SD)]	2,080.30	(234.77)	2,276.51	(977.85)	0.40
Sensorimotor striatal volume (mm^3) [mean(SD)]	4,706	(106.60)	4668.98	(443.16)	0.80

Abbreviations: AB, Asian British; BB, Black British; MDMA, 3,4-methylenedioxy-N-methylamphetamine ("Ecstasy"); ME, Mixed Ethnicity; WB, White British

[a] Independent-samples *t*-tests for variables with normal data distributions; Mann-Whitney U tests for variables with non-normal data distributions; χ^2-tests for dichotomous variables

[b] Groups were compared on a dichotomised ethnicity variable (white British vs ethnic minority).

[c] Drug use reported in 3 months prior to scan. Drug user defined as any drug use in the 3 months prior to scan.

[d] There was no reported lysergic acid diethylamide, benzodiazepine, opiate or methamphetamine use in the 3 months before scanning.

[e] 1 UK alcohol unit = 10mL (~7.88g) alcohol

There was no significant group difference in the amount of radioactivity or specific activity injected (table 3.1). There was no significant difference in whole striatal or subdivision volumes between the groups. There was no relationship between age and K_i^{cer} in the striatum or its subdivisions in the whole sample or in either group (table 3.2).

Table 3.2 The relationship between [^{18}F]-DOPA K_i^{cer} and age at PET scan

VOI	Whole sample		Controls (*n*=19)		Cannabis Users (*n*=19)	
	r	*p*	*r*	*p*	*r*	*P*
STR	0.05	0.75	0.00	1.00	-0.17	0.47
AST	0.06	0.71	0.02	0.92	-0.17	0.48
LST	-0.07	0.69	-0.14	0.56	-0.22	0.36
SMST	0.07	0.66	0.02	0.93	-0.10	0.68

Abbreviations: AST, associative striatum; LST, limbic striatum; K_i^{cer} , influx rate constant; SMST, sensorimotor striatum; STR, whole striatum; VOI, Volume of Interest.

3.4.2 Striatal Dopaminergic Function

K_i^{cer} was significantly reduced in cannabis users relative to controls in the whole striatum (figure 3.1). Secondary analysis in each striatal subdivision showed that this reduction reached significance in the limbic and associative subdivisions (table 3.3). The finding of reduced K_i^{cer} in cannabis users remained significant after co-varying for other drugs used, with the amount of use of each of the drugs listed in table 3.1 included as separate covariates in the ANCOVA, in the whole striatum ($F_{1,37}$=4.65, p=0.040) and its associative $F_{1,37}$=5.00, p=0.034) and limbic ($F_{1,37}$=7.358, p=0.011) subdivisions.

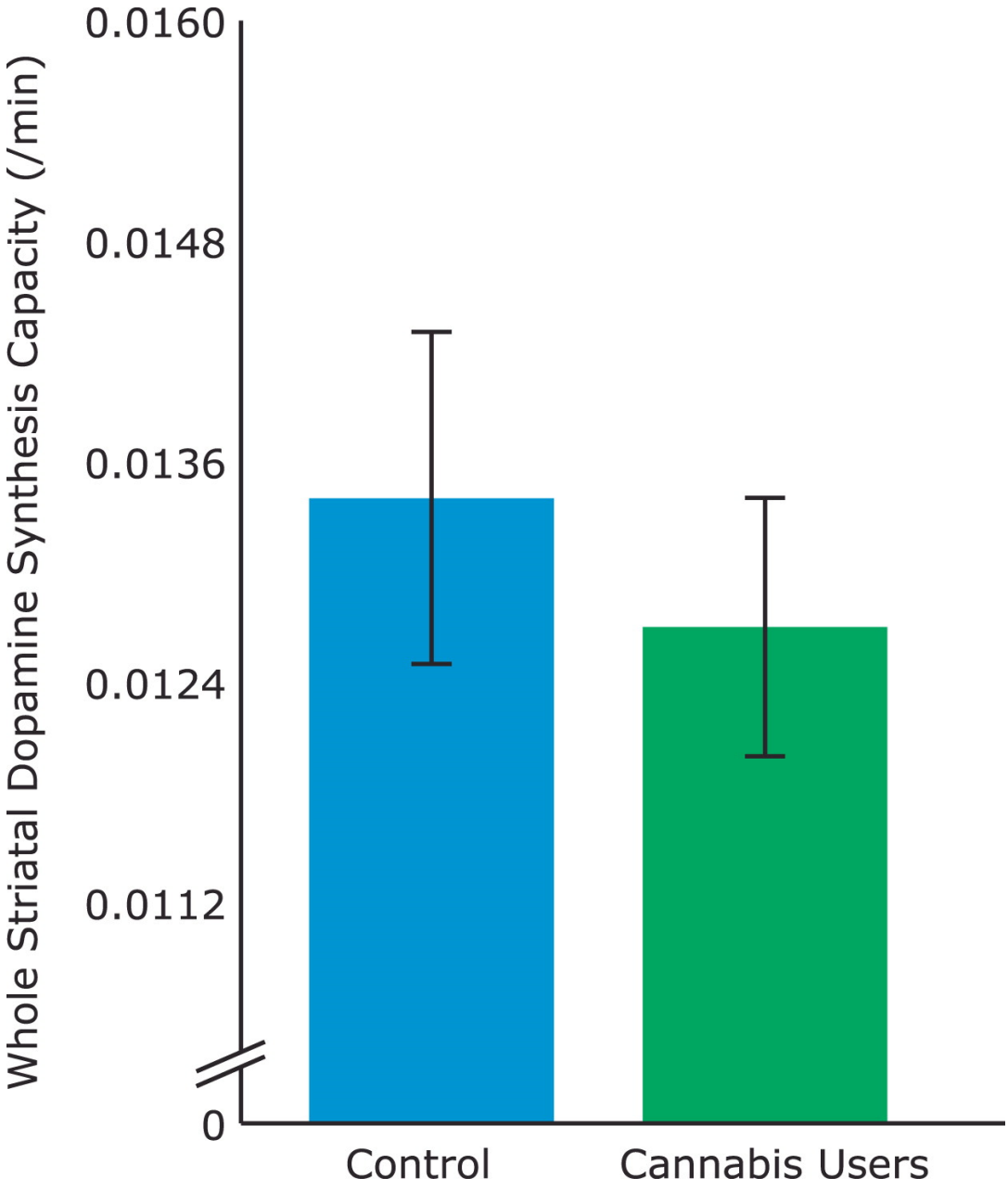


Figure 3.1 Striatal dopamine synthesis capacity in regular cannabis users (n = 19) and nonuser control subjects (n = 19). Dopamine synthesis capacity was significantly reduced in cannabis users compared with nonusers (t_{36} = 2.54, p = 0.016). Error bars indicate standard deviations.

Table 3.3 [^{18}F]-DOPA K_i^{cer} (min^{-1}) by group							
VOI	Controls (*n*=19)		Cannabis Users (*n*=19)		Group comparisons[a]		Effect size
	Mean	(SD)	mean	(SD)	t_{df}	*p*	(Cohen's *d*)
STR	0.0134	(0.0009)	0.0127	(0.0007)	2.54_{36}	0.016	0.85
AST	0.0127	(0.0009)	0.0121	(0.0007)	5.54_{36}	0.015	0.85
LST	0.0138	(0.0009)	0.0132	(0.0008)	2.23_{36}	0.032	0.74
SMST	0.0146	(0.0014)	0.0139	(0.0008)	1.85_{36}	0.070	0.62

Abbreviations: AST, associative striatum; LST, limbic striatum; K_i^{cer} , influx rate constant; SMST, sensorimotor striatum; STR, whole striatum; VOI, Volume of Interest

[a]Independent-samples *t*-tests.

Voxel-based analysis confirmed reduced Kicer in the cannabis user group relative to non-user controls with peak statistical significance in the right putamen (Figure 3.2). There were no voxels where there was a significant elevation in Kicer in cannabis users relative to controls.

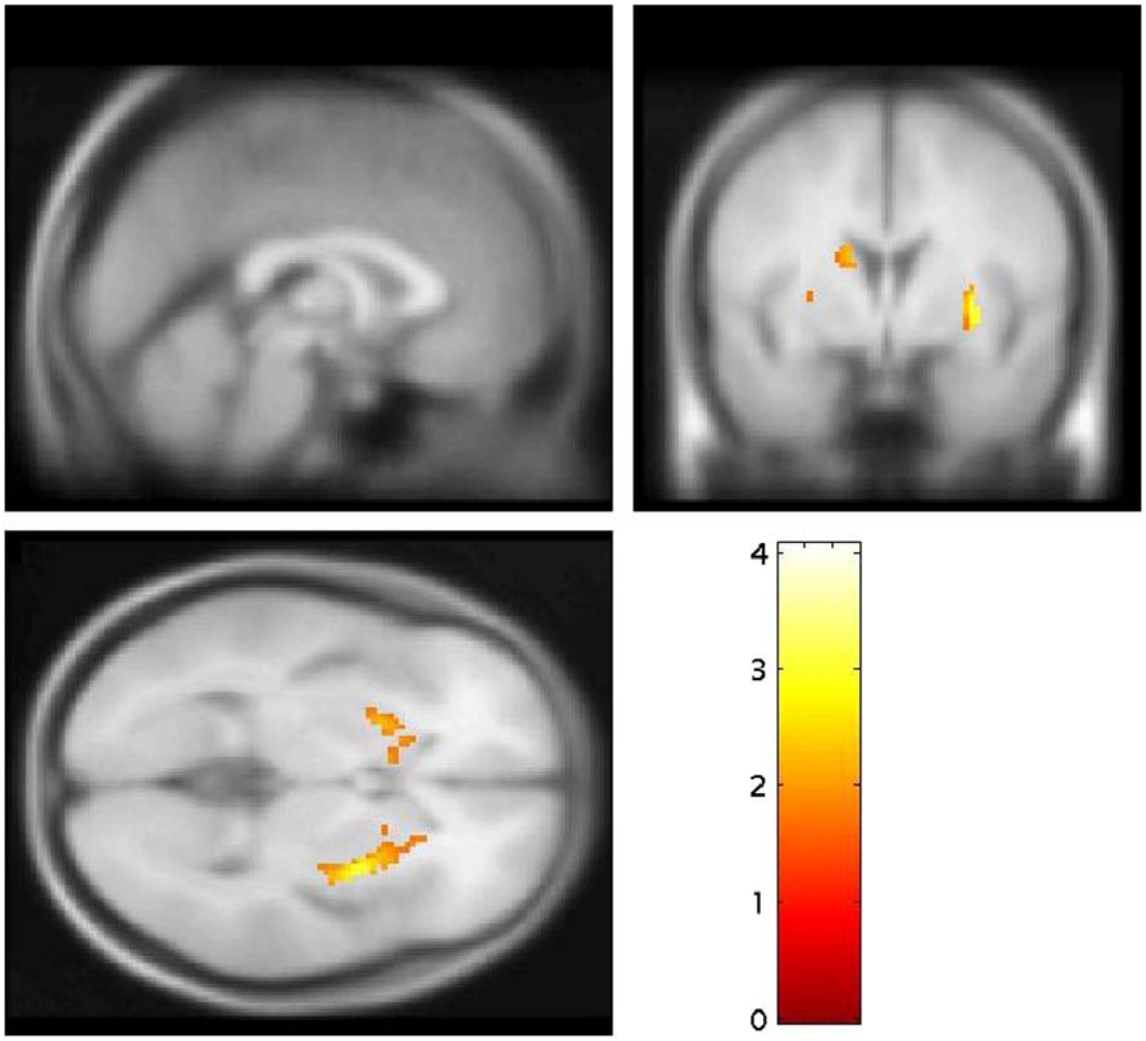


Figure 3.2 – Reduced striatal dopamine synthesis capacity in regular cannabis users relative to non-user controls. The image shows a statistical parametric map of significant reductions (p<0.05) in dopamine synthesis capacity, relative to healthy comparison subjects (n=19), in regular cannabis users who experienced transient psychotic-like symptoms (n=19). The most significant reduction reported by SPM software was in the right putamen (MNI coordinates: 28,6,-8) (p=0.048, corrected at the family-wise error rate). The colour bar indicates the t statistic for each voxel.

3.4.3 The Relationship between Striatal Dopamine Synthesis Capacity and Cannabis Use

Within the cannabis user group, greater levels of current cannabis use (less time to smoke an '*eighth'* of cannabis) were associated with lower K_i^{cer} in the whole striatum *(r*=-0.77, p<0.001; figure 3.3A). Secondary analysis in each striatal subdivision showed that this pattern reached significance in the associative (r=-0.68, p=0.001) and sensorimotor (r=-0.84*,* p<0.001) subdivisions, but not the limbic subdivision (r=-0.26, p=0.290). In addition, there was a significant correlation between age of onset of cannabis use and K_i^{cer} in the whole striatum (r=0.51, p=0.027; figure 3.3B) and in its associative subdivision (r=0.56, p=0.013), which remained significant after controlling for current age (r=0.49, p=0.04[whole striatum]; r=0.54, p=0.02 [associative])**,** with no significant correlation in the sensorimotor (r=0.34, p=0.158) or limbic (r=0.36, p=0.126) subdivisions. There was no significant correlation between age of first cannabis use and current cannabis use (r=0.16, p=0.52).

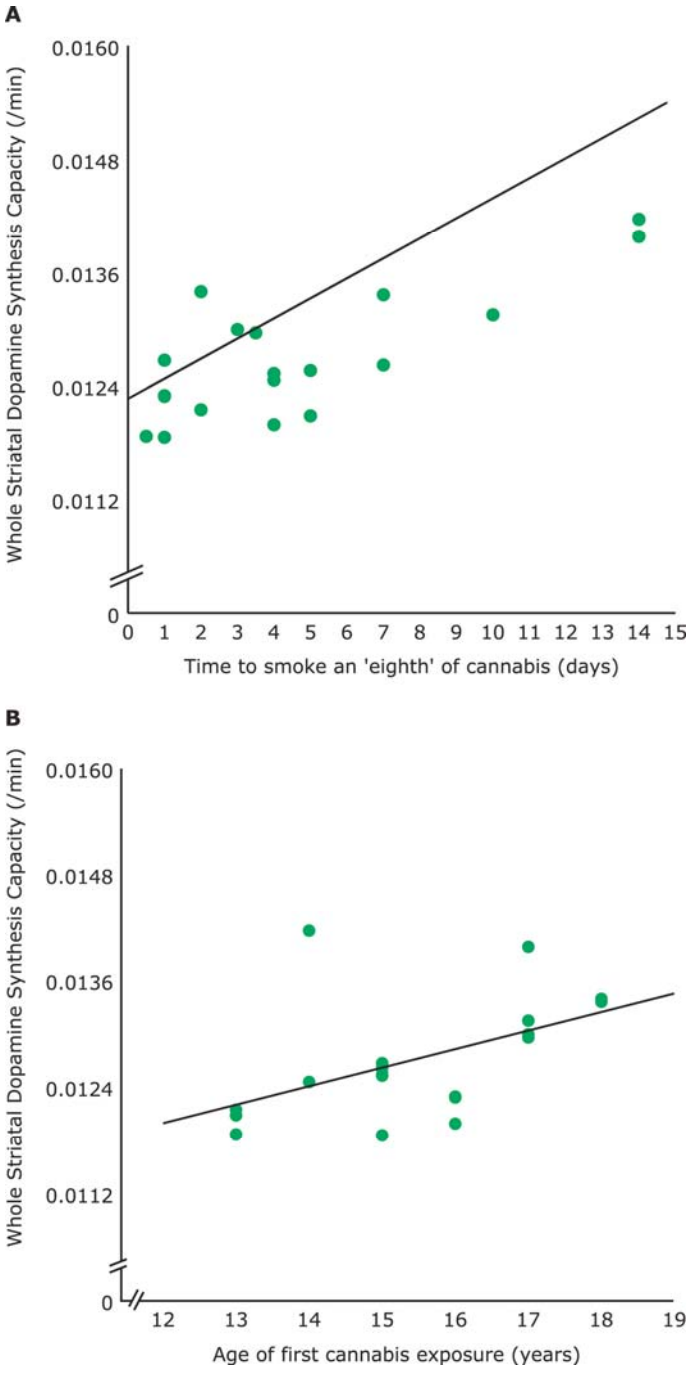


Figure 3.3A (top) - The correlation between level of cannabis use (time to smoke an *"eighth"* [~3.5g] of cannabis; days), and striatal dopamine synthesis capacity, indexed as Ki^{cer} (min-1), in cannabis users (r=-0.77, p<0.001).

Figure 3.3B (bottom) - The correlation between age of onset of cannabis use and Ki^{cer} in the whole striatum (r=0.51, p=0.027), which remained significant when controlling for current age (r=0.49, p=0.04).

Across the whole sample and within the control group there was no significant difference between K_i^{cer} in tobacco smokers and non-tobacco smokers in any of the regions examined (all *p*-values>0.1). Within the whole sample and within each group there was no relationship between K_i^{cer} and daily cigarette use amongst tobacco cigarette smokers in the whole striatum (r=0.26, p=0.91 [whole sample], r=0.10, p=0.81 [controls]; r=0.18, p=0.52 [cannabis users]) and its functional subdivisions (table 3.4). Within the whole sample and within each group there were no significant relationships (all *p*-values>0.1) between whole striatal K_i^{cer} and other substances used (listed in table 3.1).

Table 3.4 The relationship between [^{18}F]-DOPA K_i^{cer} and daily cigarette use amongst cigarette smokers

VOI	Whole sample (*n*=23)		Controls (*n*=8)		Cannabis Users (*n*=15)	
	r	*P*	*r*	*p*	*r*	*p*
STR	0.03	0.91	0.19	0.65	0.18	0.52
AST	0.12	0.60	0.15	0.73	0.31	0.26
LST	0.08	0.73	0.29	0.49	0.22	0.42
SMST	-0.16	0.46	-0.02	0.97	-0.17	0.55

To examine whether cannabis dependency/abuse was associated with reduced K_i^{cer} the cannabis user group was divided into subjects that met DSM-IV diagnostic criteria for cannabis dependency or abuse (*n*=10), and those who did not meet criteria (*n*=9). One-way ANOVA found a significant effect of group on whole striatal K_i^{cer} ($F_{2,37}$=4.02, p=0.027, Figure 3.4). *Post hoc t*-tests showed significant differences between the cannabis dependency/abuse and non-dependency/non-abuse cannabis user sub-groups (t_{17}=2.80, p=0.012) and between the cannabis dependency/abuse sub-group and controls (t_{27}=2.67, p=0.013), but not between the non-dependency/non-abuse sub-group and the control group (p=0.60). When examining the striatal subdivisions, significant differences in K_i^{cer} between the cannabis dependency/abuse and non-dependency/non-abuse cannabis user sub-groups were observed in the associative subdivision only (t_{17}=2.89, p=0.010).

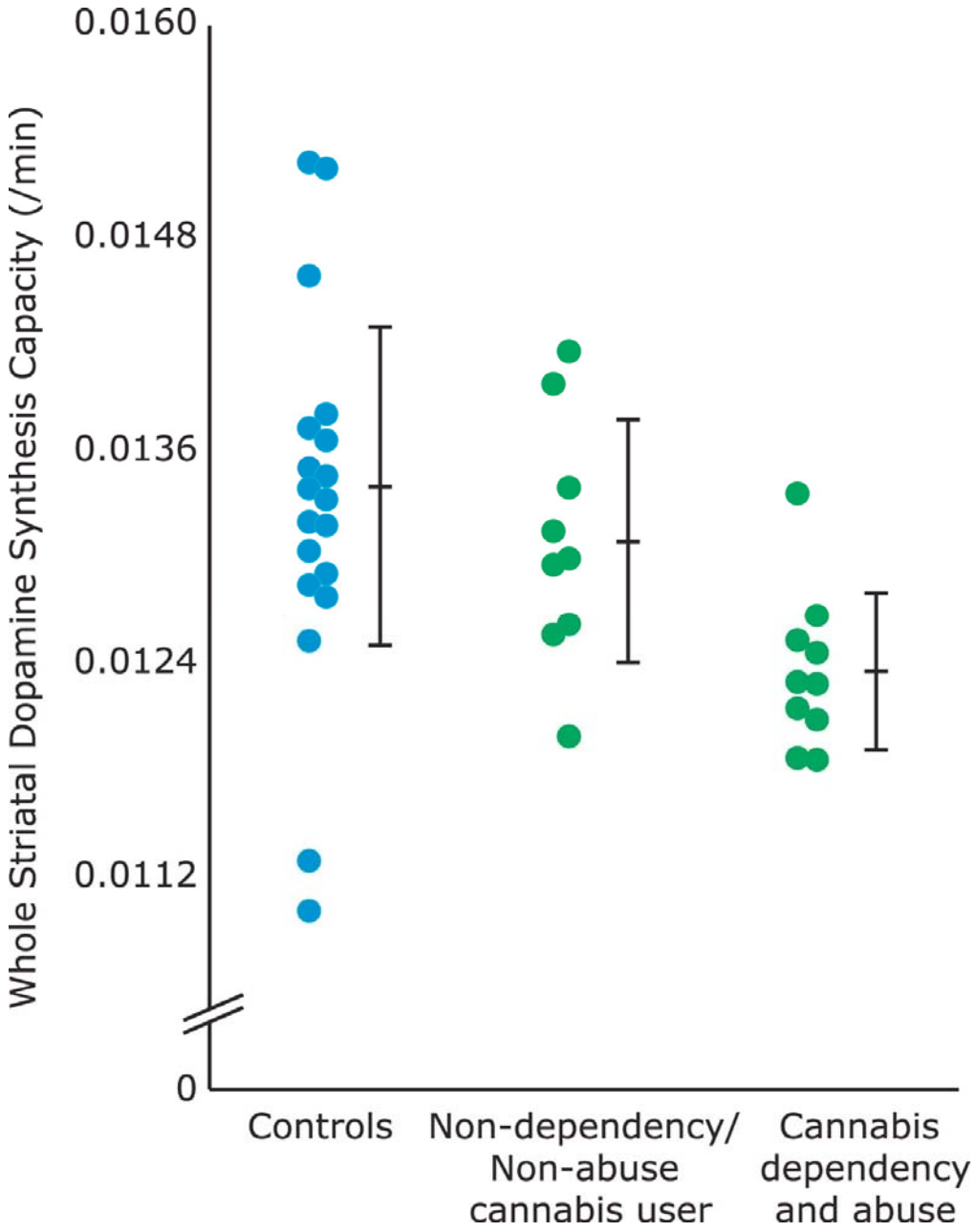


Figure 3.4 - Striatal dopamine synthesis dopamine synthesis capacity in subjects who met DSM-IV criteria for a diagnosis of Cannabis Dependence or Abuse (*n*=10), regular cannabis users who did not meet diagnostic criteria (*n*=9) and non-user controls (*n*=19). There were significant differences between cannabis dependence/abuse vs. cannabis users who did not meet criteria (t_{17}=2.80, p=0.012) and cannabis dependence/abuse vs. control group (t_{27}=2.67, p=0.013). There was no significant difference between controls vs. cannabis users who did not meet dependence/abuse criteria (t_{26}=0.54, p=0.60). Error bars indicate standard deviations.

3.4.4 The Relationship between Striatal Dopamine Synthesis Capacity and Cannabis-Induced Psychotic Symptoms

Within the cannabis user group the mean (SD) increase in PSI psychotic symptom subscale score after consuming cannabis was 9.9 (5.1). There was no significant correlation between striatal K_i^{cer} and increase in transient psychotic-like symptoms following cannabis use [r=0.32, p=0.19] (Figure 3.5). Within users there was no significant relationship between K_i^{cer} and both the positive and negative subscales of the PANSS (p>.1).

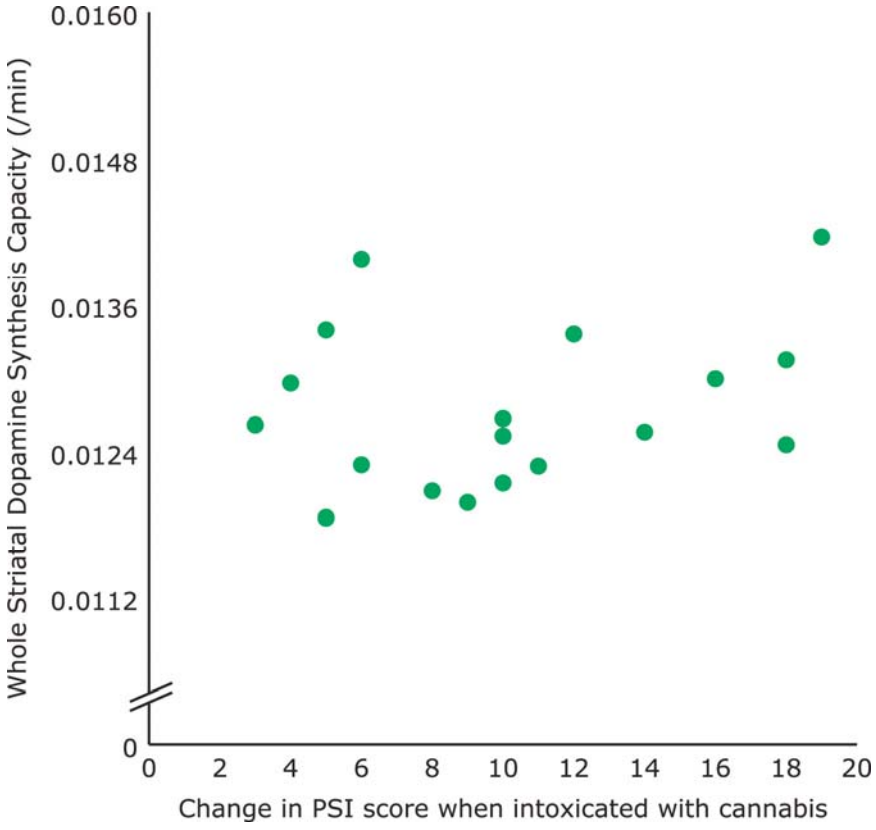


Figure 3.5 - The relationship between striatal K_i^{cer} and transient induction of cannabis-induced psychotic-like symptoms in the cannabis users. There was no significant relationship between the two variables (r=0.32, p=0.19).

3.4.5 Summary

These results show that regular long-term cannabis use is associated with a dose-dependent reduction in dopamine synthesis capacity in the corpus striatum, particularly in those meeting diagnostic criteria for cannabis abuse or dependence. However, no relationship was found between dopaminergic function and cannabis-induced psychotic-like symptoms.

Chapter 4:

Salience Attribution and its Relationship to Cannabis-Induced Psychotic Symptoms

4.1 Introduction

Cannabis is a widely used recreational drug and users are dose-dependently at increased risk of schizophrenia (Moore *et al.* 2007). Psychosis has been proposed to reflect a state of aberrant salience processing (Kapur 2003) and aberrant salience has been related to the presence of delusions in medicated patients with schizophrenia (Roiser *et al.* 2009). Likewise, individuals at ultra-high risk of psychosis demonstrate aberrant salience processing, the degree of which has been related to the severity of delusion-like symptoms (Roiser *et al.* 2013). Since long-term regular cannabis users are at epidemiological risk of psychosis (Moore *et al.* 2007), salience processing may be disrupted in this group. However, salience processing has not previously been investigated in this group.

In this chapter salience processing is investigated in cannabis users who experience transient cannabis-induced psychotic-like symptoms compared to non-user controls using the Salience Attribution Task (Roiser *et al.* 2009). The SAT is a probabilistic reward learning task utilizing cue stimuli that vary along two dimensions, one task-relevant and one task-irrelevant. During the task, participants respond to a probe after seeing 1 of 4 categories of cues (blue animals, red animals, blue household objects, and red household objects), which vary along 2 dimensions (colour and form); as described in Chapter 2. Participants receive monetary reward (£0.05 to £1.00) on 50% of trials, with more money available for faster responses. Feedback was given at the end of each trial. The probability of reward varies along one of the cue dimensions (such that if colour was the task-relevant dimension if blue stimuli were reward 87.5% of the time, red stimuli would be rewarded 12.5% of the time), but not for the other (such that if form was the task-irrelevant dimension both animals and household objects would be rewarded 50% of the time). 'Adaptive' reward learning refers to differences in reaction

times (the implicit measure of learning) and subjective ratings on a visual analogue scale (the explicit measure of learning) along the task-relevant cue dimension, i.e. for high-probability reward cue features relative to low-probability reward cue features. 'Aberrant' reward learning is defined similarly, but along the task-irrelevant dimension, i.e. differences in ratings or reaction times between cue features that are both associated with 50% probability of reward give rise to aberrant explicit and implicit measures respectively.

4.2 Hypotheses

3. Cannabis users who experience cannabis-induced psychotic-like symptoms will show elevated levels of aberrant salience compared to non-user controls, indicated by reduced implicit adaptive salience and elevated implicit salience measures of the SAT.

4. Within the cannabis user group, aberrant implicit and explicit salience will be directly related to a greater severity of transient psychotic phenomena.

Exploratory hypotheses:

A. Cannabis users who meet DSM-IV diagnostic criteria for cannabis dependence or abuse will exhibit elevated aberrant implicit salience compared to users who do not meet dependence or abuse criteria.

B. There will be a direct relationship between dopamine synthesis capacity and implicit aberrant salience processing.

4.3 Materials and Methods

4.3.1 Research Ethics

The study was conducted according to the research ethics that were described in Chapter 2.

4.3.2 Power Calculation

Explicit aberrant salience is the primary measure and the study is powered for this variable. In a previous study comparing aberrant salience in people with schizophrenia compared to controls (Roiser *et al.* 2009), explicit aberrant salience was elevated in the patient group with a Cohen's *d* effect size of 1.6. Using G*Power software, an *a priori* power calculation was conducted and it was found that in order to achieve a power of 0.8, with an expected effect size of 1.6 and *alpha* set at $p<0.05$ (two-tailed), using an independent *t*-test, it was calculated that at least 8 participants would be required in each group.

4.3.3 Study Population

The study population including inclusion and exclusion criteria were described in Chapter 2.

4.3.4 Salience Attribution Test

The Salience Attribution Test (SAT) was described in Chapter 2.

4.3.5 Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences, version 21 (IBM, Armonk, NY, USA). Demographic data were analyzed using independent samples *t* tests and χ^2 tests. SAT data were analyzed using repeated-measures analysis of variance (ANOVA). Block (1 / 2) was the within-subjects variable and Group (cannabis user / control) was the between-subjects variable. Normality of distributions was assessed using the one sample Kolmogorov-Smirnov test. Salience outcome measures were assessed for statistically significant skew. RT and VAS aberrant salience scores from the SAT were square root transformed prior to analysis to reduce skew, though untransformed values are presented in the text, figures and tables for clarity. Relationships between data were assessed using Pearson's *r* product–moment correlation coefficient for normally distributed data and Spearman's ρ rank correlation was used for non-normally distributed data. To determine whether participants consistently assigned aberrant salience to any particular stimulus feature, χ^2 tests were employed. For all analyses a *p* value of <0.05 (two-tailed) was considered significant.

4.4 Results

4.4.1 Participant Characteristics

Fifteen cannabis users who experienced a positive change in psychotic-like symptom severity in response to cannabis were recruited from the Morgan *et al.* study. Owing to data storage malfunction for three participants, complete data were available on 12 of these users who experienced a positive change in psychotic-like symptom severity in response to cannabis. An additional two users who did not experience a positive change in PSI score were recruited from the Morgan *et al.* study and a further three users were recruited by public advertisement. Therefore 17, at least weekly cannabis users are included in the present study. All cannabis users consumed the drug as a *spliff*.

The mean (SD) age of first cannabis use was 15.5 (2.0) years, and the mean (SD) duration of at least weekly use was 5.9 (3.1) years. The mean (SD) time taken to smoke an eighth and lifetime exposure to cannabis was 8.3 (7.3) days and 2,850 (2,447) spliffs, respectively. Six users met DSM-IV criteria for cannabis dependence or abuse. Mean (SD) time to smoke an eighth was 4.0 (4.3) days in users who met dependency/abuse criteria and 11.0 (8.4) days in users who did not meet criteria. Nineteen control participants were matched to the user group for age (±5 years) and sex. Participant characteristics are reported in Table 4.x. Urine drug screen was positive for THC and negative for all other substances (amphetamine, opiates, cocaine, methamphetamine, benzodiazepines) in every cannabis user and negative for all drugs (including cannabis) in every control participant. There was a

significant group difference in current cannabis consumption, as expected (Table 4.1). There was no significant group difference in age or sex.

Table 4.1 Sample characteristics

	Controls (*n*=17)	Cannabis Users (*n*=17)	p^a
Sample characteristic			
Age (years) [mean(SD)]	23.9 (4.2)	22.4 (1.9)	0.19
Sex (*n*)	6 female, 11 male	3 female, 14 male	0.44
Cannabis users (*n)*	0	17	1.00
Cannabis use (grams of cannabis/month), mean (SD)	0.0 (0.0)	31.8 (38.5)	0.00
THC content of cannabis (%), mean (SD)	-	7.5 (2.9)	-
Time to smoke an eighth of cannabis (days), mean (SD)	-	8.3 (7.3)	-
Age of onset of regular cannabis use (years), mean (SD)	-	16.3 (2.0)	-

[a] Independent-samples *t*-tests for variables with normal data distributions; Mann-Whitney *U* tests for variables with non-normal data distributions; χ^2-tests for dichotomous variables

4.4.2 Salience Attribution Task

Behavioural data are presented in Table 4.2.

Table 4.2 Behavioural Data

Test	Measure	Controls (n=17)	Cannabis Users (n=17)	p^a
Block 1				
	RT high probability (ms)	300.5 (114.9)	277.5 (111.7)	.56
	RT low probability (ms)	335.8 (51.4)	304.2 (53.2)	.10
	RT adaptive salience (ms)	11.2 (21.9)	3.8 (14.2)	.25
	RT aberrant salience (ms)	12.8 (4.7)	20.8 (19.5)	.18
	VAS high probability (mm)	55.8 (26.9)	63.0 (19.0)	.39
	VAS low probability (mm)	14.1 (8.4)	18.0 (12.1)	.41
	VAS adaptive salience (mm)	41.3 (29.4)	45.7 (25.3)	.64
	VAS aberrant salience (mm)	16.3 (14.5)	10.4 (9.6)	.30
Block 2				
	RT high probability (ms)	312.9 (56.4)	294.8 (57.5)	.38
	RT low probability (ms)	332.7 (58.1)	310.8 (67.0)	.33
	RT adaptive salience (ms)	20.3 (22.4)	14.9 (18.6)	.45
	RT aberrant salience (ms)	13.4 (15.2)	12.4 (7.7)	.81
	VAS high probability (mm)	63.3 (24.7)	66.3 (19.8)	.71
	VAS low probability (mm)	16.3 (9.7)	10.8 (7.6)	.09
	VAS adaptive salience (mm)	46.3 (26.7)	56.0 (23.1)	0.27
	VAS aberrant salience (mm)	8.7 (6.4)	8.4 (8.6)	0.62
SPQ		-	19.9 (9.1)	

Abbreviations: RT, reaction time; SPQ; Schizotypal Personality Questionnaire; VAS, visual analogue scale.
Values given as mean (standard deviation).
[a] independent samples *t* test

4.4.2.1 Reaction Time (Implicit Salience)

All participants responded faster to high relative to low probability reinforced trials ($F_{1,31}$=21.4, p<.001). There was no group x block interaction ($F_{1,32}$=0.05, p=0.82) on implicit adaptive salience. Collapsing across blocks, controls exhibited greater implicit adaptive salience than cannabis users, but this was not statistically significant ($F_{1,32}$=1.60 p=0.22, Figure 4.1). There was a main effect of block ($F_{1,32}$=5.28, p = 0.03), as for both groups implicit adaptive salience was greater in block 2 than block 1 as determined via *post-hoc* paired sample t test (t= 2.33, p=0.026) (see table 4.2).

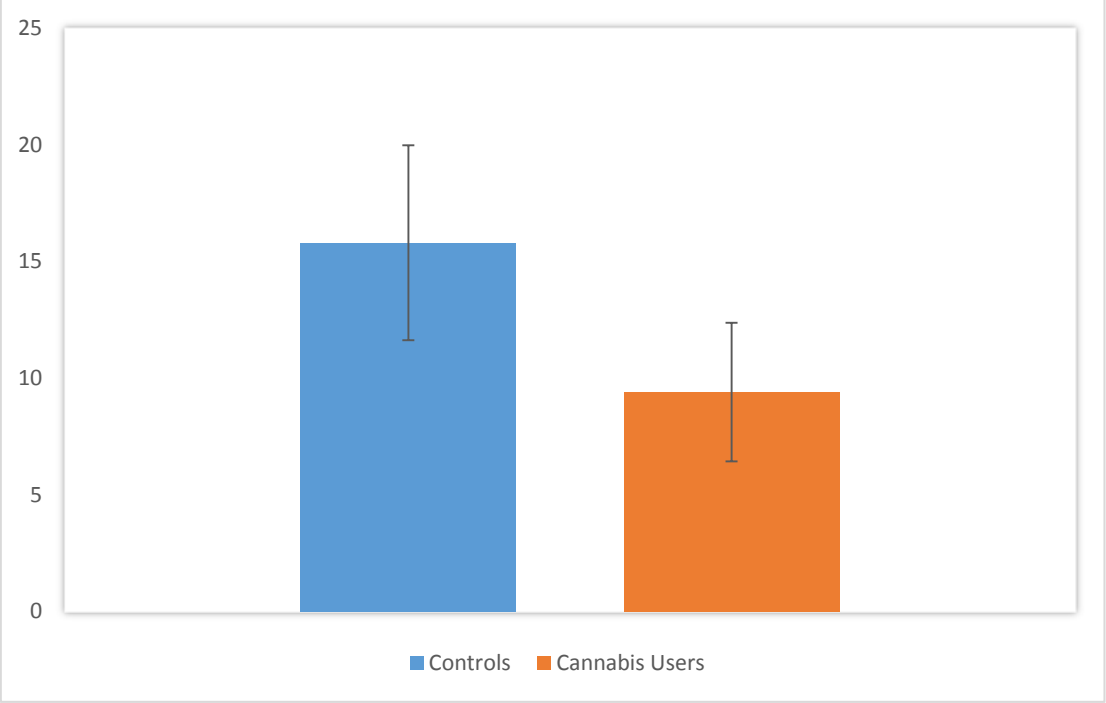


Figure 4.1 Adaptive Salience (ordinate) based on latency (ms) in cannabis users and controls. Values are means and standard errors.

There was no group x block interaction ($F_{1,32}$ = 1.08, p=0.31) on implicit aberrant salience. Collapsing across blocks, cannabis users exhibited greater implicit aberrant salience than controls, but this was not statistically significant ($F_{1,32}$ = 1.12, p=0.30, Figure 4.2). There was no effect of block on implicit aberrant salience ($F_{1,32}$ = 1.30, p = 0.26). Participants did not consistently respond faster in the context of any particular irrelevant stimulus feature (p>0.05).

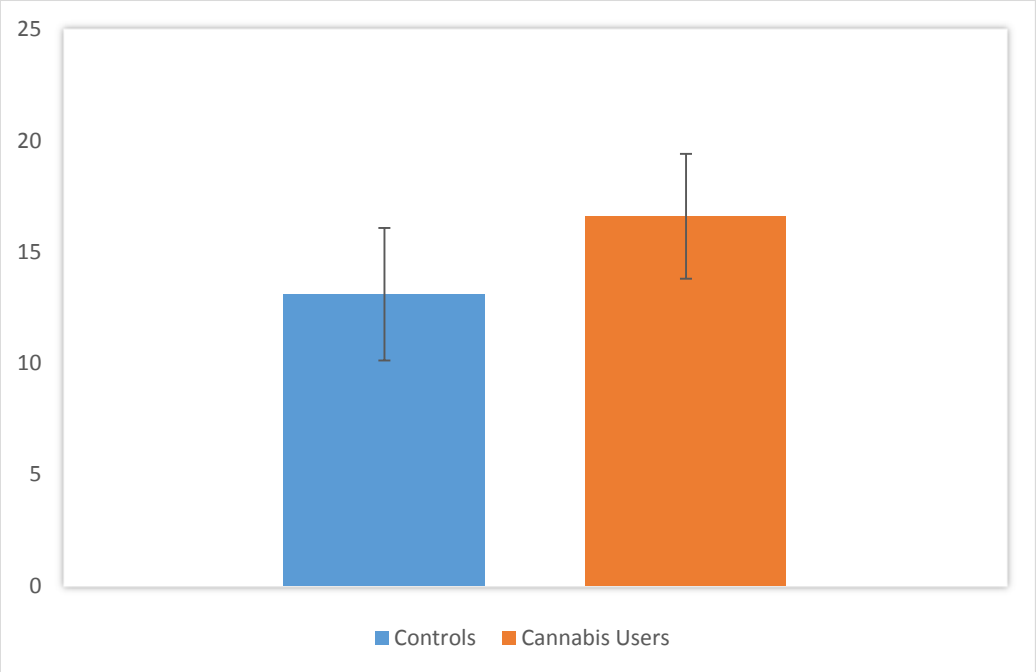


Figure 4.2 Aberrant Salience (ordinate) based on latency (ms) in cannabis users and controls. Values are means and standard errors.

4.4.2.2 Visual Analogue Scales (Explicit Salience)

Across all participants, high-probability-reinforced trails were rated as being more likely to yield reward compared to low-probability-reinforced trials ($F_{1,31}$=130.0, p<0.001). There was no group x block interaction ($F_{1,32}$=0.38 p=0.54) on explicit adaptive salience. Collapsing across blocks, there was no significant effect of group on explicit adaptive salience ($F_{1,32}$=0.80, p=0.38, Figure 4.3). There was no effect of block ($F_{1,32}$=3.18, p = 0.08).

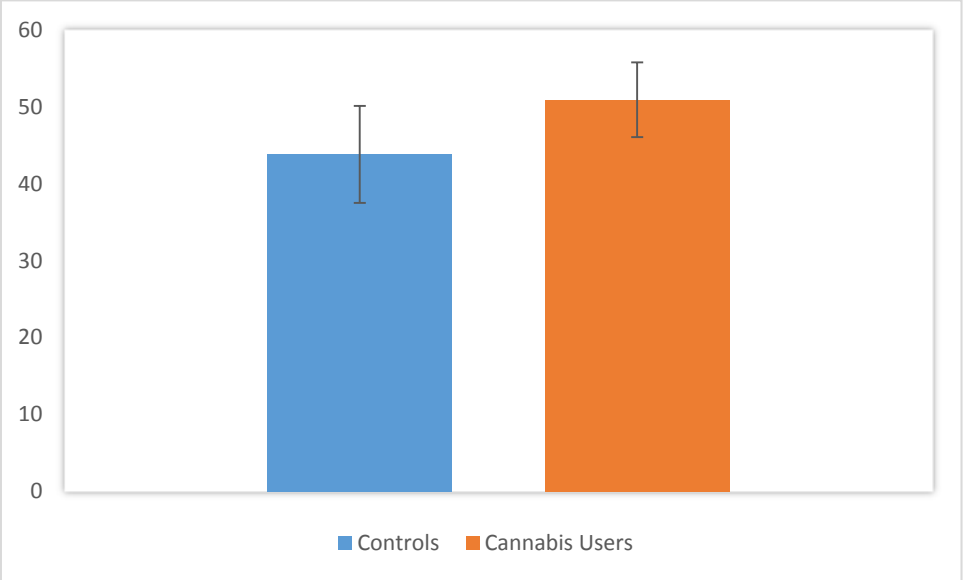


Figure 4.3 Adaptive Salience (ordinate) calculated from subjective reinforcement probability ratings (mm) in cannabis users and controls. Values are means and standard errors.

There was no group x block interaction ($F_{1,32}$=0.35 p=0.56) on explicit aberrant salience. Collapsing across blocks, there was no significant effect of group on explicit aberrant salience ($F_{1,32}$=1.09, p=0.30, figure 4). There was no effect of block ($F_{1,32}$=2.43, p = 0.13). Participants did not consistently rate any particular irrelevant stimulus feature as more likely to yield reward relative to the others.

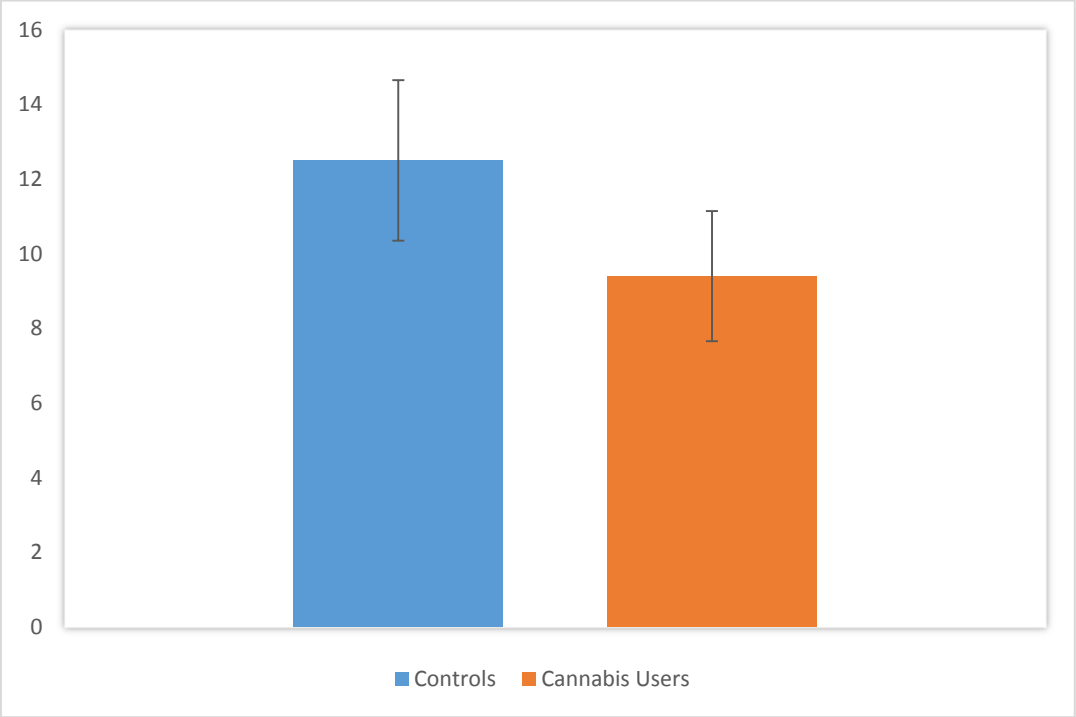


Figure 4.4 Aberrant Salience (ordinate) calculated from subjective reinforcement probability ratings (mm) in cannabis users and controls. Values are means and standard errors.

4.4.3 The Relationship between Salience Processing and Cannabis Use.

Within the cannabis user group, there were no significant relationships between current cannabis use and measures of salience processing (implicit adaptive salience: r = .07, p =.79; implicit aberrant salience r = .49, p =.06; explicit adaptive salience r = -.46, p =.07; explicit aberrant salience r = .14, p =.61). There was no significant relationship between age of onset of cannabis use and measures of salience processing (implicit adaptive salience: r = 0.32, p = 0.23; implicit aberrant salience r = -0.18, p = 0.52; explicit adaptive salience r = -0.12, p = 0.66; explicit aberrant salience r = -0.12, p = 0.65). There was no significant relationship between age of first cannabis use and current cannabis use (r = 0.02, p = 0.95).

As an exploratory analysis, to examine whether cannabis dependency/abuse was associated effects of salience processing, the cannabis user group was divided into participants that met DSM-IV-TR criteria for cannabis dependency or abuse (n=6) and those who did meet criteria (n=11). Within the cannabis users there was a significant effect of DSM-IV-TR dependency/abuse status on implicit aberrant salience ($F_{1,15}$ = 5.8, p = 0.03), but not on the other outcome measures (Figure 4.5). Across the whole sample, the effect of DSM-IV-TR dependency/abuse status on implicit aberrant salience did not reach the threshold for statistical significance ($F_{2,32}$ = 2.9, p = 0.07).

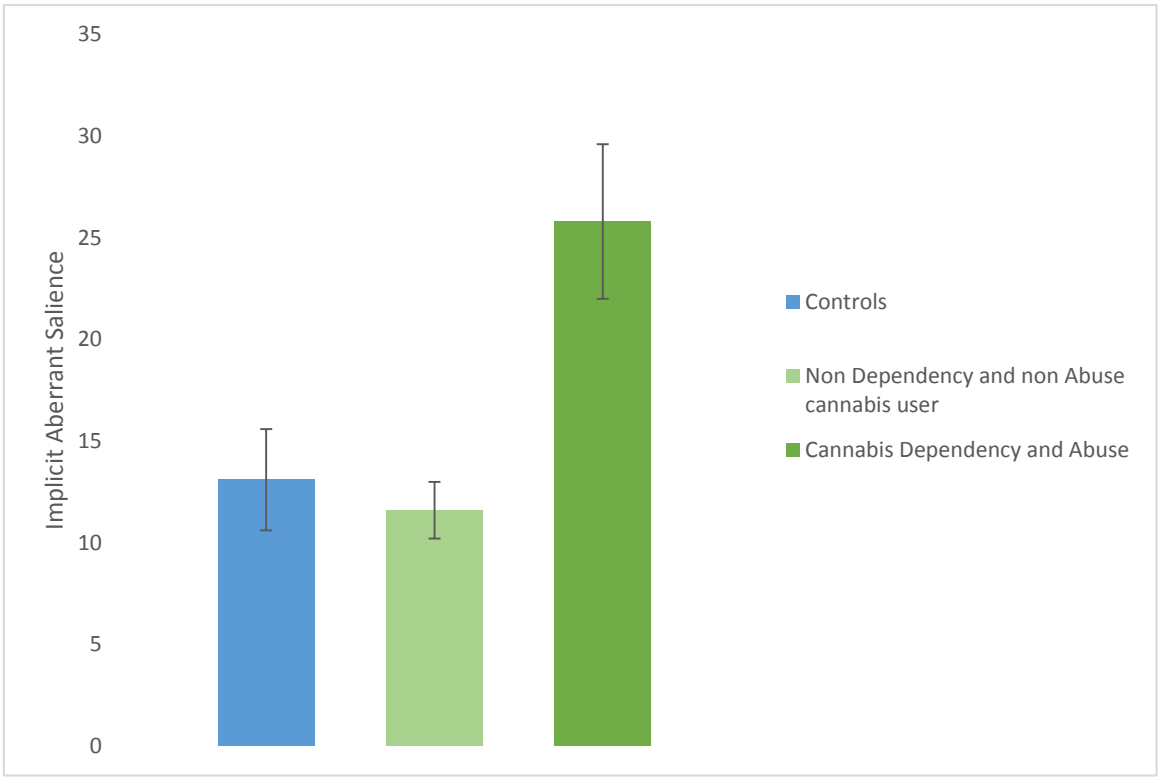


Figure 4.5 Implicit Aberrant Salience (ordinate) based in controls and in cannabis users who meet DSM-IV Dependency and Abuse (*n* = 6) and those who do not meet criteria (*n* = 11). Values (ms) are means and standard errors. Within the cannabis users there was a significant effect of DSM-IV-TR dependency/abuse status on implicit aberrant salience ($F_{1,15}$ = 5.8, p = .03), but across the whole sample, this did not reach the threshold for statistical significance ($F_{2,32}$ = 2.9, p = .07).

4.4.4 The Relationship between Aberrant Salience Processing and Cannabis-Induced Psychotic-Like Symptoms

Within the cannabis users who experienced cannabis-induced psychotic-like symptoms ($n = 12$) there was a significant relationship between cannabis-induced psychotic-like symptom severity and explicit aberrant salience ($r = 0.61$, $p = 0.04$). See figure 4.6. There were no significant relationships between cannabis-induced psychotic-like symptoms and the other salience measures ($p>0.05$), or between SPQ score and all salience measures ($p>0.05$).

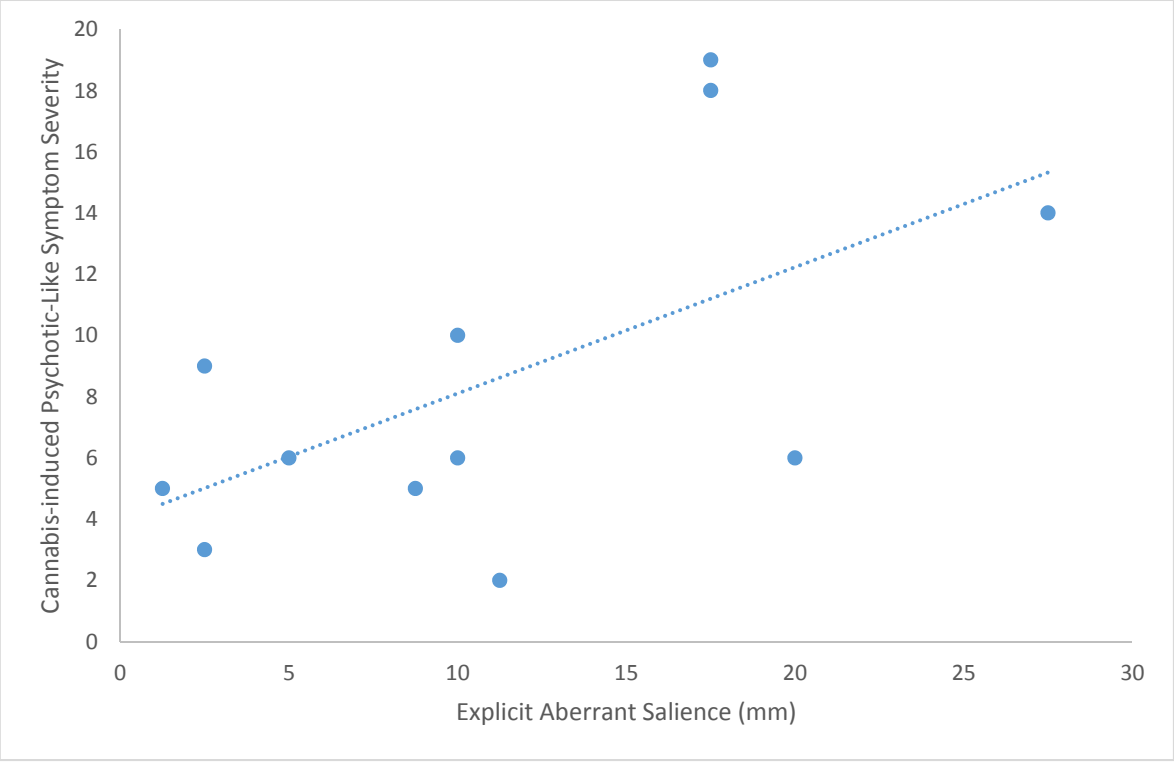
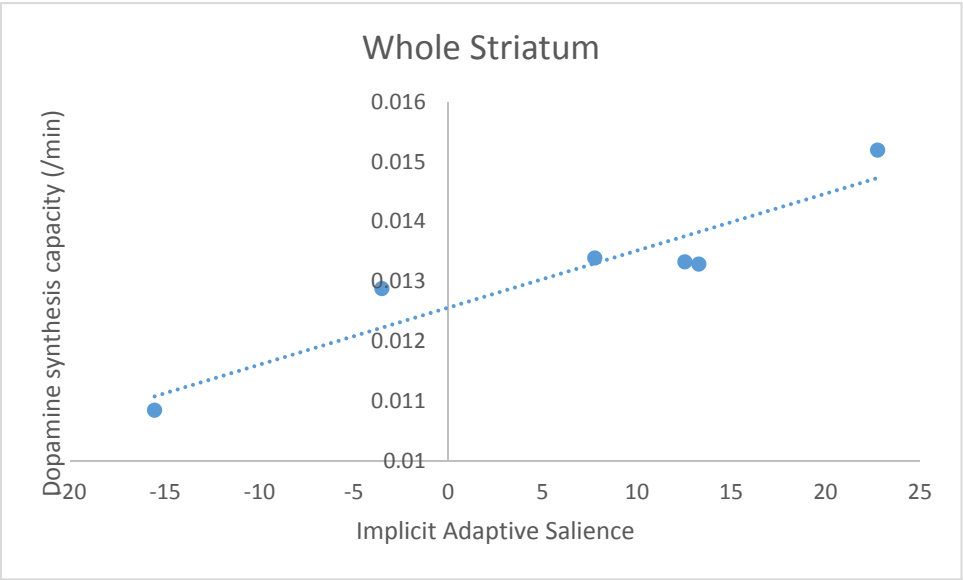


Figure 4.6 The relationship between Explicit Aberrant Salience (mm) and cannabis-induced psychotic-like symptom severity (change in Psychotomimetic States Inventory Score)

4.4.5 The Relationship between Salience Processing and Dopaminergic Function

As an exploratory analysis, data are also presented on salience processing and dopaminergic functioning. Six controls in the present study had participated in the study of dopaminergic function in cannabis users. Both implicit and explicit adaptive salience were positively correlated with whole striatal dopamine synthesis capacity, whilst implicit aberrant salience was inversely correlated with whole striatal dopamine synthesis capacity (Figure 4.7 Table 4.3).



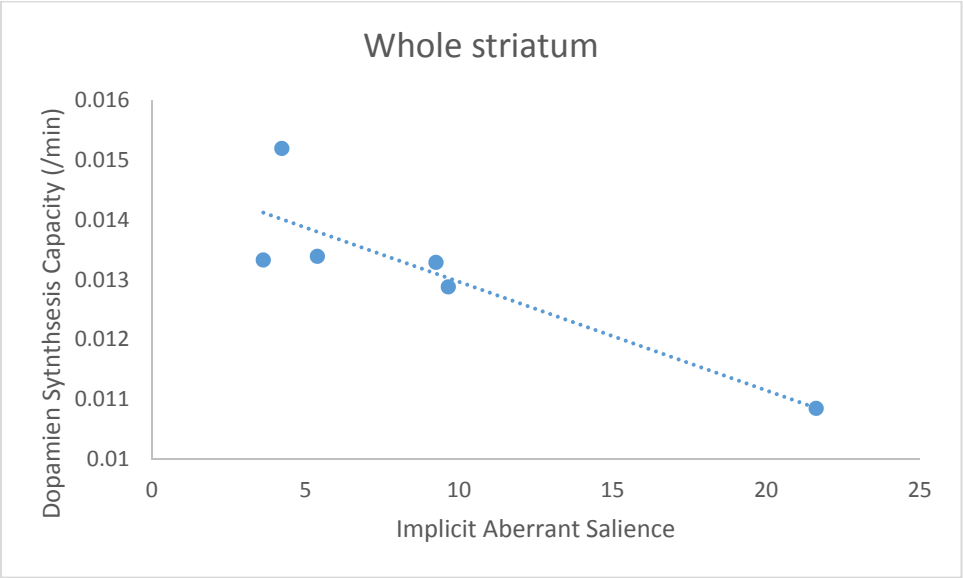


Figure 4.7 The relationships between dopamine synthesis capacity (indexed as K_i^{cer}) in the whole striatum and implicit adaptive salience (top) and implicit aberrant salience (bottom) in controls.

Table 4.3 The relationships between salience attribution and dopamine synthesis capacity (indexed as K_i^{cer}) in the striatum and each of its functional subdivisions in controls who had previously undergone PET scans (n=6).

	K_i^{cer} (min-1)		RT Adaptive Salience		RT Aberrant Salience		VAS Adaptive Salience		VAS Aberrant Salience	
ROI	Mean	(SD)	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
STR	.0132	.0014	.94	.006	-.91	.01	.82	.05	-.15	.78

Ten cannabis users in the present study had participated in the study of dopaminergic function in cannabis users. There were no significant relationships between the SAT outcome measures and dopamine synthesis capacity in the whole striatum (Table 4.3). There were no other significant relationships between salience measures and dopamine synthesis in the other striatal functional subdivisions.

Table 4.4 The relationships between salience attribution and dopamine synthesis capacity (indexed as K_i^{cer}) in the striatum and each of its functional subdivisions in cannabis users who had previously undergone PET scans (*n*=10).

	K_i^{cer} (min-1)		RT Adaptive Salience		RT Aberrant Salience		VAS Adaptive Salience		VAS Aberrant Salience	
ROI	Mean	(SD)	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
STR	.0128	.0008	.27	.45	-.11	.77	.55	.10	.22	.55

Fisher's *r*-to-*z* transformation was applied to examine for significant differences in the relationships between dopaminergic functioning and salience processing in users and controls. Significant differences were found in the relationships between both implicit adaptive and aberrant salience processing and dopamine synthesis capacity in the whole striatum. Specifically, cannabis use was associated with the loss of a positive relationship between implicit adaptive salience and dopamine synthesis capacity, and the loss of an inverse relationship between implicit aberrant salience and dopamine synthesis capacity.

Table 4.5 Fisher's *r*-to-*z* transformation to examine significant differences in the relationships between salience processing and striatal dopamine synthesis capacity in cannabis users and controls.

	RT Adaptive Salience		RT Aberrant Salience		VAS Adaptive Salience		VAS Aberrant Salience	
ROI	*z*	*p*	*z*	*p*	*z*	*p*	*z*	*p*
STR	2.12	.03	-2.05	.04	.78	.44	-.54	.59

4.5 Summary

These results demonstrate that regular long-term cannabis use is not associated with statistically significant differences in behavioural measurements of salience processing. However, these results show preliminary evidence for a difference in implicit aberrant salience between cannabis who do and do not meet DSM-IV criteria for cannabis abuse or dependence. The results above also indicate a loss of relationship between implicit salience processing and dopamine synthesis capacity in the whole striatum associated with long-term cannabis use.

Chapter 5:

The Relationship Between Apathy and

Dopamine Synthesis Capacity in Cannabis Users

5.1 Introduction

Over 100 years ago, the Indian Hemp Commission reported that heavy cannabis use was associated with apathy (Indian Hemp Drugs Commission 1893). This is particularly of interest to schizophrenia research as apathy has also long been recognised as being an important and common negative symptom of schizophrenia. Bleuler (1911) described the apathy he saw in his patients:

> *"an indifference to everything—to friends and relations, to vocation or enjoyment, to duties or rights, to good fortune or to bad."*

Likewise, Kraeplin (1919) wrote:

> *"(my) patients have lost every independent inclination for work and action; they sit about idle, trouble themselves about nothing, do not go to their work, neglect their most pressing obligations, although they are perhaps still capable of employing themselves in a reasonable way if stimulated from outside."*

Heavy cannabis use has been associated with educational and occupational under-achievement in several (Brook *et al.* 2002; Fergusson *et al.* 2003; Gruber *et al.* 2003; Kandel *et al.* 1986; Macleod *et al.* 2004; Horwood *et al.* 2010) but not all studies (Reilly *et al.* 1998). Since then, there is evidence that regular use of the drug is associated with apathy (Looby and Earleywine 2007; McGlothin & West 1968; Tennant & Groesbeck 1972; Verdejo-Garcia *et al.* 2006), defined as reduced motivation for goal-directed behaviour (Levy & Dubois 2006; Marin

1991). Thus, reduced motivation, i.e. apathy, had been proposed to be one factor potentially involved in impaired educational and occupational outcomes associated with heavy cannabis use (Fergusson *et al.* 2003). In support of this, heavy chronic cannabis use has been found to produce apathetic behaviours in rhesus monkeys (Paule *et al.* 1992). However, there is limited evidence of amotivational effects of cannabis from laboratory studies in humans using operant conditioning paradigms (Cherek *et al.* 2002) and that this may be related to heavy use of the drug (Mendelson *et al.* 1976; Lane *et al.* 2005). There is evidence both from studies in animal models and humans that THC administration disrupts reinforced behaviour (Stiglick and Kalant 1983; Kamien *et al.* 1994; Lane & Cherek 2002; Lane *et al.* 2004; Foltin *et al.* 1989). As described in Chapter 1, a key proposed function of the mesolimbic dopaminergic system is to mediate the processing of incentive stimuli by modifying their motivational value (Berridge and Robinson 1998). Under this model, the dopamine system encodes the salience, i.e. biological importance, of sensory stimuli. Since there is evidence that this process is in turn modulated by the brain's own *"cannabis"*, the endocannabinoid system (Fernandez-Ruiz *et al.* 2010; Melis *et al.* 2012; Melis and Pistis 2012), disturbance of endocannabinoid signalling via the actions of exogenous cannabinoids (i.e. THC) will therefore likely alter dopamine-mediated reward processing.

A functional magnetic resonance imaging (fMRI) study has reported attenuated striatal reward processing in chronic cannabis users (van Hell *et al.* 2010). It was therefore proposed that attenuated mesolimbic dopaminergic transmission due to long-term cannabis use results in a mesolimbic reward system that is hyporesponsive to non-drug stimuli (van Hell *et al.* 2010), in line with the reward deficiency hypothesis (Blum *et al.* 2000; Koob and Le Moal 2005). Whilst only a limited number of studies have examined processing of drug and non-drug stimuli within the same model, there is evidence to support the hypothesis that complementary

processes co-occur within an individual to give rise to both hypersensitivity to drug reward and hyposensitivity to non-drug rewards (Garavan *et al.* 2000; Wrase *et al.* 2007; Zijlstra *et al.* 2009). However, it has also been proposed that amotivational symptoms in cannabis users could be attributed to coexisting depressive symptoms (Musty and Kaback 1995).

Treatment with the dopamine precursor levodopa and pramipexole, a dopamine D2 agonist, has both been found to improve apathy in patients with Parkinson's disease (Czernecki *et al.* 2002; Lemke *et al.* 2006), where striatal hypodopaminergia has been found to be related with apathy in depressed patients with Parkinson's disease (Remy *et al.* 2005).

Studies of macaque monkeys have found that mesolimbic dopamine pathway loss, induced via 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesions, predicted apathetic behaviour (Brown *et al.* 2012; Schneider *et al.* 1988) and that apathy was inversely correlated to [^{18}F]-DOPA uptake in the nucleus accumbens and dorsal striatum (Brown *et al.* 2012). In the context of the findings from Chapter 3, a supplementary hypothesis was made that that apathy in cannabis users would be inversely correlated with striatal dopaminergic function.

5.2 Materials and Methods

5.2.1 Research Ethics & Governance

The research ethics and governance are described in Chapter 2.

5.2.2 Study Population

14 regular cannabis users (defined as at least weekly cannabis use for >1 year; mean [SD] age 20.4 [1.3] years, 13 males, one female) recruited from an ongoing cohort study (Morgan *et al.* 2011) who participated in the study described in Chapter 3. The median (interquartile range, IQR) time to smoke an 'eighth' was 3.8 (6.0) days, and the mean (SD) age of onset of regular cannabis use was 16.3 (2.2) years.

5.2.3 Study Measures

In addition to the procedures described in Chapter 3 including structured psychiatric history, detailed drug history and positron emission tomography, participants completed the Apathy Evaluation Scale (self-rated) (AES-S) (Marin *et al.* 1991).

5.2.4 Statistical analysis

Normality of distributions was assessed using the one-sample Kolmogorov–Smirnov test. Relationships between K_i^{cer}, levels of cannabis use and apathy severity were tested using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation

coefficient for non-normally distributed data. Statistical significance was defined as $p < 0.05$ (two-tailed). The primary outcome measure was K_i^{cer} in the whole striatum. Exploratory analyses in the striatal sub-divisions (presented uncorrected for multiple comparisons) were conducted. Exploratory analyses between sub-groups were conducted using independent samples *t*-tests for normally distributed data and Mann–Witney *U* tests for non-normally distributed data.

5.3 Results

It was found that all cannabis users scored in excess of 34 points on the AES-S (median [IQR] 59.5 [7.5]), indicative of significant 'apathy' based on normative data from a healthy population non-cannabis using sample (mean [SD] = 24.4 [4.5]) (Kant *et al.* 1998). There was no significant difference in apathy scores between subjects who met DSM-IV(TR) criteria for abuse or dependence and users who did not meet criteria ($p = .71$), and there was no significant difference in K_i^{cer} between those subgroups in the whole striatum ($t_{12} = 1.96$, $p = .07$). There were no significant relationships between apathy and the volumes of any of the ROIs examined (all $p > .1$).

K_i^{cer} was inversely correlated to AES-S score in the whole striatum and its associative subdivision (Spearman's $rho = -.64$, $p = .015$ [whole striatum]; $rho = -.69$, $p = .006$ [associative]) but not in the limbic or sensorimotor subdivisions (Fig. 6.1). Cook's *D* and leverage analysis indicated that these correlations were not driven by outlying data points. Our findings of an inverse relationship between apathy and K_i^{cer} in the whole striatum and its associative subdivision remained significant when co-varying for the respective striatal volume

(r_{df} = −.61$_{11}$, p = .03 [whole striatum]; r_{df} = −.62$_{11}$, p = .03 [associative]) and for recent tobacco and alcohol use (r_{df} = −.63$_{10}$, p = .03 [whole striatum]; r_{df} = −.65$_{10}$, p = .02 [associative]).

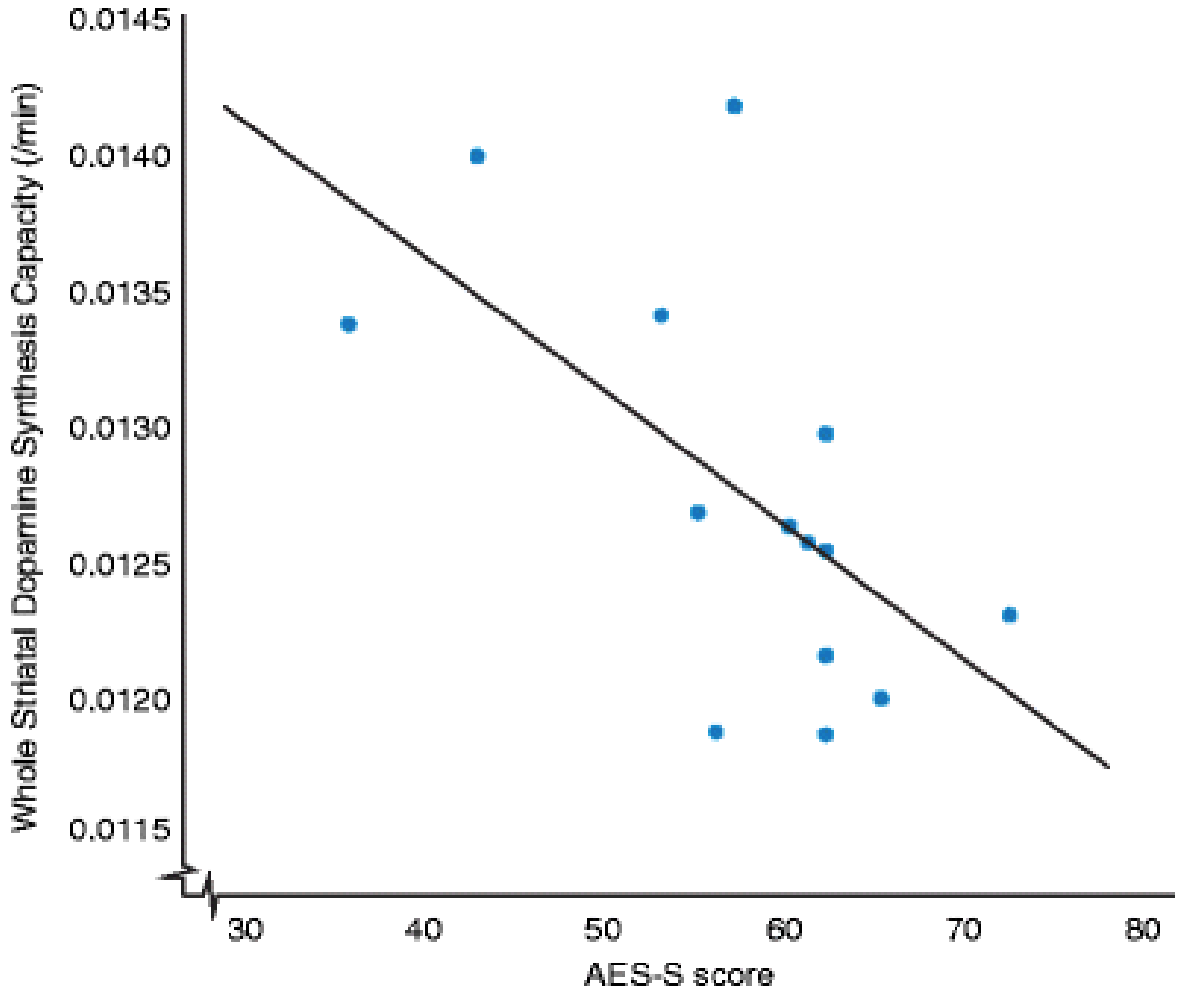


Figure 5.1 The relationship between whole striatal dopamine synthesis capacity (K_i^{cer}) and apathy (AES-S score) (*rho* = −.64, p = .015)

There were no significant relationships between AES-S and current cannabis consumption (*rho* = .28, *p* = .34) or age of first cannabis use (*rho* = .25, *p* = .40), suggesting that it is not related to recent use or age of first use of cannabis per se. In addition, there were no significant relationships between the time since last cannabis exposure and apathy (*rho* = −.39, *p* = .17) or K_i^{cer} (*rho* = −.24, *p* = .41). Furthermore, there was no relationship between apathy and psychotogenic response to cannabis (*rho* = .11, *p* = .70). However, there was no significant relationship between Apathy and the CEQ After Effects scale (*rho* = −.06, p = .83), and there was no significant relationship between the CEQ After Effects scale and whole striatal K_i^{cer} (*r* = −.46, *p* = .88). Four subjects reported a family history of depression in a first degree relative. There was no significant difference in K_i^{cer} between subjects with and without a family history of depression (t_{df} = .34$_{12}$, *p* = .74).

5.4 Summary

14 of the cannabis users who participated in the experiment described in Chapter 3 completed the AES-S. Of these, all subjects scored in excess of 34 points on the AES-S (median [interquartile range] 59.5 [7.5]), indicative of significant apathy based on normative data. K_i^{cer} was inversely correlated to AES-S score in the whole striatum and its associative functional subdivision (Spearman's *rho* = −0.64, *p* = 0.015 [whole striatum]; *rho* = −0.69, *p* = 0.006 [associative]) but not in the limbic or sensorimotor striatal subdivisions. There were no significant relationships between AES-S and current cannabis consumption (*rho* = 0.28, *p* = 0.34) or age of first cannabis use (*rho* = 0.25, *p* = 0.40).

Chapter 6:

Dopamine synthesis capacity

and its Relationship to Psychosocial Risk Factors for Schizophrenia

6.1 Introduction

Whilst a history of schizophrenia in a first degree relative is associated with the highest relative risk of having the illness at the individual level, environmental risk factors account for many more cases on a population basis (Mortensen 1999). Furthermore, environmental factors appear necessary for the manifestation of frank illness in the majority of cases (Van Os & Marcelis, 1998). Epidemiological studies have found that a number of psychosocial stressors increase the risk of schizophrenia. As discussed in chapter 1, the main identified psychosocial stressors relate to inner city dwelling, migration, childhood adversity and/or trauma, and adult adversity. These will be revisited below.

Inner city dwelling, or "urbanicity", was first associated with schizophrenia in a landmark epidemiological study where Faris and Dunham (1939) recorded the pre-admission geographical location of over 30,000 patients treated in Chicago's psychiatric hospitals and they found high rates of schizophrenia ''in the deteriorated regions in and surrounding the centre of the city, no matter what race or nationality inhabited that region.'' Subsequently, in a study of 49,191 male Swedish conscripts linked to the Swedish National Register of Psychiatric Care, Lewis *et al.* (1992) found that the incidence of schizophrenia was 1.65 times higher among men brought up in cities than in those who had had a rural upbringing. Likewise, Marcelis *et al.* (1998) followed up all live births recorded between 1942 and 1978 in the Netherlands through the National Psychiatric Case Register for first psychiatric admission for psychosis between 1970 and 1992 (n=42,115) and found that urban birth carried twice the risk of later schizophrenia than rural birth. A systematic review by Kelly *et al.* (2010) found that all but one of 18 register-based studies examining rates of psychosis according to urbanicity found a positive association. The relative risk was between 1.4 and 4.3, and in most cases was

approximately 2. There is also evidence of a dose-response relationship between degree of urbanicity and risk of schizophrenia (Pedersen & Mortensen, 2001).

Many studies have replicated the finding that migration is a risk factor for schizophrenia since Ødegaard (1932) described high rates of Norwegians undergoing a schizophrenic breakdown after migration to Minnesota. In a meta-analysis (Cantor-Craee & Selton, 2005) the relative risk (RR) of schizophrenia was higher in migrants from less economically developed countries and particularly for black migrants moving to a white-majority country. A recent meta-analysis (Bourque *et al.* 2011) has confirmed an increased risk of psychosis in both first and second generation migrants with incidence rate ratios (IRR) of 2.3 and 2.1 respectively with no significant difference between the two generations. The effect of migration may also be related to ethnic minority status as a replicated finding is that the incidence of psychosis in migrants increases as they form a decreasing proportion of the population (e.g. Boydell *et al.* 2001 & Veling *et al.* 2008b). Other factors such as urbanicity do not appear to mediate the risk associated with this (Bourque *et al.* 2011).

There is evidence that a variety of childhood stressors increase the risk of psychosis. There is an approximate two-fold increase in the risk of schizophrenia among people with unknown fathers compared with people with known fathers (Mortensen *et al.* 1999) and in individuals who have experienced the death or long-term separation from a parent before the age of 16 years (e.g. Morgan *et al.* 2007, Agid et al. 1999). Studies have also found associations between childhood abuse and/or neglect and psychotic risk although this evidence is less consistent (Morgan & Fisher 2007). Despite methodological challenges, these studies suggest traumatic events may increase the likelihood of experiencing psychotic symptoms (reviewed in Read *et al.* 2008 and van Os *et al.* 2010) and it has been suggested there may be

specific associations between different types of trauma and specific psychotic phenomena (Bentall & Fernyhough, 2008), with ongoing interest in the tentative association between childhood sexual abuse and hallucinosis (e.g. Read *et al.* 2003; Varese *et al.* 2012).

Sudden changes in an individual's life, e.g. bereavement, unemployment or moving house, are termed *life events*. In 1968, Brown and Birley reported acute life events to be associated with relapse of schizophrenia and Bebbington *et al.* (1993) found a significant relationship between life events and onset or relapse of schizophrenia. More recently, Myin-Germeys & van Os (2007) have extended this to show increased stress-reactivity, i.e. sensitivity to the small stressors of everyday life, in people with and those at genetic risk of schizophrenia. In a case-control study of the effects of cumulative social disadvantage and first-episode psychosis Morgan *et al.* (2008) found a relationship between social disadvantage and the odds of psychosis, these factors included living with relatives (OR 5.2), living alone for over a year (2.19), lacking a confidant (OR 7.74), being unemployed (3.61) and currently not being in a stable relationship (3.36).

Dopamine dysfunction is thought to underlie the development of psychosis (Howes & Kapur 2009). As described in chapter 1, there is evidence from preclinical and clinical studies that both acute and chronic stress alter mesocortical dopaminergic function. Briefly, in animals social defeat stress was found to selectively increase mesocorticolimbic dopamine release (Tidey 1996) and increase phasic dopamine neuron firing in the ventral tegmental area (Razzoli *et al.* 2011). Maternal deprivation and neonatal isolation have been associated with enduring increases in dopamine release in the nucleus accumbens (Hall *et al.* 1999) and greater cocaine-induced nucleus accumbens dopamine (Kosten 2003). However, there have been

inconsistencies in findings as separate studies found that unavoidable stress administered over one week and three weeks was associated with a decrease in nucleus accumbens dopamine output that remained evident two weeks after administration of stress had abated (Mangiavacchi *et al.* 2001) and 30 minutes of stress administered twice daily for 21 days was associated with a reduction cocaine-induced nucleus accumbens dopamine response (Shimamoto *et al.* 2011).

In terms of evidence of dopaminergic effects of psychosocial stress from human studies, elevated urinary dopamine and other catecholamine metabolites have been reported in girls with a history of sexual abuse compared to those without (De Bellis *et al.* 1994). There is fMRI evidence of striatal reward pathway dysfunction in adults who were abused as children (Dillon *et al.* 2009) and adolescents who had suffered severe early life deprivation. In PET studies, Pruessner *et al.* (2004) reported ventral striatal dopamine release was increased in response to psychosocial stress in humans who reported insufficient early life maternal care; Wand *et al.* (2007) found stress-induced cortisol levels were positively associated with amphetamine-induced dopamine release in the striatum; Mizrahi *et al.* (2011) found increased psychosocial stress-induced striatal dopamine release in antipsychotic-naïve subjects with schizophrenia and those at clinical high-risk of the illness compared to matched healthy controls; and Oswald *et al.* (2014) reported positive associations between childhood adversity and amphetamine-induced dopamine release.

To summarize, it has been proposed, though never directly tested, that psychosocial stressors may increase the risk of schizophrenia by creating a hyperdopaminergic state in the striatum (Thompson *et al.* 2004; Howes *et al.* 2004; Selten *et al.* 2013).

6.2 Hypotheses

1. Subjects with a high cumulative exposure to psychosocial risk factors for schizophrenia will exhibit elevated dopamine synthesis capacity compared to subjects with low cumulative exposure to the same environmental stressors.
2. Within subjects with a high cumulative exposure to psychosocial risk factors for schizophrenia there will be a direct relationship between dose of psychosocial stress and dopamine synthesis capacity.

6.3 Materials and Methods

6.3.1 Research Ethics

The study was approved by the National Research Ethics Service and the Administration of Radioactive Substances Advisory Committee (ARSAC). The study was conducted in accordance with the Helsinki Declaration and all subjects provided informed written consent to participate.

6.3.2 Power Calculation

The power calculation is described in Chapter 2.

6.3.3 Study Population

The study population including inclusion and exclusion criteria are described in Chapter 2.

6.3.6 Psychosocial measures

The following measures of urbanicity were taken: childhood urbanicity score, current urbanicity score, current dwelling population and population density. The urbanicity score was based on the Mortensen *et al.* (1999) categorization of urbanicity, i.e. rural area (with a population less than 10,000) = 1 point; provincial town (with a population less than 100,000) = 2 points; provincial city (with a population less than 100,000) = 3 points; the suburbs of a capital = 4 points; capital city = 5 points. Only the boroughs of Inner London (as defined by the ONS and Eurostat), listed above, were designated "capital city". Detailed histories of the location of each subject's residence were recorded throughout their lives. The childhood urbanicity score was then calculated for each subject based on the urbanicity score for each year of the subject's life from birth to age 16 years. The lowest possible score is therefore 16, and the highest possible score is 80. Where a subject had lived at more than one address during their childhood, a score was allocated based on where they had spent the majority of that year living. Current and historical populations were based on data obtained from the Office of National Statistics, for United Kingdom data, and from census data publicly available on the internet for non-United Kingdom data. Current population density was obtained from the Office of National Statistics.

The following childhood adversity measures were taken: the Childhood Trauma Questionnaire (CTQ) (Bernstein & Fink 1998), childhood experiences of care and abuse (CECA) (Bifalco *et al.* 2006), a questionnaire of bullying, and subjects were asked whether their parents had separated or died before their 17th birthday. The CTQ is a validated 28 item self-report inventory measuring physical abuse, sexual abuse, emotional abuse, physical neglect and emotional neglect. The CECA is a validated structured interview that measures

parental care (neglect and antipathy), parental physical abuse, and sexual abuse from any adult before age 17. The questionnaire of bullying was adapted from the Olweus Bullying Questionnaire (Olweus 1996) and includes five items measuring frequency and severity of physical and emotional abuse from peers during childhood.

The following demographics of adult adversity were taken: current living arrangements, relationship status, and personal and parental history of migration. Detailed histories of life events over the preceding six months were obtained via the List of Threatening Events (Brugha & Cragg 1990), and a life events score then calculated from these events based on the Holmes & Rahe life events stress scale.

The following clinical measures were taken: Brief Impact of Events Scale (IES-6) (Thoresen *et al.* 2010), Beck Depression Inventory (BDI); Beck Anxiety Inventory (BAI); The Oxford-Liverpool Inventory of Feelings and Experiences (O-LIFE), which measures schizotypy, and the Aberrant Salience Inventory (ASI).

6.3.7 PET Scans

Subjects were asked to fast 5 hours and to refrain from smoking tobacco for 2 hours before imaging. On the day of the PET scan, urine drug screen (Monitect HC12, Branan Medical Corporation, Irvine, California) confirmed no recent drug use, and a negative urinary pregnancy test was required in all female subjects. Head position was marked and monitored via laser crosshairs and a camera and minimized using a head-strap.

A Siemens Biograph 6 TruePoint PET-CT scanner (Siemens Healthcare, Erlangen, Germany) was used. A computed tomography (CT) scan (effective dose=0.36 mSv) was acquired for attenuation and model-based scatter correction prior to each PET scan. A target dose of approximately 150 MBq of [^{18}F]-DOPA was administered by bolus intravenous injection at the start of PET imaging. Emission data were acquired in list mode for 95 minutes, reconstructed in a 128 x 128 matrix with 2.6x zoom via filter back projection with a three dimensional 5mm full-width half-maximum Gaussian image filter and re-binned into 32 timeframes (comprising eight 15-second frames, three 60-second frames, five 120-second frames, and sixteen 300-second frames).

6.3.8 Image analysis

The image analysis methods are details in chapter 2 and summarized below. To correct for head movement in the scanner, non-attenuation-corrected dynamic images were de-noised using a level 2, order 64 Battle-Lemarie wavelet filter. Non-attenuation-corrected images were used for the realignment algorithm as they include greater scalp signal, improving re-alignment compared with attenuated-corrected images (Turkheimer *et al.* 1999). Frames were realigned to a single 'reference' frame, acquired 10 min post-injection, employing a mutual information algorithm (Studholme *et al.* 1996). The transformation parameters were then applied to the corresponding attenuated-corrected dynamic images. The realigned frames were then summated, creating a movement-corrected dynamic image, which was used in the analysis. The cerebellar reference region (Kumakura & Cumming 2009) was defined using a probabilistic atlas (Martinez *et al.* 2003), and as previously described, regions of interest (ROI) in the whole striatum and its functional sub-divisions were delineated to create an ROI map (Egerton *et al.* 2010). The functional subdivisions of the striatum reflect the topographical arrangement of corticostriatal projections. Projections to the sensorimotor striatum come from the motor cortex and related areas for instance the premotor cortex, primary motor cortex, and supplementary motor cortex; projections to the associative striatum start in associative regions such as dorsolateral prefrontal cortex; and projections to the limbic striatum are from limbic areas such as the amygdala and hippocampus (Haber, 2003). SPM8 (http://www.fil.ion.ucl.ac.uk/spm) was then used to normalize the ROI map together with the tracer-specific ([^{18}F]-DOPA) template (Egerton *et al.* 2010, Howes *et al.* 2009) to each individual PET summation image. This nonlinear transformation procedure allowed ROIs to be automatically placed on individual [^{18}F]-DOPA PET dynamic images. The influx constant (K_i^{cer}, written as K_i in some previous publications (Howes *et al.* 2009)) for the entire striatal

ROI and the functional subdivisions bilaterally were calculated compared with uptake in the reference region using a graphical approach adapted for a reference tissue input function (Egerton *et al.* 2010).

6.3.9 Statistical analysis

Normality of distribution and homogeneity of variance were assessed using Kolmogorov–Smirnov and Levene's tests, respectively. The primary analysis was for Group (HPSS *vs.* LPSS) differences in demographic and imaging variables were determined using independent samples *t*-tests for normally distributed data, Mann–Whitney *U*-tests for non-normally distributed data, and the χ^2 test for dichotomous variables. The primary region of interest was the whole striatum. Exploratory analyses were performed in the functional subdivisions of the striatum. Potential confounds were explored *post hoc* using analysis of covariance (ANCOVA). To examine the effects of childhood and recent psychosocial stressors a combined psychosocial stress score was derived from the CECA and recent life events score (LES). For the combined psychosocial stress score (CPSS), the CECA and recent life events score were combined with equal weight to both on a total scale from 0 to 100, such that half the score was from the CECA (maximum 32 points) and half the score from the life events score (maximum 250 points). The formula applied was:

$$Combined\ psychosocial\ stress\ score = \left(50\left(\frac{CECA}{32}\right)\right) + \left(50\left(\frac{LES}{250}\right)\right)$$

Within HPSS, the relationship between K_i^{cer} and combined psychosocial stress score was tested using Pearson's product-moment correlation coefficient. A two-tailed significance level of p=0.05 was employed throughout.

6.4 Results

6.4.1 Subject Characteristics and Scan Parameters

13 HPSS 'cases' were recruited to the study by public advertisement. All HPSS were current London residents and had spent most of their childhood, up to age 16, in London or the capital city of their country of birth. All HPSS were either first or second generation migrants to both the United Kingdom and the European Union. Nine HPSS had a history of parental separation or death during childhood. Five HPSS lived alone, two lived with their children but with no other adult, two lived with their parents, one lived with a long-term partner and three lived with others. Eight HPSS were single, one was married and four were in a stable non-cohabiting relationship.

13 LPSS 'controls' were matched to the HPSS group on the basis of age (+/- 5 years) and sex. None of LPSS were current residents of Greater London. All LPSS were born and had spent most of their childhood outside London. 11 LPSS had no history of first or second migration to the United Kingdom and the European Union. 1 LPSS was born in Sweden to British parents and moved to the United Kingdom during infancy and 1 further LPSS had a South African parent of British descent. 2 LPSS had a history of parental divorce. Seven LPSS lived with their partner, one LPSS was in a stable non-cohabiting relationship but lived with friends, and five LPSS were single and living with their family.

Table 6.1 Sample characteristics and scan parameters

	LPSS (*n*=13)	HPSS (*n*=13)	*p*[a]
Sample characteristic			
Age (years) [mean(SD)]	27.8 (7.0)	30.3 (7.7)	.39
Sex (*n*)	6 female, 7 male	6 female, 7 male	1.00
Ethnicity (*n*)	13 WB	3 BA, 3BB, 3 BC, 3 ME, 1 OE	.00
Migration (*n*)	1 FGM, 11 N, 1 SGM	4 FGM, 9 SGM	<.001
Urbanicity			
Childhood urbanicity Score [mean(SD)]	26.1 (12.0)	69.1 (13.0)	.00
Current urbanicity [mean(SD)]	7 R, 6 PC	9 ICC, 4 OCC	.00
Current dwelling population [mean(SD)]	67,012 (73,231)	8,174,000 (0)	.00
Current dwelling population density (km^{-2}) [mean(SD)]	3,891 (4,500)	9,251 (4,237)	.01
Childhood Adversity			
CTQ [mean(SD)]	2.6 (2.6)	18.4 (17.3)	.01
CECA [mean(SD)]	.8 (1.3)	6.2 (5.9)	<.01
Childhood bullying [mean(SD)]	5.0 (5.5)	8.7 (4.4)	.08
Parental Separation or death during childhood (*n*)	2	9	.02
Adult Adversity			
Current living arrangement	3 F, 5 CHP, 5 P,	5 A, 2 AWC, 1 CHP, 2 P, 1 OF, 1 F, 1 OLA	.01
Relationship status	5 S, 7MCL	8 S, 1 MCL, 4 NCS	
Adverse life events (last 6 months)	0.4 (0.8)	3.2 (1.6)	.00
Life events score (last 6 months)	8.9 (30.9)	89 (47.7)	.00
Clinical Scores			
BDI [mean(SD)]	1.8 (2.8)	7.8 (5.6)	<.001
BAI [mean(SD)]	2.5 (3.7)	11.5 (10.7)	.01
IES-6 [mean(SD)]	2.0 (2.6)	8.8 (7.9)	.01
O-LIFE [mean(SD)]	7.7 (6.8)	14.1 (9.8)	.07
ASI [mean(SD)]	4.8 (4.9)	12.4 (7.9)	.01

Table 6.1 (continued)

	LPSS (*n*=13)	HPSS (*n*=13)	*p*[a]
Current Drug Use[c,d]			
Tobacco cigarette smokers in last 3 months (*n*)	1 user, 12 non-users	4 users, 9 non-users	.32
Tobacco use in whole sample (cigarettes/day) [mean(SD)]	.1 (.3)	2.3 (4.0)	.07
Tobacco use in smokers (cigarettes/day) [median(IQR)] (tobacco users)	1.0 (-)	7.4 (3.8)	-
Alcohol use in last 3 months (*n*)	10 users, 3 non-users	13 users, 0 non-users	.22
Alcohol[c] use (UK alcohol units/week) [mean(SD)]	9.3 (10.0)	8.0 (9.6)	.73
Scan parameter			
Injected dose (MBq) [mean(SD)]	141.3 (9.3)	143.6 (7.2)	.48
Specific activity (MBq/μmol) [mean(SD)]	35.3 (6.0)	44.6 (15.3)	.06
Whole striatal volume (mm^3) [mean(SD)]	17,665 (2,143)	16,982 (1,538)	.36
Associative striatal volume (mm^3) [mean(SD)]	10,902 (1,386)	10,552 (1,042)	.47
Limbic striatal volume (mm^3) [mean(SD)]	2,138 (279)	2,050 (152)	.33
Sensorimotor striatal volume (mm^3) [mean(SD)]	4,624 (533)	4,380 (406)	.20

Abbreviations: A, alone; ASI, Aberrant Salience Inventory; AWC, alone with children; BA, black African; BAI; Beck Anxiety Inventory; BB, black British; BC, black Caribbean; BDI, Beck Depression Inventory;; CECA, Childhood experiences of care and abuse; CHP, co-habiting partner; CTQ, Childhood Trauma Questionnaire; F, friends; FGM, first generation migrant; ICC, inner capital city; IES-6, Brief Impact of Events Scale; MCL, married, in a civil partnership or living with long-term partner; ME, mixed ethnicity; N, native; NCS, non-cohabiting stable relationship; OCC, outer capital city; OE, other ethnicity; OF, other family; OLA, other living arrangement; O-LIFE, Oxford-Liverpool Inventory of Feelings and Experiences; P, parents; PC, provincial city; R, rural; S, single; SEAT, Social Environment Assessment Tool; SGM, second generation migrant; WB, White British

[a] Independent-samples *t*-tests for variables with normal data distributions; Mann-Whitney U tests for variables with non-normal data distributions; χ^2-tests for dichotomous variables

[b] Groups were compared on a dichotomised ethnicity variable (white British vs ethnic minority).

Groups were compared on a dichotomised living arrange variable (living without other adults vs living with other adults)

Groups were compared on a dichotomised relationship variable (co-habiting partner vs other status)

[c] 1 UK alcohol unit = 10mL (~7.88g) alcohol

There was no significant group difference in the amount of radioactivity injected (table 6.1). The specific activity was higher in HPSS vs. LPSS, although this did not reach the threshold for statistical significance. There was no significant difference in whole striatal or subdivision volumes between the groups. The groups did not differ in terms of substance use including alcohol, tobacco, cannabis, ecstasy (MDMA), cocaine (p>.1).

6.4.2 Striatal Dopaminergic Function

K_i^{cer} was significantly reduced in HPSS relative to LPSS in the whole striatum (figure 6.1). The group difference in whole striatal K_i^{cer} remained significant when co-varying for specific activity ($F_{2,25} = 4.6$, $p = .02$). Secondary analysis in each striatal subdivision showed that this reduction reached significance in the limbic and associative subdivisions (table 6.3).

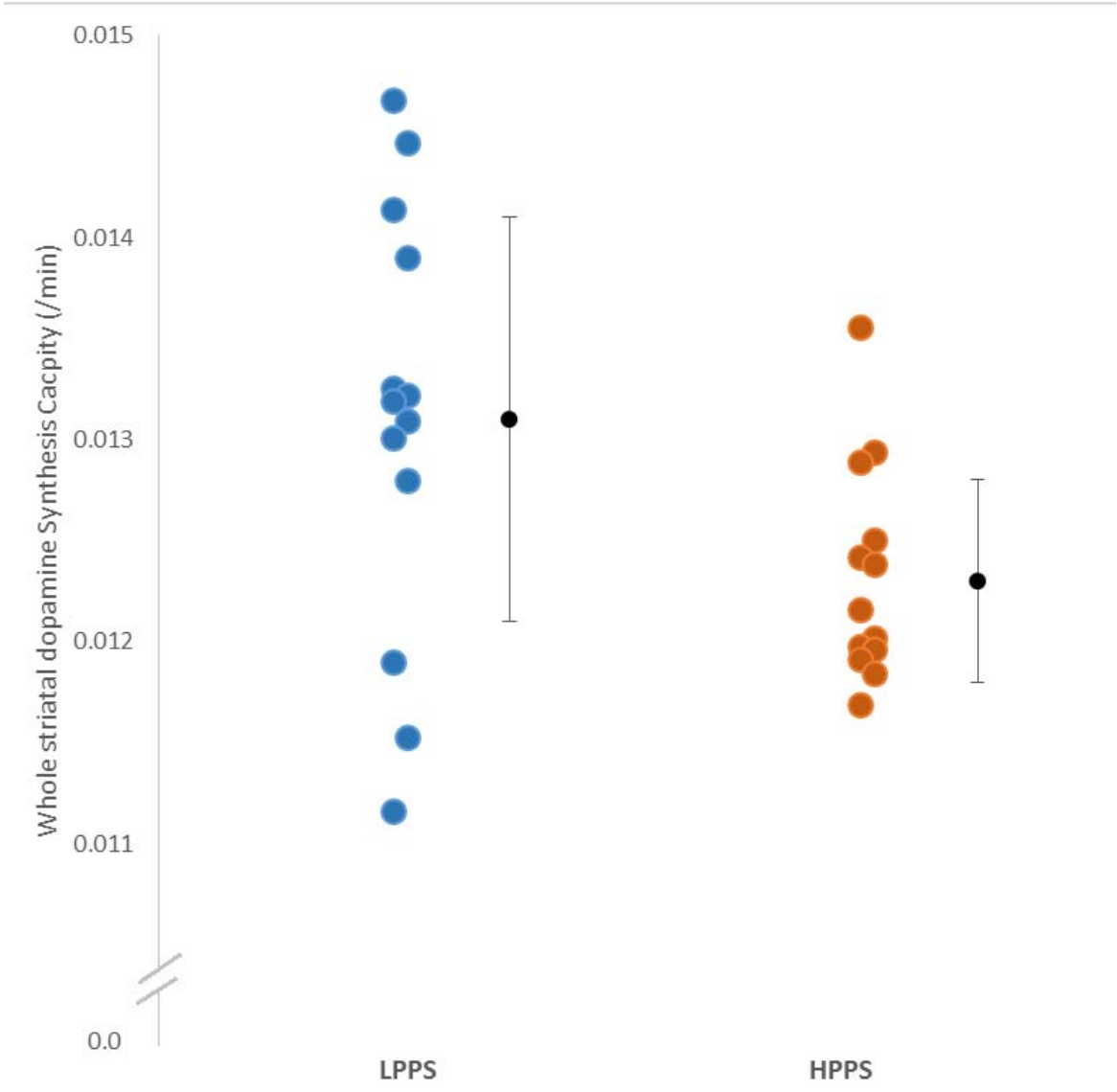


Figure 6.1 Striatal dopamine synthesis capacity in LPPS (n = 13) and HPSS subjects (n = 13). Dopamine synthesis capacity was significantly reduced in HPSS compared with LPSS (t_{24} = 2.32, p = .029). Error bars indicate standard deviations.

Table 6.2 [^{18}F]-DOPA K_i^{cer} (min^{-1}) by group							
VOI	LPSS (*n*=13)		HPSS (*n*=13)		Group comparisons[a]		Effect size
	Mean	(SD)	mean	(SD)	t_{df}	*p*	(Cohen's *d*)
STR	.0131	(.0010)	.0123	(.0005)	2.32_{24}	.029	.91
AST	.0131	(.0011)	.0122	(.0005)	2.45_{24}	.022	.96
LST	.0137	(.0010)	.0125	(.0009)	3.17_{24}	.004	1.24
SMST	.0129	(.0012)	.0125	(.0009)	1.15_{24}	.263	.45

Abbreviations: AST, associative striatum; LST, limbic striatum; K_i^{cer} , influx rate constant; SMST, sensorimotor striatum; STR, whole striatum; VOI, Volume of Interest

[a] Independent-samples *t*-tests.

6.4.3 Smoking and Dopamine Synthesis Capacity

There were higher rates of smoking in HPSS *vs.* LPSS, although these did not reach the threshold for statistical significance. When co-varying for the number of cigarettes smoked per day, the finding of reduced dopamine synthesis capacity in HPSS *vs.* LPSS did not remain significant in the whole striatum ($F_{2,24} = 2.0$, $p = .16$), but did remain significant in the limbic striatal subdivision only ($F_{2,24} = 4.2$, $p = .03$). Removing cigarette smokers from the analysis yielded *n*=11 LPSS and *n*=9 HPSS. As per the ANCOVA, the finding of reduced dopamine synthesis capacity in HPSS *vs.* LPSS did not remain significant in the whole striatum ($t_{18} = 1.3$, $p = .22$), but did remain significant in the limbic striatal subdivision only ($t_{18} = 2.1$, $p = .05$).

6.4.4 The relationship between dopamine synthesis capacity and symptoms of anxiety and depression

Clinical rating scales are reported in table 6.1. HPSS scored significantly higher than LPSS on measures of depressive and anxiety symptoms (BDI and BAI respectively), the degree to which previous stressors were having an impact on their lives in the week prior to scanning (BIE), schizotypy (O-LIFE) and aberrant salience (ASI).

Across all subjects, there was no significant relationship between dopamine synthesis capacity in the whole striatum and the BDI (r=-.28, p=.17) and BAI (r=-.24, p=.14). When co-varying for depressive and anxiety symptoms, the finding of reduced dopamine synthesis capacity in HPSS *vs.* LPSS did not remain significant in the whole striatum ($F_{3,24}$ = 1.3, p = .29), but did remain significant in the limbic striatal subdivision only ($F_{3,24}$ = 3.0, p = .05).

6.4.5 The relationship between dopamine synthesis capacity and combined childhood and adult psychosocial stressors

The relationships between dopamine synthesis capacity and combined psychosocial stress score are given in table 6.3. A significant relationship between psychosocial stress and dopamine synthesis capacity was observed in the associative striatal subdivision only (figure 6.2).

Table 6.3 The relationship between [^{18}F]-DOPA K_i^{cer} and combined psychosocial stress score in HPSS								
VOI	STR		AST		LST		SMST	
	r	*p*	*R*	*P*	*r*	*p*	*r*	*P*
CPSS	.49	.09	.68	.01	.22	.47	.03	.92
Abbreviations: AST, associative striatum; LST, limbic striatum; K_i^{cer} , influx rate constant; SMST, sensorimotor striatum; STR, whole striatum; VOI, Volume of Interest.								

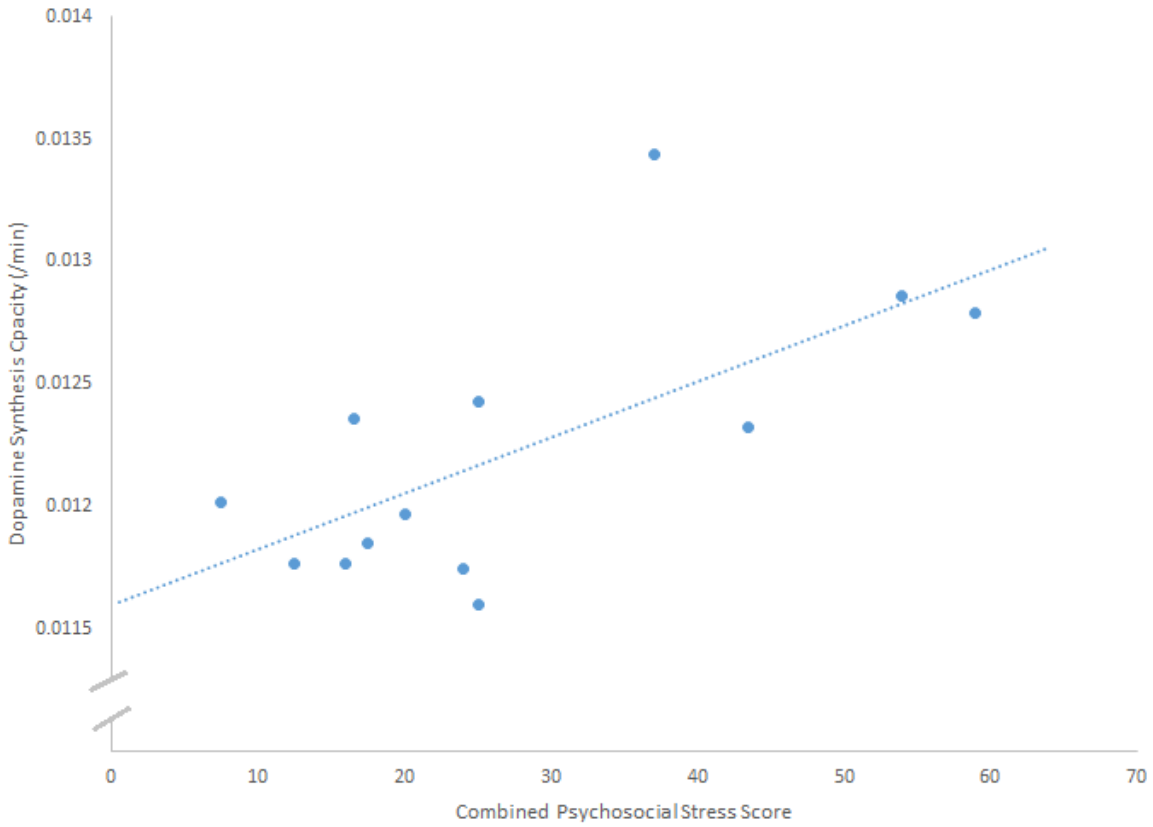


Figure 6.2 The relationship between a combined childhood and recent psychological stress and dopamine synthesis capacity in the associative subdivision of the striatum in HPSS (*r* = .68, *p* = .01).

6.5 Summary

These results show that long-term psychosocial stress is associated with a reduction in dopamine synthesis capacity in the corpus striatum and its associative and limbic subdivisions. However, the finding of reduced dopamine synthesis in the whole striatum may be related to the confounding factors of increased cigarette smoking and affective symptoms in those with high exposure to long-term psychosocial stress. The finding of reduced dopamine synthesis capacity in the limbic subdivision of the striatum remained significant when co-varying for these factors. Within individuals with a high cumulative exposure to psychosocial stress, there was a significant positive correlation between a combined score of childhood and recent stressors and dopamine synthesis in the associative subdivision of the striatum.

Chapter 7: Discussion

7.1 Introduction

The two PET studies and one behavioural study detailed herein have investigated dopaminergic mechanisms for the major environmental risk factors of psychosis. The results obtained are discussed below, beginning with the PET study in cannabis users and followed by the behavioural study of cannabis users. The next section discusses the findings of the PET study in individuals exposed to high and low cumulative psychosocial stressors. This is followed by a section discussing the effects of tobacco on the dopamine system, since this has implications for interpreting the results of the two PET studies. Finally, general conclusions and future directions are discussed.

7.2.1 Dopamine synthesis capacity and its relationship to cannabis induced psychotic symptoms

The main finding from this study is that striatal dopamine synthesis capacity is lower in ***current*** cannabis users than matched non-user controls (effect size: Cohen's $d = .85$; $t_{36} = 2.54$, $p = .016$). In users, lower dopamine synthesis capacity was associated with greater current cannabis use ($r = -.77$, $p < .001$) - explaining 59% of variance in striatal dopamine synthesis capacity - and earlier age of onset of use ($r = .51$, $p = .027$), but not with cannabis-induced psychotic-like symptoms ($r = .32$, $p = .19$). Importantly, it was also found that the lower levels of dopamine synthesis capacity in cannabis users compared to non-users were driven by users who met diagnostic criteria for abuse and dependence ($F_{2,37} = 4.02$, $p = .027$).

7.2.2 Hypothesis 1: Regular cannabis users sensitive to cannabis' psychotogenic effects exhibit elevated dopamine synthesis capacity compared with nonuser control subjects

The findings that regular cannabis users sensitive to cannabis' psychotogenic effects exhibit reduced dopamine synthesis capacity compared with nonuser control subjects are inconsistent with the hypothesis that regular cannabis users sensitive to cannabis' psychotogenic effects exhibit elevated dopamine synthesis capacity compared with nonuser control subjects.

7.2.3 Hypothesis 2: There will be a direct relationship between dopamine synthesis capacity and cannabis-induced psychotic-like symptom severity.

The findings of no significant relationship between dopamine synthesis capacity and cannabis-induced psychotic-like symptom severity are inconsistent with the hypothesis that there is a direct relationship between dopamine synthesis capacity and cannabis-induced psychotic-like symptom severity.

7.2.4 Interpretation of findings

These results extend previous findings in current (Albrecht *et al.* 2012) and recently abstinent cannabis users (Urban *et al.* 2012), which found reduced dopamine receptor density was associated with higher current cannabis use and lower dopamine release in the associative striatum was associated with earlier age of onset of cannabis. Whilst these studies (Urban *et al.* 2012, Sevy *et al.* 2008, Albrecht *et al.* 2012) and a further study in ex-users (Stokes *et al.* 2012) have reported estimates of the number of lifetime uses of cannabis, and the current sample is comparable to these, measures of the amount or type of cannabis consumed have not been reported, such that direct comparisons of cannabis use across the studies cannot be made. The findings of reduced dopamine synthesis capacity in dependent subjects may reflect a "blunted" dopamine system, as observed with other drugs of addiction (Volkow *et al.* 2011). Taken with findings from these and other studies (Heinz *et al.* 2005; Wu *et al.* 1997; Martinez *et al.* 2011; Wang *et al*. 2012), there is mounting evidence that dopaminergic dysfunction provides a biomarker of addiction severity.

Whilst the case-control design of this study is not able to detect a causative relationship between cannabis use and dopamine dysfunction, the findings suggestive of dose-effects warrant further research into potential causative mechanisms. Animal studies indicate increased dopaminergic function in response to acute THC treatment. However, there is evidence of a biphasic dose-dependent dopamine response to THC (Bloom 1982), suggesting higher cannabis exposures may reduce dopamine synthesis capacity, in line with these findings. Furthermore, with the exception of perinatal studies (Walters & Carr 1988), animal data on dopaminergic effects of long-term and high dose cannabis exposures are sparse and the longest duration of THC administration has been twenty-one days (Gorriti *et al.* 1999; Wu & French

2000; Ginovart *et al.* 2012; Behan *et al.* 2012). Of these, one study (Ginovart *et al.* 2012) in Sprague-Dawley rats reported that long-term treatment with THC was associated with reduced striatal tyrosine hydroxylase gene expression and concurrent supersensitivity of $D_{2/3}$ receptors and a separate study (Behan *et al.* 2012) in catechol-*0*-methyltransferase mutant mice found chronic treatment with THC in adolescence was associated with reduced dopaminergic cell size in the ventral tegmental area.

One explanation for these findings is that chronic cannabis use is associated with dopaminergic down-regulation of dopamine synthesis enzyme activity in response to repeated dopamine drug-induced release. This might underlie amotivation and reduced reward sensitivity in chronic cannabis users (van Hell *et al.* 2010) (see below). Alternatively, preclinical evidence suggests that low dopamine neurotransmission may predispose an individual to substance use (Nader *et al.* 2006). However, this is inconsistent with findings that recently abstinent and former cannabis users show neither altered dopamine receptor availability (Sevy *et al.* 2008; Stokes *et al.* 2012; Albrecht *et al.* 2012) nor altered dopamine release (Urban *et al.* 2012), suggesting that altered dopaminergic function during chronic cannabis use is normalised by abstinence, as is observed with amphetamine in vervet monkeys (Melega *et al.* 1997).

In the present study dopaminergic function was investigated in cannabis users who experience a transient increase in psychotic-like experiences when acutely intoxicated with cannabis. The increase in psychotic-like experience severity was of the same order of magnitude as observed in studies on the effects of cannabis and ketamine (Mason *et al.* 2009)

and the dream state (Mason & Wakerley 2012), given that dreaming has long been proposed to model psychosis (e.g. Hobson 2004). The lack of relationship between the induction of psychotic-like experiences and dopaminergic function suggests that these findings would generalise to cannabis-users in general but this requires confirmation in further studies.

These findings suggest that elevated striatal dopamine synthesis capacity is unlikely to be the mechanism underlying the link between cannabis and psychosis. This study focussed on the striatum as dopaminergic changes here have been reliably linked to psychosis (Howes *et al.* 2012) but the possibility cannot excluded that dopaminergic changes in extra-striatal regions underlie cannabis-induced psychotic symptoms. A previous study (Glenthoj *et al.* 2006) using SPECT reported a significant increase in temporal cortex $D_{2/3}$ receptor availability in antipsychotic-naïve first-episode psychosis patients who tested positive for cannabis compared to those who did not. Alternatively, the mechanism may be mediated via non-dopaminergic systems, such as direct effects on cannabinoid receptors (Hirvonen *et al.* 2012).

Whilst the results of this study find reduced dopamine synthesis in long-term heavy cannabis users, the animal data available find complex effects of cannabis/THC on the dopamine system where increases or decreases in dopaminergic function are dependent on other environmental factors – such as stress (Littleton *et al.* 1976). Nevertheless, findings that striatal dopamine release in patients with co-morbid schizophrenia and substance dependence is blunted but still associated with amphetamine-induced psychotic symptoms (Thompson *et al.* 2012) supports the possibility that other aspects of striatal dopaminergic function are altered by cannabis, for example super-sensitivity at D_2 receptors (Seeman & Seeman 2014), or that

cannabis use interacts with other risk factors for schizophrenia to induce a downstream relative hyperdopaminergia in the dopamine pathway. In parallel, early work in Wistar rats (Littleton *et al.* 1976) found THC decreased striatal dopamine uptake compared to vehicle, but increases in striatal dopamine uptake were observed when THC-treated rats were housed under "stressful" *vs.* "normal" conditions. Earlier age of onset of cannabis use increases psychosis risk and may interfere with normal brain development (Ashtari *et al.* 2009). Another possibility is thus that cannabis use during key developmental periods alters the regulation of dopaminergic function to make it more susceptible to subsequent stressors which could underlie an increased risk of psychosis. Further prospective studies on the effects of chronic cannabis exposure are therefore warranted.

7.2.5 The relationship between subjective apathy and dopamine synthesis capacity in cannabis users

The main finding from this study was that within chronic cannabis users, there was inverse relationship between striatal dopamine synthesis capacity and apathy. This was the first study to investigate the relationship between apathy and striatal dopamine synthesis capacity in regular active cannabis users. The results suggest that the reduction in striatal dopamine synthesis capacity associated with regular cannabis use may indeed underlie the reduced reward sensitivity and amotivation associated with chronic cannabis use (van Hell *et al.* 2010), accounting for 40 % of the variance in apathy. Whilst the study was cross-sectional so that causality cannot be inferred, this interpretation is supported by preclinical lesion studies (Schneider *et al.* 1988) that show lowering dopamine results in apathy and that apathy is inversely related to reduced dopamine function in both patients with Parkinson's disease (Remy *et al.* 2005) and lesioned animals (Brown et al. 2012). A further possibility could be that the cannabis users were in a state of withdrawal; however, THC and its metabolites have an elimination half-life of about 7 days (Maykut 1985), and all our cannabis users were regular, long-term users who had consumed cannabis within the past 7 days (median time since last consumption = 14.1 h) and so this would be unlikely. The results extend previous findings of attenuated striatal response to reward anticipation activity in cannabis users (van Hell *et al.* 2010). Long-term cannabis use has been associated with apathy (Looby and Earleywine 2007; McGlothin & West 1968; Tennant & Groesbeck 1972). The very high apathy scores in this sample are striking, the scale we used has not previously been administered in cannabis users. One study has, however, attributed amotivational symptoms to coexisting depressive symptoms (Musty and Kaback 1995). Yet, none of the subjects in that study met the threshold for a DSM-IV (TR) diagnosis of major depressive episode or disorder, or indeed any DSM-IV

(TR) diagnosis except for Cannabis Use Disorders. However, as depressive symptom scales were not included, the possibility that sub-clinical depressive symptoms have contributed to the findings cannot be excluded. Since apathy is also a symptom of depression, further studies to disentangle the relationship between apathy and depressive symptoms and their possible effects on the day-to-day lives of heavy chronic cannabis users are therefore warranted. The striatum has been conceptualised as the interface between motivation and action (Mogenson & Yang 1991). Whilst no relationship was found between apathy and dopamine synthesis capacity in the limbic striatal subdivision which has been described as being involved in motivation (Martinez *et al.* 2003), the findings of a significant relationship between apathy and dopamine synthesis capacity in the dorsal (associative) striatum fit with findings from the Brown *et al.* (2012) study. The associative subdivision of the striatum is part of the corticostriatal–thalamo-cortical loop projecting to and from associative areas of cortex including the dorsolateral prefrontal cortex (Joel and Weiner 2000) which has dense interconnections with premotor areas involved in motor planning (Barbas 2000) and therefore goal-directed behaviour.

7.2.6 Limitations of the study of Dopamine synthesis capacity and its relationship to cannabis induced psychotic symptoms

One potential limitation of this study is that subjects consumed their own cannabis, rather than a standard preparation. However, individuals were tested whilst intoxicated and the levels of THC in samples of the cannabis subjects were using were measured and it was confirmed that the cannabis contained high levels of THC in all subjects (mean THC content = 8.7%). There was no fixed interval between cannabis exposure and PET, meaning that heavier cannabis users may have had a shorter interval between exposure and scan. It therefore remains possible that differences in the time since last cannabis use contribute to the differences between the dependent/abuser and non-dependent groups, rather than dependency or abuse per se. In addition, the lack of association between cannabis-induced psychotic symptoms may be due to variable interval between cannabis exposure and PET. However, in terms of acute effects of cannabis, only 1 of 3 PET studies of the acute effects of THC in healthy volunteers has found evidence of dopamine release (Bossong *et al.* 2009; Stokes *et al.* 2009; Barkus *et al.* 2012), suggesting that the acute effects of THC on dopaminergic function may not be large or consistent in humans. As a challenge in these earlier studies is one of relatively low sample size, Bossong *et al.* (2015) have recently combined data from their 2009 study with those from the Stokes *et al.* (2009) study so that data are available on n=20 participants. In this new analysis, [^{11}C]raclopride binding in the limbic striatum is reduced by -3.65 %, consistent with THC-induced dopamine release. However, Nutt *et al.* (2015) are of the opinion that this is relatively smaller than the drug-induced dopamine release caused by other recreational agents, particularly the stimulants. Given that THC and its metabolites have an elimination half-life of about 7 days (Maykut *et al.* 1985), and all the cannabis users were regular, long-term users

who had consumed cannabis within the last 7 days (median time since last consumption = 14 hours), subjects were unlikely to be acutely withdrawing.

The measures of substance use rely on self-report and it was not possible to independently verify substance use histories beyond on-going cannabis use in the user group and no recent use of other drugs in all participants. As would be expected, higher rates of other substance use were reported in cannabis users, although, with the exception of tobacco, the use of other substances was low in both groups. The findings remained significant after co-varying for all other drug use suggesting that use of other substances does not underlie the findings, although it should be noted that the analysis of co-variance may be less able to adjust for factors when groups differ significantly in covariates (Miller *et al.* 2001) and should be considered exploratory. It is therefore impossible to exclude the possibility that group differences in other drug use contributed to the results observed.

Whilst cannabis users in the sample reported higher levels of ecstasy use than controls, ecstasy has been associated with increased dopamine synthesis capacity (Tai *et al.* 2011), so this is unlikely to explain the findings. More of the cannabis users smoked cigarettes than controls. The effects of cigarette smoking on presynaptic dopamine function are unclear - tobacco use has been associated with reduced amphetamine-induced dopamine release (Busto et al. 2009), but increased dopamine synthesis capacity (Salokangas *et al.* 2000). In addition, tobacco smoking may influence [^{18}F]-DOPA kinetics via cerebral blood flow effects (Domino et al. 2004), which, if regionally selective could affect the outcome measure (K_i^{cer}). However, a relationship was not found between levels of cigarette consumption and dopamine synthesis

capacity, suggesting this does not influence the results, although further research is needed to determine the effect of tobacco smoking on dopaminergic function.

7.2.7 Summary of Findings from the Study of Dopamine Synthesis Capacity and its Relationship to Cannabis-Induced Psychotic Symptoms

These results show that regular long-term cannabis use is associated with a dose-dependent reduction in dopamine synthesis capacity in the corpus striatum, particularly in those meeting diagnostic criteria for cannabis abuse or dependence. However, no relationship was found between dopaminergic function and cannabis-induced psychotic-like symptoms. These findings question the prevailing assumption that cannabis increases the risk of schizophrenia by inducing the same dopaminergic alterations seen in schizophrenia (Bowers & Kantrowitz 2007).

7.3 Salience Attribution and its Relationship to Cannabis-Induced Psychotic Symptoms

7.3.1 Key Results

The main finding from this study is that regular long-term cannabis use is not associated with statistically significant differences in behavioural measurements of salience processing. However, these results show preliminary evidence for a difference in implicit aberrant salience between cannabis who do and do not meet DSM-IV criteria for cannabis abuse or dependence ($F_{1,15}$ = 5.8, p = .03, effect size: Cohen's d = 1.2). The relationships between current cannabis use and salience processing approached the threshold for statistical significance (r =.49, p = .06 [implicit aberrant]; r = -.46, p = .07[explicit adaptive]) suggesting that increased use of the drug may be associated with increased aberrant and concomitant decreased adaptive salience. Within cannabis users who experienced a cannabis-induced psychotic-like symptoms, there was a significant relationship between cannabis-induced psychotic-like symptom severity and explicit aberrant salience processing (r = .61, p = .01). In an exploratory analysis, within controls there were positive relationships between both measures of adaptive salience and whole striatal dopamine synthesis capacity (r = .94, p = .006[implicit]; r = .82, p = .05 [explicit]), whilst there was inverse relationship between implicit aberrant salience and whole striatal dopamine synthesis capacity (r = -.91, p = .01). However, no significant relationships between whole striatal dopamine synthesis capacity and salience processing were observed in cannabis users. The results also indicate a loss of relationship between implicit salience processing and dopamine synthesis capacity in the whole striatum associated with long-term cannabis use (z = 2.12, p = .03 [adaptive], z = -2.12, p = .04[aberrant]).

7.3.2 Hypothesis 3: Regular cannabis users sensitive to cannabis' psychotogenic effects exhibit elevated aberrant salience compared with nonuser control subjects

The findings that regular cannabis users sensitive to cannabis' psychotogenic effects exhibit no significant difference in aberrant salience compared with non-user control subjects is inconsistent with the hypothesis that regular cannabis users sensitive to cannabis' psychotogenic effects exhibit elevated aberrant salience compared with nonuser control subjects.

7.3.3 Hypothesis 4: There will be a direct relationship between aberrant salience attribution and cannabis-induced psychotic-like symptom severity.

The finding of a significant correlation between cannabis-induced psychotic-like symptom severity and explicit aberrant salience provides evidence to support the hypothesis that there will be a direct relationship between aberrant salience attribution and cannabis-induced psychotic-like symptom severity.

7.3.4 Exploratory hypothesis A. Cannabis users who meet DSM-IV diagnostic criteria for cannabis dependence or abuse will exhibit elevated aberrant salience compared to users who do not meet dependence or abuse criteria.

The finding of elevated implicit aberrant salience in cannabis users who met DSM-IV(TR) criteria for cannabis dependence or abuse compared to users who do not meet criteria supports this hypothesis.

7.3.5 Exploratory hypothesis B. There will be a direct relationship between dopamine synthesis capacity and aberrant salience processing.

The findings of an inverse relationship between dopamine synthesis capacity and implicit aberrant salience in healthy controls and no significant relationship between the two measures in cannabis users is inconsistent with this hypothesis.

7.3.6 Interpretation of Findings from the Study of Salience Attribution and its Relationship to Cannabis-Induced Psychotic Symptoms

This is the first study to examine aberrant salience processing in cannabis users. Whilst there was no significant difference in aberrant salience between the cannabis users and controls, findings of increased implicit aberrant salience in cannabis users who meet DSM-IV(TR) criteria for abuse or dependence compared to those who do not, together with the trends toward a positive and negative relationship between current cannabis use and measures of aberrant and adaptive salience, respectively are consistent with the prediction that heavy cannabis use is associated with a state of aberrant salience (i.e. as described by Kapur [2003]) and this may account for the association between cannabis use and increased psychosis-proneness (Nunn *et al.* 2001). This is further supported by the finding that cannabis-induced psychotic-like symptom severity and explicit aberrant salience are significantly positively correlated, in line with findings of a positive relationship between explicit aberrant salience and delusion-like symptoms in people at ultra-high risk of psychosis (Roiser *et al.* 2013) (Figure 7.1) and delusional symptoms in people with schizophrenia (Roiser *et al.* 2009), which in turn provide evidence supporting the face validity of the SAT. Since pre-existing high schizotypy (psychosis-proneness) increases the likelihood of a psychotogenic response to cannabis (e.g. Nunn *et al.* 2001), increased explicit aberrant salience may underlie this process.

In addition, there were some novel findings not predicted by the aberrant salience hypothesis. These were that in healthy controls, whole striatal dopamine synthesis capacity was positively correlated with both measures of adaptive salience processing and negatively correlated with implicit aberrant salience.

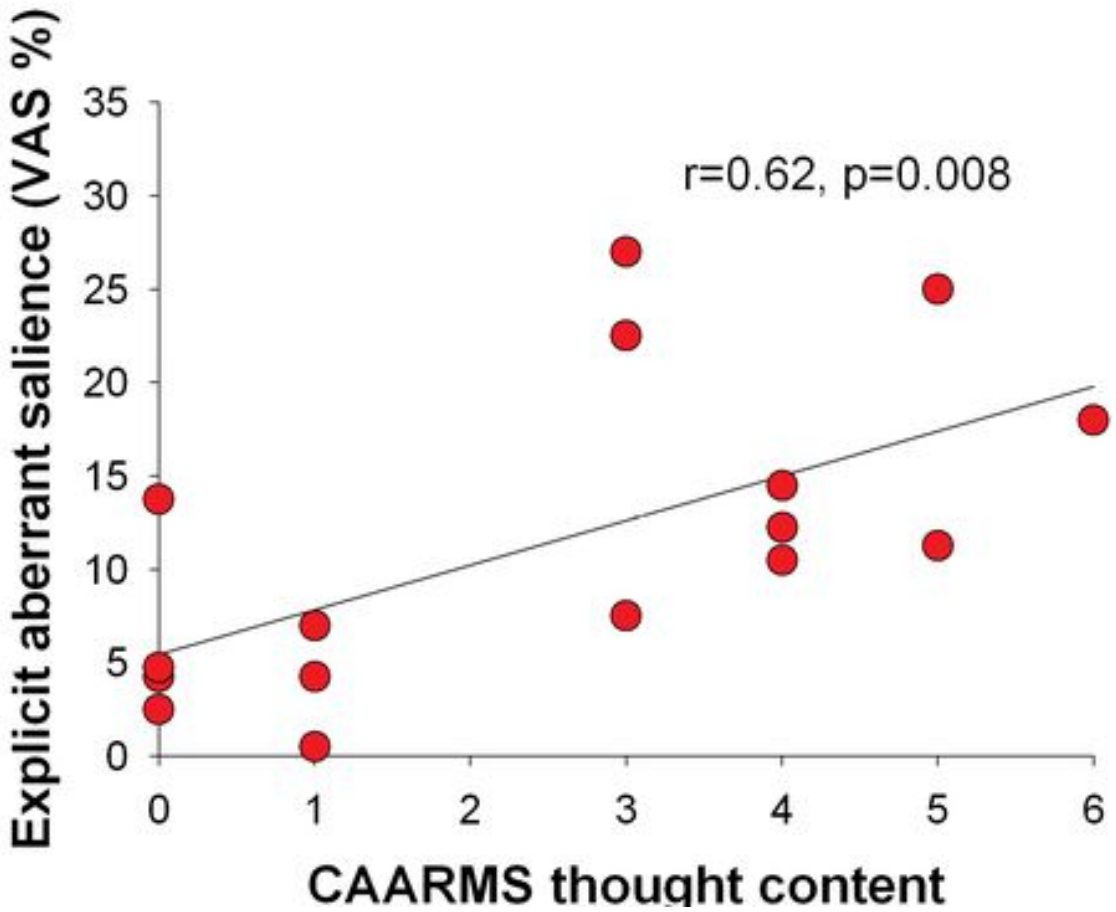


Figure 7.1 Explicit aberrant salience was positively correlated with delusion-like symptoms in individuals at ultra-high risk of risk psychosis. (Abbreviations: VAS, visual analogue scale; CAARMS, Comprehensive Assessment for the At Risk Mental State)

The finding of opposite relationships between dopamine synthesis capacity and salience processing in healthy controls is not predicted by the aberrant salience hypothesis, where increased dopamine synthesis capacity is predicted to be related to increased aberrant salience and not vice versa. The only study to have previously assessed dopamine synthesis and aberrant salience processing did not find significant relationships between the measures (Roiser *et al.* 2013). However, that study did report that higher dopamine synthesis capacity predicted greater adaptive reward prediction haemodynamic responses in controls, whereas the opposite relationship applied in the individuals at ultra-high risk of psychosis, in line with the findings in control subjects in the current study. Roiser *et al.* (2013) speculated that the positive impact of high dopamine synthesis capacity on motivational salience signalling may depend on the baseline state of the dopamine system, such that in healthy volunteers, high dopamine synthesis capacity may facilitate the transmission of motivational salience, potentiating appropriate phasic signals against a background of relatively low gain or tonic dopamine release. Taken together with findings that there is a loss of relationship between implicit salience processing and dopamine synthesis capacity in the whole striatum associated with long-term cannabis use, and given that the mesolimbic dopamine system plays a central role in normal salience processing (Zink *et al.* 2003) which is modulated by the endocannabinoid signalling (Fernandez-Ruiz *et al.* 2010; Melis *et al.* 2012; Melis & Pistis 2012), this would suggest that long-term cannabis use may give rise to aberrant salience by disrupting dopaminergic salience processing. Whilst the effects of acute THC on aberrant salience processing using the SAT have yet to be reported in the literature, and the case-control design of this study is not able to infer causality, there is evidence from a study using the oddball task (Bhattacharyya *et al.* 2012) that THC reduces latency to non-salient *vs.* salient stimuli in healthy volunteers, consistent with this interpretation. However, this phenomena may not be restricted to reward-based learning only, as increased speed and error rates observed were observed with THC challenge in a

learning and episodic memory task (Curran *et al.* 2002). Nonetheless, long-term cannabis use has been associated with impairments in filtering out non-salient information during a selective attention task (Solowij *et al.* 1991) and THC resulted in irrelevant background visual and auditory stimuli becoming more salient during the performance of a visual processing task (D'Souza *et al.* 2004).

7.3.7 Cannabis, Cognition and Schizophrenia

Impaired cognition is a key feature of schizophrenia and this is more predictive of functional outcome than any other symptomatic measure (Green 1996). Endophenotypes are internal markers of a disorder and can be present across multiple domains e.g. biochemical, anatomical, or cognitive markers of functional capacity. In complex disorders, such as schizophrenia, endophenotypes have been thought of as quantitative traits intermediate between the predisposing causative mechanisms (e.g. genotype) and overt clinical signs and expressed symptoms, i.e. the phenotype (Gottesman & Gould 2003, Gottesman & Hanson 2005). Adolescence is a period of vulnerability to the development of neurocognitive effects associated with cannabis use and there is also growing evidence that cannabis use is associated with multiple cognitive endophenotypes that are in common with schizophrenia such as response inhibition (i.e. more effortful response inhibition on Go/No-Go, stop-signal and stroop tasks, suggestive of impaired anterior cingulate and inhibitory control prefrontal networks[Kiehl *et al.* 2000; Weisbrod *et al.* 2000; Badcock *et al.* 2002; Braff *et al.* 2004; Kerns *et al.* 2005]); sustained attention (i.e. poor performance on continuous performance tasks [Cornblatt *et al.* 1994; Michie *et al.* 2000]), working memory (i.e. impaired active maintenance and manipulation of stored information critical for planning and guiding behaviour, suggestive of deficits in prefrontal, parietal and subcortical networks) and executive function (as measured with tasks of frontal lobe functions such as verbal fluency, Tower of London task and Wisconsin Card Sorting Test [Heinrichs *et al*. 2005; Pantelis *et al.* 1997]) (Solowij & Michie 2007).

Yet, behavioural studies have demonstrated that acute THC challenge produces transient, acute psychotic reactions, the extent of which are unrelated to the degree of cognitive impairment or anxiety. There is a large body of evidence describing the vulnerability of adolescents to impaired cognition, across a range of domains, associated with cannabis use (reviewed by Jager & Ramsey 2008). Animal studies indicate that brain CB_1 receptor levels peak in early adolescence (Belue *et al.* 1995) and humans exposed to cannabis in adolescence are more likely to have impaired neurocognitive function than individuals exposed in adult life (Fontes *et al.* 2011). Furthermore, there is evidence that neurocognitive deficits (such as impaired reaction times, attention and memory) associated with adolescent cannabis use can persist after abstinence (Medina *et al.* 2007). As described by Schmidt & Roiser (2009) in order to perform the SAT, participants must be able to attend continuously for an extended period, use working memory, learn probabilistic associations and guide responses on the basis of such associations, all of which may be impaired with cannabis use (Scholes & Martin-Iverson 2009; Pope *et al.* 2001), therefore potentially explaining poorer measures in the cannabis-dependent group because learning and memory are impaired. In a study assessing the construct validity of the SAT, in order to examine whether other cognitive processes (including working memory, sustained attention, probabilistic reversal learning) were influencing measures on the SAT, Schmidt & Roiser (2009) performed a factor analysis using the SAT with a battery of cognitive tasks. They found that the SAT could dissociate aberrant salience processing from other aspects of reward learning and attention (including operant/explicit learning, general cognitive ability, contingency-based speeding and attentional vigilance), although adaptive salience and learned irrelevance were associated with each other. It is therefore unlikely that other aspects of cognitive function that are affected by cannabis use are influencing the current results. Although, these were not verified in the current study, and so the findings may be due to impaired learning and memory in the cannabis group. Likewise,

findings of increased implicit aberrant salience in cannabis users who were dependent on the drug may be related to THC effects on memory and motivation. However, the cannabis users in this study had faster reaction times than non-users on both high and low probability items in both blocks of the SAT, suggesting that general psychomotor slowing in cannabis users is unlikely to account for the current results.

7.3.8 Limitations of the Study of Salience Attribution and its Relationship to Cannabis-Induced Psychotic Symptoms

A potential limitation of the current study is that subjects consumed their own cannabis, rather than a standard preparation. However, individuals were tested whilst intoxicated and the levels of THC in samples of the cannabis subjects were using were measured and it was confirmed that the cannabis contained high levels of THC. There was no fixed interval between cannabis exposure and SAT session, meaning that heavier cannabis users may have had a shorter interval between exposure and scan. It therefore remains possible that differences in the time since last cannabis exposure, and therefore acute *vs.* chronic effects of cannabis, contribute to the differences between the dependent/abuser and non-dependent groups, rather than dependency or abuse per se. The measures of substance use rely on self-report and it was not possible to independently verify substance use histories beyond on-going cannabis use in the user group and no recent use of other drugs in all participants.

Given that a relatively small sample of cannabis users was tested and multiple statistical comparisons were performed, there is a raised possibility of type I errors. It is also important

to consider the possibility of type II error in these findings. The sample had 99% power to detect the effect size (Cohen's *d* = 1.6) seen in a previous study of salience processing in patients with schizophrenia, which is above the 80% power threshold conventionally considered adequate. Whilst at this power there is a 1% chance that a true effect at this magnitude has been missed, consideration should be given to the possibility that a weaker effect has been missed. Sensitivity analysis indicates that this study would be able to detect an effect side of Cohen's *d* = .99 with 80 % power. Furthermore, since the SAT continues four measures of salience processes, in order for these to survive Bonferroni correction, applying an *alpha* = .0125 would decrease the sensitivity of this study to detect an effect size of *d* = 1.2 with 80 % power. Therefore, a larger sample size would be required to detect less pronounced effects of cannabis use on salience processing, which may indeed be the case given that none of the cannabis users experienced psychotic symptoms when not intoxicated with the drug and therefore at the time of undertaking the SAT.

A recently published large randomized, placebo-controlled study found that THC increased paranoia by increasing negative affect (i.e. depression and anxiety). A further limitation of this study would therefore be that measures of depression and anxiety, such as the Beck Depression Inventory and the Beck Anxiety Inventory, were not recorded. Future work should therefore assess the relationships between both long-term cannabis use and acute THC on salience processing, psychotic symptoms, paranoia and negative affect.

7.3.9 Summary of the Findings of the Study of Salience Attribution and its Relationship to Cannabis-Induced Psychotic Symptoms

These results suggest that cannabis dependence and abuse is associated with increased implicit aberrant salience processing, and that within cannabis users there is a positive relationship between explicit aberrant salience and cannabis-induced psychotic-like symptom severity. There is also evidence that long-term cannabis use was associated with altered relationships between striatal dopamine synthesis capacity and salience processing. Long-term cannabis use may therefore be increase the risk of psychotic like symptoms by increasing aberrant salience via non-dopaminergic mechanisms of reward-processing.

7.4 Dopamine Synthesis Capacity and its Relationship to Psychosocial Risk Factors for Schizophrenia

7.4.1 Key Results

The main finding from this study is that striatal dopamine synthesis capacity is lower in individuals with a high cumulative exposure to psychosocial stress than those with a low cumulative exposure to psychosocial stress (effect size: Cohen's $d = .91$; $t_{24} = 2.32$, $p = .029$). Within individuals with a high cumulative exposure to psychosocial stress, the combination of childhood stressors and recent adult stressors was positively correlated with dopamine synthesis capacity in the associative subdivision of the striatum ($r = .68$, $p = .01$), explaining 46 % of the variance in dopamine synthesis capacity.

7.4.2 Hypothesis 5: Participants with a high cumulative exposure to psychosocial risk factors for schizophrenia will exhibit elevated dopamine synthesis capacity compared to participants with low cumulative exposure to the same environmental stressors.

The finding that participants with a high cumulative exposure to psychosocial stressors exhibit reduced dopamine synthesis capacity compared to participants with low cumulative exposure is inconsistent with this hypothesis.

7.4.3 Hypothesis 6: Within participants with a high cumulative exposure to psychosocial stressors, there will be a direct relationship between dose of psychosocial stress and dopamine synthesis capacity.

The findings of a significant correlation between dopamine synthesis capacity and a combined score of childhood and recent stressors is consistent with this hypothesis.

7.4.4 Interpretation of the Study of Dopamine Synthesis Capacity and its Relationship to Psychosocial Risk Factors for Schizophrenia

This is the first study to directly compare dopamine synthesis capacity in healthy participants with and without long-term exposure to psychosocial stress. Childhood adversity increases the risk of adult mental illness and psychosis in particular, accounting for up to 33 % of cases (Varese *et al.* 2012). Although the case–control design of this study is not able to detect a causative relationship between psychosocial stress and dopamine dysfunction, the findings suggestive of a potential dose relationship between psychosocial stress and dopamine synthesis capacity warrant further research into potential causative mechanisms.

One explanation for the group difference is that long-term exposure to psychosocial stress is associated with dopaminergic down-regulation, particularly in the limbic striatum. This would be consistent with an fMRI study with found that adolescents who had suffered severe early life deprivation exhibited ventral striatal hyporesponsivity during anticipation of monetary reward (Mehta *et al.* 2010). However, this would not be consistent with the finding of a relationship between dopamine synthesis capacity in the associative striatum and combined childhood and recent adult stress. Yet, a finding in a [^{11}C]-raclopride study (Pruessner *et al.*

2004) in which ventral striatal dopamine release was increased in response to psychosocial stress in humans who reported insufficient early life maternal care would suggest that dopamine system can become sensitised to psychosocial stressors. Likewise, Oswald *et al.* (2014) reported positive associations between childhood adversity and amphetamine-induced dopamine release. Furthermore, in a subsequent [^{11}C]-raclopride study Wand *et al.* (2007), stress-induced cortisol levels were positively associated with amphetamine-induced dopamine release in the striatum. The current findings of a relationship between childhood stressors and recent adult life events and associative striatal dopamine synthesis capacity is consistent with the a [^{11}C]-PHNO study in which Mizrahi *et al.* (2011) found increased psychosocial stress-induced dopamine release in the associative and sensorimotor functional subdivisions of the striata of antipsychotic-naïve subjects with schizophrenia and those at clinical high-risk of the illness compared to matched healthy controls, possibly indicative of a sensitised dopaminergic stress response. Furthermore, the finding of a positive relationship between childhood and adult recent psychosocial stressors in the associative (dorsal) subdivision of the striatum is pertinent, the region in which dopamine dysfunction has been observed most frequently in psychosis (Howes *et al.* 2009). However, this interpretation should be considered highly speculative given the overall group reduction in dopamine synthesis capacity in the high stress group compared to low stress group.

The study examined a variety of known psychosocial stressors and was not powered to perform multiple analyses to examine any potential differential effects. One possibility is that continual low level stressors, as would be encountered by urbanicity for example, have a dampening effect on the dopamine system, whilst repeated sudden traumatic stressors, as would be the case with child abuse or adult life events potentiate the dopamine system. In support of this interpretation is animal evidence that finds both increases and decreases in

dopaminergic response depending on the type of stressor, its duration and the age of the animal at the time of stressor. For example, maternal deprivation and isolation of neonatal rats was associated with increased dopamine release (Hall *et al.* 1999; Kosten 2003), whilst unavoidable stress administered over one week and three weeks was associated with a decrease in nucleus accumbens dopamine output (Mangiavacchi *et al.* 2001). Furthermore, rats under a ten day episodic defeat paradigm had a sensitised dopamine response in the nucleus accumbens, whilst when under a 5 week continuous subordination paradigm they exhibited a suppressed dopamine response (Miczek *et al.* 2011).

7.4.5 Limitations of the Study of Dopamine Synthesis Capacity and its Relationship to Psychosocial Risk Factors for Schizophrenia

The measures of psychosocial stress rely on self-report and it was therefore not possible to independently verify the histories of psychosocial stressors. Furthermore, the assessment of childhood trauma may be liable to recall bias in depressed patients (Lewinsohn & Rosenbaum, 1987). However, measures of childhood trauma have also been demonstrated to remain stable over time and to be independent of the current degree of abuse-related psychopathology (Paivio 2001). Despite ongoing concerns that retrospective reporting overestimates associations between abuse and adult psychopathology (Gilbert *et al.* 2009), there is evidence that prospective and retrospective measures of abuse predict similar rates of mental illness (Scott *et al.* 2012) and recall bias accounts for less than 1% of variance in measures of child abuse (Fergusson *et al.* 2011). Yet, difficulties remain in measuring and quantifying emotional neglect due in part to its highly subjective nature (Watson *et al.* 2014). As this was the first study to mention brain dopamine synthesis capacity in human participants exposed to a range of psychosocial risk factors for the disorder, the categorisation is arguably a *catch-all*,

containing a range of experiences from the most serious psychological trauma to demographic factors alone. Along these lines there is no single measure that is used in the literature to index both historical and current psycho-social risk factors in the same scale. Furthermore, relationships and interactions between these risk factors are likely to be complex. As such the use of the combined psychosocial stress score in this study should be considered exploratory. Given the performance of multiple correlations, a further limitation of this study is that a regression model was not conducted.

7.4.6 Potential Confounders

There was a trend for the groups to differ in terms of the specific activity of the injected radiotracer ($p = .06$), with higher specific activity in the high stress group. The group difference in whole striatal K_i^{cer} remained significant when co-varying for specific activity ($F_{2,25} = 4.6$, $p = .02$). However, across all participants, there was a negative correlation between specific activity and dopamine synthesis capacity, suggesting that this could be having an effect on the main result. The specific activity is the physical decay-corrected proportion of radioactive *vs*. non-radioactive tracer concentration. Yet, a higher specific activity would in theory result in greater signal in the striatum vs. the cerebellar reference region, thereby increasing the K_i^{cer}, which suggests that this potential confound could be spurious. In other words, a higher specific activity in the high stress group would result in an over-estimation of K_i^{cer}, thereby increasing the magnitude of the observed effect, but not changing the overall finding of this study.

As would be expected, higher rates of smoking were reported in the high psychosocial stress group. The findings did not remain significant in the whole striatum when co-varying for cigarette use or removing smokers from the analysis, however the findings did remain significant in the limbic striatal subdivision. Likewise, higher rates of depressive and anxiety symptoms were reported in the high psychosocial stress group. Similarly, the findings did not remain significant in the whole striatum when co-varying for depression and anxiety symptoms, but did remain significant in the limbic striatal subdivision only. Whilst the finding of reduced limbic striatal dopamine synthesis capacity appears consistent when attempting to adjust for confounds, it must be noted again that analysis of covariance may be less able to adjust for factors when groups differ significantly in their covariates (Miller & Chapman 2001) and should therefore be considered exploratory.

As per the above study in cannabis users, the precise effects of tobacco smoking on striatal dopamine function remain to be fully elucidated (see below). Tobacco has been associated with increased dopamine synthesis capacity in a study of heavy smokers (Salokangas *et al.* 2000) with no effect seen in a subsequent study of moderate smokers (Bloomfield *et al.* 2014), as well as reduced amphetamine-induced dopamine release (Busto *et al.* 2009).

The dopamine system has been implicated in both the pathophysiology of depression and in its treatment (e.g. Nutt 2006). Higher rates of depression are seen in Parkinson's disease (Cummings & Masterman 1999), which is characterized by reduced dopaminergic transmission and both reduced striatal dopamine synthesis capacity and DAT availability have been associated with depressive symptoms in Parkinson's (Koerts *et al.* 2007; Weintraub *et al.* 2005). Reduced dopamine synthesis capacity has also been found in depressed patients without Parkinson's (Martinot *et al.* 2001). One study did find a relationship between trait depression severity and D_2 receptor availability (Kestler *et al.* 2000) but in a subsequent study, patients with major depressive disorder did not exhibit altered striatal D_2 availability and there was no relationship between depressive symptoms and D_2 receptor availability (Hirvonen *et al.* 2008). However, reduced striatal DAT availability has been observed in depression (Meyer *et al.* 2001) and has been found to be associated with the duration of dysthymia (Lehto *et al.* 2008). Although some studies have not found altered DAT availability in depression (Moresco *et al.* 2000). There is also evidence that patients with depression exhibit greater monoamine oxidase activity (Meyer e*t al.* 2006). Given the evidence that dopaminergic function may be reduced in depression together with the finding that negative affect may be influencing the result from the present study, further work is therefore warranted to investigate causative mechanisms,

given that both childhood adversity and recent life events are risk factors for depression (Kendler *et al.* 2004; Biondi & Picardi 1999).

Lastly, the groups in this study differed significantly in their ethnicity. It is not known if ethnicity has an effect on dopamine synthesis capacity. However, even though there is no available evidence to suggest that this may be the case, a further study assessing dopaminergic function in people of different ethnicities would be needed in order to exclude this possibility.

7.4.7 Summary of Findings of the Study of Dopamine Synthesis Capacity and its Relationship to Psychosocial Risk Factors for Schizophrenia

These findings show that long-term exposure to psychosocial stressors may be associated with reduced striatal dopamine synthesis capacity, particularly in the limbic subdivision of the striatum, although this may be associated with affective symptoms and tobacco. However, seemingly contradictorily, these findings also indicate that there is a positive relationship between childhood and adult recent psychosocial stressors in the associative (dorsal) subdivision of the striatum, the region in which dopamine dysfunction has been observed most frequently in psychosis. The disparity in these findings may reflect a differential effects of psychosocial stressors on the striatal dopamine system.

7.5. Cigarette Smoking and Dopamine Synthesis Capacity

The potential confounding effects of cigarette smoking on dopamine synthesis capacity have been outlined above and will be given detailed consideration here.

Tobacco addiction has been proposed to involve the effects of nicotine on the dopaminergic system (Balfour *et al.* 2000; Pidoplichko *et al.* 1997) since nicotinic receptors have been identified on nigrostriatal and mesolimbic dopaminergic neurons (Clarke & Pert 1985). Studies in rodents and non-human primates show that tobacco or nicotine increase dopamine neuron firing (Grenhoff *et al.* 1986; Zhang & Sulzer 2004), increase dopamine release (Di Chiara & Imperato 1988; Dewey *et al.* 1999; Gallezot *et al.* 2013; Marenco *et al.* 2004; Pontieri *et al.* 1996; Cumming *et al.* 2003), and increase dopamine synthesis (Tsukada *et al.* 2005) in the mesolimbic system. There is also evidence that nicotine alters the ratio of phasic bursts relative to tonic firing in the nucleus accumbens, thereby increasing the signal-to-noise relationship of dopamine system (Zhang *et al.* 2009).

In humans, tobacco use has been associated with both increased striatal dopamine synthesis capacity (Salokangas *et al.* 2000) and dopamine release in response to acute cigarette use in smokers (Brody *et al.* 2004; Le Foll *et al.* 2013). However, two studies found that acute nicotine use did not elicit a significant dopamine release in smokers (Barrett *et al.* 2004; Montgomery *et al.* 2007), although these did find that the subjective hedonic response to acute nicotine was related to dopamine release. Yet, a subsequent study found that nicotine did result in dopamine release in smokers, but not in non-smokers (Takahashi *et al.* 2008). Smoking-

induced dopamine release has been associated with a reduction in craving and the severity of tobacco dependence (Brody *et al.* 2004). Yet, unlike other drugs of addiction, drug-related (i.e. smoking-related) cues did not result in significant dopamine release in smokers when compared with neutral images (Chiuccariello *et al.* 2013). However, since the plasma half-life of nicotine is approximately two hours (Hukkanen *et al.* 2005) and that PET study was conducted one hour after nicotine exposure, it likely that the findings could be explained by the persistence of nicotine resulting in minimized craving and the ability to detect cue-induced dopamine release. This is especially relevant given that images of tobacco and tobacco advertising are highly salient to cigarette smokers. An alternative possibility would therefore be that nicotine disrupts reward processing via non-dopaminergic mechanisms.

Interestingly, one study (Busto *et al.* 2009) found that tobacco dependence was associated with reduced amphetamine-induced striatal dopamine release, although this is likely exacerbated by comorbid depression. Also of interest, are findings from one study using decotinised cigarettes which suggest that there may be lateralisation effects of the dopaminergic system relating to nicotine addiction, such that the left striatum has been associated with nicotine-induced striatal dopamine release, whilst the right striatum was not (Domino *et al.* 2013).

In terms of receptor availability, one study found that there were differences in D_2 receptor availability between male smokers *vs*. non-smokers, whilst no differences were observed between female smokers *vs*. non-smokers (Brown *et al.* 2012). This study also found that there were differences in caudate and putamen D_2 receptor availability between male and female smokers, but not between male and female non-smokers. In a separate study (Fehr *et*

al. 2008), reduced striatal D_2 receptor availability was reported in smokers compared to non-smokers, with a positive correlation between nicotine craving and ventral striatal D_2 availability. Reduced ventral striatal D_1 receptor availability has likewise been reported in smokers vs. non-smokers (Dagher *et al.* 2001; Yasuno *et al.* 2007). Although other studies reported no significant difference in D_2 availability between smokers and non-smokers (Yang *et al.* 2006; Yang *et al.* 2008).

These findings may reflect differences in the study design (Gallezot *et al.* 2013), the influence of other factors, such as sex effects, co-morbidity, or genetic variants (Dierker *et al.* 2002; Kendler *et al.* 1993; Lerman *et al.* 1998; Zhang *et al.* 2006), or the difficulty of imaging dopamine changes that are comparatively small (Egerton *et al.* 2010). In terms of other aspects of dopaminergic function, whilst three studies did report reduced dopamine transporter availability in smokers vs. non-smokers (Yang *et al.* 2008; Newberg 2007; Leroy *et al.* 2012), a large study on the dopamine transporter did not find an association between cigarette smoking and dopamine transporter availability (Thomsen *et al.* 2013), in line with earlier findings (Staley *et al.* 2001).

These studies indicate that striatal dopamine synthesis may be altered by nicotine exposure. Only one study had previously investigated dopamine synthesis capacity in cigarette smokers. This study, exclusively in male volunteers, found that striatal uptake of [^{18}F]-DOPA was 16–29% higher in smokers than non-smokers (Salokangas *et al.* 2000). However, as this sample was exclusively of males and there is evidence of sex differences in the release of dopamine in response to nicotine (Dluzen and Anderson, 1997), dopamine synthesis capacity

was examined in a larger sample of cigarette smokers that included females and did not have a history of psychiatric co-morbidity including depression and alcohol use disorders, compared to controls, to further examine whether tobacco smoking was influencing the findings of reduced dopamine synthesis capacity in cannabis users (Chapter 3) and individuals exposed to long-term psychosocial stressors (Chapter 5).

Dopamine synthesis capacity was measured in 15 cigarette smokers compared to 15 non-smoker controls (Bloomfield *et al.* 2014) using the same methods employed for the measurement of K_i^{cer} as described in Chapter 3. This study found no evidence for altered striatal dopamine synthesis in tobacco smokers compared with non-smokers (figure 7.2), or relationship between the levels of daily cigarette smoking and dopamine synthesis capacity ($r = -.23$, $p = .41$). Furthermore, an effect of nicotine dependence on dopamine synthesis capacity was not observed.

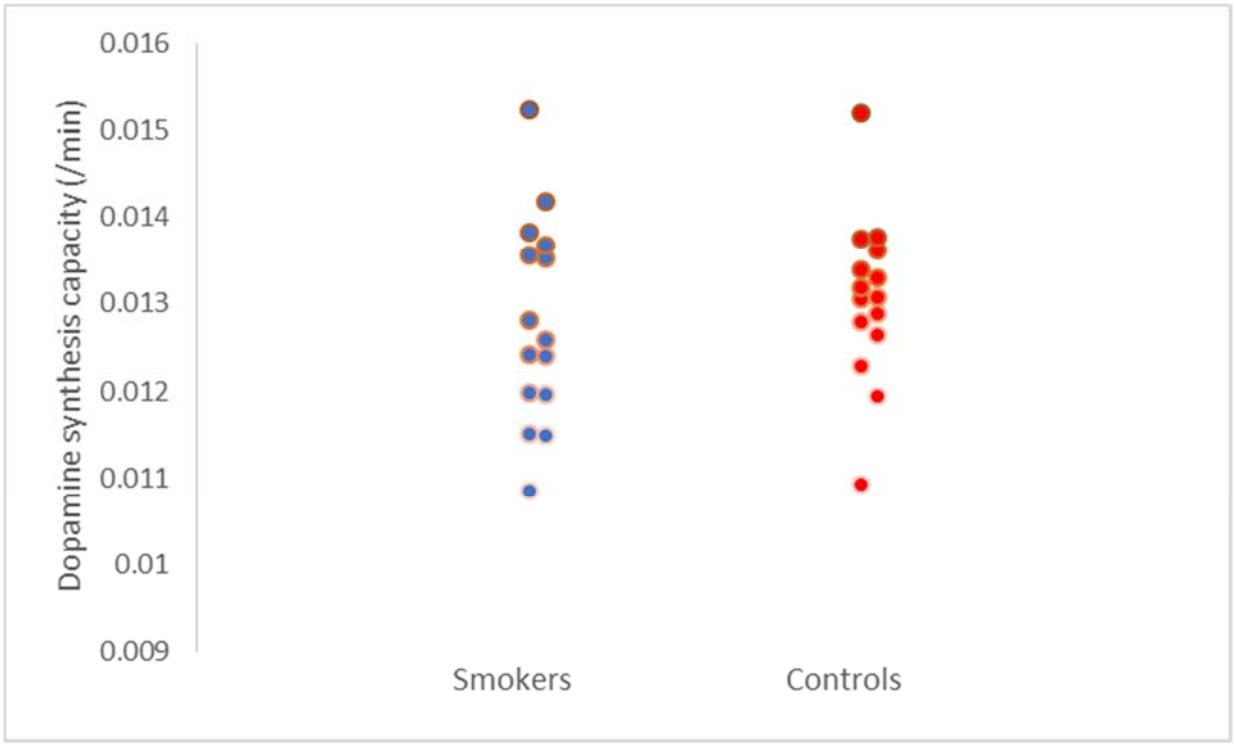


Figure 7.2 Whole striatal dopamine synthesis capacity (indexed K_i^{cer}) in smokers compared to non-smokers ($t_{df} = .64_{28}$, $p = .53$).

These negative findings are in contrast to a previous report of elevated dopamine synthesis capacity in 9 male smokers compared with 10 non-smokers (Salokangas *et al.* 2000). Although striatal dopamine synthesis capacity may be higher in females (Laakso *et al.* 2002), this was not evident in this sample. The subjects in the study by Salokangas *et al.* were heavy smokers (at least 15 cigarettes/day, mean 19.8 cigarettes/day compared with mean 8.1 cigarettes/day in this study), which could explain the difference with these findings. Although there was no evidence of a relationship between K_i^{cer} and the level of daily cigarette consumption, this may indicate that elevations in presynaptic dopamine synthesis capacity are only apparent in heavy smokers.

The study by Salokangas *et al.* (2000) used the same methodology as a previous study by Hietala *et al.* (1999), i.e. Carbidopa 100 mg only was administered 90 min before PET scan (Personal Communication from Professor Salokangas), followed by measurement of radiolabeled metabolites in the arterial input function. This is in comparison to the carbidopa 150 mg and entacapone 400 mg administered one hour before PET scan followed by a cerebellar reference region approach in this study. Data on the effects of smoking on the pharmacodynamics of entacapone are lacking. Entacapone undergoes rapid hepatic metabolism via the uridine 5'-diphospho-glucuronosyltransferase pathway (Lautala *et al.* 2000). Data from studies in humans (Bock & Köhle 2004) and mice models (Villard *et al.* 1998) indicate that cigarette smoke is a potent inducer of uridine 5'-diphospho-glucuronosyltransferase, which would thus lead to a faster elimination of entacapone and therefore a potential reduction in plasma [^{18}F]-DOPA in smokers vs controls whom have had entacapone administered. As the reference region approach theoretically eliminates the plasma input function, it would be predicted that the main effect of any potential reduction in plasma [^{18}F]-DOPA would result in increased variability of K_i^{cer} without altering mean K_i^{cer}. However, there are limited data directly comparing Ki values obtained via a tissue reference region and arterial plasma input function method, as was the case in the study by Salokangas *et al.* (2000). Sossi *et al.* (2003) reported a comparison between a reference region and arterial input function approach. In that study, Sossi *et al.* used the occipital lobe as the reference region, rather than the cerebellum used in this study. Therefore, it remains possible that a difference in entacapone metabolism in smokers may be underlying the disparity in results between this study and that of Salokangas *et al.* (2000). Nevertheless, the entry into and exit from the brain of radiolabeled plasma metabolites may affect graphical analysis (Boyes *et al.* 1986; Cumming *et al.* 1987) and could bias results if metabolism is selectively altered in one group. Compared with non-smokers, smokers have reduced cerebrospinal levels of the dopamine metabolite homovanillic acid

(Geracioti *et al.* 1999) and there is evidence of reduced MAO-A and MAO-B activity in smokers (Fowler *et al.* 1996a, 1996b). However, as these differences would, if anything, reduce the production of radiolabeled metabolites in smokers, they are unlikely to explain the failure to detect an elevation in smokers. In summary, even if entacapone clearance is higher in this smoker group, it cannot be concluded that it would be sufficient to impact metabolite production within the experimental window. Furthermore, even if there was a sufficient impact on metabolite production, the use of a reference region approach would be expected to be sufficiently robust to overcome this problem.

Survey data in Great Britain indicate that over the last few decades there has been a gradual decline in the number of cigarettes consumed per day among smokers, such that the average number of cigarettes smoked per day is now 12 for men and 11 for women (Office for National Statistics, 2013). The sample of moderate smokers is therefore in the same range as that of the general population. Overall the findings and those of Salokangas *et al.* thus indicate that there is no markedly altered dopamine function in moderate smokers, but alterations are apparent in heavy smokers.

The role of the dopamine system in drug reinforcement has long been accepted from animal studies (e.g. Koob 1992) and there is mounting evidence that dysregulated dopamine function is central to addiction behaviours in humans (as reviewed by Volkow *et al.* 2011), although there is emergent evidence of parallel opioid-dopaminergic abnormalities in addiction (e.g. Scott *et al.* 2007). There is growing evidence that chronic drug abuse is associated with abnormal striatal dopaminergic functioning in humans, as has been found with alcohol (Heinz et al. 2005), cannabis (Chapter 3), cocaine (Wu *et al.* 1997), methamphetamine (Wang *et al.*

2012), and ecstasy (Tai *et al.* 2011). However, the study suggests that dopamine dysregulation may only become apparent at higher levels of use, either because it is below the level of detection with more moderate use, or because it is a cumulative consequence of heavy use. It must be emphasised that like all of the cross-sectional studies on dopamine transmission, that the presence or absence of dopaminergic abnormalities may be due to interactions between a trait (i.e. addiction proneness) and a state (e.g. addiction, withdrawal or abstinence).

Therefore, as dopamine synthesis capacity has been found to be elevated in heavy smokers vs. controls, and found to be the same in moderate smokers vs. controls, it is extremely unlikely that smoking is contributing to the findings of reduced dopamine synthesis in both cannabis users *vs.* controls (Chapter 3) and individuals with high exposure to long-term psychosocial stressors vs. individuals with low exposure (Chapter 6).

7.6 General conclusions and future directions

The studies detailed above indicate that there was a relationship between cannabis-induced psychotic-like symptoms and aberrant salience processing and long-term cannabis use is associated with an altered relationship between salience processing and dopamine synthesis capacity. However, the lack of a significant relationship between dopamine synthesis capacity and cannabis-induced psychotic-like symptoms and the finding that cannabis use was associated with reduced, rather than increased, striatal dopamine synthesis capacity is not consistent with the model that cannabis increases the risk of psychosis by creating a state of aberrant salience via increased dopamine synthesis capacity.

In line with these findings, Thompson *et al.* (2013) found that patients with comorbid schizophrenia and substance dependence, including cannabis, had reduced amphetamine-induced dopamine release. Yet, despite a blunted dopamine response, this study found the previously described relationship between dopamine release and increase in psychotic symptoms (as per Laruelle *et al.* 1999), which they suggested may be due to super-sensitivity of the D_2 receptor in line with a recent hypothesis advanced by Seeman & Seeman (2014), findings from animal studies that chronic THC sensitises the D_2 receptor (Ginovart *et al.* 2012) and that genetic variation of AKT1 genotype may alter D_2 receptor signalling to increase the risk of cannabis-induced psychosis (Di Forti *et al.* 2012). This therefore calls into question the final common pathway hypothesis, which has gained traction since its publication. As Murray *et al.* (2014) commented :

"... it is becoming clear is that there are different pathways to schizophrenia-like psychosis (Howes & Murray 2013). These pathways include neurodevelopmental impairment, affective disturbance, and abuse of drugs. Furthermore, it seems that excess striatal dopamine synthesis is not the final common pathway in all psychotic individuals."

Whilst the Aberrant Salience hypothesis has heuristic value, one key phenomenological aspect of schizophrenic psychosis that is not accounted for by the Final Common Pathway model is paranoia. Paranoia is both central to a diagnosis of paranoid schizophrenia, although it exists on a continuum of severity in the general population (Bebbington *et al.* 2013), and has been identified by factor analysis to be independent of other psychotic experiences (Ronald *et al.* 2013). In simple terms, paranoia is about reading threat in neutral settings. Whereas this is entirely compatible with the aberrant salience hypothesis in terms of increased threat-related aberrant salience, it does not take into account reward-based aberrant salience. To put this another way – why would increased dopamine synthesis only result in paranoia and not positive incentive motivation? If this alternative mechanism (i.e. increased positive incentive salience) occurs, might this explain manic episodes in bipolar affective disorder? Whilst dopamine antagonism is used clinically in the treatment of manic episodes and some antipsychotic agents have mood stabilizing properties, the initial hypothesis as advanced by Kapur does not take these issues into account and further research is needed to explore these possibilities.

In addition to the link between cannabis-induced psychotic symptoms and aberrant salience, THC has also been proposed to increase anxiety via amygdala endocannabinoid receptors (Bhattacharyya *et al.* 2010). In a large randomized, placebo-controlled, study of

THC, Freeman *et al.* (2014) found that increases in paranoia caused by THC were accounted for by increases in negative emotions such as anxiety. There is novel evidence that dopaminergic mechanisms may be underlying this process too as a recent study by Volkow *et al.* (2014) found that, compared to controls, cannabis users had a blunted methylphenidate-induced dopamine release and that within cannabis uses, dopamine release was inversely with negative emotions and addiction severity.

Furthermore, the finding of an inverse relationship between apathy and dopamine synthesis capacity in cannabis users could provide an indirect route to increase psychosis risk, by increasing social withdrawal resulting in reduced data gathering (i.e. jumping to conclusions) (Garety & Freeman 1999) and reducing motivation to generate or considerate alternative explanations for experiences (Freeman *et al.* 2004), in line with the threat anticipation model of persecutory delusions (Freeman 2007). However, this is speculative and requires further investigation.

It must of course be held in mind that outside the association of cannabis and frank schizophreniform psychosis, long-term heavy use cannabis is associated with a number of adverse outcomes that themselves resemble the different phenotypic domains of the illness. These are increased positive psychotic symptoms (as reviewed in chapter 1), cognitive impairment (Crane *et al.* 2013; Fried *et al.* 2005; Meier *et al.* 2012) and negative symptoms such as reduced motivation (as described above). One possible explanation is that heavy cannabis use increases the risk of each of those domains to varying degrees and it is the relationships between the effects in these domains that accounts for some of the risk of developing a frank schizophrenia phenotype. However, this hypothesis needs testing in large studies.

It must also be recalled that in view of the complex effects of THC on the dopamine system, as reviewed earlier, cannabis may well have differential effects on different parts of the mesolimbic dopamine pathway. Therefore, it is not beyond the realms of possibility that long-term cannabis exposure results in pre-synaptic hypodopaminergia (i.e. decreased dopamine synthesis capacity) and at the same time is associated with a post-synaptic hyperdopaminergia (i.e. increased D_2 receptor sensitivity). Taken together, an albeit paradoxical possibility could therefore be: the increased risk of psychosis associated with cannabis may be occurring at various levels, such that dopaminergic mechanisms of salience attribution are disrupted whilst D_2 sensitivity is increased, a reduction in dopamine synthesis capacity may contribute to negative affect which increases the risk of paranoia, and reduced motivation associated with hypodopaminergia increases the likelihood that cognitive distortions arise which contribute to the development of persecutory beliefs.

To complicate the picture, there has also recently been evidence to suggest that part of the association between schizophrenia and cannabis is due to a shared genetic aetiology (Power *et al.* 2014), suggestive of potential bi-directionality. Future understanding of how cannabis increases the risk of psychosis therefore would benefit from studies that are able to assess the relationships between acute and chronic cannabis and cannabinoid effects on the dopamine system, and how these relate to salience processing, negative affect and paranoia, and how these interact with genetic risk.

In terms of understanding psychosocial risk, whilst dopamine synthesis capacity was not elevated in a relatively small sample size, a finding of interest from the above study is that

there is a relationship between childhood adversity and recent adult stress in the associative striatum, in line with the schizophrenia literature. Furthermore, findings of increased negative emotions and schizotypy require further investigation. Likewise, further work is necessary to disentangle which psychosocial stressors are driving these presumptive effects. In particular, the relationships between these stressors and aberrant salience, the development of psychotic-like symptoms, stress reactivity and other aspects of dopaminergic function including dopamine release and dopamine receptor availability are needed.

The dopaminergic mechanisms underlying the association between psychosocial stressors and psychosis perhaps share similarities with above proposed model of dopaminergic mechanisms of psychosis in cannabis use. These have already been proposed by Howes & Murray (2013) in an integrated sociodevelopmental-cognitive model of schizophrenia. This suggests that childhood psychosocial stressors sensitise, and indeed cross-sensitize, the dopamine system (Jezierski *et al.* 2007; Prasad *et al.* 1995; de Jong *et al.* 2005). Psychosocial stressors also increase the risk of developing cognitive schemas that perceive the world as threatening (Bentall *et al.* 2009), and this is likely compounded by negative affects which would increase the risk of paranoia, as above. In support of this are well replicated associations between childhood maltreatment and negative emotion (Bifulco *et al.* 1991; Kendler *et al.* 2004), findings that urbanicity alters neural stress response and increases negative affect (Lederbogen *et al.* 2011), and adverse life events being risk factors for depression (Biondi & Picardi 1999). The potential effect of psychosocial stress on dopaminergic function may only increase risk if there are interactions with other risk factors, in particular genetic risks (Modinos *et al.* 2013), consistent with current models of gene-environment interactions in psychosis (van Winkel *et al.*)

Despite the above, a key answered question remains. Given that increased striatal dopamine synthesis capacity is such a well replicated finding in psychosis (and at least the hypothesised type A schizophrenia [Howes & Kapur 2014]), and that the main environmental risk factors addressed in this thesis are not associated with elevated dopamine synthesis capacity in the absence of psychotic symptoms, does the key to understanding the neurobiology of these risks and future preventative interventions for psychosis lie in answering the question "What are the adaptive neurochemical mechanisms that *prevent* individuals exposed to these risks from developing psychosis?" For now, the answers lie outside this thesis.

7.7 Final conclusion

This thesis investigated the dopaminergic mechanisms underlying the relationship between chronic cannabis use and long-term psychosocial stress, two important epidemiological risk factors for schizophrenia, in line with the Aberrant Salience and Final Common Pathway hypotheses. Environmental risk factors are by their very nature modifiable, and so this thesis examined whether these environmental risk factors are associated with the same dopaminergic abnormalities that have been observed in schizophrenia. Contrary to the hypotheses, both long-term cannabis use and exposure to psychosocial stressors were not associated with increased dopamine synthesis capacity. Furthermore, cannabis-induced psychotic-like symptoms were not related to dopamine synthesis capacity, yet they were related to a behavioural measure of aberrant salience processing. Although, compared to controls, cannabis users did not exhibit altered aberrant salience processing, there was preliminary evidence to suggest that dopaminergic mechanisms of salience processing may indeed be altered by long-term cannabis use. There was, however, evidence to suggest a similar relationship between certain psychosocial risk factors for schizophrenia and dopaminergic function to that which has been observed in the illness. Future research should investigate how these findings relate to other aspects of dopaminergic function, cognitive models of psychosis and genetic risks for schizophrenia.

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Appendix 1 – Papers published in peer reviewed journals arising from this thesis

Bloomfield MAP, Morgan CJA, Egerton A, Kapur S, Curran HV, Howes OD (2014) Dopaminergic function in cannabis users and its relationship to cannabis-induced psychotic symptoms. *Biol Psychiatry.* 75(6):470-8.

Bloomfield MAP, Morgan CJA, Egerton A, Kapur S, Curran HV, Howes OD (2014) The link between dopamine function and apathy in cannabis users: an [18F]-DOPA PET imaging study. *Psychopharmacology (Berl).* 231(11):2251-9.

Bloomfield MAP, Pepper F, Egerton A, Demjaha A, Tomasi G, Mouchlianitis E, Maximen L, Veronese M, Turkheimer F, Selvaraj S, Howes OD (2014) Dopamine Function in Cigarette Smokers: An [^{18}F]-DOPA PET Study. *Neuropsychopharmacology* 39: 2397–2404.

Dopaminergic Function in Cannabis Users and Its Relationship to Cannabis-Induced Psychotic Symptoms

Michael A.P. Bloomfield, Celia J.A. Morgan, Alice Egerton, Shitij Kapur, H. Valerie Curran, and Oliver D. Howes

Background: Cannabis is the most widely used illicit drug globally, and users are at increased risk of mental illnesses including psychotic disorders such as schizophrenia. Substance dependence and schizophrenia are both associated with dopaminergic dysfunction. It has been proposed, although never directly tested, that the link between cannabis use and schizophrenia is mediated by altered dopaminergic function.

Methods: We compared dopamine synthesis capacity in 19 regular cannabis users who experienced psychotic-like symptoms when they consumed cannabis with 19 nonuser sex- and age-matched control subjects. Dopamine synthesis capacity (indexed as the influx rate constant K_i^{cer}) was measured with positron emission tomography and 3,4-dihydroxy-6-[^{18}F]-fluoro-*l*-phenylalanine ([^{18}F]-DOPA).

Results: Cannabis users had reduced dopamine synthesis capacity in the striatum (effect size: .85; $t_{36} = 2.54$, $p = .016$) and its associative (effect size: .85; $t_{36} = 2.54$, $p = .015$) and limbic subdivisions (effect size: .74; $t_{36} = 2.23$, $p = .032$) compared with control subjects. The group difference in dopamine synthesis capacity in cannabis users compared with control subjects was driven by those users meeting cannabis abuse or dependence criteria. Dopamine synthesis capacity was negatively associated with higher levels of cannabis use ($r = -.77$, $p < .001$) and positively associated with age of onset of cannabis use ($r = .51$, $p = .027$) but was not associated with cannabis-induced psychotic-like symptoms ($r = .32$, $p = .19$).

Conclusions: These findings indicate that chronic cannabis use is associated with reduced dopamine synthesis capacity and question the hypothesis that cannabis increases the risk of psychotic disorders by inducing the same dopaminergic alterations seen in schizophrenia.

Key Words: Addiction, dependence, dopamine, drugs, imaging, psychosis

Cannabis is the most widely used illicit drug globally (1), and the prevalence of cannabis abuse or dependence in the United States is 4.4% (2). Cannabis can induce transient psychotic symptoms in healthy individuals (3,4), and there is consistent epidemiologic evidence that cannabis dose-dependently increases the risk of psychotic disorders (5,6).

Dopaminergic dysfunction is linked to drug dependence (7–11) and psychosis (12–17). Increased dopamine synthesis capacity and release have been reported in psychotic patients (18–26), drugs that increase dopamine release can induce or worsen psychosis (15,27,28), and elevated dopamine synthesis capacity has been reported in people who subsequently develop a frank psychotic disorder (29–32). Patients with cannabis-induced psychosis have elevated peripheral dopamine metabolites (33), and a case report found striatal dopamine release and symptom exacerbation in a schizophrenic patient following cannabis use (34). Thus, cannabis has been proposed to increase psychosis risk by causing striatal hyperdopaminergia (32).

Supporting this, preclinical studies indicate acute administration of Δ9-tetrahydrocannabinol (THC), the main psychoactive ingredient of cannabis (35), increases mesolimbic dopaminergic neuron firing rates via endocannabinoid CB_1 receptor agonism (36). CB_1 agonists inhibit striatal dopamine reuptake (37), selectively increase tyrosine hydroxylase expression (38), and increase dopamine release (39) and synthesis (40) in the majority of, although not all, studies (41).

Dopaminergic sensitisation to THC occurs in animals (42), suggesting that dopaminergic effects are greater with regular cannabis exposures. Studies in recently abstinent and ex-cannabis users have not found abnormal striatal dopamine release (43) or $D_{2/3}$ receptor availability (44,45), but this may be due to normalization of dopaminergic function with abstinence, as has been observed with alcohol (46). One study reported reduced dopamine transporter availability in cannabis users (47), although this was related to concurrent tobacco use, rather than cannabis. However, to our knowledge, no study has examined dopamine synthesis capacity in cannabis users or whether acute psychotic response to cannabis is related to dopaminergic function.

We therefore sought to study presynaptic dopaminergic function in active cannabis users who experienced cannabis-induced psychotic-like symptoms because these individuals are most at risk of psychosis (48). We hypothesized that regular cannabis users sensitive to cannabis' psychotogenic effects would exhibit elevated dopamine synthesis capacity compared with nonuser control subjects, and this would be directly related to cannabis-induced psychotic-like symptom severity.

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Methods and Materials

The study was approved by the National Research Ethics Service and the Administration of Radioactive Substances Advisory Committee. The study was conducted in accordance with the Declaration of Helsinki. All subjects provided informed written consent to participate.

Study Population

Inclusion criteria for all subjects were as follows: minimum age 18 years, good physical health with no history of major medical condition, and capacity to give written informed consent. Exclusion criteria for all subjects were current or past psychiatric illness (except cannabis use disorders in the cannabis user group and nicotine use disorder in all subjects) using the Structured Clinical Interview for DSM-IV (49), history of serious mental illness (including psychosis) in a first-degree relative determined via the Family Interview for Genetic Studies (50), evidence of an At Risk Mental State for psychosis (51), DSM-IV-TR (52) substance dependency or abuse (other than cannabis in the cannabis user group and tobacco for all subjects), and contraindications to positron emission tomography (PET; including pregnancy and breast-feeding). None of the subjects were taking psychotropic medication at the time of study participation.

Detailed drug histories were obtained from all subjects using the Cannabis Experience Questionnaire (53), structured interview and timeline follow-back. Lifetime cannabis use was estimated as the total number of "spliffs" (cannabis cigarettes; "joints") consumed. The time taken to smoke an "eighth" of cannabis (one-eighth ounce; approximately 3.5 g, representing the standard unit of sale in Britain) was chosen as the primary index of cannabis use because this provides a measure of the amount of current drug consumption (shorter time indicating greater consumption). This is likely to be more accurate than subjective recall of the number of spliffs consumed because of variability in cannabis dose between spliffs and inconsistencies in self-reported cannabis use (54).

Cannabis User Group

We recruited cases from an ongoing cohort study in which more than 400 cannabis users were tested when intoxicated with cannabis and when not intoxicated (55). Subjects met the following criteria: current, at least weekly use of cannabis and the induction of psychotic-like symptoms in response to smoking cannabis, which was defined as a positive change on the psychotic items score of the Psychotomimetic States Inventory (PSI) (56) measured 5 minutes after smoking their usual amount of cannabis (i.e., when acutely intoxicated) compared with when not intoxicated with the drug. Cannabis users consumed their own cannabis, and testing occurred in the presence of a researcher in the environment where users habitually consumed cannabis in their usual drug-taking context (e.g., at home) because drug effects are typically larger in naturalistic as opposed to laboratory environments (53). Cannabis-induced psychotic-like symptoms abated within 2 hours of consumption, and no subject met the DSM-IV TR criteria for a diagnosis of a psychotic disorder. The psychotic items from the PSI covered "Delusional Thinking," "Perceptual Distortions," "Cognitive Disorganization" (thought disorder), and "Paranoia." Each item is rated on a 4-point scale from "not at all" (score = 0) to "strongly" (score = 3). Examples of items include "People can put thoughts into your mind" and "You can sense an evil presence around you, even though you cannot see it." A sample of the cannabis that each participant smoked was taken on the day of testing and analyzed for levels of THC (Forensic Science Service, Birmingham, United Kingdom).

Control Group

Nonuser control subjects were recruited from the same geographic area by public advertisement. Controls were required to have no lifetime history of cannabis dependence or abuse (DSM-IV), no more than 10 total uses of cannabis in their lifetime, no report of the induction of psychotic symptoms by cannabis, and no history of cannabis use in the preceding 3 months. Community surveys indicate that more than 30% of young adults in England report trying cannabis in their lifetime (57). We therefore permitted control subjects to have had a minimal exposure to cannabis to ensure the control group was representative of the same general population from which we recruited the cannabis users.

PET Data Acquisition

All subjects underwent a 3,4-dihydroxy-6-[^{18}F]-fluoro-/-phenylalanine ([^{18}F]-DOPA) scan on an ECAT HR+ 962 PET scanner (CTI/Siemens, Knocksville, Tennessee) in three-dimensional mode, with an axial field of view of 15.5 cm, performed as previously reported (28). Subjects were asked to fast and abstain from cannabis for 12 hours and to refrain from smoking tobacco for 2 hours before imaging. On the day of the PET scan, urine drug screen (Monitect HC12, Branan Medical Corporation, Irvine, California) confirmed no recent drug use (other than cannabis in the user group), and a negative urinary pregnancy test was required in all female subjects. A research clinician assessed psychotic symptoms using the Positive and Negative Syndrome Scale at the time of scanning. No subjects had psychotic symptoms at the time of scanning (mean [SD] Positive and Negative Syndrome Scale positive score cannabis users = 7.3 [.5]; control subjects = 7.2 [.4]). Subjects received carbidopa 150 mg and entacapone 400 mg orally 1 hour before imaging (58) to reduce the formation of radiolabeled [^{18}F]-DOPA metabolites (59,60). Head position was marked and monitored via laser crosshairs and a camera and minimized using a head-strap. A 10-minute transmission scan was performed before radiotracer injection for attenuation and scatter correction. Approximately 180 MBq of [^{18}F]-DOPA was administered by bolus intravenous injection 30 seconds after the start of PET imaging. We acquired emission data in list mode for 95 minutes, rebinned into 26 timeframes (30-second background frame, four 60-second frames, three 120-second frames, three 180-second frames, and fifteen 300-second frames).

Volume of Interest Analysis

To correct for head movement, nonattenuation-corrected dynamic images were denoised using a level 2, order 64 Battle-Lemarie wavelet filter (61), and individual frames were realigned to a single frame acquired 10 minutes after the [^{18}F]-DOPA injection using a mutual information algorithm (62). Transformation parameters were then applied to the corresponding attenuation-corrected frames, and the realigned frames were combined to create a movement-corrected dynamic image (from 6 to 95 minutes following [^{18}F]-DOPA administration) for analysis.

After movement correction, we defined standardized volumes of interest (VOIs) bilaterally in the whole striatum, the limbic (ventral), associative (precommisural dorsal caudate, precommisural dorsal putamen, and postcommisural caudate), and sensorimotor (postcommisural putamen) striatal functional subdivisions and the cerebellar reference region in Montreal Neurologic Institute space (63,64). An [^{18}F]-DOPA template was normalized with the VOI map to each individual PET summation

Table 1. Sample Characteristics and Scan Parameters

	Controls ($n = 19$)	Cannabis Users ($n = 19$)	p[a]
Sample Characteristic			
Age (years), mean (SD)	22.3 (2.8)	20.8 (1.7)	.07
Sex (*n*)	2 female, 17 male	2 female, 17 male	1.00
Handedness (*n*)	2 left, 17 right	4 left, 15 right	.37
Ethnicity (*n*)	4 AB, 3 BB, 1 ME, 11 WB	4 AB, 15 WB	.16[b]
Current Drug Use[c,d]			
Cannabis users (*n*)	0 users, 19 nonusers	19 users, 0 nonusers	1.00
Cannabis use (grams of cannabis/month), median (IQR)	.0 (.0)	26.3 (90.0)	.00
THC content of cannabis (%), mean (SD)	—	8.7 (3.8)	—
Time since last cannabis exposure (hours), median (IQR)	—	14.0 (23.8)	—
Time taken to smoke an "eighth" of cannabis (days), median (IQR)	—	4.0 (13.5)	—
Age of onset of regular cannabis use (years), mean (SD)	—	15.5 (1.6)	—
Tobacco cigarette smokers (*n*)	8 users, 11 nonusers	15 users, 4 nonusers	.02
Tobacco use in whole sample (cigarettes/day), median (IQR)	.0 (.0)	4.0 (7.0)	.01
Tobacco use in smokers (cigarettes/day), median (IQR) (tobacco users)	1.0 (9.0)	7.0 (8.0)	—
Alcohol use in past 3 months (*n*)	19 users, 0 nonusers	19 users, 0 nonusers	1.00
Alcohol[e] use (United Kingdom alcohol units/week), median (IQR)	9.0 (12.0)	12.0 (21.0)	.34
MDMA use in past 3 months (*n*)	5 users, 14 nonusers	11 users, 8 nonusers	.05
MDMA use in whole sample (grams of MDMA/month), median (IQR)	.0 (.0)	.3 (1.0)	.02
MDMA use in MDMA users (grams of MDMA/month), median (IQR)	.3 (.8)	1.0 (1.7)	—
Cocaine use in past 3 months (*n*)	3 users, 16 nonusers	3 users, 16 nonusers	1.00
Cocaine use in whole sample (grams of cocaine/month), median (IQR)	.0 (.0)	.0 (.0)	.60
Cocaine use in cocaine users (grams of cocaine/month), median (IQR)	<.1 (<.1)	<.1 (1.0)	—
Amphetamine use in past 3 months (*n*)	1 user, 18 nonusers	4 users, 15 nonusers	.15
Amphetamine use in whole sample (grams of amphetamine/month), median (IQR)	.0 (.0)	.0 (.0)	.27
Amphetamine use in amphetamine users (grams of amphetamine/month), median (IQR)	<.1	.5 (.3)	—
Ketamine use in past 3 months (*n*)	1 user, 18 nonusers	6 users, 13 nonusers	.04
Ketamine use in whole sample (grams of ketamine/month), median (IQR)	.0 (.0)	<.1 (.5)	.10
Ketamine use in ketamine users (grams of ketamine/month), median (IQR)	<.1	1.5 (2.9)	—
Psilocybin use in past 3 months (*n*)	1 user, 18 nonusers	1 user, 18 nonusers	1.00
Psilocybin use in whole sample (grams of "magic mushrooms"/month), median (IQR)	.0 (.0)	.0 (.0)	.80
Psilocybin use in psilocybin users (grams of "magic mushrooms"/month)	<.1	2.0	—
Scan Parameter			
Injected dose (MBq), mean (SD)	180.6 (7.2)	184.4 (5.2)	.11
Specific activity (MBq/μmol), mean (SD)	31.1 (17.3)	30.5 (14.0)	.92
Whole striatal volume (mm^3), mean (SD)	17,587.82 (1729.50)	17,942.90 (1286.73)	.48
Associative striatal volume (mm^3), mean (SD)	10,801.19 (1134.46)	10,772.76 (1161.24)	.94
Limbic striatal volume (mm^3), mean (SD)	2080.30 (234.77)	2276.51 (977.85)	.40
Sensorimotor striatal volume (mm^3), mean (SD)	4706 (106.60)	4668.98 (443.16)	.80

AB, Asian British; BB, black British; IQR, interquartile range; MDMA, 3,4-methylenedioxy-N-methylamphetamine ("Ecstasy"); ME, mixed ethnicity; WB, white British.

[a]Independent-samples *t* tests for variables with normal data distributions; Mann-Whitney *U* tests for variables with nonnormal data distributions; χ^2 tests for dichotomous variables.

[b]Groups were compared on a dichotomized ethnicity variable (white British vs. ethnic minority).

[c]Drug use reported in 3 months before scan. Drug user defined as any drug use in the 3 months before scan.

[d]There was no reported lysergic acid diethylamide, benzodiazepine, opiate, or methamphetamine use in the 3 months before scanning.

[e]1 UK alcohol unit = 10 mL (~7.88 g) alcohol.

(add) image using statistical parametric mapping software (SPM5, http://fil.ion.ucl.ac.uk/spm), allowing VOIs to be placed automatically on individual [^{18}F]-DOPA PET images without observer bias. We calculated [^{18}F]-DOPA uptake, relative to the cerebellum [K_i^{cer} (min^{-1})], for each VOI using the Patlak graphic analysis adapted for a reference tissue input function (65–68). We have previously demonstrated good test–retest reliability for striatal K_i^{cer} determined this way (64).

Voxelwise Analysis

We complemented the VOI analysis with an independent voxelwise analysis using a wavelet-based Patlak method (69) as previously described (29). The parametric image for each participant was then normalized into standard space using the participants PET summation image and the [^{18}F]-DOPA template (29). Statistical parametric mapping was conducted using SPM5 and a striatal mask defined according to previously described criteria (64) to compare groups. Results are presented corrected for multiple comparisons using random field theory as applied in SPM5 ($p < .05$, corrected at the family-wise error rate).

Statistical Analysis

We assessed normality of distributions using the one-sample Kolmogorov-Smirnov test. Between-group comparisons were made with two-tailed independent *t* tests for normally distributed data and Mann-Whitney *U* tests for nonnormally distributed data.

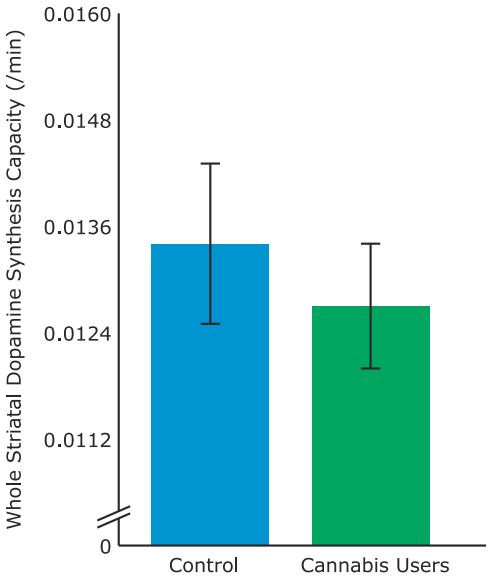

Figure 1. Striatal dopamine synthesis capacity in regular cannabis users ($n = 19$) and nonuser control subjects ($n = 19$). Dopamine synthesis capacity was significantly reduced in cannabis users compared with nonusers ($t_{36} = 2.54$, $p = .016$). Error bars indicate standard deviations.

Relationships among K_i^{cer}, levels of cannabis use, and cannabis-induced psychotic-like symptom severity were tested using Pearson's product–moment correlation coefficient. Potential confounding effects of other substance use were explored using a single analysis of covariance (ANCOVA) with subject group as the fixed factor; K_i^{cer} as the dependent variable and levels of use of each substance other than cannabis as separate covariates, and using Pearson's product–moment correlation coefficient to determine if there was a relationship between K_i^{cer} and levels of tobacco smoking. Statistical significance was defined as $p < .05$ (two-tailed). Our primary outcome measure was K_i^{cer} in the whole striatum. Exploratory analyses were conducted in the striatal subdivisions (presented uncorrected for multiple comparisons).

Results

Subject Characteristics and Scan Parameters

Twenty cannabis users were recruited to the study. Owing to tomograph malfunction during one scan, complete data were available on nineteen users. All cannabis users consumed the drug as a spliff. The mean (SD) age of first cannabis use was 15.5 (1.6) years, and the mean (SD) duration of at least weekly use was 4.7 (3.1) years. The median (interquartile range) time taken to smoke an eighth and lifetime exposure to cannabis was 4.0 (13.5) days and 2340 (6240) spliffs, respectively. Within the user group, the median (interquartile range) time between the scan and the last cannabis exposure and self-reported cannabis-induced psychotic-like symptoms was 14.0 (23.8) hours. Ten users met DSM-IV criteria for cannabis dependence ($n = 5$) or abuse ($n = 5$). Mean (SD) time to smoke an eighth was 2.3 (2.2) days in users who met dependency/abuse criteria and 6.9 (4.7) days in users who did not meet criteria. Mean (SD) age of first cannabis consumption was 14.8 (1.6) years in users who met dependency/abuse criteria, and 16.2 (1.3) years in users who did not meet criteria. Nineteen control subjects were matched to the user group for age (±5 years) and sex. Subjects' characteristics are reported in Table 1. Urine drug screen was positive for THC and negative for all other substances (amphetamine, opiates, cocaine, methamphetamine, benzodiazepines) in every cannabis user and negative for all drugs (including cannabis) in every control subject. There was a significant group difference in current cannabis consumption, as expected, and also in tobacco and ecstasy use (Table 1).

There was no significant group difference in the amount of radioactivity or specific activity injected (Table 1). There was no significant difference in whole striatal or subdivision volumes between the groups. There was no relationship between age and K_i^{cer} in the striatum or its subdivisions in the whole sample or in either group (data available on request).

Striatal Dopaminergic Function

K_i^{cer} was significantly reduced in cannabis users relative to controls in the whole striatum (Figure 1). Secondary analysis in each striatal subdivision showed that this reduction reached significance in the limbic and associative subdivisions (Table 2). The finding of reduced K_i^{cer} in cannabis users remained significant after covarying for other drugs used, with the amount of use of each of the drugs listed in Table 1 included as separate covariates in the ANCOVA, in the whole striatum ($F_{1,37} = 4.65$, $p = .040$) and its associative ($F_{1,37} = 5.00$, $p = .034$) and limbic ($F_{1,37} = 7.358$, $p = .011$) subdivisions.

Voxel-based analysis confirmed reduced K_i^{cer} in the cannabis user group relative to nonuser control subjects with peak statistical significance in the right putamen (Montreal Neurological Institute coordinates: 28, 6, −8; $p = .048$ [corrected for familywise error]; Figure 2). There were no voxels where there was a significant elevation in K_i^{cer} in cannabis users relative to control subjects.

The Relationship Between Striatal Dopamine Synthesis Capacity and Cannabis Use

Within the cannabis user group, greater levels of current cannabis use (less time to smoke an eighth of cannabis) were associated with lower K_i^{cer} in the whole striatum ($r = -.77$, $p < .001$; Figure 3A). Secondary analysis in each striatal subdivision showed that this pattern reached significance in the associative ($r = -.68$, $p = .001$) and sensorimotor ($r = -.84$, $p < .001$) subdivisions but not the limbic subdivision ($r = -.26$, $p = .290$). In addition, there was a significant correlation between age of onset of cannabis use and K_i^{cer} in the whole striatum ($r = .51$, $p = .027$; Figure 3B) and in its associative subdivision ($r = .56$, $p = .013$), which remained significant after controlling for current age ($r = .49$, $p = .04$ [whole striatum]; $r = .54$, $p = .02$ [associative]), with no significant correlation in the sensorimotor ($r = .34$, $p = .158$) or limbic ($r = .36$, $p = .126$) subdivisions. There was no significant correlation between age of first cannabis use and current cannabis use ($r = .16$, $p = .52$).

Table 2. [^{18}F]-DOPA K_i^{cer} (min^{-1}) by Group

	Control Subjects ($n = 19$)		Cannabis Users ($n = 19$)		Group Comparisons[a]		Effect Size
VOI	Mean	SD	Mean	SD	t_{df}	p	(Cohen's d)
STR	.0134	.0009	.0127	.0007	2.54_{36}	.016	.85
AST	.0127	.0009	.0121	.0007	5.54_{36}	.015	.85
LST	.0138	.0009	.0132	.0008	2.23_{36}	.032	.74
SMST	.0146	.0014	.0139	.0008	1.85_{36}	.070	.62

AST, associative striatum; K_i^{cer}, influx rate constant; LST, limbic striatum; SMST, sensorimotor striatum; STR, whole striatum; VOI, volume of interest.

[a]Independent-samples t tests.

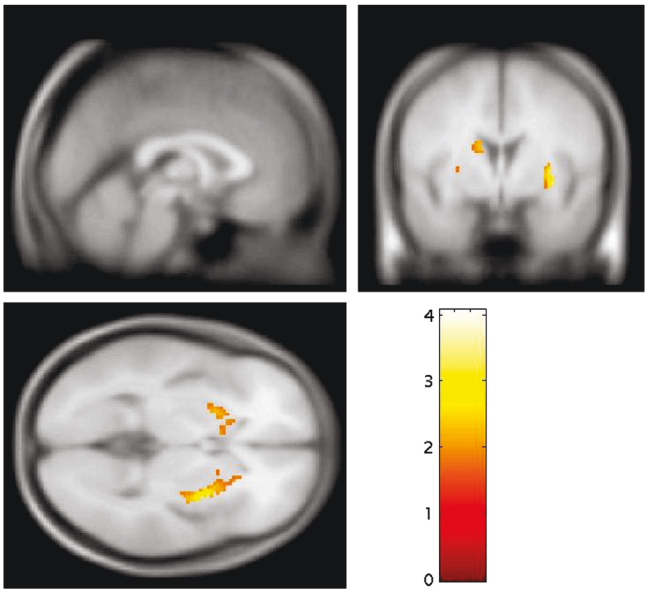

Figure 2. Reduced striatal dopamine synthesis capacity in regular cannabis users relative to nonuser controls. The image shows a statistical parametric map of significant reductions ($p < .05$) in dopamine synthesis capacity, relative to healthy comparison subjects ($n = 19$), in regular cannabis users who experienced transient psychotic-like symptoms ($n = 19$). The most significant reduction was in the right putamen (Montreal Neurological Institute coordinates: 28, 6, −8; $p = .048$, corrected at the family-wise error rate). The color bar indicates the *t* statistic for each voxel.

Across the whole sample and within the control group, there was no significant difference between K_i^{cer} in tobacco smokers and nontobacco smokers in any of the regions examined (all *p*s >.1). Within the whole sample and within each group, there was no relationship between K_i^{cer} and daily cigarette use among tobacco cigarette smokers in the whole striatum ($r = .26$, $p = .91$ [whole sample], $r = .10$, $p = .81$ [control subjects]; $r = .18$, $p = .52$ [cannabis users]) and its functional subdivisions (data available on request). Within the whole sample and within each group, there were no significant relationships (all *p*s > .1) between whole striatal K_i^{cer} and other substances used (listed in Table 1).

To examine whether cannabis dependency/abuse was associated with reduced K_i^{cer}, we divided the cannabis user group into subjects that met DSM-IV diagnostic criteria for cannabis dependency or abuse ($n = 10$) and those who did not meet criteria ($n = 9$). One-way analysis of variance found a significant effect of group on whole striatal K_i^{cer} ($F_{2,37} = 4.02$, $p = .027$, Figure 4). Post hoc *t* tests showed significant differences between the cannabis dependency/abuse and nondependency/nonabuse cannabis user subgroups ($t_{17} = 2.80$, $p = .012$) and between the cannabis dependency/abuse subgroup and control subjects ($t_{27} = 2.67$, $p = .013$), but not between the nondependency/nonabuse subgroup and the control group ($p = .60$). When examining the striatal subdivisions, significant differences in K_i^{cer} between the cannabis dependency/abuse and nondependency/nonabuse cannabis user subgroups were observed in the associative subdivision only ($t_{17} = 2.89$, $p = .010$).

The Relationship Between Striatal Dopamine Synthesis Capacity and Cannabis-Induced Psychotic Symptoms

Within the cannabis user group, the mean (SD) increase in PSI psychotic symptom subscale score after consuming cannabis was 9.9 (5.1). There was no significant correlation between striatal K_i^{cer} and increase in transient psychotic-like symptoms following cannabis use ($r = .32$, $p = .19$; Figure 5).

Discussion

Our main finding is that striatal dopamine synthesis capacity is lower in current cannabis users than matched nonuser control subjects. In users, lower dopamine synthesis capacity was associated with greater current cannabis use, which explained 59% of variance in striatal dopamine synthesis capacity, and

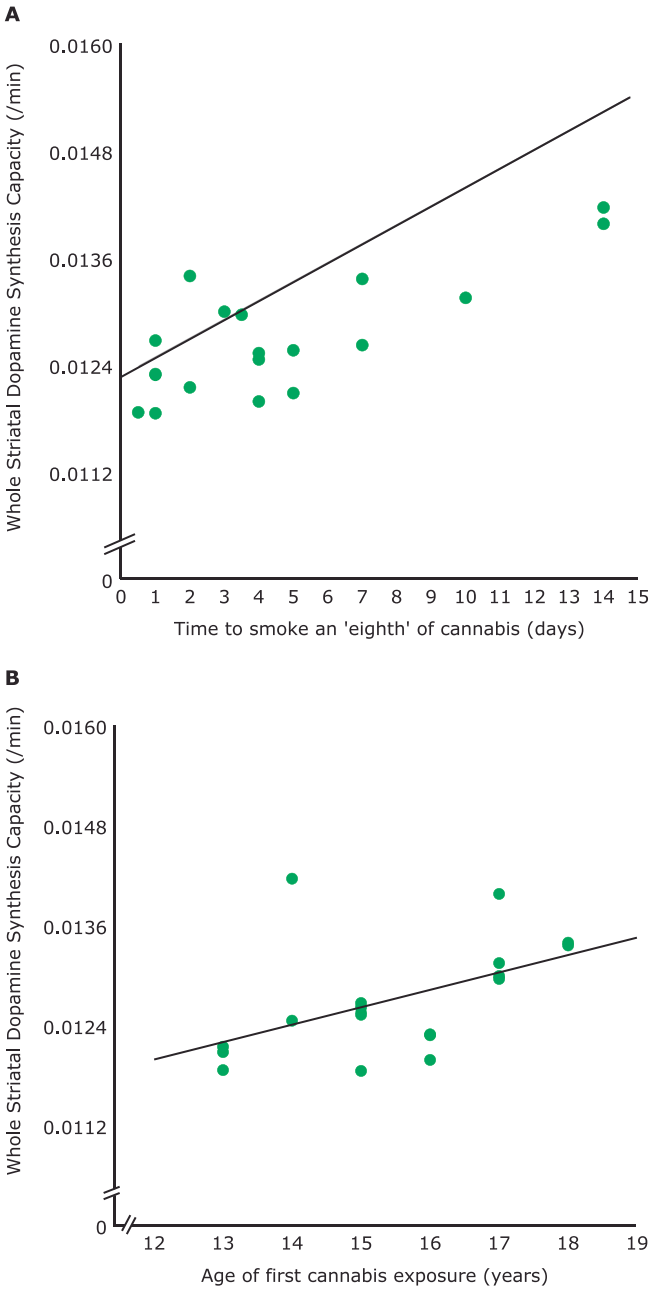

Figure 3. (A) The correlation between level of cannabis use (time to smoke an "eighth" [~3.5 g] of cannabis; days) and striatal dopamine synthesis capacity, indexed as K_i^{cer} (min^{-1}), in cannabis users ($r = -.77$, $p < .001$). **(B)** The correlation between age of onset of cannabis use and K_i^{cer} in the whole striatum ($r = .51$, $p = .027$), which remained significant when controlling for current age ($r = .49$, $p = .04$).

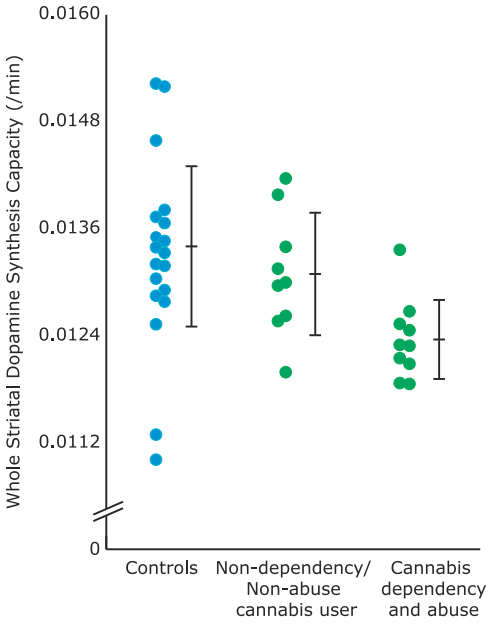

Figure 4. Striatal dopamine synthesis dopamine synthesis capacity in subjects who met DSM-IV criteria for a diagnosis of cannabis dependence or abuse ($n = 10$), regular cannabis users who did not meet diagnostic criteria ($n = 9$), and nonuser control subjects ($n = 19$). There were significant differences between cannabis dependence/abuse versus cannabis users who did not meet criteria ($t_{17} = 2.80$, $p = .012$) and cannabis dependence/abuse versus control group ($t_{27} = 2.67$, $p = .013$). There was no significant difference between controls versus cannabis users who did not meet dependence/abuse criteria ($t_{26} = .54$, $p = .60$). Error bars indicate standard deviations.

earlier age of onset of use, but not with cannabis-induced psychotic-like symptoms.

Importantly, we also found that the lower levels of dopamine synthesis capacity in cannabis users compared with nonusers were driven by users who met diagnostic criteria for abuse and dependence. These findings are inconsistent with our hypothesis that elevated dopamine synthesis capacity underlies the link between cannabis and risk of psychosis.

Our results extend previous findings in current (70) and recently abstinent cannabis users (43), which found reduced dopamine receptor density was associated with higher current cannabis use and lower dopamine release in the associative striatum was associated with earlier age of onset of cannabis. Although these studies (43,44,70) and a further study in ex-users (45) have reported estimates of the number of lifetime uses of cannabis and our sample is comparable to these, measures of the amount or type of cannabis consumed have not been reported, such that direct comparisons of cannabis use across the studies cannot be made. Our findings of reduced dopamine synthesis capacity in dependent subjects may reflect a "blunted" dopamine system, as observed with other drugs of addiction (7). Taken with findings from these and other studies (8–11), there is mounting evidence that dopaminergic dysfunction provides a biomarker of addiction severity.

Although the case–control design of this study is not able to detect a causative relationship between cannabis use and dopamine dysfunction, our findings suggestive of dose effects warrant further research into potential causative mechanisms. Animal studies indicate increased dopaminergic function in response to acute THC treatment. However, there is evidence of a biphasic dose-dependent dopamine response to THC (71), suggesting higher cannabis exposures may reduce dopamine synthesis capacity, in line with our findings. Furthermore, with the exception of perinatal studies (72), animal data on dopaminergic effects of long-term and high dose cannabis exposures are sparse, and the longest duration of THC administration has been 21 days (42,73–75). Of these, one study (74) in Sprague-Dawley rats reported that long-term treatment with THC was associated with reduced striatal tyrosine hydroxylase gene expression and concurrent supersensitivity of $D_{2/3}$ receptors, and a separate study (75) in catechol-*O*-methyltransferase mutant mice found chronic treatment with THC in adolescence was associated with reduced dopaminergic cell size in the ventral tegmental area.

One explanation for our findings is that chronic cannabis use is associated with dopaminergic down-regulation. This might underlie amotivation and reduced reward sensitivity in chronic cannabis users (76). Alternatively, preclinical evidence suggests that low dopamine neurotransmission may predispose an individual to substance use (77). However, this is inconsistent with findings that recently abstinent and former cannabis users show neither altered dopamine receptor availability (44,45,70) nor altered dopamine release (43), suggesting that altered dopaminergic function during chronic cannabis use is normalized by abstinence, as is observed with amphetamine in vervet monkeys (78).

In this study, we investigated dopaminergic function in cannabis users who experience a transient increase in psychotic-like experiences when acutely intoxicated with cannabis. The lack of relationship between the induction of psychotic-like experiences and dopaminergic function suggests that our findings would generalize to cannabis users in general, but this requires confirmation in future studies.

Our findings suggest that elevated striatal dopamine synthesis capacity is unlikely to be the mechanism underlying the link between cannabis and psychosis. Our study focused on the striatum because dopaminergic changes there have been reliably linked to psychosis (22) but we cannot exclude the possibility that dopaminergic changes in extrastriatal regions underlie

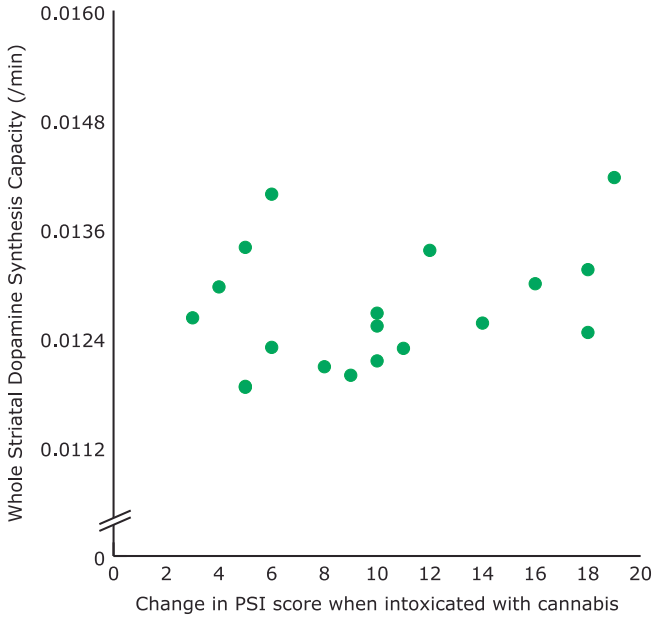

Figure 5. The relationship between the striatal influx rate constant K_i^{cer} and transient induction of cannabis-induced psychotic-like symptoms in the cannabis users. There was no significant relationship between the two variables ($r = .32$, $p = .19$). PSI, Psychotomimetic States Inventory.

cannabis-induced psychotic symptoms. A previous study (79) using single photon emission computed tomography reported a significant increase in temporal cortex $D_{2/3}$ receptor availability in antipsychotic-naive first-episode patients with psychosis who tested positive for cannabis compared with those who did not. Alternatively, the mechanism may be mediated via non-dopaminergic systems, such as direct effects on cannabinoid receptors (80).

Nevertheless, findings that striatal dopamine release in patients with comorbid schizophrenia and substance dependence is blunted but still associated with amphetamine-induced psychotic symptoms (81) supports the possibility that other aspects of striatal dopaminergic function are altered by cannabis or that cannabis use interacts with other risk factors for schizophrenia to induce hyperdopaminergia. In support of this, early work in Wistar rats (82) found THC decreased striatal dopamine uptake compared with vehicle, but increases in striatal dopamine uptake were observed when THC-treated rats were housed under "stressful" versus "normal" conditions. Earlier age of onset of cannabis use increases psychosis risk and may interfere with normal brain development (83). Another possibility is thus that cannabis use during key developmental periods alters the regulation of dopaminergic function to make it more susceptible to subsequent stressors that could underlie an increased risk of psychosis. Additional prospective studies on the effects of chronic cannabis exposure are therefore warranted.

Study Limitations

One potential limitation of this study is that subjects consumed their own cannabis rather than a standard preparation. However, we tested individuals while intoxicated, measured levels of THC in samples of the cannabis our subjects were using, and confirmed it contained high levels of THC in all subjects (mean THC content = 8.7%). There was no fixed interval between cannabis exposure and PET, meaning that heavier cannabis users may have had a shorter interval between exposure and scan. It therefore remains possible that differences in the time since last cannabis use contribute to the differences between the dependent/abuser and nondependent groups, rather than dependency or abuse per se. In addition, lack of association between cannabis-induced psychotic symptoms may be due to variable interval between cannabis exposure and PET. However, in terms of acute effects of cannabis, only one of three molecular imaging studies of the acute effects of THC in healthy volunteers have found evidence of dopamine release (84–86), suggesting that acute effects of THC on dopaminergic function may not be large or consistent in humans. Given that THC and its metabolites have an elimination half-life of about 7 days (87) and all our cannabis users were regular, long-term users who had consumed cannabis within the past 7 days (median time since last consumption = 14 hours), our subjects were unlikely to be acutely withdrawing.

Our measures of substance use rely on self-report, and we were not able to independently verify substance use histories beyond ongoing cannabis use in the user group and no recent use of other drugs in all participants. As would be expected, higher rates of other substance use were reported in cannabis users, although, with the exception of tobacco, the use of other substances was low in both groups. Our findings remained significant after covarying for all other drug use, suggesting that use of other substances does not underlie our findings, although it should be noted that ANCOVA may be less able to adjust for factors when groups differ significantly in covariates (88) and should be considered exploratory. We therefore cannot exclude the possibility that group differences in other drug use contributed to the results observed.

Although cannabis users in our sample reported higher levels of ecstasy use than control subjects, ecstasy has been associated with increased dopamine synthesis capacity (89), so this is unlikely to explain our findings. More of the cannabis users smoked cigarettes than control subjects. The effects of cigarette smoking on presynaptic dopamine function are unclear; tobacco use has been associated with reduced amphetamine-induced dopamine release (90) but increased dopamine synthesis capacity (91). In addition, tobacco smoking may influence [^{18}F]-DOPA kinetics via cerebral blood flow effects (92), which, if regionally selective, could affect our outcome measure. However, we did not find a relationship between levels of cigarette consumption and dopamine synthesis capacity, suggesting this did not influence our results, although additional research is needed to determine the effect of tobacco smoking on dopaminergic function.

Conclusion

Our results show that regular long-term cannabis use is associated with a dose-dependent reduction in dopamine synthesis capacity in the corpus striatum, particularly in those meeting diagnostic criteria for cannabis abuse or dependence. However, we found no relationship between dopaminergic function and cannabis-induced psychotic-like symptoms. These findings question the prevailing assumption that cannabis increases the risk of schizophrenia by inducing the same dopaminergic alterations seen in schizophrenia.

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ORIGINAL INVESTIGATION

The link between dopamine function and apathy in cannabis users: an [^{18}F]-DOPA PET imaging study

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Abstract
Rationale Cannabis is the most widely used illicit drug in the world, and regular use has been associated with reduced motivation, i.e. apathy. Regular long-term cannabis use has been associated with reduced dopamine synthesis capacity. The mesolimbic dopaminergic system mediates the processing of incentive stimuli by modifying their motivational value, which in turn is modulated by endocannabinoid signalling. Thus, it has been proposed that dopaminergic dysfunction underlies the apathy associated with chronic cannabis use.
Objectives The aim of this study was to examine the relationship between dopaminergic function and subjective apathy in cannabis users.
Methods We measured dopamine synthesis capacity (indexed as the influx rate constant K_i^{cer}) via 3,4-dihydroxy-6-[^{18}F]-fluoro-*l*-phenylalanine positron emission tomography and subjective apathy using the self-rated Apathy Evaluation Scale (AES-S) in 14 regular cannabis users.
Results All subjects scored in excess of 34 points on the AES-S (median [interquartile range] 59.5 [7.5]), indicative of significant apathy based on normative data. K_i^{cer} was inversely correlated to AES-S score in the whole striatum and its associative functional subdivision (Spearman's rho=−0.64, p=0.015 [whole striatum]; rho=−0.69, p=0.006 [associative]) but not in the limbic or sensorimotor striatal subdivisions. There were no significant relationships between AES-S and current cannabis consumption (rho=0.28, p=0.34) or age of first cannabis use (rho=0.25, p=0.40).
Conclusions These findings indicate that the reduction in striatal dopamine synthesis capacity associated with chronic cannabis use may underlie reduced reward sensitivity and amotivation associated with chronic cannabis use.

Keywords Apathy · Cannabis · Drugs · Dopamine · Imaging · Motivation · PET

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Introduction

Cannabis is a widely used recreational drug (Anthony et al. 1994; United Nations Office on Drugs and Crime 2010). Heavy cannabis use has been associated with educational and occupational under-achievement in several (Brook et al. 2002; Fergusson et al. 2003; Gruber et al. 2003; Kandel et al. 1986; Macleod et al. 2004; Horwood et al. 2010) but not all studies (Reilly et al. 1998). Over 100 years ago, the Indian Hemp Commission reported that heavy cannabis use was associated with apathy (Indian Hemp Drugs Commission 1893). Since then, there is evidence that regular use of the drug is associated with apathy (Looby and Earleywine 2007; McGlothin and West 1968; Tennant and Groesbeck 1972; Verdejo-Garcia et al. 2006), defined as reduced motivation for goal-directed behaviour (Levy and Dubois 2006; Marin 1991). Thus, reduced motivation, i.e. apathy, has been

proposed to be one factor potentially involved in impaired educational and occupational outcomes associated with heavy cannabis use (Fergusson et al. 2003).

In support of this, heavy chronic cannabis use has been found to produce apathetic behaviours in rhesus monkeys (Paule et al. 1992). However, there is limited evidence of amotivational effects of cannabis from laboratory studies in humans using operant conditioning paradigms (Cherek et al. 2002) and that this may be related to heavy use of the drug (Mendelson et al. 1976; Lane et al. 2005).

The main psychoactive substance in cannabis is Δ9-tetrahydrocannabinol (THC) (Wachtel et al. 2002). THC was originally described as an agonist of endocannabinoid CB_1 receptors (Felder et al. 1992); however, there is growing evidence of partial agonist properties from both in vitro (Sim et al. 1996; Petitet et al. 1998; Shen and Thayer 1999; Breivogel and Childers 2000; Govaerts et al. 2004; Kelley and Thayer 2004) and in vivo (Paronis et al. 2012) studies. There is evidence both from studies in animal models and humans that THC administration disrupts reinforced behaviour (Stiglick and Kalant 1983; Kamien et al. 1994; Lane and Cherek 2002; Lane et al. 2004; Foltin et al. 1989).

A key function of the mesolimbic dopaminergic system is to mediate the processing of incentive stimuli by modifying their motivational value (Berridge and Robinson 1998), which is modulated by endocannabinoid signalling (Fernandez-Ruiz et al. 2010; Melis et al. 2012; Melis and Pistis 2012). Animal studies indicate that the acute effects of THC include increase dopaminergic neuron firing rates (French 1997), whilst the chronic effects of THC include reduced presynaptic dopaminergic function (Ginovart et al. 2012). However, only two of four molecular imaging studies in human volunteers found that acute THC resulted in striatal dopamine release (Barkus et al. 2011; Bossong et al. 2009; Kuepper et al. 2013; Stokes et al. 2009), suggesting that the effect is not consistent and that it may be greater for those who are at clinical risk of psychotic disorder. Yet, studies in recently abstinent and current chronic cannabis users have found that reduced striatal dopamine release was associated with earlier age of onset of cannabis use and reduced dopamine receptor density is associated with greater current use (Albrecht et al. 2013; Urban et al. 2012). A functional magnetic resonance imaging (fMRI) study has reported attenuated striatal reward processing in chronic cannabis users (van Hell et al. 2010). Therefore, it has been proposed that attenuated mesolimbic dopaminergic transmission due to long-term cannabis use results in a mesolimbic reward system that is hyporesponsive to non-drug stimuli (van Hell et al. 2010), in line with the reward deficiency hypothesis (Blum et al. 2000; Koob and Le Moal 2005). Whilst only a limited number of studies have examined processing of drug and non-drug stimuli within the same model, there is evidence to support the hypothesis that complementary processes co-occur within an individual to give rise to both hypersensitivity to drug reward and hyposensitivity to non-drug rewards (Garavan et al. 2000; Wrase et al. 2007; Zijlstra et al. 2009). However, it has also been proposed that amotivational symptoms in cannabis users could be attributed to coexisting depressive symptoms (Musty and Kaback 1995).

Treatment with the dopamine precursor levodopa and pramipexole, a dopamine D_2 agonist, has both been found to improve apathy in patients with Parkinson's disease (Czernecki et al. 2002; Lemke et al. 2006), where striatal hypodopaminergia has been found to be related with apathy in depressed patients with Parkinson's disease (Remy et al. 2005). We have recently found that cannabis users exhibit reduced striatal dopamine synthesis capacity indexed using positron emission tomography (PET) and 3,4-dihydroxy-6-[^{18}F]-fluoro-*l*-phenylalanine ([^{18}F]-DOPA) uptake compared to non-users (Bloomfield et al. 2013). Studies of macaque monkeys have found that mesolimbic dopamine pathway loss, induced via 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) lesions, predicted apathetic behaviour (Brown et al. 2012; Schneider et al. 1988) and that apathy was inversely correlated to [^{18}F]-DOPA uptake in the nucleus accumbens and dorsal striatum(Brown et al. 2012). We therefore hypothesised that apathy in cannabis users would be inversely correlated with striatal dopaminergic function.

Methods

Our study was approved by the Administration of Radioactive Substances Advisory Committee (ARSAC) and the National Research Ethics Service. We conducted the study in accordance with the Declaration of Helsinki and Good Clinical Practice. All our subjects provided informed written consent to participate.

Cannabis users

We included 14 regular cannabis users (defined as at least weekly cannabis use for >1 year; mean [SD] age 20.4 [1.3] years, 13 males, one female) recruited from an ongoing cohort study (Morgan et al. 2011) who participated in a larger study investigating whether cannabis users exhibit elevated dopamine synthesis capacity (Bloomfield et al. 2013). Inclusion criteria for all subjects were: minimum age 18 years; good physical health with no history of major medical condition; capacity to give written informed consent; current, at least weekly use of cannabis and the induction of psychotic-like symptoms in response to smoking cannabis, which was defined as a positive change on the psychotic items score of the Psychotomimetic States Inventory (PSI) (Mason et al. 2008) measured 5 min after smoking their usual amount of cannabis (i.e. when acutely intoxicated) compared to when not intoxicated with the drug. All users consumed their own cannabis

and testing occurred in the presence of a researcher in the environment where users normally consumed cannabis in their usual drug-taking context (e.g. at home). Cannabis-induced psychotic-like symptoms ceased within 2 h of consumption, and no subject met the Diagnostic and Statistical Manual of Mental Disorders, fourth edition, Text Revision (DSM-IV TR) criteria for a diagnosis of a psychotic disorder. The psychotic items from the PSI covered 'Perceptual Distortions', 'Delusional Thinking', 'Cognitive Disorganization' and 'Paranoia'. Each item is rated on a four-point scale from 'not at all' (score=0) to 'strongly' (score=3). A sample of the cannabis that each participant smoked was taken on the day of testing and analysed for levels of THC (Forensic Science Service, Birmingham, UK). Exclusion criteria for all subjects were current or past psychiatric illness, substance dependency or abuse (except Cannabis and Nicotine Use Disorders) using the Structured Clinical Interview for DSM-IV (SCID) (First et al. 1996), a history of serious mental illness (including psychosis) in a first degree relative determined via the Family Interview for Genetic Studies (FIGS) (NIMH Genetics Initiative 1992), evidence of an at-risk mental state for psychosis (Phillips et al. 2000), DSM-IV TR (American Psychiatric 2005) and contra-indications to PET (including pregnancy and breast-feeding).

We obtained detailed drug histories from all subjects using structured interview, the Cannabis Experience Questionnaire (CEQ) (Barkus et al. 2006), and timeline follow-back. We estimated lifetime cannabis use as the total number of *spliffs* (cannabis cigarettes; *joints*) consumed. We chose the time taken to smoke an *eighth* of cannabis ($^1/_8$oz; approximately 3.5 g, representing the standard unit of sale in Britain) as the primary index of cannabis use as this provides a measure of the amount of current drug consumption (shorter time indicating greater consumption). Reporting the actual amount of cannabis used (Temple et al. 2011) is likely to be more accurate than subjective recall of the number of *spliffs* consumed because of variability in cannabis dose between *spliffs* (Temple et al. 2011) but also inconsistencies in self-reported cannabis use (Akinci et al. 2001; Buchan et al. 2002) although there is some evidence of agreement between self-reported drug use and drug tests (Harrison et al. 2007). The median (interquartile range, IQR) time to smoke an 'eighth' was 3.8 (6.0) days, and the mean (SD) age of onset of regular cannabis use was 16.3 (2.2) years.

PET data acquisition

All subjects underwent an [^{18}F]-DOPA scan using an ECAT HR+ 962 PET scanner (CTI/Siemens, Knoxville, TN, USA) in 3D mode, with an axial field of view of 15.5 cm, which we performed as previously reported (Egerton et al. 2010). We asked subjects to abstain from cannabis and fast for 12 h (mean [SD] time since last cannabis exposure=30.1 [34.4]h) and to refrain from smoking tobacco for 2 h before imaging. Subjects were instructed to fast for a fixed interval as a precautionary approach to minimise variations in plasma large neutral amino acids which compete with tyrosine for uptake into the central nervous system (Oldendorf 1973) and stimulate hepatic incorporation of tyrosine into proteins (Harper et al. 1970). Large neutral amino acids therefore are capable of markedly lowering plasma tyrosine concentrations (Moja et al. 1996; Palmour et al. 1998) and catecholamine metabolites in both the cerebrospinal fluid (Palmour et al. 1998) and striatum (Biggio et al. 1976) within hours. The resultant increased variability in tyrosine and phenylalanine availability increases the likelihood of altered responses to dopamine-mediated pharmacological challenges (reviewed in Milner and Wurtman 1986; Tam and Roth 1997).

All subjects reported no use of drugs other than cannabis, tobacco, alcohol and caffeine in the 1 week before PET scan. On the day of the PET scan, urine drug screen (Monitect HC12, Branan Medical Corporation, Irvine, CA, USA) confirmed no recent drug use, other than cannabis, and a negative urinary pregnancy test was required in all female subjects. A research clinician (MAPB) assessed psychotic symptoms using the Positive and Negative Syndrome Scale (PANSS) and Comprehensive Assessment of At-Risk Mental States (CAARMS) at the time of scanning. No subjects had psychotic symptoms at the time of scanning (mean [SD] PANSS positive score cannabis users=7.3 [0.5]) nor met CAARMS criteria for an At Risk Mental State. Thirty minutes before PET scanning, subjects completed the 18-item self-rated Apathy Evaluation Scale (AES-S) (Marin et al. 1991).

We administered oral carbidopa 150 mg and entacapone 400 mg 1 h before imaging (Sawle et al. 1994) to all subjects in order to reduce the formation of radiolabelled [^{18}F]-DOPA metabolites(Cumming et al. 1993; Guttman et al. 1993). We restricted head movement using a head strap and then marked and monitored head position via laser crosshairs and a camera. We performed a 10-min transmission scan before radiotracer injection for attenuation and scatter correction. We injected approximately 180 MBq of [^{18}F]-DOPA as an intravenous bolus 30 s after the start of emission recording. We acquired emission data in 3D mode for 95 min, re-binned into 26 time-frames (30-s background frame, four 60-s frames, three 120-s frames, three 180-s frames and 15 300-s frames).

Region-of-interest analysis

We denoised the nonattenuation-corrected dynamic images using a level 2, order 64 Battle–Lemarie wavelet filter (Turkheimer et al. 1999) and realigned individual frames to a single frame acquired 10 min after the [^{18}F]-DOPA injection using a mutual information algorithm (Studholme et al. 1996) to correct for head movement. We then applied the transformation parameters to the corresponding attenuation-corrected

frames and combined the realigned frames to create a movement-corrected dynamic image (from 6 to 95 min following [^{18}F]-DOPA administration) for analysis.

The region-of-interest (ROI) analysis was performed blind to group status by one of us (M.A.P.B). A summation (add) image was created from each movement-corrected dynamic image using RPM (Gunn et al. 1997).

We defined standardised ROIs bilaterally in the whole striatum and its functional subdivisions, i.e. the associative (precommissural dorsal caudate, precommissural dorsal putamen and postcommissural caudate), limbic (ventral) and sensorimotor (postcommissural putamen) subdivisions and the cerebellum (as the reference region) in Montreal Neurologic Institute space (Egerton et al. 2010; Martinez et al. 2003) to create an ROI map.

We normalised an [^{18}F]-DOPA template from a previous study (McGowan et al. 2004) with the ROI map to each individual PET summation (add) image using statistical parametric mapping software (SPM5, http://fil.ion.ucl.ac.uk/spm). This allowed us to place ROIs automatically on individual [^{18}F]-DOPA PET images, thus removing observer bias. ROIs were then double-checked on each subject's PET scan to verify anatomical accuracy blind to group.

We calculated the influx rate constant of [^{18}F]-DOPA uptake in each ROI relative to the cerebellum [K_i^{cer} (min^{-1})] using the Patlak graphical analysis adapted for a reference tissue input function (Hartvig et al. 1991, 1997; Hoshi et al. 1993; Patlak and Blasberg 1985). We have previously demonstrated good test–retest reliability for striatal K_i^{cer} determined this way (Egerton et al. 2010).

Statistical analysis

We assessed normality of distributions using the one-sample Kolmogorov–Smirnov test. Relationships between K_i^{cer}, levels of cannabis use and apathy severity were tested using Pearson's correlation coefficient for normally distributed data and Spearman's rank correlation coefficient for non-normally distributed data. Statistical significance was defined as $p<0.05$ (two-tailed). Our primary outcome measure was K_i^{cer} in the whole striatum. We conducted exploratory analyses in the striatal sub-divisions (presented uncorrected for multiple comparisons). Exploratory analyses between sub-groups were conducted using independent samples *t* tests for normally distributed data and Mann–Witney *U* tests for non-normally distributed data.

Results

Subject characteristics and scan parameters

All users consumed the drug as a *spliff* with tobacco. The mean (SD) age of initiation of at least weekly cannabis use was 16.3 (2.2) years. The median (IQR) lifetime exposure to cannabis and time taken to smoke an *eighth* was 2,080 (4,641) *spliffs* and 3.8 (6.0) days, respectively. Seven users met DSM-IV criteria for cannabis abuse or dependence. Subjects' characteristics are reported in Table 1. Urine drug screen was positive for THC and negative for all other substances (amphetamine; opiates; cocaine; methamphetamine; benzodiazepines) in every user.

Striatal dopaminergic function

The relationship between striatal dopamine synthesis capacity and apathy

All subjects scored in excess of 34 points on the AES-S (median [IQR] 59.5 [7.5]), indicative of significant 'apathy'

Table 1 Sample characteristics and scan parameters

Sample characteristic	Cannabis users (n=14)
Age (years) [mean (SD)]	20.4 (1.3)
Sex (n)	13 male, 1 female
Handedness (n)	12 right, 2 left
Ethnicity (n)	11 WB, 3AB
AES-S [median (IQR)]	59.5 (7.5)
Cannabis use[a]	
Cannabis use (g of cannabis/month) [median (IQR)]	28.1 (90.0)
THC content of cannabis (%) [mean (SD)]	8.6 (4.0)
Time since last cannabis exposure (h) [median (IQR)]	14.1 (19.1)
Time taken to smoke an *eighth* of cannabis (days) [median (IQR)]	3.8 (6.0)
Duration of at least weekly cannabis use (years) [mean (SD)]	4.9 (2.0)
Age of onset cannabis use (years) [mean (SD)]	15.5 (1.6)
Increase in PSI psychotic symptom subscale [mean (SD)]	8.4 (4.5)
CEQ Immediate Effects Subscale [mean (SD)]	90.1 (9.4)
CEQ After Effects Subscale [mean (SD)]	30.2 (13.3)
Scan parameter	
Injected dose (MBq) [mean (SD)]	185.1 (5.4)
Specific activity (MBq/μmol) [mean (SD)]	27.9 (13.1)
Whole striatal volume (mm^3) [mean (SD)]	17,811.11 (1,325.85)
Associative striatal volume (mm^3) [mean (SD)]	10,787.73 (1,336.46)
Limbic striatal volume (mm^3) [mean (SD)]	2,354.99 (1,136.64)
Sensorimotor striatal volume (mm^3) [mean (SD)]	4,668.38 (486.64)

AB Asian British, *CEQ* Cannabis Experiences Questionnaire, *PSI* Psychotomimetic States Inventory, *WB White British*

[a] Drug use reported in 3 months prior to scan

based on normative data from a healthy population non-cannabis using sample (mean [SD]=24.4 [4.5]) (Kant et al. 1998). There was no significant difference in apathy scores between subjects who met DSM-IV criteria for abuse or dependence and users who did not meet criteria (p=0.71), and there was no significant difference in K_i^{cer} between those subgroups in the whole striatum (t_{12}=1.96, p=0.07) or any of its subdivisions (data available on request). Dopamine synthesis capacity in each ROI and the relationships between dopamine synthesis capacity and apathy for each of the ROIs are given in Table 2. There were no significant relationships between apathy and the volumes of any of the ROIs examined (all p>0.1). We found that K_i^{cer} was inversely correlated to AES-S score in the whole striatum and its associative subdivision (Spearman's rho=−0.64, p=0.015 [whole striatum]; rho=−0.69, p=0.006 [associative]) but not in the limbic or sensorimotor subdivisions (Fig. 1). Cook's D and leverage analysis indicated that these correlations were not driven by outlying data points. Our findings of an inverse relationship between apathy and K_i^{cer} in the whole striatum and its associative subdivision remained significant when co-varying for the respective striatal volume (r_{df}=−0.61$_{11}$, p=0.03 [whole striatum]; r_{df}=−0.62$_{11}$, p=0.03 [associative]) and for recent tobacco and alcohol use (r_{df}=−0.63$_{10}$, p=0.03 [whole striatum]; r_{df}=−0.65$_{10}$, p=0.02 [associative]).

There were no significant relationships between AES-S and current cannabis consumption (rho=0.28, p=0.34) or age of first cannabis use (rho=0.25, p=0.40), suggesting that it is not related to recent use or age of first use of cannabis per se. In addition, there were no significant relationships between the time since last cannabis exposure and apathy (rho=−0.39, p=0.17) or K_i^{cer} (rho=−0.24, p=0.41). Furthermore, there was no relationship between apathy and psychotogenic response to cannabis (rho=0.11, p=0.70). However, there was no significant relationship between Apathy and the CEQ After Effects scale (rho=−0.06, p=0.83), and there was no significant relationship between the CEQ After Effects scale and whole striatal K_i^{cer} (r=−0.46, p=0.88). Four subjects reported a family history of depression in a first degree relative.

Table 2 Dopamine synthesis capacity in each ROI and the relationships between dopamine synthesis capacity and apathy for each of the ROIs

	K_i^{cer} (min^{-1})		Relationship between K_i^{cer} and AES-S	
ROI	Mean	SD	rho	p
STR	0.0127	0.0007	−0.636	0.015
AST	0.0121	0.0008	−0.691	0.006
LS	0.0133	0.0008	−0.364	0.200
SMST	0.0140	0.0009	−0.244	0.400

AST associative striatum, *LST* limbic striatum, *SMST* sensorimotor striatum, *STR* whole striatum

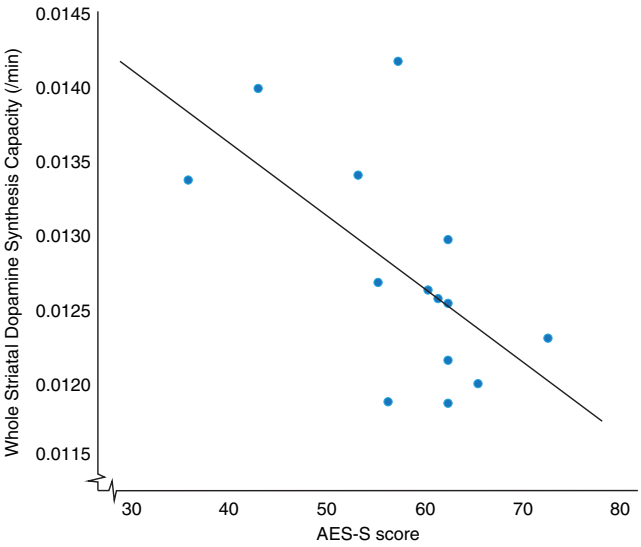

Fig. 1 The relationship between whole striatal dopamine synthesis capacity (K_i^{cer}) and apathy (AES-S score) (rho=−0.64, p=0.015)

There was no significant difference in K_i^{cer} between subjects with and without a family history of depression (t_{df}=0.34$_{12}$, p=0.74).

Discussion

Our main finding is that within chronic cannabis users, there is an inverse relationship between striatal dopamine synthesis capacity and apathy. To our knowledge, this is the first study to investigate the relationship between apathy and striatal dopamine synthesis capacity in regular active cannabis users. Our results suggest that the reduction in striatal dopamine synthesis capacity associated with regular cannabis use may underlie the reduced reward sensitivity and amotivation associated with chronic cannabis use (van Hell et al. 2010), accounting for 40 % of the variance in apathy. Whilst our study was cross-sectional so we cannot infer causality, this interpretation is supported by preclinical lesion studies (Schneider et al. 1988) that show lowering dopamine results in apathy and that apathy is inversely related to reduced dopamine function in both patients with Parkinson's disease (Remy et al. 2005) and lesioned animals (Brown et al. 2012). A further possibility could be that our cannabis users were in a state of withdrawal; however, THC and its metabolites have an elimination half-life of about 7 days (Maykut 1985), and all our cannabis users were regular, long-term users who had consumed cannabis within the past 7 days (median time since last consumption=14.1 h) and so this would be unlikely.

Our results extend previous findings of attenuated striatal response to reward anticipation activity in cannabis users (van Hell et al. 2010). Long-term cannabis use has been associated with apathy (Looby and Earleywine 2007; McGlothin and

West 1968; Tennant and Groesbeck 1972). The very high apathy scores in our sample are striking, and to our knowledge, the scale we used has not previously been administered in cannabis users. One study has, however, attributed amotivational symptoms to coexisting depressive symptoms (Musty and Kaback 1995). Yet, none of the subjects in our study met the threshold for a DSM-IV (TR) diagnosis of major depressive episode or disorder, or indeed any DSM-IV (TR) diagnosis except for Cannabis Use Disorders. However, as we did not include depressive symptom scales in this study, we cannot exclude the possibility that sub-clinical depressive symptoms have contributed to our findings. Since apathy is also a symptom of depression, further studies to disentangle the relationship between apathy and depressive symptoms and their possible effects on the day-to-day lives of heavy chronic cannabis users are therefore warranted.

The striatum has been conceptualised as the interface between motivation and action (Mogenson and Yang 1991). Whilst we did not find a relationship between apathy and dopamine synthesis capacity in the limbic striatal subdivision which has been described as being involved in motivation (Martinez et al. 2003), our findings of a significant relationship between apathy and dopamine synthesis capacity in the dorsal (associative) striatum fit with findings from the Brown et al. (2012) study. The associative subdivision of the striatum is part of the corticostriatal–thalamo-cortical loop projecting to and from associative areas of cortex including the dorsolateral prefrontal cortex (Joel and Weiner 2000) which has dense interconnections with premotor areas involved in motor planning (Barbas 2000) and therefore goal-directed behaviour.

One limitation of our study is that the cannabis users were long-term regular users who were sensitive to the psychotogenic effects of cannabis, as indicated by both the CEQ Immediate Effects scale and increase in PSI psychotic scale, and so our findings may not generalise to less heavy users. However, we previously found no relationship between dopamine synthesis capacity and cannabis-induced psychotic-like symptoms (Bloomfield et al. 2013) and in the present study found no relationship between apathy and psychotogenic response to cannabis, suggesting that this is unlikely to be influencing our results. Our measures of apathy were subjective, and we did not record observed behavioural data. Therefore, we cannot exclude the possibility that long-term cannabis use is associated with bias when completing this scale, although data exploring this possibility are lacking. Likewise, our measures of cannabis use relied on self-report, and we were not able to verify substance use histories beyond active current cannabis use. Although there is some heterogeneity in our sample in terms of DSM-IV criteria, cannabis users in the study were at least weekly users of the drug with mean duration of regular use was 4.9 years and within this sample there was no DSM-IV subgroup effect on apathy score. We did not find relationships between the CEQ After Effects scale and dopamine synthesis capacity or apathy. This may reflect the fact that CEQ asks users about experiences after the initial effects of cannabis have worn off but which are felt to be directly related to using cannabis vs. the AES-S asking subjects to report how they have been feeling over the last 4 weeks. In addition, the After Effects scale includes items that are unrelated to amotivation including disinhibition, paranoia and depersonalisation. A further limitation of our study is that we did not examine apathy in non-user controls.

Conclusion

Cannabis is now second only to heroin as the most frequently reported primary illicit drug among those entering specialised treatment for the first time (European Monitoring Centre for Drugs and Drug Addiction 2013) and half of young people reporting any cannabis use in the preceeding 12 months feel they should either stop or reduce their use (Terry-McElrath et al. 2008). However, in terms of Prochaska and DiClemente's (1982) trans-theoretical model, the motivation for change amongst cannabis users is low (Fernandez-Artamendi et al. 2013). Our findings of an inverse relationship between striatal dopamine synthesis capacity and apathy in cannabis users suggest that reduced dopamine synthesis capacity may underlie this and represent a biologically driven self-perpetuating barrier to treatment. Cannabis use is associated with a variety of mental illness outcomes, and our findings may also have implications for understanding the biology of the psychiatric sequelae of cannabis use.

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Dopamine Function in Cigarette Smokers: An [^{18}F]-DOPA PET Study

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Tobacco addiction is a global public health problem. Addiction to tobacco is thought to involve the effects of nicotine on the dopaminergic system. Only one study has previously investigated dopamine synthesis capacity in cigarette smokers. This study, exclusively in male volunteers, reported increased dopamine synthesis capacity in heavy smokers compared with non-smokers. We sought to determine whether dopamine synthesis capacity was elevated in a larger sample of cigarette smokers that included females. Dopamine synthesis capacity was measured in 15 daily moderate smokers with 15 sex- and age-matched control subjects who had never smoked tobacco. Dopamine synthesis capacity (indexed as the influx rate constant K_i^{cer}) was measured with positron emission tomography and 3,4-dihydroxy-6-[^{18}F]-fluoro-*l*-phenylalanine. There was no significant group difference in dopamine synthesis capacity between smokers and non-smoker controls in the whole striatum ($t_{28} = 0.64$, $p = 0.53$) or any of its functional subdivisions. In smokers, there were no significant relationships between the number of cigarettes smoked per day and dopamine synthesis capacity in the whole striatum ($r = -0.23$, $p = 0.41$) or any striatal subdivision. These findings indicate that moderate smoking is not associated with altered striatal dopamine synthesis capacity.

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INTRODUCTION

Tobacco addiction is a major global public health problem (Ezzati and Lopez, 2003). Only 3–5% of self-quitters achieve prolonged abstinence for 6–12 months after quitting (Hughes *et al*, 2004). Treatment of tobacco addiction is successful in less than 19% of cases (West *et al*, 2000), indicating that there is a pressing need to develop improved treatments (Menossi *et al*, 2013). The development of better therapies for tobacco addiction is likely to need greater understanding of the neurobiological changes associated with tobacco use. Addiction to tobacco is thought to involve the effects of nicotine, its main addictive component (Stolerman *et al*, 1995), on the dopaminergic system (Balfour *et al*, 2000; Pidoplichko *et al*, 1997) as nicotinic receptors have been identified on nigrostriatal and mesolimbic dopaminergic neurons (Clarke and Pert, 1985). Supporting this, studies in rodents and non-human primates show that tobacco or nicotine increase dopamine neuron firing (Grenhoff *et al*, 1986; Zhang and Sulzer, 2004), increase dopamine release (Dewey *et al*, 1999; Gallezot *et al*, 2013; Marenco *et al*, 2004; Pontieri *et al*, 1996), and increase dopamine synthesis (Tsukada *et al*, 2005) in the striatum.

In humans, tobacco use has been associated with both increased striatal dopamine synthesis capacity (Salokangas *et al*, 2000) and dopamine release in response to acute cigarette use in smokers (Brody *et al*, 2004; Le Foll *et al*, 2013). However, two studies found that acute nicotine use did not elicit a significant dopamine release in smokers (Barrett *et al*, 2004; Montgomery *et al*, 2007), although these did find that the subjective hedonic response to acute nicotine was related to dopamine release. Smoking-induced dopamine release has been associated with a reduction in craving and the severity of tobacco dependence (Brody *et al*, 2004). Yet, unlike other drugs of addiction, drug-related (ie, smoking-related) cues did not result in significant dopamine release in smokers when compared with neutral images (Chiuccariello *et al*, 2013). Interestingly, one study (Busto *et al*, 2009) found that tobacco dependence was

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associated with reduced amphetamine-induced striatal dopamine release, although this is likely exacerbated by comorbid depression. These findings may reflect differences in the study design (Gallezot *et al*, 2013), the influence of other factors, such as sex effects, co-morbidity, or genetic variants (Dierker *et al*, 2002; Kendler *et al*, 1993; Lerman *et al*, 1998; Zhang *et al*, 2006), or the difficulty of imaging dopamine changes that are comparatively small (Egerton *et al*, 2010). In terms of other aspects of dopaminergic function, a large study on the dopamine transporter did not find an association between smoking and dopamine transporter availability (Thomsen *et al*, 2013).

As discussed above, studies indicate that striatal dopamine synthesis may be altered by nicotine exposure. Dopamine synthesis capacity can be indexed in humans using a radiolabeled dopamine precursor, L-3,4-dihydroxyphenylalanine (L-DOPA) with positron emission tomography (PET; Kumakura and Cumming, 2009). Our primary hypothesis was, therefore, that cigarette smokers would have increased dopamine synthesis capacity compared with non-smoker controls. To our knowledge, only one study has investigated dopamine synthesis capacity in cigarette smokers. This study, exclusively in male volunteers, found that striatal uptake of [^{18}F]-DOPA was 16–29% higher in smokers than non-smokers (Salokangas *et al*, 2000). However, as this sample was exclusively of males and there is evidence of sex differences in the release of dopamine in response to nicotine (Dluzen and Anderson, 1997), we sought to determine whether dopamine synthesis capacity was elevated in a larger sample of cigarette smokers that included females and did not have a history of psychiatric co-morbidity including depression and alcohol use disorders.

MATERIALS AND METHODS

A case–control design was used to compare striatal [^{18}F]-DOPA uptake in smokers to that in non-smokers. The study was conducted in accordance with the Declaration of Helsinki and followed National Research Ethics Service and the Administration of Radioactive Substances Advisory Committee approval. All participants received full information about the study and gave informed written consent to take part.

Participants

Participants were recruited through advertisements in the press. The non-smokers were matched to the smokers on the basis of age (within 5 years) and sex. Inclusion criteria for all subjects were: minimum age 18 years, good physical health with no history of major medical condition, and capacity to give written informed consent. The exclusion criteria for all participants were: presence of any significant current medical disorder or treatment including history of head injury resulting in loss of consciousness and any neurological disorder; contraindications to PET including pregnancy or breast-feeding; a diagnosis of past or current psychiatric disorders including personality disorder using the Structured Clinical Interview for DSM-IV (SCID; First *et al*, 1996) including alcohol or any other substance dependence or abuse (apart from Nicotine Use Disorders in cigarette smokers); evidence of an At Risk Mental State for Psychosis; drug use other than alcohol or cigarettes in the 3 months before PET scanning; a family history of any psychotic disorder in first- or second-degree relatives. All participants provided urine samples to screen for drug use (Monitect HC12, Branan Medical Corporation, Irvine, California), and in women, for pregnancy test. Participants were excluded if either sample came back with positive result on the day of the scan. No subject was taking psychotropic medication at the time of study participation.

Smoking Data

Smoking data were collected via a semi-structured questionnaire for assessing exposure to cigarettes and alcohol (from the Cannabis Experiences Questionnaire interview; Barkus *et al*, 2006). The non-smoker group was defined as those with no lifetime use of tobacco.

PET

All participants were asked to not to eat or drink (except water), and refrain from alcohol for 12 h before the scan. The smokers were allowed tobacco 3 h before the scan. This time period was selected so that nicotine levels were at steady state (Benowitz *et al*, 1982). In addition, this is a similar time period to the only other study that has investigated dopamine synthesis capacity in smokers (Salokangas *et al*, 2000). Less than 2 h would coincide with peak plasma nicotine levels and longer durations would likely be measuring the dopamine system in a state of nicotine withdrawal. Imaging data from the PET scans were obtained on a Siemens CTI ECAT HR + 962 PET scanner (Siemens, Erlanger, Germany) in three-dimensional mode with an axial field of view of 15.5 cm. One hour before the scan, participants received 400 mg entacapone, a peripheral catechol-0-methyltransferase inhibitor, and 150 mg carbidopa, a peripheral aromatic acid decarboxylase inhibitor, in order to increase specific signal detection, as these compounds decrease the formation of radiolabeled metabolites that may cross the blood–brain barrier (Cumming *et al*, 1993; Guttman *et al*, 1993). A 10 min transmission scan was conducted before the radiotracer injection using a 150-MBq cesium-137 rotating point source to correct for scatter and attenuation. Participants were positioned in the scanner with the orbitomeatal line parallel to the transaxial plane of the tomograph. Head position was marked and monitored via laser crosshairs and a camera, and movement was minimized using a head strap.

A 17 MeV GE PET-trace cyclotron was used for radionuclide production. The gas target was filled with $^{18}O_2$ and bombarded at 40 μA for 30 min followed by a passivation bombardment of 0.1% F_2 in argon at 20 μA for 20 min. This produced [^{18}F]-F by the ^{18}O(p,n) ^{18}F reaction. An electrophilic fluorination procedure was then used to synthesize 6-[^{18}F]fluoro-L-DOPA. In brief, [^{18}F]F_2 was bubbled through a solution of 6-trimethylstannyl-L-DOPA (60 mg) stirring in Deutero-chloroform (5 ml) over 20 min at 5 °C. 6 M HCl (2 ml) was added and the chloroform evaporated at 70 °C. The resulting aqueous mixture was heated at reflux for 10 min before allowing to cool. The cooled crude mixture was purified by semi-prep high-pressure liquid chromato-

graphy polymer X column eluting with ammonium acetate buffer. The peak corresponding to [^{18}F]-L-DOPA eluted at 15 min was stabilized with 1 mg ascorbic acid and sodium phosphate dibasic. Typical yields were 2.96–3.33 GBq. For quality assurance purposes, a sample was taken from each synthesis and analyzed by reverse phase high-pressure liquid chromatography to confirm identity and purity. To be able to proceed with the injection, a radiochemical purity of 95.0% or higher was required. Approximately 180 MBq of [^{18}F]-DOPA was administered as a bolus intravenous injection 30 s after the start of the emission scan. Emission data were acquired as 26 frames of increasing duration over the 90 min scan. This comprised a 30-s background frame, 4 60-s frames, 3 120-s frames, 3 180-s frames, and finally 15 300-s frames.

Image Analysis

To correct for head movement in the scanner, non-attenuation-corrected dynamic images were denoized using a level 2, order 64 Battle-Lemarie wavelet filter. We used nonattenuation-corrected images used for the realignment algorithm as they include greater scalp signal, improving re-alignment compared with attenuated-corrected images (Turkheimer *et al,* 1999). Frames were realigned to a single 'reference' frame, acquired 10 min post-injection, employing a mutual information algorithm (Studholme *et al*, 1996). The transformation parameters were then applied to the corresponding attenuated-corrected dynamic images. The realigned frames were then summated, creating a movement-corrected dynamic image, which was used in the analysis. The cerebellar reference region (Kumakura and Cumming, 2009) was defined using a probabilistic atlas (Martinez *et al,* 2003), and as previously described, regions of interest (ROI) in the whole striatum and its functional sub-divisions were delineated to create an ROI map (Egerton *et al,* 2010). The functional subdivisions of the striatum reflect the topographical arrangement of corticostriatal projections. Projections to the sensorimotor striatum come from the motor cortex and related areas for instance the premotor cortex, primary motor cortex, and supplementary motor cortex; projections to the associative striatum start in associative regions such as dorsolateral prefrontal cortex; and projections to the limbic striatum are from limbic areas such as the amgydala and hippocampus (Haber, 2003). SPM5 (http://www.fil.ion.ucl.ac.uk/spm) was then used to normalize the ROI map together with the tracer-specific ([^{18}F]-DOPA) template (Egerton *et al,* 2010, Howes *et al,* 2009) to each individual PET summation image. This nonlinear transformation procedure allowed ROIs to be automatically placed on individual [^{18}F]-DOPA PET dynamic images. The influx constant (K_i^{cer}, written as *Ki* in some previous publications (Howes *et al*, 2009)) for the entire striatal ROI and the functional subdivisions bilaterally were calculated compared with uptake in the reference region using a graphical approach adapted for a reference tissue input function (Egerton *et al*, 2010).

Statistical Analysis

Normality of distribution and homogeneity of variance were assessed using Kolmogorov–Smirnov and Levene's tests, respectively. Group (smoker *vs* non-smoker) differences in demographic and imaging variables were determined using independent samples *t*-tests for normally distributed data, Mann–Witney *U*-tests for non-normally distributed data, and the χ^2 test for dichotomous variables. The influence of sex on group differences in K_i^{cer} was determined using a two-way analysis of variance. Within the smoker group, the relationship between K_i^{cer} and level of cigarette consumption was tested using Pearson's product-moment correlation coefficient. A two-tailed significance level of $p = 0.05$ was employed throughout. A power calculation determined that we needed a sample size of 15 per group to have 80% or greater power to detect the effect size reported in the Salokangas *et al*, study (Cohen's $d = 1.1$) at this significance level.

RESULTS

Subject Characteristics and Scan Parameters

Fifteen smokers were recruited into the study. All smokers consumed tobacco as a cigarette. Mean (SD) cigarette consumption was 8.1 (4.1) per day (range: 1–17). Twelve smokers met DSM-IV(TR) criteria for Nicotine Dependence. Mean (SD) cigarette consumption was 9.6 (2.9) per day in smokers who met Dependency criteria (range: 5–17). Mean (SD) cigarette consumption was 2.0 (1.0) per day in smokers who did not meet criteria.

Fifteen non-smoker control subjects were matched to the smoker group for age (± 5 years) and sex. Group demographics are reported in Table 1. Urine drug screens were negative for all substances (cannabis, amphetamine, opiates, cocaine, methamphetamine, benzodiazepines) in every subject.

Subjects were well matched for age and sex. There was no significant group difference in the amount of radioactivity or specific activity of [^{18}F]-DOPA administered (Table 1). No subjects had a history of alcohol dependence or abuse according to DSM-IV(TR) criteria and subjects were well matched for alcohol use. There was no relationship between age and K_i^{cer} in the whole striatum ($r = -0.10$, $p = 0.62$) or its subdivisions in the whole sample or in either group (data available on request).

Striatal Dopaminergic Function

There was no significant group difference in K_i^{cer} in the whole striatum (Figure 1), or any striatal subdivision (Table 2). No significant differences in K_i^{cer} were detected after removing the three smokers who did not meet DSM-IV(TR) diagnostic criteria for nicotine dependency from the analysis ($t_{25} = 0.85$, $p = 0.40$).

The Relationship between Striatal Dopamine Synthesis Capacity and Tobacco Use

In smokers, there were no significant relationships between the number of cigarettes smoked per day and K_i^{cer} in the whole striatum ($r = -0.23$, $p = 0.41$; Figure 2), or any striatal subdivision (associative: $r = -0.16$, $p = 0.57$; sensorimotor: $r = -0.33$, $p = 0.23$; limbic: $r = -0.22$, $p = 0.44$).

To examine whether nicotine dependency was specifically associated with elevated K_i^{cer}, we divided the tobacco user

Table 1 Sample Characteristics and Scan Parameters

	Non-smoker controls	Smokers	P[a]
Sample characteristic			
Age (years), mean (SD)	29.5 (11.1)	29.9 (10.3)	0.92
Sex (m:f) (*n*)	10:5	10:5	1.00
Ethnicity[b] (*n*)	8WB, 3BB, 2AB, 1ME, 1OE	12WB, 1BB, 1AB1 1ME	0.12
Tobacco smokers (*n*)	0	15	0.00
Tobacco use (cigarettes/day), mean(SD)	0.0 (0.0)	8.1 (4.1)	0.00
Cigarettes smoked before PET scan, mean (SD)	0.0 (0.0)	0.8 (0.4)	0.00
Alcohol users (*n*)	13	11	0.36
Alcohol[c] use (UK alcohol units/week), mean (SD)	7.1 (8.7)	11.2 (8.7)	0.21
Scan parameter			
Injected dose (MBq), mean (SD)	182.4	182.7	0.87
Specific activity (MBq/μmol), mean (SD)	30.1	27.5	0.62

Abbreviations: AB, Asian British; BB, black British; ME, mixed ethnicity; OE, other ethnicity; WB, white British.
[a]Independent-samples *t*-tests for variables with normal data distributions; Mann–Whitney *U*-tests for variables with non-normal data distributions; χ^2 tests for dichotomous variables.
[b]Groups were compared on a dichotomized ethnicity variable (white British *vs* ethnic minority).
[c]1 UK alcohol unit = 10 ml (~7.88 g) alcohol.

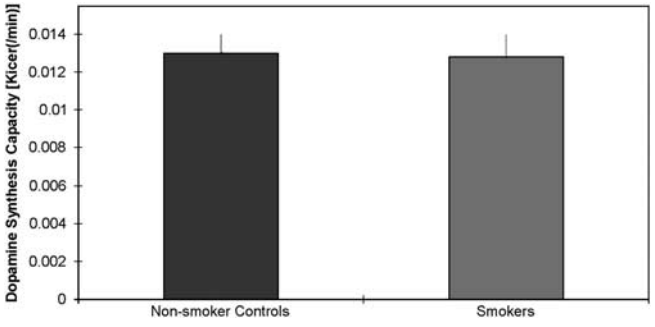

Figure 1 Whole striatal dopamine synthesis capacity (indexed as the influx rate constant K_i^{cer}) in smokers compared to non-smokers. Error bars indicate standard deviations.

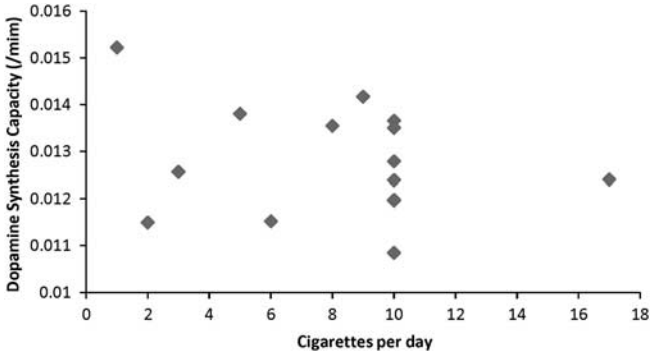

Figure 2 The relationship between tobacco use and dopamine synthesis capacity (indexed as the influx rate constant K_i^{cer}) in smokers.

Table 2 [18F]-DOPA K_i^{cer} (min^{-1}) by Group

ROI	Non-smoker controls ($n=15$)		Smokers ($n=15$)		Group comparison[a]	
	Mean	SD	Mean	SD	t_{df}	p
STR	0.0130	0.0010	0.0128	0.0012	0.64_{28}	0.53
AST	0.0126	0.0010	0.0122	0.0011	0.84_{28}	0.41
LST	0.0132	0.0017	0.0135	0.0012	0.63_{28}	0.53
SMST	0.0141	0.0011	0.0138	0.0016	0.67_{28}	0.51

Abbreviations: AST, associative striatum; K_i^{cer}, influx rate constant; LST, limbic striatum; ROI, region of interest; SMST, sensorimotor striatum; STR, whole striatum.
[a]Independent-samples *t*-tests.

group into subjects who met DSM-IV(TR) diagnostic criteria for nicotine dependency ($n=12$), and those who did not meet criteria ($n=3$). Mean (SD) K_i^{cer} was 0.0127 (0.0010) min^{-1} in smokers meeting Nicotine Dependence criteria and 0.0131 (0.0019) min^{-1} in smokers who did not meet criteria. *t*-Tests showed no significant differences between the nicotine dependency sub-group and non-smoker controls ($t_{25}=0.85$, $p=0.40$). There was no significant relationship between tobacco use and dopamine synthesis capacity in the whole striatum ($r=0.18$, $p=0.57$) or any of its functional subdivisions (data available on request) within the nicotine-dependent sub-group.

Sex

We performed a further explorative analysis to examine for possible sex effects. Mean (SD) K_i^{cer} was 0.0127 (0.0008) min^{-1} in females and 0.0130 (0.0012) min^{-1} in males. Among males, mean (SD) K_i^{cer} was 0.0130 (0.0013) min^{-1} in smokers and 0.0131 (0.0010) min^{-1} in non-smokers. Among females, mean (SD) K_i^{cer} was 0.0125 (0.0010) min^{-1}

in smokers and 0.0130 (0.0006) min^{-1} in non-smokers. Two-way analysis of variance did not reveal a significant interaction between smoking status and sex on dopamine synthesis capacity in the whole striatum ($F_{1,26} = 0.23$, $p = 0.64$) or any functional subdivision (associative striatum: $F_{1,26} = 0.08$, $p = 0.79$; sensorimotor striatum: $F_{1,26} = 0.34$, $p = 0.54$; limbic striatum: $F_{1,26} = 0.73$, $p = 0.40$).

DISCUSSION

This study found no evidence for altered striatal dopamine synthesis in tobacco smokers compared with non-smokers, or relationship between the levels of daily cigarette smoking and dopamine synthesis capacity. Furthermore, we did not find an effect of nicotine dependence on dopamine synthesis capacity. Our findings are therefore not consistent with our hypothesis that dopamine synthesis capacity would be elevated in smokers compared with non-smokers.

These negative findings are in contrast to a previous report of elevated dopamine synthesis capacity in 9 male smokers compared with 10 non-smokers (Salokangas *et al,* 2000). Although striatal dopamine synthesis capacity may be higher in females (Laakso *et al*, 2002), this was not evident in our sample. The subjects in the study by Salokangas *et al* were heavy smokers (at least 15 cigarettes/day, mean 19.8 cigarettes/day compared with mean 8.1 cigarettes/day in our study), which could explain the difference with our findings. Although we found no evidence of a relationship between K_i^{cer} and the level of daily cigarette consumption, this may indicate that elevations in presynaptic dopamine synthesis capacity are only apparent in heavy smokers.

The study by Salokangas *et al* (2000) used the same methodology as a previous study by Hietala *et al* (1999), ie, Carbidopa 100 mg only was administered 90 min before PET scan (Personal Communication from Professor Salokangas), followed by measurement of radiolabeled metabolites in the arterial input function. This is in comparison to the carbidopa 150 mg and entacapone 400 mg administered 1 h before PET scan followed by a cerebellar reference region approach in the present study. To our knowledge, data on the effects of smoking on the pharmacodynamics of entacapone are lacking. Entacapone undergoes rapid hepatic metabolism via the uridine 5'-diphospho-glucuronosyltransferase pathway (Lautala *et al*, 2000). Data from studies in humans (Bock and Köhle, 2004) and mice models (Villard *et al*, 1998) indicate that cigarette smoke is a potent inducer of uridine 5'-diphospho-glucuronosyltransferase, which would thus lead to a faster elimination of entacapone and therefore a potential reduction in plasma [^{18}F]-DOPA in smokers *vs* controls whom have had entacapone administered. As our reference region approach theoretically eliminates the plasma input function, we would predict the main effect of any potential reduction in plasma [^{18}F]-DOPA to result in increased variability of K_i^{cer} without altering mean K_i^{cer}. However, there are limited data directly comparing K_i values obtained via a tissue reference region and arterial plasma input function method, as was the case in the study by Salokangas *et al* (2000). Sossi *et al* (2003) reported a comparison between a reference region and arterial input function approach. In that study, Sossi *et al* used the occipital lobe as the reference region, rather than the cerebellum used in our study. Therefore, it remains possible that a difference in entacapone metabolism in smokers may be underlying the disparity in results between our study and that of Salokangas *et al* (2000). Nevertheless, the entry into and exit from the brain of radiolabeled plasma metabolites may affect graphical analysis (Boyes *et al,* 1986; Cumming *et al*, 1987) and could bias results if metabolism is selectively altered in one group. Compared with non-smokers, smokers have reduced CSF levels of the dopamine metabolite homovanillic acid (Geracioti *et al*, 1999) and there is evidence of reduced MAO-A and MAO-B activity in smokers (Fowler *et al*, 1996a, b). However, as these differences would, if anything, reduces the production of radiolabeled metabolites in smokers, they are unlikely to explain the failure to detect an elevation in smokers. In summary, even if entacapone clearance is higher in our smoker group, we remain unable to conclude that it would be sufficient to impact metabolite production within our experimental window. Furthermore, even if there was a sufficient impact on metabolite production, our use of a reference region approach would be expected to be sufficiently robust to overcome this problem.

Survey data in Great Britain indicate that over the last few decades there has been a gradual decline in the number of cigarettes consumed per day among smokers, such that the average number of cigarettes smoked per day is now 12 for men and 11 for women (Office for National Statistics, 2013). Our sample of moderate smokers is therefore in the same range as that of the general population. Overall our findings and those of Salokangas *et al* thus indicate that there is no markedly altered dopamine function in moderate smokers, but alterations are apparent in heavy smokers. A further contributing factor for the discrepancy in findings from those of the study by Salokangas *et al* and the present study is that the nicotine content of cigarettes has decreased since the former. However, we did not collect data on the type of cigarette consumed by each tobacco-smoking subject, which would have enabled an estimation of the amount of nicotine consumed. Despite proposals for nicotine reduction policies (Benowitz and Henningfield, 1994), which have intended for the nicotine content of cigarettes to be reduced, there is some evidence that the nicotine yield of cigarettes in American brands may, in fact, have increased since the Salokangas study (Connolly *et al*, 2007). Future studies assessing both the effects of heavy *vs* moderate smoking and nicotine dose of cigarettes on dopaminergic function are therefore warranted.

Study Limitations

Although our sample size was larger than the only other study to investigate dopamine synthesis capacity in smokers, it is important to consider the possibility of a type II error in our findings. We had 84% power to detect the effect size (Cohen's $d = 1.12$) seen in the Salokangas *et al* study, which is above the 80% power threshold conventionally considered adequate. Nevertheless, even at this power, there is a 16% chance that a true effect of this magnitude has been missed. As we only included moderate smokers, a limitation of our study would be that we did not include a group of heavy smokers for comparison. A potential limitation of the correlation between cigarette use and

dopamine synthesis capacity is that seven of the smokers in our study consumed 10 cigarettes per day, which likely reflects the fact that tobacco in the United Kingdom is sold in packets of 10 and 20 cigarettes, but limits the power of the correlational analysis. Our sample contained five female subjects per group and so was underpowered to detect sex–group interactions, indicating that these analyses should only be considered exploratory. Gonadal hormones may influence dopaminergic function. However, we did not assess this or phase of the menstrual cycle in the females.

A further limitation of our study is that we did not include the Fagerström Test for Nicotine Dependence, which would have enabled the exploration of the relationships between dopamine synthesis capacity and subjective craving. Likewise, we did not measure plasma nicotine, cotinine, or carbon dioxide levels. Future studies would therefore benefit from including these measures in larger sample sizes.

Implications for Understanding Tobacco Addiction

The role of the dopamine system in drug reinforcement has long been accepted from animal studies (eg, Koob, 1992) and there is mounting evidence that dysregulated dopamine function is central to addiction behaviors in humans (as reviewed by Volkow *et al*, 2011). There is growing evidence that chronic drug abuse is associated with abnormal striatal dopaminergic functioning in humans, as has been found with alcohol (Heinz *et al*, 2005), cannabis (Bloomfield *et al*, 2013), cocaine (Wu *et al*, 1997), methamphetamine (Wang *et al*, 2012), and ecstasy (Tai *et al*, 2011). However, our study suggests that dopamine dysregulation may only become apparent at higher levels of use, either because it is below the level of detection with more moderate use, or because it is a cumulative consequence of heavy use.

CONCLUSIONS

This study found that moderate smoking was not associated with marked effects on striatal dopamine synthesis capacity, in contrast to a previous finding of elevated dopamine synthesis capacity in heavy smokers. Further studies in smokers of presynaptic dopaminergic function using heavy and moderate smokers are warranted to determine whether dopaminergic effects only become evident with heavier use.

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Appendix 2 – Key Psychometric Assessments

Aberrant Salience Inventory (ASI)

Apathy Evaluation Scale (self-rated) (AES-S)

Back Anxiety Inventory (BAI)

Beck Depression Inventory (BDI)

Cannabis Experience Questionnaire (CEQ)

Childhood Experiences of Care and Abuse (CECA)

Childhood Trauma Questionnaire (CTQ)

Comprehensive Assessment for At Risk Mental State (CAARMS)

Impact of Invents Scale (IES-6)

Oxford-Liverpool Inventory of Feelings and Experiences (O-LIFE)

Psychotomimetic States Inventory (PSI)

Structured Clinical Interview for DSM-IV (SCID)

ASI

Instructions: Please read the following statements and answer them yes (Y) or no (N) as they apply to you. Please do not consider thoughts or experiences that you have had only under the influence of drugs or alcohol.

	Yes	No
1) Do certain trivial things ever suddenly seem especially important or significant to you?	Y	N
2) Do you sometimes feel like you are on the verge of something really big, but you're not sure what it is?	Y	N
3) Do your senses sometimes seem sharpened?	Y	N
4) Do you ever feel like you are rapidly approaching the height of your intellectual powers?	Y	N
5) Do you sometimes notice small details that you have not noticed before that seem important?	Y	N
6) Do you sometimes feel like it is important for you to figure something out, but you're not sure what it is?	Y	N
7) Do you ever go through periods where you feel especially religious or mystical?	Y	N
8) Do you ever have difficulty telling if you are thrilled, frightened, pained, or anxious?	Y	N
9) Do you ever go through periods of heightened awareness?	Y	N
10) Do you ever feel the need to make sense of seemingly random situations or occurrences?	Y	N
11) Do you sometimes feel like you are finding the missing piece to a puzzle?	Y	N
12) Do you sometimes feel that you can hear with a greater clarity?	Y	N
13) Do normally trivial observations sometimes take on an ominous significance?	Y	N
14) Do you go through periods in which songs sometimes seem to have an important meaning for your life?	Y	N
15) Do you sometimes attribute importance to objects which you normally would not?	Y	N
16) Do you sometimes feel like you are on the verge of figuring out something really big or important, but you aren't sure what it is?	Y	N
17) Has your sense of taste ever seemed more acute?	Y	N
18) Do you ever feel like the mysteries of the universe are revealing themselves to you?	Y	N
19) Do you go through periods in which you feel over-stimulated by things or experiences that are normally manageable?	Y	N
20) Do you often become fascinated by the little things around you?	Y	N
21) Do your senses ever seem extremely strong or clear?	Y	N
22) Do you ever feel like a whole world is opening up to you?	Y	N
23) Do you ever feel that your boundaries between inner and outer sensations have been removed?	Y	N

24) Do you sometimes feel like the world is changing and you are searching for an explanation?	Y	N
25) Do you ever perceive an overwhelming significance to things that are usually not significant to you?	Y	N
26) Do you ever have a feeling of inexpressible urgency, and you are not sure what to do?	Y	N
27) Have you sometimes become interested in people, events, places, or ideas that normally would not make an impression on you?	Y	N
28) Do your thoughts and perceptions ever come faster than can be assimilated?	Y	N
29) Do you sometimes notice things that you haven't noticed before that take on special significance?	Y	N

Apathy Evaluation Scale (Self-rated)

Name: _____ Date: ___/___/___

For each statement, circle the answer that best describes the subject's thoughts, feelings, and activity in the past 4 weeks.

1. I am interested in things.

   NOT AT ALL SLIGHTLY SOMEWHAT A LOT

2. I get things done during the day.

   NOT AT ALL SLIGHTLY SOMEWHAT A LOT

3. Getting things started on my own is important to me.

   NOT AT ALL SLIGHTLY SOMEWHAT A LOT

4. I am interested in having new experiences.

   NOT AT ALL SLIGHTLY SOMEWHAT A LOT

5. I am interested in learning new things

   NOT AT ALL SLIGHTLY SOMEWHAT A LOT

6. I put little effort into anything.

   NOT AT ALL SLIGHTLY SOMEWHAT A LOT

7. I approach life with intensity.

   NOT AT ALL SLIGHTLY SOMEWHAT A LOT

8. Seeing a job through to the end is important to me.

   NOT AT ALL SLIGHTLY SOMEWHAT A LOT

9. I spend time doing things that interest me.

   NOT AT ALL SLIGHTLY SOMEWHAT A LOT

10. Someone has to tell me what to do each day.

    NOT AT ALL SLIGHTLY SOMEWHAT A LOT

11. I am less concerned about my problems than I should be.

NOT AT ALL SLIGHTLY SOMEWHAT A LOT

12. I have friends.

NOT AT ALL SLIGHTLY SOMEWHAT A LOT

13. Getting together with friends is important to me.

NOT AT ALL SLIGHTLY SOMEWHAT A LOT

14. When something good happens, I get excited.

NOT AT ALL SLIGHTLY SOMEWHAT A LOT

15. I have an accurate understanding of my problems.

NOT AT ALL SLIGHTLY SOMEWHAT A LOT

16. Getting things done during the day is important to me.

NOT AT ALL SLIGHTLY SOMEWHAT A LOT

17. I have initiative.

NOT AT ALL SLIGHTLY SOMEWHAT A LOT

18. I have motivation.

NOT AT ALL SLIGHTLY SOMEWHAT A LOT

The Apathy Evaluation Scale was developed by Robert S. Marin, M.D. Development and validation studies are described in RS Marin, RC Biedrzycki, S Firinciogullari: "Reliability and Validity of the Apathy Evaluation Scale," *Psychiatry Research*, 38:143-162, 1991

Beck Anxiety Inventory

Below is a list of common symptoms of anxiety. Please carefully read each item in the list. Indicate how much you have been bothered by that symptom during the past month, including today, by circling the number in the corresponding space in the column next to each symptom.

	Not At All	Mildly but it didn't bother me much.	Moderately - it wasn't pleasant at times	Severely – it bothered me a lot
Numbness or tingling	0	1	2	3
Feeling hot	0	1	2	3
Wobbliness in legs	0	1	2	3
Unable to relax	0	1	2	3
Fear of worst happening	0	1	2	3
Dizzy or lightheaded	0	1	2	3
Heart pounding/racing	0	1	2	3
Unsteady	0	1	2	3
Terrified or afraid	0	1	2	3
Nervous	0	1	2	3
Feeling of choking	0	1	2	3
Hands trembling	0	1	2	3
Shaky / unsteady	0	1	2	3
Fear of losing control	0	1	2	3
Difficulty in breathing	0	1	2	3
Fear of dying	0	1	2	3
Scared	0	1	2	3
Indigestion	0	1	2	3
Faint / lightheaded	0	1	2	3
Face flushed	0	1	2	3
Hot/cold sweats	0	1	2	3
Column Sum				

Scoring - Sum each column. Then sum the column totals to achieve a grand score. Write that score here _____ .

Interpretation

A grand sum between **0 – 21** indicates very low anxiety. That is usually a good thing. However, it is possible that you might be unrealistic in either your assessment which would be denial or that you have learned to "mask" the symptoms commonly associated with anxiety. Too little "anxiety" could indicate that you are detached from yourself, others, or your environment.

A grand sum between **22 – 35** indicates moderate anxiety. Your body is trying to tell you something. Look for patterns as to when and why you experience the symptoms described above. For example, if it occurs prior to public speaking and your job requires a lot of presentations you may want to find ways to calm yourself before speaking or let others do some of the presentations. You may have some conflict issues that need to be resolved. Clearly, it is not "panic" time but you want to find ways to manage the stress you feel.

A grand sum that **exceeds 36** is a potential cause for concern. Again, look for patterns or times when you tend to feel the symptoms you have circled. Persistent and high anxiety is not a sign of personal weakness or failure. It is, however, something that needs to be proactively treated or there could be significant impacts to you mentally and physically. You may want to consult a counselor if the feelings persist.

Appendix 14
Beck Depression Inventory

BDI-II

Date:

Name: _____ Marital Status: _____ Age: _____ Sex: _____

Occupation: _____ Education: _____

Instructions: This questionnaire consists of 21 groups of statements. Please read each group of statements carefully, and then pick out the **one statement** in each group that best describes the way you have been feeling during the **past two weeks, including today**. Circle the number beside the statement you have picked. If several statements in the group seem to apply equally well, circle the highest number for that group. Be sure that you do not choose more than one statement for any group, including Item 16 (Changes in Sleeping Pattern) or Item 18 (Changes in Appetite).

1. Sadness
0 I do not feel sad.
1 I feel sad much of the time.
2 I am sad all the time.
3 I am so sad or unhappy that I can't stand it.

2. Pessimism
0 I am not discouraged about my future.
1 I feel more discouraged about my future than I used to be.
2 I do not expect things to work out for me.
3 I feel my future is hopeless and will only get worse.

3. Past Failure
0 I do not feel like a failure.
1 I have failed more than I should have.
2 As I look back, I see a lot of failures.
3 I feel I am a total failure as a person.

4. Loss of Pleasure
0 I get as much pleasure as I ever did from the things I enjoy.
1 I don't enjoy things as much as I used to.
2 I get very little pleasure from the things I used to enjoy.
3 I can't get any pleasure from the things I used to enjoy.

5. Guilty Feelings
0 I don't feel particularly guilty.
1 I feel guilty over many things I have done or should have done.
2 I feel quite guilty most of the time.
3 I feel guilty all of the time.

6. Punishment Feelings
0 I don't feel I am being punished.
1 I feel I may be punished.
2 I expect to be punished.
3 I feel I am being punished.

7. Self-Dislike
0 I feel the same about myself as ever.
1 I have lost confidence in myself.
2 I am disappointed in myself.
3 I dislike myself.

8. Self-Criticalness
0 I don't criticize or blame myself more than usual.
1 I am more critical of myself than I used to be.
2 I criticize myself for all of my faults.
3 I blame myself for everything bad that happens.

9. Suicidal Thoughts or Wishes
0 I don't have any thoughts of killing myself.
1 I have thoughts of killing myself, but I would not carry them out.
2 I would like to kill myself.
3 I would kill myself if I had the chance.

10. Crying
0 I don't cry anymore than I used to.
1 I cry more than I used to.
2 I cry over every little thing.
3 I feel like crying, but I can't.

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_____ Subtotal Page 1

Continued on Back

11. Agitation

0 I am no more restless or wound up than usual.

1 I feel more restless or wound up than usual.

2 I am so restless or agitated that it's hard to stay still.

3 I am so restless or agitated that I have to keep moving or doing something.

12. Loss of Interest

0 I have not lost interest in other people or activities.

1 I am less interested in other people or things than before.

2 I have lost most of my interest in other people or things.

3 It's hard to get interested in anything.

13. Indecisiveness

0 I make decisions about as well as ever.

1 I find it more difficult to make decisions than usual.

2 I have much greater difficulty in making decisions than I used to.

3 I have trouble making any decisions.

14. Worthlessness

0 I do not feel I am worthless.

1 I don't consider myself as worthwhile and useful as I used to.

2 I feel more worthless as compared to other people.

3 I feel utterly worthless.

15. Loss of Energy

0 I have as much energy as ever.

1 I have less energy than I used to have.

2 I don't have enough energy to do very much.

3 I don't have enough energy to do anything.

16. Changes in Sleeping Pattern

0 I have not experienced any change in my sleeping pattern.

1a I sleep somewhat more than usual.

1b I sleep somewhat less than usual.

2a I sleep a lot more than usual.

2b I sleep a lot less than usual.

3a I sleep most of the day.

3b I wake up 1–2 hours early and can't get back to sleep.

17. Irritability

0 I am no more irritable than usual.

1 I am more irritable than usual.

2 I am much more irritable than usual.

3 I am irritable all the time.

18. Changes in Appetite

0 I have not experienced any change in my appetite.

1a My appetite is somewhat less than usual.

1b My appetite is somewhat greater than usual.

2a My appetite is much less than before.

2b My appetite is much greater than usual.

3a I have no appetite at all.

3b I crave food all the time.

19. Concentration Difficulty

0 I can concentrate as well as ever.

1 I can't concentrate as well as usual.

2 It's hard to keep my mind on anything for very long.

3 I find I can't concentrate on anything.

20. Tiredness or Fatigue

0 I am no more tired or fatigued than usual.

1 I get more tired or fatigued more easily than usual.

2 I am too tired or fatigued to do a lot of the things I used to do.

3 I am too tired or fatigued to do most of the things I used to do.

21. Loss of Interest in Sex

0 I have not noticed any recent change in my interest in sex.

1 I am less interested in sex than I used to be.

2 I am much less interested in sex now.

3 I have lost interest in sex completely.

_____ Subtotal Page 2

_____ Subtotal Page 1

_____ Total Score

Appendix 15
Mood/Depression Assessment Questionnaire

Mood/Depression Assessment Questionnaire

1. Since your last visit have you felt depressed, sad or blue much of the time?
   yes ☐
   no ☐

2. Since your last visit have you often felt helpless about the future?
   yes ☐
   no ☐

3. Since your last visit have you had little interest or pleasure in doing things?
   yes ☐
   no ☐

4. Since your last visit have you had trouble sleeping many nights?
   yes ☐
   no ☐

Are two (2) or more of the above questions marked YES while undergoing treatment in this protocol?

yes ☐ → *complete a Beck Depression Inventory. If score is 30 or less, patient may continue in the study. If score is ≥ 31, patient will need to complete all final assessments and be dropped from the study. The investigator may recommend that the patient be referred for a professional psychiatric assessment.*

no ☐

CEQ(v6)

The following questionnaire has been developed to try to build a picture of the feelings, emotions and experiences which people typically have, both as they smoke cannabis… and afterwards (for a few hours or even days).

Please answer all **four** sections if you are a current or past user.

If you have never used cannabis (in any form) circle NO in section 1a and complete section 2 if appropriate.

Please answer **ALL** questions as honestly and accurately as possible. Responses will remain confidential.

EB, JS, & SL (2007)

Personal identification (if required)

Four digit PIN_____

Memorable password_____

Section 1.

a) Have you ever smoked/used cannabis? YES / NO (circle as appropriate)

<u>If NO, skip the rest of section 1 and complete section 2 if appropriate.</u>

b) Are you a current* past* cannabis user? (*circle as appropriate)

c) How old were you when you first tried cannabis? _____ (give approx age in years)

d) If you are a current user, indicate how often you use cannabis. If you are a past user indicate your usage pattern when you were using cannabis (Circle as appropriate.)

Everyday
More than once a week
About once a week
About once/twice a month
A few times each year
About once a year
Only once or twice

e) Do you/did you mostly smoke/use cannabis;

Socially (with friends)
On your own
Other (please state)

f) How much money per week do you/did you usually spend on cannabis? (if this figure varies, indicate average expenditure).

Less than £2.50 £2.50- £5 £5-£10 £10-£15 £15-£20 Above £20

g) When do/did you mostly smoke cannabis:

During the day
During the evening
Frequently during the day and evening

h) What type of cannabis do you/did you usually buy? (if this varies, indicate the most frequent)

Hash (cannabis resin/solid)
Home-grown skunk
White widow
Super skunk
Other (please state)

j) Approximately how many times have you smoked/used cannabis in your life? _____
(a guesstimate will do)

Section 2.
Please indicate in the table below any other drug(s) **including alcohol and nicotine** which you use/have used recreationally, the frequency with which you use/have used this drug, your age when you first tried the drug(s) and whether you are a past or current user. Use a new box for each additional drug: Circle your response(s) as appropriate.

Drug	Frequency	Age	Use	When
	Everyday More than once a week A few times each month A few times each year Only once or twice		Current Past	Day Night Both day and night
	Everyday More than once a week A few times each month A few times each year Only once or twice		Current Past	Day Night Both day and night
	Everyday More than once a week A few times each month A few times each year Only once or twice		Current Past	Day Night Both day and night
	Everyday More than once a week A few times each month A few times each year Only once or twice		Current Past	Day Night Both day and night

Drug	Frequency	Age	Use	When
	Everyday More than once a week A few times each month A few times each year Only once or twice		Current Past	Day Night Both day and night
	Everyday More than once a week A few times each month A few times each year Only once or twice		Current Past	Day Night Both day and night
	Everyday More than once a week A few times each month A few times each year Only once or twice		Current Past	Day Night Both day and night
	Everyday More than once a week A few times each month A few times each year Only once or twice		Current Past	Day Night Both day and night

Section 3.
How often do you have/have you had these experiences while **smoking** cannabis?
(please be sure to tick your answer for each item)

	Rarely or never	From time to time	Sometimes Yes & sometimes No	More often than not	Almost always or always
Feeling Happy					
Feeling fearful					
Enhanced perceptual awareness					
Paranoid					
Uncomfortably sleepy					
Anxious for no reason					
Like there was something which you had to do no matter what (compulsive)					
Feeling all-powerful (like you could do anything)					
Deluded (believed in something which afterwards you knew not to be true)					
Looking for excitement					
Feeling threatened by an unknown force					
Lethargic					
Sentimental					
Energized					
Nervy					
Speech becomes slurred					
Slowing of time					
Hearing things other people couldn't hear (auditory hallucinations)					
Powerful (strong)					
Able to understand the world better					
Losing your sense of reality					
Having visions (like visual hallucinations)					
Fearful that you are going crazy/mad					
Depressed					

	Rarely or never	From time to time	Sometimes Yes & sometimes No	More often than not	Almost always or always
Increased appetite					
Obsessive (or fixated on something)					
Being relaxed					
Sleepy					
Disturbed in your thinking					
Feeling like you no longer know yourself					
Laid back					
Sad					
Excited					
Religious					
Full of plans					
Ecstatic					
Feeling more creative					
Things not feeling ‘right’ on your skin or in your body					
Angry					
Rapid flow of thoughts					
Having out of body experiences					
Feeling full of ideas					
Reduced level of consciousness					

Section 4.
How often have you had/did you have these experiences **AFTER** the initial effects of cannabis have worn off (ie; experiences which you feel are directly related to using cannabis)? (Please answer each item)

	Rarely or never	**From time to time**	**Sometimes Yes & sometimes No**	**More often than not**	**Almost always or always**
Dis-inhibited					
Not wanting to do anything					
Feeling generally slowed down (physically)					
Feeling a lack of motivation					
Feeling that your thinking has been slowed down					
Being unable to concentrate					
Having a sense of slowing of time					
Paranoid without reason					
Suspicious of people, events or things without reason					
Feeling depersonalised					
Being unable to remember things					
Having reduced attention					

If you are a current or past cannabis user, please make sure that you have provided a response to all items in sections 3 and 4.

Thanks for your co-operation and time!

Emma Barkus, John Stirling and Shon Lewis
Manchester, UK,
2007

CHILDHOOD EXPERIENCES OF CARE AND ABUSE

I would now like to ask you some questions about your childhood and adolescence. We are interested in different experiences you may have had before you were 17 years of age. Some of the experiences I want to ask about may bring back upsetting or painful memories, so if at any time you do not wish to answer a question please say so. Of course, all information you provide will be treated in the strictest confidence.

1. Who were your main parent figures, before age 17? [If necessary, continue on a separate sheet]

0 = No mother, father figure	**1 = Natural mother, father**	**2 = Step-mother, father**	**3 = Grandmother, father**	**4 = Other**

1. Family arrangement	**A. Mother figure**					**B. Father figure**					**C. Your age at start**
1. First	O0	O1	O2	O3	O4	O0	O1	O2	O3	O4	0
2. Second	O0	O1	O2	O3	O4	O0	O1	O2	O3	O4	
3. Third	O0	O1	O2	O3	O4	O0	O1	O2	O3	O4	
4. Fourth	O0	O1	O2	O3	O4	O0	O1	O2	O3	O4	
5. Fifth	O0	O1	O2	O3	O4	O0	O1	O2	O3	O4	
6. Sixth	O0	O1	O2	O3	O4	O0	O1	O2	O3	O4	

2. Did one or both of your parents die, before age 17?

2.a. Did your mother die? **O0 No** **O1 Yes**

2.b. Did your father die? **O0 No** **O1 Yes**

2.c. If yes, how old were you when your mother died? | | |

2.d. If yes, how old were you when your father died? | | |

3. Were you ever separated from a parent (longer than six months), before age 17?

3.a. Separated from mother? **O0 No** **O1 Yes**

3.b. Separated from father? **O0 No** **O1 Yes**

3.c. If yes, how old were you at your first separation from mother? | | |

3.d. If yes, how old were you at your first separation from father? | | |

3.e. How long were you separated, in months? | | |

3.f. What was the main reason for the separation?

O1 Parental Illness	O2 Divorce, Separation	O3 Work	O4 Never knew parent
O5 Own illness	O6 Boarding school	O7 Migration	O8 Other

3.g. Specify: ……..

Before the age of 17 years …

	0-11 years			12-16 years		
4. Did you ever change schools? (other than change from primary to secondary)	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
5. Were you ever expelled from school?	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
6. Did you ever run away from home? (i.e., stayed away for more than two nights)	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
7. Were you ever taken into care? (i.e., children's home, fostered)	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
8. Were there ever times when your family was significantly short of money?	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
9. Were your basic needs ever neglected? (for food, clean clothing, etc.)	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
10. Were there ever frequent arguments or extreme tensions between your parents?	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
11. Were you ever tormented or treated cruelly by a parent or a member of household?	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
12. Were you ever hit or slapped on a number of occasions, sufficient to cause harm?	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
13. Ever had any unwanted sexual experiences?	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
14. Any adults could go to with problems or to discuss feelings?	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
15. Others own age could go to with problems or to discuss feelings?	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer
16. Ever felt lonely for a significant period? (i.e., 6 months or more)?	O0 No	O1 Yes	O2 Refused to answer	O0 No	O1 Yes	O2 Refused to answer

Notes:

(Note for DATA ENTRY: open **EU_BULL,** Bullying)

CHILDHOOD TRAUMA QUESTIONNAIRE

STUDIE: EU GEI	**Date of Birth**
Subject number: |__| EU |__|__| - |__|__|__|__|	|__|__|-|__|__|-|1|9|__|__|
Time interval: lifetime	**Period – Replicat** |0|__|-|0|__|
Interviewer: ……	**Date** |__|__|-|__|__|-|2|0|__|__|

For each question, tick the box under the response that best describes how you feel.

[ALL participants should be given the CTQ to complete on their own. Assistance may be provided if the person has difficulty reading the questions.]

Before the age of 17:	**Never**	**Rarely**	**Sometimes**	**Often**	**Very often**
1. I didn't have enough to eat	O1	O2	O3	O4	O5
2. I knew that there was someone to take care of me and protect me	O1	O2	O3	O4	O5
3. People in my family called me things like "stupid", "lazy", or "ugly"	O1	O2	O3	O4	O5
4. My parents were too drunk or high to take care of the family	O1	O2	O3	O4	O5
5. There was someone in my family who helped me feel that I was important or special	O1	O2	O3	O4	O5
6. I had to wear dirty clothes	O1	O2	O3	O4	O5
7. I felt loved	O1	O2	O3	O4	O5
8. I thought that my parents wished I had never been born	O1	O2	O3	O4	O5
9. I got hit so hard by someone in my family that I had to see a doctor or go to the hospital	O1	O2	O3	O4	O5
10. People in my family hit me so hard that it left me with bruises or marks	O1	O2	O3	O4	O5
11. I was punished with a belt, a board, a cord, or some other hard object	O1	O2	O3	O4	O5
12. People in my family looked out for each other	O1	O2	O3	O4	O5
13. People in my family said hurtful or insulting things to me	O1	O2	O3	O4	O5
14. I believe that I was physically abused	O1	O2	O3	O4	O5
15. I got hit or beaten so badly that it was noticed by someone like a teacher, neighbor, or doctor	O1	O2	O3	O4	O5
16. I felt that someone in my family hated me	O1	O2	O3	O4	O5
17. People in my family felt close to each other	O1	O2	O3	O4	O5
18. Someone tried to touch me in a sexual way, or tried to make me touch them	O1	O2	O3	O4	O5
19. Someone threatened to hurt me or tell lies about me unless I did something sexual with them	O1	O2	O3	O4	O5
20. Someone tried to make me do sexual things or watch sexual things	O1	O2	O3	O4	O5

Before the age of 17:	**Never**	**Rarely**	**Sometimes**	**Often**	**Very often**
21. Someone molested me	O1	O2	O3	O4	O5
22. I believe that I was emotionally abused	O1	O2	O3	O4	O5
23. There was someone to take me to the doctor if I needed it	O1	O2	O3	O4	O5
24. I believe that I was sexually abused	O1	O2	O3	O4	O5
25. My family was a source of strength and support	O1	O2	O3	O4	O5

CAARMS

COMPREHENSIVE ASSESSMENT OF AT RISK MENTAL STATES

A. Yung, L. Phillips, M.B. Simmons, J. Ward, K. Thompson, P. French, P. McGorry

NAME: _____

ID #: _____

Rater: _____

Date: _____ / _____ / _____

pace
the PACE Clinic
ORYGEN Research Centre
University of Melbourne
Department of Psychiatry
Parkville Victoria, Australia

OVERVIEW OF THE CAARMS

Aims:

- To determine if an individual meets the criteria for 'Ultra High Risk' status.
- To rule out, or confirm criteria for acute psychosis.
- To map a range of psychopathology and functioning factors, over time in young people at ultra high-risk of psychosis.

Structure of the CAARMS:

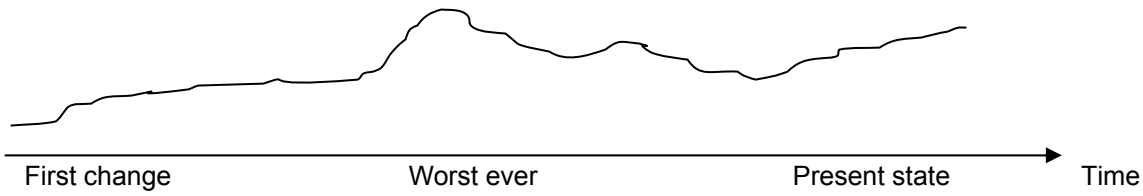
- Ratings are made on a range of subscales that target different areas of psychopathology and functioning. From these ratings it is then possible to extract information relating to the above aims.

Overview of Symptoms and Functioning - Longitudinal Change:

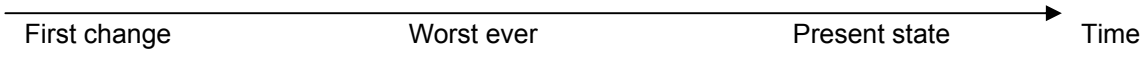
- At the first interview (not follow-up interviews), the CAARMS aims to obtain a general overview of the history of change from the premorbid state in the respondent. All available information should be used.
- Record the **time of first noted change** - date and age of respondent in years:

  Date: ……
  Age: ……

- Note first ever symptoms or signs:
  ……..……
  ……..……
  ……..……
  ……..……
  ……..……
- Overview of course since then - map on timeline e.g.:



- Current time line:



INDEX

1: POSITIVE SYMPTOMS

1.1 UNUSUAL THOUGHT CONTENT

Delusional Mood and Perplexity ('Non Crystallized Ideas')

- Have you had the feeling that something odd is going on that you can't explain? What is it like?
- Do you feel puzzled by anything? Do familiar surroundings feel strange?
- Do you feel that you have changed in some way?
- Do you feel that others, or the world, have changed in some way?

Ideas of Reference

- Ideas of Reference: Have you felt that things that were happening around you had a special meaning, or that people were trying to give you messages? What is it like? How did it start?

Bizarre Ideas ('Crystallized Ideas')

- Made thoughts, feelings, impulses: Have you felt that someone, or something, outside yourself has been controlling your thoughts, feelings, actions or urges? Have you had feelings or impulses that don't seem to come from yourself?
- Somatic Passivity: Do you get any strange sensations in your body? Do you know what causes them? Could it be due to other people or forces outside yourself?
- Thought Insertion: Have you felt that ideas or thoughts that are not your own have been put into your head? How do you know they are not your own? Where do they come from?
- Thought Withdrawal: Have you ever felt that ideas or thoughts are being taken out of your head? How does that happen?
- Thought Broadcasting: Are your thoughts broadcast so that other people know what you are thinking?
- Thoughts Being Read: Can other people read your mind?

UNUSUAL THOUGHT CONTENT- GLOBAL RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Psychotic and Severe**
No unusual thought content.	Mild elaboration of conventional beliefs as held by a proportion of the population	Vague sense that something is different, or not quite right with the world, a sense that things have changed but not able to be clearly articulated. Subject not concerned/ worried about this experience.	A feeling of perplexity. A stronger sense of uncertainty regarding thoughts than 2.	Referential ideas that certain events, objects or people have a particular and unusual significance. Feeling that experience may be coming from outside the self. Belief not held with conviction, subject able to question. Does not result in change in behaviour.	Unusual thoughts that contain completely original and highly improbable material. Subject can doubt (not held with delusional conviction), or which the subject does not believe all the time. May result in some change in behaviour, but minor.	Unusual thoughts containing original and highly improbable material held with delusional conviction (no doubt). May have marked impact on behaviour.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

Level of Distress (In Relation to Symptoms)

0 **100**

Not At All Distressed Extremely Distressed

1.2 NON-BIZARRE IDEAS

Non-Bizarre Ideas ('Crystallized Ideas')

- Suspiciousness, Persecutory Ideas: Has anybody been giving you a hard time or trying to hurt you? Do you feel like people have been talking about you, laughing at you, or watching you? What is it like? How do you know this?
- Grandiose Ideas: Have you been feeling that you are especially important in some way, or that you have powers to do things that other people can't do?
- Somatic Ideas: Have you had the feeling that something odd is going on with your body that you can't explain? What is it like? Do you feel that your body has changed in some way, or that there is a problem with your body shape?
- Ideas of Guilt: Do you feel you deserve punishment for anything you have done wrong?
- Nihilistic Ideas: Have you ever felt that you, or a part of you, did not exist, or was dead? Do you ever feel that the world does not exist?
- Jealous Ideas: Are you a jealous person? Do you worry about relationships that your spouse/girlfriend/boyfriend has with other people?
- Religious Ideas: Are you very religious? Have you had any religious experiences?
- Erotomanic Ideas: Is anyone in love with you? Who? How do you know this? Do you return his/her feelings?

NON-BIZARRE IDEAS - GLOBAL RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Psychotic and Severe**
No non-bizarre ideas.	Subtle changes that could be reality based. Eg. Very self-conscious.	Increased self-consciousness. Eg. Feeling that others look at the subject, or talk about the subject. Or feeling of increased self-importance. Subject able to question.	Odd or unusual thoughts but whose content is not entirely implausible- may be some logical evidence. More evidence than rating of 4. Content of thoughts not original i.e. jealousy, mild paranoia.	Clearly idiosyncratic beliefs, which although 'possible' have arisen without logical evidence. Less evidence than rating of 3. Eg. Thoughts that others wish the subject harm, which can be easily dismissed. Thoughts of having special powers, which can be easily dismissed.	Unusual thoughts about which there is some doubt (not held with delusional conviction), or which the subject does not believe all the time. May result in some change in behaviour, but minor.	Unusual thoughts containing original and highly improbable material held with delusional conviction (no doubt). May have marked impact on behaviour.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use/stress noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

Level of Distress (In Relation to Symptoms)

0 **100**
Not At All Distressed Extremely Distressed

1.3 PERCEPTUAL ABNORMALITIES

Visual Changes

- Distortions, illusions: Is there a change in the way things look to you? Do things somehow look different, or abnormal? Are there alterations in colour, or brightness of objects (things seeming brighter, or duller in colour)? Are there alterations in the size and shape of objects? Do things seem to be moving?
- Hallucinations: Do you have visions, or see things that may not really be there? Do you ever seen things that others can't, or don't seem to? What do you see? At the time that you see these things, how real do they seem? Do you realise they are not real at the time, or only later?

Auditory Changes

- Distortions, illusions: Is there any change in the way things sound to you? Do things somehow sound different, or abnormal? Does your hearing seem more acute, or have increased sensitivity? Does your hearing seem muted, or less acute?
- Hallucinations: Do you ever hear things that may not really be there? Do you ever hear things that other people seem not to (such as sounds or voices)? What do you hear? At the time you hear these things, how real do they seem? Do you realise they are not real at the time, or only later?

Olfactory Changes

- Distortions, illusions: Does your sense of smell seem to be different, such as more, or less intense, than usual?
- Hallucinations: Do you ever smell things that other people don't notice? At the time, do these smells seem real? Do you realise they are not real at the time, or only later?

Gustatory Changes

- Distortions, illusions: Does your sense of taste seem to be different, such as more, or less intense, than usual?
- Hallucinations: Do you ever get any odd tastes in your mouth? At the time that you taste these things, how real do they seem? Do you realise they are not real at the time, or only later?

Tactile Changes

- Distortions, illusions, hallucinations: Do you ever get strange feelings on, or just beneath, your skin? At the time that you feel these things, how real do they seem? Do you realise they are not real at the time, or only later?

Somatic Changes

NOTE: Probes also used to rate Impaired Bodily Sensation, p.26

- Distortions, illusions: Do you ever get strange feelings in your body (eg feel that parts of your body have changed in some way, or that things are working differently)? Do you feel/think that there is a problem with some part, or all of your body, i.e. that it looks different to others, or is different in some way? How real does this seem?
- Hallucinations: Have you noticed any change in your bodily sensations, such as increased, or reduced intensity? Or unusual bodily sensations such as pulling feelings, aches, burning, numbness, vibrations?

PERCEPTUAL ABNORMALITIES - GLOBAL RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Psychotic but not severe**	6 **Psychotic and severe**
No abnormal perceptual experience.		Heightened, or dulled perceptions, distortions, illusions (eg lights/ shadows). Not particularly distressing. Hypnogogic/ hypnopompic experiences	More puzzling experiences: more intense/vivid distortions/ illusions, indistinct murmuring, etc. Subject unsure of nature of experiences. Able to dismiss. Not distressing. Derealisation/ depersonalis[n]	Much clearer experiences than 3 such as name being called, hearing phone ringing etc, but may be fleeting/ transient. Able to give plausible explanation for experience. May be associated with mild distress.	True hallucinations i.e. hearing voices or conversation, feeling something touching body. Subject able to question experience with effort. May be frightening or associated with some distress.	True hallucinations which the subject believes are true at the time of, and after, experiencing them. May be very distressing

Onset date: _____ ***Offset date*****:** _____

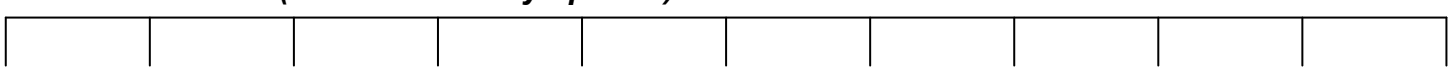
Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

Level of Distress (In Relation to Symptoms)



0 **100**

Not At All Distressed Extremely Distressed

1.4 DISORGANISED SPEECH

NOTE: Probes also used to rate Alogia, p. 16

Subjective Change:

- Do you notice any difficulties with your speech, or ability to communicate with others?
- Do you have trouble finding the correct word at the appropriate time?
- Do you ever use words that are not quite right, or totally irrelevant?
- Have you found yourself going off on tangents when speaking and never getting to the point? Is this a recent change?
- Are you aware that you are talking about irrelevant things, or going off the track?
- Do other people ever seem to have difficulty in understanding what you are trying to say/trouble getting your message across?
- Do you ever find yourself repeating the words of others?
- Do you ever have to use gesture or mime to communicate due to trouble getting your message across? How bad is this?
- Does it ever make you want to stay silent and not say anything?

Objective Rating of Disorganised Speech

- Is it difficult to follow what the subject is saying at times due to using incorrect words, being circumstantial or tangential?
- Is the subject vague, overly abstract or concrete? Can responses be condensed?
- Do they go off the subject often and get lost in their words? Do they appear to have difficulty finding the right words?
- Do they repeat words that you have used or adopt strange words (or 'non-words') in the course of regular conversation?

DISORGANISED SPEECH- GLOBAL RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Psychotic**
Normal logical speech, no disorganisation, no problems communicating or being understood.		Slight subjective difficulties eg problems getting message across. Not noticeable by others.	Somewhat vague, some evidence of circumstantiality, or irrelevance in speech. Feeling of not being understood.	Clear evidence of mild disconnected speech and thought patterns. Links between ideas rather tangential. Increased feeling of frustration in conversation.	Marked circumstantiality, or tangentiality in speech, but responds to structuring in interview. May have to resort to gesture, or mime to communicate.	Lack of coherence, unintelligible speech, significant difficulty following line of thought. Loose associations in speech.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

Level of Distress (In Relation to Symptoms)

0 **100**
Not At All Distressed Extremely Distressed

2: COGNITIVE CHANGE - ATTENTION/CONCENTRATION

2.1 SUBJECTIVE EXPERIENCE (HUBER'S BASIC SYMPTOM)

Concentration and Attention Problems:

- Have you had difficulty concentrating (difficulty listening to others, watching television, reading)?
- Is it more of an effort to think about, or concentrate on things?

Selective Attention Problems:

- Is it difficult to pay attention to just one thing?
- Are you distracted by other things easily?
- Have you been feeling overwhelmed, or confused by all the things that have been happening in the environment around you?

Thought Form Problems:

NOTE: See also Alogia, p. 16

- Do your thoughts ever seem to stop, get blocked, or disappear (e.g. do you have 'trances', or 'blank spells')? Can you describe this more fully?
- Do you ever experience racing or confused, jumbled thoughts?
- Do other things, as well as your thoughts, seem to stop e.g. attention, hearing, sight, memory, speech, or movement?
- Do you ever lose your sense of personal identity? What do you think was the cause of this?

Comprehension Difficulties:

- Do you have trouble following what others are saying?
- Do you sometimes require sentences to be repeated, especially long sentences?
- Do you sometimes not understand figures of speech and so on?
- Is this a change for you, or have you always had trouble with this?
- Do you ever have trouble picking up the emotional tone of conversations (eg. not recognising sarcasm, or irony)?
- Is it ever hard to understand non-verbal forms of communication i.e. gestures? How bad is this?

Memory Problems:

NOTE: See also Dissociative Symptoms, p.36

- Have you had memory problems?
- Have you ever felt as if there were large gaps in your memory?
- Are they present all the time, or do they come and go? Have you noticed if the memory problems come at times of stress?

SUBJECTIVE COGNITIVE CHANGE- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No subjective difficulty with concentration /attention.	Subject aware of some changes, but attributable perhaps to extraneous factors. Subject has difficulty in pinpointing changes.	Mild, but definite problems eg some difficulty concentrating while reading, or watching TV. Concentrating requires more effort. **OR** Slight impairment in memory, but passing.	Subjectively feeling muddled, or confused, racing, or slowed thoughts, difficulty understanding conversations. Occ. episodes of thought blocking. **OR** Memory problems more evident but do not interfere with everyday functioning.	Subjective feeling of being unable to think properly, confused, unable to understand others. More regular episodes of thought blocking **OR** Memory difficulties impair conversation, results in frequent misplacing of items.	Marked inattentiveness, feeling confused and overwhelmed at times, distracted by other things in the environment. Frequent episodes of thought block. **OR** Memory difficulties noted by others, distressing.	Subject reports extreme difficulty focussing on interview. Interview suspended due to impossibility of patient to concentrate or severe thought blocking. **OR** Severe memory problems.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

2.2 OBSERVED COGNITIVE CHANGE

Observed Inattentiveness During Interview

- Subject appears inattentive - looks away during interview, does not pick up the topic during a discussion, shifts focus of attention.
- Attention may be drawn to noise in adjoining room, objects around the room, interviewer's clothing etc

Observed Inattentiveness During Mental Status Testing

- The subject may perform poorly on simple tests of intellectual functioning in spite of adequate education and intellectual ability.
- This is assessed by having the subject spell the word 'world' backwards and by serial 7s or serial 3s for a series of 5 subtractions.
- **D L R O W**
- **100, 93, 86, 79, 72**
- **100, 97, 94, 91, 88**

OBSERVED COGNITIVE CHANGE – SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No abnormalities observed.	Some questionable inattentiveness - may be explained by other events.	Mild problems with concentration. Objectively may be observed to shift focus of attention from interview 1 to 3 times. Not quite understanding what others are saying or the emotional tone of the conversation.	Moderate concentration problems during interview. Mild disruption to flow of interview as a result.	Poor concentration and attention significantly affect ability to perform tasks. Distractibility clearly observed to interfere with flow of the interview.	Severe concentration and attention difficulties Extremely difficult to conduct interview, or pursue a topic due to preoccupation with irrelevant stimuli.	Inability to concentrate at all. Impossible to conduct interview due to preoccupation with irrelevant stimuli.

3: EMOTIONAL DISTURBANCE

3.1 SUBJECTIVE EMOTIONAL DISTURBANCE (HUBER'S BASIC SYMPTOM)

Impaired Emotional Functioning:

NOTE: See also Anhedonia, p. 18; Depression, p.29

- Have you noticed any change in your feelings, or emotions e.g. feel like you have no feelings, feel your emotions are 'empty', or that your emotions are somehow not genuine?
- Has there been any change in the way you are using your emotions?
- Have you still been able to enjoy things, or experience pleasure?
- Do you find that even when something sad happens, you are no longer able to feel sadness? Or when something happy happens, you can no longer feel happy?

Change in Affect:

Facial expressions:

- Have you noticed any change in your facial expressions?
- Have people commented on your facial expression, saying it is blank, or hard to know what you are thinking?

Eye contact:

- Has there been a change in the way you interact with other people e.g. do you find it hard to look at people when you speak to them?
- Has anyone commented on this?

Speech:

- Have you noticed a change in the way you talk, such as your voice becoming monotonous?
- Have people told you that you have a monotonous way of talking?
- Do they seem to find you boring?

Inappropriate affect:

- Have you ever felt different on the inside from the way you look to others?
- Like your appearance was uncoordinated with your emotions? Would you smile, or laugh when talking about something that was sad, or not funny at all?

SUBJECTIVE EMOTIONAL DISTURBANCE - SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No subjective change in feelings, or emotions.		Subjectively sporadic, mild, but definite problems reported eg not able to enjoy things as much as previously. Some feeling of blunting of emotional responses. Affect is inappropriate, but not sustained.	Subjectively, more frequent, or continuous problems. Some feeling of blunting of emotional responses. More pervasive feeling of inappropriate affect, but subject able to control somewhat.	Subject describes more marked change in emotions eg not able to express, or experience feelings as before. Sense of distance when with others. Inappropriate affect more difficult to hide from others.	Subject describes feeling of having no feelings, or emotions feel empty, or not genuine. Unable to feel sad at all. Severe degree of distance from others. Inappropriate affect interferes with relationships.	Subject reports constant emotional blunting, OR Inappropriate affect.

Onset date: _____ ***Offset date*:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

3.2 OBSERVED BLUNTED AFFECT

NOTE: Incorporate informant information as well as interviewer's impression

- Rate observed evidence of blunting of affect. For example, diminished facial expressions, reduced emotional tone in speech, reduced expressive movements and gestures.
- The rater may also feel a diminished ability to engage the subject.

OBSERVED BLUNTED AFFECT – SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe, not psychotic**	6 **Extreme/ psychotic**
No abnormalities observed by interviewer, or others.		Slight degree of constriction of affect may be observed.	Observable constriction of emotional field. Avoidance or failure to display feelings. Reduced emotional expressivity. Interviewer feels a sense of 'distance', or decreased rapport.	More marked degree of dullness or blockade. Definite decrease in sense of rapport observed by interviewer. May have been reported, or commented on by informants.	Minimal evidence of affective display	Gross blunting of affect. No spontaneous emotional expression observed during interview. Definitely reported by informants.

Onset date: _____ ***Offset date*****:** _____
(Do not score if relying on interviewer's report only- -3 on database)

Frequency and Duration

(Do not score if relying on interviewer's report only- -3 on database)

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

3.3 OBSERVED INAPPROPRIATE AFFECT

NOTE: Incorporate informant information as well as interviewer's impression

- Also rate clear-cut inappropriate affect (affect clearly discordant from the content of speech, or ideation (e.g. giggling when speaking of something sad).

OBSERVED INAPPROPRIATE AFFECT- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No abnormalities observed by interviewer, or others.		Mild inappropriate affect during interview, or reported occasionally by others. Subject appears able to control.	More pervasive inappropriate emotion displayed. Does not dominate interview. Subject appears able to control somewhat.	More often reported by others- distracting during interview.	Inappropriate affect reported frequently. Interferes with social relationships and flow of interview.	Inappropriate affect throughout interview. Severely impacts on ability to conduct interview. Reported by others as occurring most of the time.

Onset date: _____ ***Offset date*****:** _____
(Do not score if relying on interviewer's report only- Enter-3 on database)

Frequency and Duration

(Do not score if relying on interviewer's report only- Enter -3 on database)

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

4: NEGATIVE SYMPTOMS

4.1 ALOGIA

NOTE: Refer also to Cognitive Change, p.9; Disorganised Speech, p. 7

- Have you noticed problems trying to form conversations - i.e. hard to find words, thought blocking?
- Are the subject's responses to questions vague, or convey little information? Does the subject take a long time to respond to questions, but when prompted, displays an awareness of the question?

ALOGIA - SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No observed, or reported changes in speech.	Subject unsure about recent changes. Changes may be attributable to external factors, but subject unsure.	Very mild changes in ability to speak spontaneously Subject reports feeling "blocked" in their thinking. Difficulty finding words for thoughts. Not reported by others.	Difficulty expressing self in words - finding words, or more regular instances of thought blocking Observable by others, but not constant difficulty. Subject responds to prompting.	More marked poverty of speech, or thought blocking Does not significantly interfere with school, or work functioning.	Unable to express oneself adequately, or severe thought blocking May experience infrequent periods of mutism as a result of word finding and expression difficulties.	Marked poverty of speech or thought blocking. Seriously hinders flow of interview. Subject may be mute at times. Interferes significantly with ability to perform in social, occupation and educational settings.

Onset date: _____ ***Offset date*:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

4.2 AVOLITION/APATHY (HUBER'S BASIC SYMPTOM)

Subjective Experience:

- Have you felt lacking in energy- mental and physical? Are you tired, or lacking in motivation, or 'get up and go'? Lack of will power? Lack of physical strength?
- To what extent does this interfere with activities such as going to school/work and other everyday tasks? How are you spending your days?

Observed Avolition/Apathy:

NOTE: Refer also to Disorganising/Odd/Stigmatising Behaviours, p.21

- Has the subject indicated difficulty maintaining the level of his/her usual social, or occupational/educational commitments?
- Does the subject appear to be looking after him/herself adequately- cleanliness/hygiene/general self-care?

Avolition/Apathy - Severity Rating Scale

0 Never, absent	1 Questionable	2 Mild	3 Moderate	4 Mod. Severe	5 Severe	6 Extreme
No observed, or reported changes in energy.	Subject unsure about recent changes. Changes may be attributable to external factors, but unclear.	Feeling fatigued, things are an effort. May not initiate activities as much as previously. Still able to perform everyday tasks. Does not interfere with schoolwork, or work attendance.	Feeling of reduced energy, or will power. Decreased attendance at school/work, or not performing usual tasks to usual ability. Not everyday and not reported by others.	More marked reduction in energy/ motivation. Some interference with normal functioning eg tasks take longer to do, subject doesn't bother to do some things. May miss school, or work a few times a week or frequently run late. May be unable to attend to personal hygiene as usual,	Daily reduction in energy, drive, will power, physical strength, or motivation. Interferes with normal functioning eg missing school, or work most day. Spends significant portions of time lying around. Clear impact on personal hygiene	Extreme and continuous disability eg unable to perform normal tasks, confined to house, no will power, or volition. Unable to attend school/work at all due to motivation. Marked impact on personal hygiene

Onset date: _____ ***Offset date*:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week – **more** than one hour per occasion **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

4.3 ANHEDONIA

NOTE: Refer also to Depression, p. 29

- Have you been able to enjoy social activities/work/study as much as usual?
- Have you noticed a decrease in your level of interest in things you usually enjoy?
- Has this interfered with your ability to perform activities, e.g. going to school/work/participating in events?

ANHEDONIA- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No observed, or reported changes in affect, speech, activity level, or attentiveness.	Some mild decrease in interest in events, but may be attributable to external cause (i.e. dislikes topic at school).	Some mild decrease in interest or enjoyment of activities. Not interfering with ability to perform them.	Moderate reduction in interest or enjoyment of activities such as school/work. May affect school/work performance.	Some regular experience of pleasure or humour but decreased in extent and quality. May impact on work/school attendance. Others concerned by associated withdrawal and isolation.	Rarely gains sense of enjoyment/ interest from tasks. At times able to enjoy something, but short lived. Poor attendance at school/work. Very noticeable by others.	No enjoyment or interest at all in tasks. Marked lack of interest. Isolated and withdrawn.

Onset date: _____ ***Offset date*:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

5: BEHAVIOURAL CHANGE

Consider informant information as well as subjective report

5.1 SOCIAL ISOLATION

- Have you stayed at home more often than usual recently? Has this been by choice?
- Have you felt uncomfortable around others recently?
- Have you wanted to be alone more than usual recently? Has there been a reason for this? Have others commented on this?
- Have you missed important social events/school/work due to this?

Questions for informants:

- Has the subject been staying at home, perhaps in their room alone, more often than in the past? If so, do you know the reason for this?
- Have they missed social events/work/school due to this?
- Do they appear to want to spend time alone at present (more so than usual)?

SOCIAL ISOLATION- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No change in level of social activity.		Subject feels that she/he does not want to fulfill all social/role functions. Wanting to be alone, but able to motivate self.	Isolating self at times, but not marked. Able to fulfill main role functions involving interactions with others. May miss some social activities.	Intolerant of being around others for long periods of time. Social withdrawal commented n by others. May miss 2-3 days week of school/work because of wanting to be alone.	Missing more days than not of work/school, spending greater part of day alone.	Isolated from others for extended periods (i.e. days)

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

5.2 IMPAIRED ROLE FUNCTION

NOTE: See also Depression, p. 29

- Have you been able to attend school/work as usual recently?
- Has your school/work performance dropped recently?
- Have you been less interested in your work/school recently? Have others commented on this? Is there a reason for this? (Phrase questions appropriately i.e. for job seekers etc)

Questions for Informants:

- Have you noticed a change in attendance at work/school recently?
- Does the subject appear as capable at achieving normal tasks as usual?

IMPAIRED ROLE FUNCTION- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No recent change in role function.		Subject reports mild impairment in performance of usual activities. Not noted by informants.	Usual tasks performed with less care than usual. Missing occasional day of work/school. Noted as mild by informants.	Around half of usual time spent on normal daily tasks. Decreased quality of task performance noted by others.	Marked impairment of role functioning. Spending about half of day in aimless activity.	Subject attempting no role function whatsoever

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

5.3 DISORGANISING/ODD/STIGMATISING BEHAVIOUR

NOTE: See also Avolition, p.17; OCD, p.34; Social Isolation, p. 19

- Has there been anything about your lifestyle recently that others might regard as unusual, or odd? (Attempt to sensitively assess peculiar behaviours such as hoarding, talking to self, odd movements etc.)
- Have you been able to look after yourself as well as usual (Bathing, eating etc)? Has this been reported by others?

Questions for Informants:

- Have you noticed the subject behaving in an odd manner recently?
- Have you felt there is something strange about their behaviour? Has this been commented on by others?
- Have you noticed that they are hoarding goods, talking to self, moving in a bizarre fashion etc?

DISORGANISED/ODD/STIGMATISING BEHAVIOUR- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No change in behaviour noted by subject, informants, or in interview.		Some reduction in self care, social isolation, but not marked. Subject able to motivate self to rectify this change. Slightly odd behaviour that would not normally attract attention of others, or conducted in private.	May require pressure from others to maintain social/ occupational commitments, or self care. Able to be motivated. Occasional odd behaviour that is noticeable by others (ie. giggling to self).	Mildly eccentric behaviour - clearly noticeable by others (ie talking to self/hoarding Not constant.	Clearly bizarre behaviour that attracts attention of others. Sometimes resulting in intervention by others.	Very poor self-care. Eccentric behaviours dominate clinical picture. May result in intervention by others. Odd behaviours may have negative impact on physical health. Extreme social isolation.

Onset date: _____ ***Offset date*:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

5.4 AGGRESSION/DANGEROUS BEHAVIOUR

- Have you been feeling angry, or irritable recently? Has there been a reason for this? Have you felt more irritated than usual at small things? Have you been in more arguments with others than usual recently? Have you been taking more risks (i.e. when driving) recently than usual? Have others commented that your behaviour is becoming risky, or unsafe? Have you felt like striking out at people or objects recently (more so than usual)?
- Have you become so angry at someone that you have had thoughts of hurting them, or destroying their property? Have you acted on these thoughts?

Questions for Informants:

- Has the subject been acting in an aggressive or dangerous manner recently? Have there been any recent episodes of anger outbursts/physical confrontation? Is this how the subject normally behaves? Have others commented on a change in their level of anger, or irritability? Has the subject destroyed property lately (in association with anger)? Have you felt safe with the subject recently (i.e. when driving, at otherwise normal times)?

AGGRESSION/DANGEROUS BEHAVIOUR- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No aggressive, or dangerous behaviour reported by the subject or others.		Slight irritability but not associated with rise in aggressive behaviour. May be attributed to events by subject.	More marked increase in irritability/anger towards self/others. May be expressed verbally, or physically in restrained manner (i.e punching pillow etc). May be noted by subject only.	Marked increase in irritability towards others expressed in increased propensity to verbal confrontations with threat of physical aggression. Noted by others and subject.	Aggressive behaviour results in property damage, or harm to others. Subject reports some level of control over anger.	Dangerousness in conjunction with anger at very destructive level, resulting in some considerable physical damage to others, or property. Dominates clinical picture. May attract attention of police etc.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

6: MOTOR/PHYSICAL CHANGES

6.1 SUBJECTIVE COMPLAINTS OF IMPAIRED MOTOR FUNCTIONING
(HUBER'S BASIC SYMPTOM)

Disorganised Movement:

- Have you noticed any change in the way you are moving e.g. clumsiness, lack of coordination, trouble organising your activities, or movements, loss of spontaneous movements?
- Have you noticed if your ability to perform some movements is distracted by other things?
- Does it require more effort or energy to perform some movements?

Mannerisms, Posturing:

- Have you developed any new movements, or poses (e.g. developed a nervous habit, a characteristic way of doing something, mimicking others, assuming certain postures)? What is your explanation for this?

SUBJECTIVE MOTOR CHANGE- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No abnormal movements, or somatic difficulties reported by subject.		Mild changes only. Feeling clumsier, more uncoordinated than usual, feeling slightly slowed down. Occasional grimace, or mildly unusual gait	Experien-ces noted in column 1, but the subject feels a more noticeable change. Reports control over	Changes such as loss of coordination. Movements distracted by other things. Different gait, new poses, tics or mannerisms Loss of some previous abilities.	Experiences noted in column 4, but more distressing. May include episodes of mutism, bizarre postures, copying others movements.	Clearly distorted, or idiosyncratic movements, which dominate the clinical picture. Gross mannerisms, bizarre postures. Mute, or almost mute, with only very occasional spontaneous movements.

Onset date: _____ ***Offset date*:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

6.2 INFORMANT REPORTED OR OBSERVED CHANGES IN MOTOR FUNCTIONING

Disorganised Movement:

- Have you noticed any change in the way they are moving e.g. clumsiness, lack of coordination, trouble organising activities, or movements, loss of spontaneous movements?
- Have you noticed if their ability to perform some movements is distracted by other things?
- Does it require more effort or energy for them to perform some movements?

Mannerisms, Posturing:

- Have they developed any new movements, or poses (e.g. developed a nervous habit, a characteristic way of doing something, mimicking others, assuming certain postures)? What is their explanation for this?

OBSERVED MOTOR CHANGE- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No abnormal movements, or somatic difficulties observed or reported by others.		Others report mild changes such eg. more clumsy, uncoordinated than usual, occasional grimace, or mildly unusual gait.	Experiences noted in column 2, but more marked. Subject appears to have some control over them.	Others report that subject having difficulty performing usual tasks i.e. driving. Has also developed new movements i.e. gait, new stance/ mannerisms. Some mimicking may also be reported.	Episodes of mutism and bizarre posturing reported. Not sustained- subject able to stop with assistance and effort.	Clearly distorted, or idiosyncratic movements, which dominate the clinical picture. Gross mannerisms, bizarre postures. Mute, or almost mute, with only very occasional spontaneous movements.

6.3 SUBJECTIVE COMPLAINTS OF IMPAIRED BODILY SENSATION
(HUBER'S BASIC SYMPTOM)

NOTE: Refer also to p. 5 Perceptual Abnormalities

- Subjects say that there is something wrong with their bodily sensations.
- This includes disagreeable, but qualitatively normal sensations e.g pulling sensations, aches, pains, itching, burning, numbness, or qualitatively abnormal, unusual, or bizarre sensations may be described such as 'rustling' sensations in the eyes, vibrations, crawling sensations
- Do you ever get strange feelings in your body (eg feel that parts of your body have changed in some way, or that things are working differently)?
- Do you feel/think that there is a problem with some part, or all of your body, i.e. that it looks different to others, or is different in some way? How real does this seem?

IMPAIRED BODILY SENSATION- SEVERITY RATING SCALE

0 **Absent**	1 **Questionable**	2 **Mild,**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
Subject reports no change noticed in bodily sensations.		Subject notices occasional slight differences in bodily sensations. Not constant, able to ignore.	More intense changes to bodily sensations reported. Less able to ignore.	Occasional bizarre bodily sensation. Subject unsure of experience.	Subject reports more unusual, or bizarre sensations. Very distracting,	Subject reports extremely bizarre and unusual bodily sensations. May be distressing.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

6.4 SUBJECTIVE COMPLAINTS OF IMPAIRED AUTONOMIC FUNCTIONING
(HUBER'S BASIC SYMPTOM)

Subjects may complain of something wrong with one, or more of their autonomic systems such as:

- The feeling of the heart racing, or going too slow, breathing too fast, or too deeply,
- Nausea,
- Increased sensitivity to the weather,
- Having to urinate more often, constipation,
- Poor sleep etc.

IMPAIRED AUTONOMIC FUNCTIONING: SEVERITY RATING SCALE

0 **Absent**	1 **Questionable**	2 **Mild,**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
Nothing reported.		Subject reports occasional change to autonomic functioning – e.g. fleeting panic sensations. No real impact on usual activities.	More enduring changes perceived – e.g. poor sleep over a number of nights. Mild interference with usual activities.	Numerous changes may be experienced simultaneously. Moderate interference with usual activities	Changes in autonomic functioning are distressing. Results in more marked disruption to usual activities	Subject reports constant and intense changes to autonomic functions. Very distressing.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

7: GENERAL PSYCHOPATHOLOGY

7.1 MANIA

NOTE: See also Dangerous Behaviour/Aggression, p. 22

- Would you describe your mood as 'high', or 'hyper' recently?
- Have you been feeling excessively cheerful and had more energy than usual? How long has this feeling lasted?
- Have you felt out of control at these times?
- Has this feeling been in response to a substance, or event that has occurred (i.e. finished exams, new boyfriend/girlfriend etc)?
- Have you been able to stay awake doing things for longer periods of time than usual?
- Have you been sleeping less than usual?
- Have you found yourself spending more money than usual, or acting in ways you would not normally (i.e. heightened sexual drive, reckless behaviour etc)?
- Have you found your self, or have others described you, talking more than usual and faster than usual?
- Have people commented on your mood, or energy, saying you seem more energetic than usual, or out of control?
- Have you been feeling more irritable than usual recently? Has there been a reason for this?
- Have you been feeling better about yourself recently?
- Have you felt that you are special in some way, or have special powers, or skills?

MANIA- SEVERITY RATING SCALE

0 Never, absent	1 Questionable	2 Mild	3 Moderate	4 Moderately severe	5 Severe	6 Extreme
No observed, or reported elevation in mood. No change in self - opinion/ energy.		Cheerful without much reason. Unaccountable feelings of well-being that persist or Mild lability in mood Evidence of over-confidence with no real reason –within normal limits **&/OR** Some mild irritability	Reports excessive feelings of well-being, or cheerfulness without underlying reason Inappropriate to circumstances sometimes. More marked level of excitement. More prominent feels of self-importance. Overvalued ideas not delusional **&/OR** Moderate irritability	More persistent feelings of optimism, happiness, or elevated mood. Mood able to be shifted only with difficulty. Subject aware of inappropriateness of feelings. Behaviour may reflect the heightened mood. Clear cut grandiosity/belief in special powers - not all the time. More marked irritability evident/reported by others.	Mood elevated and inappropriate most of the time. Some delusional beliefs about own powers/ abilities. Highly distractable/ loosening of associations. Interview difficult.	Subject reports feeling elated, euphoric, marked increase in energy, restlessness. Behaviour may be destructive- excessive spending of money/sexual activity etc. Delusional beliefs of grandiosity/ power. Easily distractable, interview very difficult. Subject obviously irritable.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

7.2 DEPRESSION

NOTE: Refer also to: Avolition, p.17; Anhedonia, p.18; Role Functioning, p.20; Suicidality, p.31

- How would you describe your mood recently?
- Have you been feeling sad, or low? How often have you felt this way?
- Out of 10, what would be your average mood? Your lowest mood?
- Have you been able to enjoy activities, or feel good about yourself at all?
- How have you been feeling about the future (assess helplessness/hopelessness)?
- Has your interest in activities/events been lower than usual?
- Have you been able to complete, or start tasks you have been set (assess motivation)?
- How has your sleep been recently (assess change in sleep pattern/insomnia)?
- What has your appetite been like recently? Have you lost any weight?
- Have any events occurred recently that might account for these feelings (death/relationship issues/job/school)?

DEPRESSION- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No reported depressed mood. No physical signs of depression.		Some feelings of sadness. Does not dominate clinical picture. Able to distract self from depressive thoughts. Depressive themes not spontaneously volunteered.	Evidence of more sustained lowered mood. More difficult to shift mood. Lowered mood may be impacting on level of motivation, but not significantly interfering with role functioning. May be slightly tearful, or sad expression in interview.	Stronger observational evidence of lowered mood. Reduced ability to react to pleasurable events. More regular 'tearful episodes'.	Severe depression - mood not able to be shifted. No evidence of delusional component. Some suicidality, but not acted upon. Biological changes consistent with lowered mood evident (appetite/sleep disturbance). Very low energy.	Abject misery. Delusional component to mood – i.e. nihilistic. More marked feelings of suicidality and associated behaviour.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

7.3 SUICIDALITY AND SELF HARM

- Have you had any thoughts recently about harming, or killing yourself? How often have you felt this way?
- Have you had any thoughts of what you would do to achieve this?
- Have you acted on those thoughts at all? What happened?

SUICIDALITY- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
Not present.		Occasional thoughts of being tired of living. Occasional thought of self-harm. No suicidal thoughts, or plans.	Feeling of being better off dead. Suicidal thoughts, with only vague plan. Able to be distracted from thoughts with some effort. **OR** Minor actions of self-harm (slight scratches etc).	Thoughts of suicide more frequent with associated plan. May be more seriously considering attempt with specific plan. **OR** Impulsive attempts using non-lethal method, or with knowledge of potential for being found.	Clear expression of wanting to kill self. **OR** Potentially serious, or lethal attempt with knowledge of possible rescue.	Specific plan and attempt. **OR** Serious attempt that clearly could have been fatal.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

7.4 MOOD SWINGS/LABILITY

- Have you experienced mood swings recently?
- Have you felt that your moods have been up and down for no apparent reason?
- Do you find yourself happy one moment, and sad the next (or irritable), with no explanation?
- How often does this happen?
- Has this occurred in response to drugs, or events that have happened? Have others commented on this?
- How often has this occurred?

MOOD SWINGS- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No evidence, or reported mood swings.		Subject reports feeling mood changes more easily than usual. More marked changes in response to external events. Not noticed/report-ed by others.	Subject reports more extreme changes in mood. Feeling that mood is out of control some of the time.	More pervasive experience of mood swings. Noted by others. Distressing. Interferes with normal activities.	Mood swings experienced more days than not. Significant interference with normal activities.	Subject reports that mood changes constantly and completely out of control. Unable to maintain normal level of activity.

Onset date: _____ ***Offset date*:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

7.5 ANXIETY

- Have you been feeling nervous, or anxious recently? Has there been a reason for this? How often have you felt this way?
- How long does this feeling remain for?
- Have you felt panicky lately?
- Have you had times when you have felt breathless, heart racing, sweaty palms, tingling fingers, for no apparent reason?
- Do you have a phobia/are you afraid of dogs, spiders, enclosed places, crowds etc?
- Have you felt nervous around others recently (differentiate social anxiety from suspiciousness)?

ANXIETY- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No evidence, or reporting of anxiety.		Minor worries. Able to distract self from these. **&/OR** Mild physical signs of anxiety.	Moderate concerns, but level of anxiety is within appropriate range for event **&/OR** Moderate physical symptoms of anxiety.	Level of anxiety interfering slightly with normal activities. Some preoccupation with trigger. **&/OR** More marked physical signs.	More marked preoccupation with fears, sense of dread. **&/OR** Intrusive, distressing physical symptoms of anxiety	Level of anxiety disabling, feeling of panic, terrified.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

7.6 OCD SYMPTOMS

- Do you have distressing, or intrusive thoughts that go round and round in your head that you cannot stop?
- Do you have any repetitive behaviours that you feel compelled to perform?
- Do you have anything that you do to stop 'bad things' from occurring (rituals/superstitions etc)?
- Do you have to have things a certain way, or you feel extremely anxious?
- Do you repeatedly check things, like light switches/gas/electrical appliances are switched off/doors locked etc?

OCD SYMPTOMS- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No obsessional thoughts, or ruminations. No compulsive behaviour.		Some reported ruminating or compulsions, but not interfering with normal activities. Not time consuming Able to be distracted.	Some compulsive behaviours in response to obsessional thinking, but subject able to control. **&/OR** Compulsions do not distract from other activities.	Obsessional thinking distracting. interferes with ability to perform normal work/study. **&/OR** Compulsions not restricted to home, or private environment	Obsessional thinking or compulsions markedly distressing. **&/OR** Compulsions almost constantly - noticed by others.	Obsessional thoughts have quasi-delusional quality. **&/OR** Compulsions interfere with other activities, or are threatening to physical health (ie, hoarding garbage, excessive cleansing of body).

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

7.7 DISSOCIATIVE SYMPTOMS

Depersonalisation:
Have you experienced yourself as being unreal, as if you were outside your own body?
Or that part of your body did not belong to you?

Derealisation:
NOTE: See also Nihilistic Ideas, p.3
Have you had the feeling that things around you were unreal?

Dissociative Memory Problems:
NOTE: See also Cognitive Change, p.9
Have you ever found yourself a long way from your usual range of travel without any memory of how you got there?
Were you under stress then?

DISSOCIATIVE SYMPTOMS- SEVERITY RATING SCALE

0 Never, absent	1 Questionable	2 Mild	3 Moderate	4 Moderately severe	5 Severe	6 Extreme
No reported feelings of depersonalis-ation/ dissociation.		Mild feeling of depersonalis-ation/ derealisation. Not distressing, or distracting.	More marked dissociative experiences. Some concern expressed by subject about these, but not marked concern.	Dissociative experiences associated with heightened concern/ Distress about these experiences.	Distress as a result of dissociative experiences. Interferes somewhat with usual activities (i.e. has to leave work/school/ social situation).	Feelings of depersonalis-ation/derealisation extremely distressing. Feeling of extreme distance from others. Marked periods of time when subject not able to describe what they have been doing, where they have been etc.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

7.8 IMPAIRED TOLERANCE TO NORMAL STRESS
(HUBER'S BASIC SYMPTOM)

- Have you noticed a change in the way you have been coping with everyday stress?
- Have you felt less able to cope with, or tolerate everyday stress than before?
- When subjected to everyday stressors have you found yourself becoming excitable, uneasy, tense, nervous or anxious?
- Have you found that ordinary stressors increase other difficulties you have been experiencing?

IMPAIRED TOLERANCE TO STRESS- SEVERITY RATING SCALE

0 **Never, absent**	1 **Questionable**	2 **Mild**	3 **Moderate**	4 **Moderately severe**	5 **Severe**	6 **Extreme**
No subjectively impaired tolerance to normal stress.		Mild, or rare feeling of not coping as well as before.	Feeling mildly stressed in response to situations which would normally be coped with easily. Mild anxiety with everyday stressors, but still able to cope with them.	More marked feeling of high anxiety, or tension with everyday stressors, but able to perform everyday tasks. Feeling unable to cope with more stressful situations. May feel anxious for no reason infrequently.	Feelings of high anxiety, or tension with everyday stressors. Sometimes anxious for no reason at all.	Extreme disability eg. even trivial events, or minor concerns result in feelings of being overwhelmed and panicked. Very anxious all of the time, even if there is no apparent reason. Unable to adapt to novel situations.

Onset date: _____ ***Offset date*****:** _____

Frequency and Duration

0	1	2	3	4	5	6
Absent	Less than once a month	Once a month to twice a week **– less** than one hour per occasion	Once a month to twice a week – **more** than one hour per occasion **OR** 3 to 6 times a week - **less** than one hour per occasion	3 to 6 times a week - **more** than an hour per occasion **OR** daily – **less** than an hour per occ.	Daily – **more** than an hour per occ. **OR** several times a day	Continuous

Pattern of Symptoms

0	1	2
No relation to substance use noted	Occurs in relation to substance use and at other times as well	Noted only in relation to substance use

8: INCLUSION CRITERIA

INTAKE CRITERIA CHECKLIST

Group 1: Vulnerability Group

This criterion identifies young people at risk of psychosis due to the combination of a trait risk factor and a significant deterioration in mental state and/or functioning

	YES	NO
• **Family history of psychosis** in first degree relative **OR Schizotypal Personality Disorder** in identified patient	☐	☐
PLUS		
• **30% drop in SOFAS** score from premorbid level, sustained for a month, occurred within past 12 months **OR SOFAS score of 50 or less** for past 12 months or longer	☐	☐
CRITERION MET FOR GROUP 1 – Vulnerability Group	☐	☐

Group 2: Attenuated Psychosis Group

This criterion identifies young people at risk of psychosis due to a subthreshold psychotic syndrome. That is, they have symptoms which do not reach threshold levels for psychosis due to subthreshold intensity (the symptoms are not severe enough) or they have psychotic symptoms but at a subthreshold frequency (the symptoms do not occur often enough).

	YES	NO
2a) Subthreshold intensity:		
• **Global Rating Scale Score of 3-5** on *Unusual Thought Content* subscale, **3-5** on *Non-Bizarre Ideas* subscale, **3-4** on *Perceptual Abnormalities* subscale **and/or 4-5** on *Disorganised Speech* subscales of the CAARMS	☐	☐
PLUS		
• **Frequency Scale Score of 3-6** on *Unusual Thought Content, Non-Bizarre Ideas*, *Perceptual Abnormalities* **and/or** *Disorganised Speech* subscales of the CAARMS for **at least a week**	☐	☐
2b) Subthreshold frequency:		
• **Global Rating Scale Score of 6** on *Unusual Thought Conten*t, **6** on Non-Bizarre Ideas, **5-6** on *Perceptual Abnormalities* **and/or 6** on *Disorganised Speech* subscales of the CAARMS		
PLUS		
• **Frequency Scale Score of 3** on *Unusual Thought Content*, *Non-Bizarre Ideas*, *Perceptual Abnormalities* **and/or** *Disorganised Speech* subscales of the CAARMS	☐	☐
PLUS (for both categories)		
• **Symptoms present in past year**	☐	☐
PLUS (for both categories)		
• **30% drop in SOFAS** score from premorbid level, sustained for a month, occurred within past 12 months **OR SOFAS score of 50 or less** for past 12 months or longer	☐	☐
CRITERION MET FOR GROUP 2 – Attenuated Psychosis Group	☐	☐

Group 3: BLIPS Group

This criterion identifies young people at risk of psychosis due to a recent history of frank psychotic symptoms that resolved spontaneously (without antipsychotic medication) within one week.

	YES	NO
• **Global Rating Scale Score of 6** on *Unusual Thought Content* subscale, **6** on *Non-Bizarre Ideas*, **5 or 6** on *Perceptual Abnormalities* subscale **and/or 6** on *Disorganised Speech* subscales of the CAARMS	☐	☐
PLUS		
• **Frequency Scale Score of 4-6** on *Unusual Thought Content*, *Non-Bizarre Ideas*, *Perceptual Abnormalities* **and/or** *Disorganised Speech* subscales	☐	☐
PLUS		
• **Each episode of symptoms is present for less than one week** and symptoms spontaneously remit on every occasion.	☐	☐
PLUS		
• **Symptoms occurred during last year**	☐	☐
PLUS		
• **30% drop in SOFAS** score from premorbid level, sustained for a month, occurred within past 12 months **OR SOFAS score of 50 or less** for past 12 months or longer	☐	☐
CRITERION MET FOR GROUP 3 – BLIPS Group	☐	☐

9: PSYCHOSIS THRESHOLD /ANTI-PSYCHOTIC TREATMENT THRESHOLD

	YES	NO
• **Severity Scale Score of 6** on *Unusual Thought Content* subscale, **6** on *Non-Bizarre Ideas*, **5 or 6** on *Perceptual Abnormalities* subscale **and/or 6** on *Disorganised Speech* subscales of the CAARMS	☐	☐
PLUS		
• **Frequency Scale Score of greater than or equal to 4** on *Unusual Thought Content*, *Non-Bizarre Ideas, Perceptual Abnormalities* **and/or** *Disorganised Speech* subscales	☐	☐
PLUS		
• Symptoms present for **longer than one week**	☐	☐
PSYCHOSIS THRESHOLD CRITERION MET	☐	☐

SOCIAL AND OCCUPATIONAL FUNCTIONING ASSESSMENT SCALE (SOFAS)[1].

Consider social and occupational functioning on a continuum from excellent functioning to grossly impaired functioning. Include impairments in functioning due to physical limitations, as well as due to mental impairments. to be counted, impairment must be a direct consequence of mental and physical health problems: the effects of lack of opportunity and other environmental limitations are not to be considered.

Code (Note: use intermediate codes when appropriate e.g., 45, 68, 72). RATING: _____

100 Superior functioning in a wide range of activities
|
91

90 Good functioning in all areas, occupational and socially effective
|
81

80 No more than a slight impairment in social, occupational, or school functioning (e.g.
| infrequent interpersonal conflict, temporarily falling behind in schoolwork).
71

70 Some difficulty in social, occupational or school functioning, but generally functioning
| well, has some meaningful interpersonal relationships
61

60 Moderate difficulty in social, occupational or school functioning (e.g. few friends, conflicts
| with peers, co-workers).
51

50 Serious impairment in social, occupational, or school functioning (e.g., no friends, unable
| to keep a job)
41

40 major impairment in several areas such as work or school, family relations (e.g. depressed
| man avoids friends, neglects family and is unable to work: child frequently beats up
31 younger children, is defiant at home, and is failing school)

30 Inability to function in almost all areas (e.g. stays in bed all say, no job, home or friends)
|
21

20 Occasionally fails to maintain minimal personal hygiene. Unable to function
| independently.
11

10 Persistent inability to maintain minimal personal hygiene. Unable to function without
| harming self or others without considerable external support (e.g. nursing care and
1 supervision)

0 Inadequate information

[1] **Note:** the rating of overall psychological functioning on a scale of 0-100 was operationalized by Luborsky in the Health-Sickness Rating Scale (Luborsky L: "Clinicians Judgements of Mental Health" *Archives of General Psychiatry* 7: 401-417, 1962). Spitzer and colleagues developed a revision of the Health-Sickness Rating Scale called the Global Assessment Scale (GAS) (Endicott J, Spitzer RL, Fleiss JL et al: "The Global Assessment Scale: A Procedure for Measuring Overall Severity of Psychiatric Disturbance" *Archives of General Psychiatry* 33: 766-771, 1976). The SOFAS is derived from the GAS and its development is described in Goldman HH, Skodol AF, Lave TR: "Revising Axis V for DSM-IV: A Review of Measures of Social Functioning: *American Journal of Psychiatry* 149; 1148-1156, 1992

BRIEF IMPACT OF EVENTS (IES-6)

The following is a list of difficulties people sometimes have after stressful life events. In relation to any of the above events you have experienced in both childhood and the 12 months pre-onset [researcher summarize events] - or indeed for any event or difficulty that occurred at another time (PROBE!!!) - Please indicate the extent to which you have experienced the following difficulties in the PAST SEVEN DAYS. How much were you distressed or bothered by these difficulties? Check *one* number for each item. If it has not occurred at all in the last seven days then please tick 'not at all'. **If no events, do not complete.**

	Not at all	**A little bit**	**Moderately**	**Quite a bit**	**Extremely**
1. I thought about it when I didn't mean to	O0	O1	O2	O3	O4
2. I felt watchful or on-guard	O0	O1	O2	O3	O4
3. Other things kept making me think about it	O0	O1	O2	O3	O4
4. I was aware that I still had a lot of feelings about it, but I didn't deal with them.	O0	O1	O2	O3	O4
5. I tried not to think about it	O0	O1	O2	O3	O4
6. I had trouble concentrating	O0	O1	O2	O3	O4
7. I had dreams about it	O0	O1	O2	O3	O4
8. Pictures about it popped into my mind	O0	O1	O2	O3	O4

8a. Which of the events, difficulties that you have mentioned, doe these experiences mostly relate to? (specify and note whether occurred before onset)

……

(Note for DATA ENTRY: open **EU_SEAT,** Social Environment Assessment Tool)

Please read these instructions before completing the questionnaire:

These questions relate to your thoughts, feelings, experiences and preferences. There are no right or wrong answers or trick questions so please be as honest as possible.

For each question please choose either YES or NO and circle this on the form. Please do not spend too much time thinking about it – choose the answer closest to your own.

1. Do you often hesitate when you are going to say something in a group of people whom you more or less know?
2. Do you often overindulge in alcohol or food?
3. Are the sounds you hear in your daydreams really clear and distinct?
4. Do you enjoy many different kinds of play and recreation?
5. Do your thoughts sometimes seem as real as actual events in your life?
6. Does it often happen that nearly every thought immediately and automatically suggests an enormous number of ideas?
7. When in a group of people do you usually prefer to let someone else be the centre of attention?
8. Do you frequently have difficulty in starting to do things?
9. Has dancing or the idea of it always seemed dull to you?
10. When you catch a train do you often arrive at the last minute?
11. Is trying new foods something you have always enjoyed?
12. Do you often change between intense liking and disliking of the same person?
13. Have you ever cheated at a game?
14. Are there very few things that you have ever really enjoyed doing?
15. Do you at times have an urge to do something harmful or shocking?
16. Do you often worry about things you should not have done or said?
17. Are your thoughts sometimes so strong that you can almost hear them?
18. Are you usually in an average sort of mood, not too high and not too low?
19. Would you take drugs which may have strange or dangerous effects?
20. Do you think you could learn to read other's minds if you wanted to?
21. When in a crowded room, do you often have difficulty in following a conversation?
22. No matter how hard you try to concentrate do unrelated thoughts creep into your mind?
23. Are you easily hurt when people find fault with you or the work you do?
24. Do you stop to think things over before doing anything?
25. Have you ever felt that you have special, almost magical powers?

26. Are you much too independent to really get involved with other people?
27. Do ideas and insights sometimes come to you so fast that you cannot express them all?
28. Do you easily lose your courage when criticised or failing in something?
29. Can some people make you aware of them just by thinking about you?
30. Does a passing thought ever seem so real it frightens you?
31. Have you ever blamed someone for doing something you know was really your fault?
32. Are you a person whose mood goes up and down easily?
33. Does your voice ever seem distant or faraway?
34. Do you think having close friends is not as important as some people say?
35. Are you rather lively?
36. Are you sometimes so nervous that you are `blocked'?
37. Do you find it difficult to keep interested in the same thing for a long time?
38. Do you dread going into a room by yourself where other people have already gathered and are talking?
39. Does it often feel good to massage your muscles when they are tired or sore?
40. Do you sometimes feel that your accidents are caused by mysterious forces?
41. Do you like mixing with people?
42. On seeing a soft thick carpet have you sometimes had the impulse to take off your shoes and walk barefoot on it?
43. Do you often have difficulties in controlling your thoughts?
44. Do the people in your daydreams seem so true to life that you sometimes think they are real?
45. Are people usually better off if they stay aloof from emotional involvements with people?
46. Can just being with friends make you feel really good?
47. Is your hearing sometimes so sensitive that ordinary sounds become uncomfortable?
48. Have you often felt uncomfortable when your friends touch you?
49. When things are bothering you do you like to talk to other people about it?
50. Do you have many friends?
51. Would being in debt worry you?
52. Do you think people spend too much time safeguarding their future with savings and insurance?
53. Do you ever have the urge to break or smash things?
54. Do you often feel that there is no purpose to life?

55. Do you worry about awful things that might happen?
56. Have you ever felt the urge to injure yourself?
57. Would it make you nervous to play the clown in front of other people?
58. Have you felt that you might cause something to happen just by thinking too much about it?
59. Have you had very little fun from physical activities like walking, swimming, or sports?
60. Do you feel so good at controlling others that it sometimes scares you?
61. Are you easily distracted from work by daydreams?
62. Are you easily confused if too much happens at the same time?
63. Do you ever have a sense of vague danger or sudden dread for reasons that you do not understand?
64. Is it true that your relationships with other people never get very intense?
65. Have you sometimes had the feeling of gaining or losing energy when certain people look at you or touch you?
66. Do you worry too long after an embarrassing experience?
67. Do you love having your back massaged?
68. Do you consider yourself to be pretty much an average kind of person?
69. Have you ever taken advantage of someone?
70. Would you like other people to be afraid of you?
71. Have you ever thought you heard people talking only to discover that it was in fact some nondescript noise?
72. Have you occasionally felt as though your body did not exist?
73. Do you often feel lonely?
74. Do you often have an urge to hit someone?
75. Do you often experience an overwhelming sense of emptiness?
76. On occasions, have you seen a person's face in front of you when no one was in fact there?
77. Is it fun to sing with other people?
78. Do you often have days when indoor lights seem so bright that they bother your eyes?
79. Have you wondered whether the spirits of the dead can influence the living?
80. Do people who try to get to know you better usually give up after a while?
81. Do you often feel `fed up'?
82. Have you felt as though your head or limbs were somehow not your own?
83. When you look in the mirror does your face sometimes seem quite different from usual?

84. Do people who drive carefully annoy you?

85. Would you call yourself a nervous person?

86. Can you usually let yourself go and enjoy yourself at a lively party?

87. Do you ever suddenly feel distracted by distant sounds that you are not normally aware of?

88. Do you sometimes talk about things you know nothing about?

89. When in the dark do you often see shapes and forms even though there's nothing there?

90. Have you sometimes sensed an evil presence around you, even though you could not see it?

91. Is it hard for you to make decisions?

92. Do you find the bright lights of a city exciting to look at?

93. Does your sense of smell sometimes become unusually strong?

94. Do you usually have very little desire to buy new kinds of food?

95. Do you ever feel that your speech is difficult to understand because the words are all mixed up and don't make sense?

96. Do you often feel like doing the opposite of what other people suggest, even though you know they are right?

97. Do you like going out a lot?

98. Do you feel very close to your friends?

99. Do you ever feel sure that something is about to happen, even though there does not seem to be any reason for you thinking that?

100. Do you often feel the impulse to spend money which you know you can't afford?

101. Are you easily distracted when you read or talk to someone?

102. Do you feel that making new friends isn't worth the energy it takes?

103. Do you believe in telepathy?

104. Do you prefer watching television to going out with other people?

Thank you very much for taking the time and effort to fill this in.

O-LIFE ID_____ Date ____/ _____ / _____

1.	Y	N	36.	Y	N	71.	Y	N
2.	Y	N	37.	Y	N	72.	Y	N
3.	Y	N	38.	Y	N	73.	Y	N
4.	Y	N	39.	Y	N	74.	Y	N
5.	Y	N	40.	Y	N	75.	Y	N
6.	Y	N	41.	Y	N	76.	Y	N
7.	Y	N	42.	Y	N	77.	Y	N
8.	Y	N	43.	Y	N	78.	Y	N
9.	Y	N	44.	Y	N	79.	Y	N
10.	Y	N	45.	Y	N	80.	Y	N
11.	Y	N	46.	Y	N	81.	Y	N
12.	Y	N	47.	Y	N	82.	Y	N
13.	Y	N	48.	Y	N	83.	Y	N
14.	Y	N	49.	Y	N	84.	Y	N
15.	Y	N	50.	Y	N	85.	Y	N
16.	Y	N	51.	Y	N	86.	Y	N
17.	Y	N	52.	Y	N	87.	Y	N
18.	Y	N	53.	Y	N	88.	Y	N
19.	Y	N	54.	Y	N	89.	Y	N
20.	Y	N	55.	Y	N	90.	Y	N
21.	Y	N	56.	Y	N	91.	Y	N
22.	Y	N	57.	Y	N	92.	Y	N
23.	Y	N	58.	Y	N	93.	Y	N
24.	Y	N	59.	Y	N	94.	Y	N
25.	Y	N	60.	Y	N	95.	Y	N
26.	Y	N	61.	Y	N	96.	Y	N
27.	Y	N	62.	Y	N	97.	Y	N
28.	Y	N	63.	Y	N	98.	Y	N
29.	Y	N	64.	Y	N	99.	Y	N
30.	Y	N	65.	Y	N	100.	Y	N
31.	Y	N	66.	Y	N	101.	Y	N
32.	Y	N	67.	Y	N	102.	Y	N
33.	Y	N	68.	Y	N	103.	Y	N
34.	Y	N	69.	Y	N	104.	Y	N
35.	Y	N	70.	Y	N	Thanks for completing this		

Please complete the following questions by circling the number that best describes your experience <u>at the moment</u>

	Not at all	*Slightly*	*Moderately*	*Strongly*
1. You enjoy mixing with people	0	1	2	3
2. You hesitate even when you know what you are going to say	0	1	2	3
3. Your mood is going up and down a lot	0	1	2	3
4. You feel that you can predict what is about to happen	0	1	2	3
5. You feel more sensitive to light or the colour or brightness of things	0	1	2	3
6. You feel close to people	0	1	2	3
7. You think you are being talked about	0	1	2	3
8. It is more difficult than normal to follow conversations with people	0	1	2	3
9. You feel rather indifferent about things	0	1	2	3
10. Your mind jumps a lot from one thing to another	0	1	2	3
11. You think people are saying or doing things to annoy you	0	1	2	3
12. You think other people can read your mind	0	1	2	3
13. You find it more difficult than usual to start doing things	0	1	2	3
14. You are bothered by the idea that people are watching you	0	1	2	3
15. You find activities less enjoyable than usual	0	1	2	3
16. Your mind is so full of ideas that you can’t concentrate on one thing	0	1	2	3
17. You feel that people have it in for you	0	1	2	3
18. It is fun to do things with other people	0	1	2	3
19. You feel that you have special or magical powers	0	1	2	3
20. Your sense of smell is unusually strong or different	0	1	2	3
21. You want to be the centre of attention more than usual	0	1	2	3
22. Your experience of time is unnaturally fast or slow	0	1	2	3
23. You feel that no one understands you	0	1	2	3

Please turn over

	Not at all	*Slightly*	*Moderately*	*Strongly*
24. You feel rather uninvolved with other people	0	1	2	3
25. People can put thoughts into your mind	0	1	2	3
26. You are experiencing something very special or important	0	1	2	3
27. Your hearing has become very sensitive	0	1	2	3
28. You find it difficult to think clearly	0	1	2	3
29. You stop to think things over before doing them	0	1	2	3
30. Your speech is difficult to understand because your words are all mixed up	0	1	2	3
31. You feel that you might cause something to happen just by thinking about it	0	1	2	3
32. You feel as though your head, limbs or body have somehow changed	0	1	2	3
33. You feel that you deserved to be punished in some way	0	1	2	3
34. When you try to concentrate many unrelated thoughts pop into your mind	0	1	2	3
35. Your thoughts are sometimes so strong that you can almost hear them	0	1	2	3
36. You have seen a person's face in front of you when no one was in fact there	0	1	2	3
37. Your thoughts stop suddenly, interrupting what you are saying	0	1	2	3
38. You have a vague sense of danger or sudden dread for reasons you don’t understand	0	1	2	3
39. You would feel uncomfortable if your friends touch you	0	1	2	3
40. You feel that you can read other people’s minds	0	1	2	3
41. Ideas and insights come to you so fast that you can’t express them all	0	1	2	3
42. You think people are laughing about you behind your back	0	1	2	3
43. You have the feeling of gaining or losing energy when people look at or touch you	0	1	2	3
44. You can sense an evil presence around you, even though you cannot see it	0	1	2	3
45. You can see shapes and forms even though they aren’t there	0	1	2	3
46. You are easily distracted when doing something or talking to someone	0	1	2	3
47. You are confused by too much happening at the same time	0	1	2	3
48. You believe you are a special person with an important mission	0	1	2	3

Highlighted pink are reversed.

Example Structured Clinical Interview for DSM-IV (SCID)

Psychiatric History

Have you ever seen a mental health professional or your doctor for psychiatric reasons? Y / N

Have you or anyone in your family been diagnosed with any of the following (by a mental health professional)?

Depression Y / N _____

Bipolar Disorder Y / N _____

Anxiety Disorders Y / N _____

Schizophrenia Y / N _____

ADHD Y / N _____

Have you ever been hospitalized for a psychiatric illness? Y / N

Details:_____

_____ _____

DSM-IV Screening

Instructions:

? = Inadequate information 1 = false/absent 2 = sub-threshold 3 = true/present

Mood Disorder Questions

Has there ever been a period of time when you were feeling down or depressed most of the day, nearly every day? How long did it last? ? 1 2 3

Have you ever lost interest or pleasure in things you usually enjoyed? ? 1 2 3

Was it nearly every day? How long did it last?

If yes, check symptoms present at the time:

- Low mood
- Loss of enjoyment most of time
- Sleeping difficulties
- Loss of appetite or weight
- Lack of energy
- Lack of concentration
- Feelings of guilt
- Pessimism about things
- Suicidal ideas or attempts

Has there ever been a time when you were feeling so good, “high” or hyper that other people thought you were not your normal self, or you were so hyper that you got into trouble? Or were you so irritable that you found yourself shouting at people or starting fights? How long did it last? ? 1 2 3

<u>Anxiety Screening Questions</u>

Have you ever had a panic attack when you suddenly felt frightened or suddenly developed a lot of physical symptoms? ? 1 2 3

Were you ever afraid of going out of the house alone, being in crowds, standing in a line, or traveling on buses or trains? ? 1 2 3

In the last 6 months, have you been particularly nervous or anxious? ? 1 2 3

Psychosis Screening Questions

Has it ever seemed like people were talking about you or taking special notice of you? What about anyone going out of his/her way to give you a hard time, or trying to hurt you? ? 1 2 3

Did you ever feel you were especially important in some way, or had special powers to do things others couldn't? Have you ever been able to hear things that others couldn't, such as noises or the voices of people whispering or talking? ? 1 2 3

Pharmacotherapy:

Are you taking, or have you ever taken, any prescription medications for depression or anxiety? Anything to help you sleep? Any other medications for any emotional problems?

Medication	Dose	Date/Response

SUMMARISED SCID MKv3

Depressions

A1- Has there ever been a period of time when you were feeling depressed or down most of the day nearly every day – as long as 2 weeks? ? 1 2 3

A2 – Lost interest or pleasure in things you usually enjoyed – most of the day nearly every day – as long as 2 weeks? ? 1 2 3

Mania

A16 – Have you had a period of time when you were feeling so good, “high” or hyper that other people thought you were not your normal self, or you got into trouble? - as long as one week? ? 1 2 3

If no: What about a period of time when you were so irritable that you found yourself shouting at people or starting arguments? - as long as one week? (4 days for hypomania) ? 1 2 3

Dysthymic

A45 – For the past couple of years have you been bothered by depressed mood most of the day,

more days than most? ? 1 2
3

Psychotic Symptoms

B1 - Has it ever seemed like people were talking about you or taking special

notice of you? (*Delusion of reference)* ? 1 2
3

B2 - What about anyone going out of his/her way to give you a hard

time, or trying to hurt you? (*Persecutory delusion)* ? 1 2
3

B3 - Did you ever feel you were especially important in some way, or had special

powers to do things that other people couldn't do? (*Grandiose delusion)* ? 1 2
3.

B5 – Did you ever believe someone could read your mind? (*Other delusions)*

B6 – Have you ever been able to hear things that other people couldn't hear, such as noises

or the voices of people whispering or talking? (*Auditory hallucinations*) ? 1 2
3

B7 – Did you ever have visions or see things that people couldn't see? ? 1 2
3

B8 – What about strange sensations on your body or skin? ? 1 2 3

B9 – What about smelling or tasting things that other people couldn't smell or taste? ? 1 2 3

B10-15 (Catatonic, Disorganised, Inappropriate Behaviour, Disorganised speech, Negative symptoms). ? 1 2 3

E2: If possible alcohol abuse: (all these should be recurrent)

Did you ever miss work or school because intoxicated or hung over? ? 1 2 3

Did you ever drink in a situation in which it might have been dangerous to drink at all? ? 1 2 3

Did your drinking ever get you into trouble with the law? ? 1 2 3

Did your drinking ever cause problems with other people such as family members? ? 1 2 3

E17 Any drug taking? Use above

Did you ever miss work or school because intoxicated or hung over? ? 1 2 3

Did you ever drink in a situation in which it might have been dangerous to drink at all? ? 1 2 3

Did your drinking ever get you into trouble with the law? ? 1 2 3

Did your drinking ever cause problems with other people such as family members? ? 1 2 3

Panic attacks

F1: Have you ever had a panic attack when you suddenly felt frightened or suddenly developed a lot of physical symptoms? ? 1 2 3

Obsessions

F25: Bothered by thoughts that don't make sense and kept coming back, even whe you try not to have them – like hurting someone when you don't want to, or being contaminated by germs or dirt? ? 1 2 3

PTSD

F39 – Sometimes things happen to people that are very upsetting – things such as being in a life-threatening situation such as a major disaster, a very serious accident or fire, being physically assaulted or raped, seeing another person killed or badly hurt or hearing about something horrible that has happened to someone you are very close to. At any time during your life have any of these things happened to you? ? 1 2 3

If trauma experienced, ask if affects the way they get on with work, life and socializing in their day-to-day activities.

Other Anxiety disorders

F65 - Were you ever afraid of going out of the house alone, being in crowds, standing in a line, or standing on buses or trains? (*Agrophobia)* ? 1 2 3

F66 – Is there anything that you have been afraid to do for felt uncomfortable doing in front of other people, such as speaking, eating or writing? (*Social*) ? 1 2 3

F67 – Are there any other things that you are especially afraid of seeing such as blood, heights, closed places, or certain kinds of animals or insects? ? 1 2 3

F68 – Over the last 6 months have you been particularly nervous or anxious? ? 1 2 3

Somatoform

F72 and F73 – Do you worry much about your physical health and does you doctor think you worry too much? ? 1 2 3

F74 – Some people are very bothered about the way they look.
Is that a problem for you? ? 1 2 3

Eating disorders

F75 – Have you ever had a time when you weighed much less than other people thought you ought to weigh? (*Anorexia)* ? 1 2 3

F76 – Have you often had times when your eating was out of control (*Bulimia)* ? 1 2 3