STUDIES OF GENETIC INFLUENCES ON NICOTINE DEPENDENCE UTILISING FUNCTIONAL NEUROIMAGING

Sean P. David, M.D., S.M.

A thesis submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy in the University of Oxford

Green College
Department of Pharmacology
University of Oxford

Trinity Term 2005

Supervisors:
Professor Edith Sim and Dr. Robert Walton

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ABSTRACT

A major contributor to relapse following smoking cessation is nicotine craving triggered by environmental cues, such as the sight of a lighted cigarette. Therefore, three integrated functional neuroimaging studies were conducted to examine the biological mechanisms underling cue-elicited craving for cigarettes.

(1) First, I examined the effect of smoking-related pictorial cues on neural activation in brain regions of interest (ROI) associated with reward signalling using functional magnetic resonance imaging (fMRI). Voxel-wise analysis demonstrated that smokers, but not non-smokers, demonstrated significant activation associated with smoking-related pictorial cues in the anterior cingulate cortex, orbitofrontal cortex, and ventral striatum. Upon ROI analysis of the ventral striatum including the nucleus accumbens (VS/NAc), smokers exhibited significantly greater VS/NAc activation than non-smokers.

(2) Next, I examined whether pre-specified serotonergic polymorphisms would affect binding potential (BP) to a serotonin (5-HT) receptor implicated in the behavioural sensitisation process to nicotine (5-HT_{1A} receptor). Healthy volunteers who had undergone positron emission tomography (PET) with a 5-HT_{1A}-specific ligand [^{11}C]WAY-100635 were genotyped for the 5-HT_{1A} –1018 G>C and 5-HT transporter (5-HTT) 5-HTT gene-linked polymorphic region (5-HTTLPR) polymorphisms. Participants carrying the 5-HTTLPR S allele (SS or SL genotypes) demonstrated significantly lower global presynaptic and postsynaptic BP compared to subjects with LL genotypes.

(3) Finally, I triangulated the two initial studies to examine whether pre-specified trait (5-HTTLPR genotype) and/or state (smoking vs. abstinence) variables would influence cue-elicited activation of the VS/NAc. There was greater activation to smoking-related cues in the VS/NAc of smokers during the smoking condition than the abstinent condition and a significant correlation between tobacco craving and VS/NAc activation in the smoking condition. The 5-HTTLPR polymorphism was not associated with VS/NAc activation. Power calculations are presented as the basis for future examination of genetic hypotheses. These data have implications for the ultimate goal of enhancing the efficacy of smoking cessation pharmacotherapy.

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PREFACE

The research reported in this thesis examines the complex relationship between genetic and environmental influences on brain function and behavioural phenotypes associated with nicotine dependence. The ultimate goal of this research is to inform the process of developing more efficacious smoking cessation pharmacotherapies by advancing understanding of the molecular and cellular mechanisms underlying nicotine dependence. Three specific aims were achieved through the design, execution, and analyses of three integrated functional neuroimaging studies. Specifically, my three specific aims (S.A.) were to:

1. **(S.A.1). Identify novel nicotine dependence endophenotypes using functional magnetic resonance imaging;**
2. **(S.A.2). Examine the functional significance of polymorphisms in the serotonin 5-HT_{1A} receptor and serotonin transporter genes utilising positron emission tomography; and**
3. **(SA.3.) Triangulate results from both arms of research in order to examine whether or not a trait variable (5-HTTLPR genotype) or a state variable (smoking condition) would significantly influence cue-elicited activation of the ventral striatum including the nucleus accumbens.**

Chapter 1 provides a broad overview of the neurobiology of nicotine dependence resulting from a comprehensive literature review. Sections 1.1 through 1.5 review the following topics: The public health impact and natural history of tobacco use and the current state of the art of smoking cessation therapies (Section 1.1); the neuropharmacology of

nicotine and neurotransmitter systems implicated in nicotine dependence (Section 1.2); evidence suggesting genetic influences on nicotine dependence (Section 1.3); a review of functiononal neuroimaging techniques and their utility for the study of nicotine dependence (Section 1.4); and a theoretical framework for combining genetics and functional neuroimaging to test specific hypotheses emanating from my specific aims, and proposing a role for interactions between the 5-HT_{1A} receptor and nicotine in the development of incentive sensitisation to smoking-related environmental cues (Section 1.5). I have published portions of the review in one manuscript[1] and one book chapter.[2]

Chapters 2 through 4 describe three studies I performed, under the guidance of mentors, to test specific hypotheses consistent with my specific aims.

Chapter 2 **(S.A.1)** describes a functional magnetic resonance imaging (fMRI) pilot study of abstinent smokers and non-smokers that examined the effect of smoking-related pictorial cues on neural activation in brain reward-signalling regions. In this study, smokers but not non-smokers, demonstrated significant activation associated with smoking-related pictorial cues in the ventral striatum, anterior cingulate cortex, orbitofrontal cortex, and posterior fusiform gyrus. Region of interest (ROI) analysis demonstrated that the effect size of the activation (associated with smoking-related pictorial cues) in the ventral striatum was significantly greater in smokers than non-smokers.

My role in this investigation was as follows: Under the supervision and guidance of mentors, I planned, wrote the protocol, recruited the participants, worked with the radiographer in conducting the fMRI scans for every participant, analysed the fMRI and

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[1] David, S.P. (2004). 'Pharmacogenetics', *Prim Care*, **31**(3): pp. 543-559.

[2] David, S.P., Munafo', M.R., Walton, R.T. (2005). 'Chapter 21. Pharmacogenomics Research in Nicotine Addiction and Smoking Cessation', in *Clinical Pharmacogenomics and Introduction to PharmacoProteomics*, ed. by Wong, S.H.Y., Linder, M., Valdes, R. (Washington, D.C., U.S.A: American Association for Clinical Chemistry (AACC) Press).

behavioural data, and published the results, now in press, in a peer-reviewed journal.[3] My mentors for this study were Professor Paul Matthews, Dr. Robert Walton, Dr. Marcus Munafo', and Dr. Robert Rogers.

Chapter 3 **(S.A.2)** is an examination of the influence of genetic variation in genes associated with serotonin (5-HT) neurotransmission [5-HT_{1A} receptor and 5-HT transporter (5-HTT) genes] on 5-HT_{1A} receptor function as evaluated with positron emission tomography (PET). A polymorphism in the promoter region of the 5-HTT gene was significantly associated with pre-synaptic (raphe nucleus) and global post-synaptic 5-HT_{1A} binding potential.

My role in this study was as follows: Working with mentors (RW, MM, Professor Edith Sim, and Professor Paul Grasby), I planned and wrote the study protocol and ethics application; contacted all subjects who had undergone PET with the 5-HT_{1A} radioligand [^{11}C]-N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)-cyclohexane-carboxamide ('WAY')-100635 and arranged for blood collection at general practice surgeries and genotyping at the Cancer Research UK General Practice Research Group; performed the statistical analysis of the genetic and PET data; and published the results of this study in a peer-reviewed journal.[4] All PET scans were conducted by staff at the Medical Research Council Cyclotron Unit located at the Hammersmith Hospital in London, and under the supervision of Professor Paul Grasby. I conducted a literature review and selected

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[3] David, S.P., Munafo', M., Johansen-Berg, H., Smith, S.M., Rogers, R.D., Matthews, P.M., Walton, R.T. (2005). 'Ventral Striatum/Nucleus Accumbens Activation to Smoking-Related Pictorial Cues in Smokers and Non-Smokers: An fMRI study', *Biol Psychiatry*, **58**(6):488-94.

[4] David, S.P., Murthy, N. V., Rabiner, E. A., Munafo', M. R., Johnstone, E. C., Jacob, R., Walton, R. T., Grasby, P. M. (2005). 'A Functional Genetic Variation of the Serotonin (5-HT) Transporter Affects 5-HT1A Receptor Binding in Humans', *J Neurosci.* **25**(10): pp. 2586-90.

single nucleotide polymorphisms (SNPs) for analysis. Dr. Elaine Johnstone and Mrs. Robyn Jacob performed the DNA extraction and genotyping.

Chapter 4 **(S.A.3)** describes a study triangulating the results from the fMRI pilot study and the 5-HT_{1A} PET study to examine potential interactions between the 5-HTTLPR polymorphism (a trait variable) and nicotine satiation (following *ad libitum* cigarette smoking vs. overnight abstinence from smoking)(a state variable) on cue-elicited activation associated with smoking-related pictorial cues in the ventral striatum including the nucleus accumbens (VS/NAc). The rationale for this study is based on a proposed interaction between the 5-HT_{1A} receptor and nicotine in the development of nicotine dependence, as discussed in Chapter 1. The results from this study indicated a main effect of smoking condition on VS/NAc activation (associated with smoking-related pictorial cues compared with neutral cues) and a significant and positive correlation between cigarette craving and activation in the VS/NAc. In addition, there was a significant effect of smoking condition on reaction times (RT) to a gender discrimination task suggesting an effect of nicotine deprivation on RT. Furthermore, there was a statistical trend suggesting a condition by cue type interaction such that the RTs for smoking cues were significantly greater than neutral cues in the abstinent condition but not in the nicotine satiated condition. There was not a significant effect of the 5-HTTLPR polymorphism on VS/NAc activation. However, notable trends and observed statistical power are described with implications for future work.

My role in this investigation was as follows: Under the supervision and guidance of mentors (PM, RW, MM, RR), I planned, wrote the protocol, recruited the participants, was involved in conducting the fMRI scans for each session for every participant, analysed the

fMRI and behavioural data, and am now in the process of writing up the results for submission to a peer-reviewed journal.

I describe the materials and methods for each study within the chapters describing each investigation. The rationale for incorporating the methods in each chapter rather than in a separate chapter is that multiple methods are used and there are unique aspects to each of the studies. As such, incorporating materials and methods within each investigational chapter provided a theoretical context for use of each method, and permitted description of how methodological approaches progressed based on the observations and lessons learned in the process of my studies.

Chapter 5 is a synthesis of the results of these three studies in which I propose a theoretical biological mechanism underlying these results. Furthermore, I discuss the strengths and weaknesses of the studies I conducted, potential translational applications for the development of novel smoking cessation therapies, and my plans for future work as a logical extension of my doctoral studies.

PUBLICATIONS RESULTING FROM MY DOCTORAL RESEARCH

Original Manuscripts in Peer-Reviewed Journals.

1. David, S.P., Munafo', M., Johansen-Berg, H., Smith, S.M., Rogers, R.D., Matthews, P.M., Walton, R.T. (2005). 'Ventral Striatum/Nucleus Accumbens Activation to Smoking-Related Pictorial Cues in Smokers and Non-Smokers: An fMRI study', *Biol Psychiatry*, 58(6): pp. 488-94.
2. David, S.P., Murthy, N. V., Rabiner, E. A., Munafo', M. R., Johnstone, E. C., Jacob, R., Walton, R. T., Grasby, P. M. (2005). 'A Functional Genetic Variation of the Serotonin (5-HT) Transporter Affects 5-HT1A Receptor Binding in Humans', *J Neurosci.* 25(10): pp. 2586-90.

Reviews and Book Chapters.

3. David, S.P. (2004). 'Pharmacogenetics', *Prim Care*, 31(3): pp. 543-559.
4. David, S.P., Munafo', M.R., Walton, R.T. (2005). 'Chapter 21. Pharmacogenomics Research in Nicotine Addiction and Smoking Cessation', in *Clinical Pharmacogenomics and Introduction to Pharmacoproteomics*, ed. by Wong, S.H.Y., Linder, M., Valdes, R. (Washington, D.C., U.S.A: American Association for Clinical Chemistry Press).

DEDICATION

This thesis is dedicated my parents, Jack and Joy David.

ACKNOWLEDGMENTS

I wish to thank Professor Edith Sim, Professor Paul Matthews, Dr. Robert Walton, Dr. Heidi Johansen-Berg, Professor Paul Grasby, Professor Neville Osborne, and the late Professor Sir Richard Doll, for academic supervision and mentoring of immeasurable value. I would especially like to thank Dr. Marcus Munafò for his generous contribution of many additional hours of mentoring and content expertise. In addition, I wish to thank Dr. Elaine Johnstone and Mrs. Robyn Jacob for their work in genotyping, the Department of Family Medicine at Brown Medical School, and the Warden, staff, and students of Green College.

LIST OF ABBREVIATIONS

ACC. anterior cingulate cortex

Ach. acetylcholine

ACTH. adrenocorticotropin hormone

ANOVA. analysis of variance

AP. action potential

Am. amygdala

B_{Avail}. concentration of available binding sites

BET. FMRIB's Brain Extraction Tool

bp. base pairs

BP. binding potential

BOLD. blood oxygen level dependent

BP. binding potential

c^2. quantified shared environmental effects

Ca^{2+}. calcium

cAMP. cyclic 3',5' –adenosine monophosphate

CBF. cerebral blood flow

CBV. cerebral blood volume

Cl^-. chloride

CI. confidence interval

CO. carbon monoxide

COG. centre of gravity

COMT. catechol-O-methyltransferase

COPE. contrast of parameter estimates

COOH. carboxy terminus

COREC. Central Office for Research Ethics

CPP. conditioned place preference

CRH. corticotropin-releasing hormone

CYP2A6. cytochrome P450 2A6

CYP2B6. cytochrome P450 2B6

DA. dopamine

DAT. dopamine transporter

DH βE. dihyrdo-beta-erythroidine

DBH. dopamine beta hydroxylase

DNA. deoxyribonucleic acid

df. degrees of freedom

DOF. degrees of freedom for linear (also known as 'affine') transformation

DOPA. dihydroxyphenylalanine

DOPAC. dihyrdroxyphenylacetic acid

DRN. dorsal raphe nucleus

DSM-IV. Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition

DSM-IV-TR. DSM-IV, Text Revision

DZ. dizygotic

e^2. quantified unique environmental effects

ECAT. Electronic Card Assembly and Test

EEG. electro-encephalogram

ED_{50}. effective dose in 50% of animals tested experimentally

EPI. echo planar imaging

ERP. event-related potential

EV. explanatory variable

f_2. free fraction of radioligand in tissue

FEAT. FMRI Expert Analysis Tool

FLAME. FMRIB's Local Analysis of Mixed Effects

FLASH. fast low angle shot

FLIRT. FMRIB's Linear Image Registration Tool

FOV. field of view

fMRI. functional magnetic resonance imaging

GPRG. Cancer Research UK General Practice Research Group

FMRIB. Oxford Centre for Functional Magnetic Resonance Imaging of the Brain

FTND. Fagerström Test of Nicotine Dependence

FWHM. full-width half maximum

g. gram

GABA. γ-aminobutyric acid

GLM. general linear model

GTP. guanine tri-phosphate

[^{3}H]-9-DPAT. [^{3}H]-8-hydroxy-(di-n-propylamino)-tetralin

HVA. homovanillic acid

IR. inversion recovery

ISIS. International Smoking Image Series

IV. intravenous

I-RISA. Impaired Response Inhibition and Salience Attribution

h^2. quantified heritability

Hbr. deoxyhaemoglobin

HBO_2. oxyhaemoglobin

5-HIAA. 5-hydroxyindoleacetic acid

5-HT. 5-hydroxytryptamine 'serotonin'

5-HTT. serotonin transporter

5-HTTLPR. 5-HTT gene-linked polymorphic region

HPA. hypothalamic-pituitary-adrenal

ICSS. intracranial self-stimulation

K^+. potassium

K_D. equilibrium dissociation rate constant of the radioligand

Kg. kilogram (10^3 grams)

KO. knockout (mice)

L. long allele of 5-HTTLPR

LD_{50}. lethal dose to 50% of animals tested experimentally

LG. lingual gyrus

MANOVA. multivariate analysis of variance

MAO. monoamine oxidase

MCFLIRT. motion correction using FLIRT

ME. median eminence

MEG. magnetoencephalography

MFB. medial forebrain bundle

mg. milligram (10^{-3} grams)

μg. microgram (10^{-6} grams)

MNI. Montreal Neurological Institute

mm. millimetre (10^{-3} metres)

ms. millisecond (10^{-3} seconds)

MRC. Medical Research Council

MRI. magnetic resonance imaging

MRS. magnetic resonance spectroscopy

mRNA. messenger ribonucleic acid

MT. medial temporal cortex

MST. medial superior temporal cortex

MZ. monozygotic

NA^+. sodium

NAc. nucleus accumbens

NAChR. nicotinic acetylcholine receptors

NET. norepinephrine transporter

ng. nanogram (10^{-9} grams)

NH_2. amino terminus

NIC. nicotine

NMDA. *N*-methyl-D-aspartate

NRT. nicotine replacement therapy

NUDR. nuclear deformed epidermal autoregulatory factor/DEAF-1

OFC. orbitofrontal gyrus

8-OH-DPAT. (+/-)-8-hydroxy-2-(di-n-propylamino)tetralin

OR. odds ratio

PE. parameter estimate

PFC. prefrontal cortex

PFG. posterior fusiform gyrus

pKa. The 'dissociation constant', equal to the negative logarithm (base 10) of the equilibrium coefficient of the neutral and charged forms of a compound expressed in moles per litre.

pH. A measure of the acidity or alkalinity of a solution, equal to the negative logarithm (base 10) of the effective concentration of hydrogen ions expressed in moles per litre.

POMS. Profile of Mood States

ppm. parts per million

QTL. quantitative trait loci

ROI. region of interest

rCBF. regional cerebral blood flow

PET. positron emission tomography

s. second(s)

S. short allele of 5-HTTLPR

SD. standard deviation

SERT. serotonin transporter

SFG. superior frontal gyrus

SN. substantia nigra pars compacta

SNP. single nucleotide polymorphism

SPECT. single photon emission tomography

SPSS. Statistical Package for the Social Sciences

SR. sustained release

SSRI. selective serotonin reuptake inhibitor

T. Tesla

T1. longitudinal (z-direction) magnetization recovery time constant

T2. transverse (xy-direction) magnetization decay time constant

T2*. transverse decay time constant including magnetic field inhomogeneity effects.

TE. time to echo (from the radiofrequency excitation pulse)

TH. tyrosine hydroxylase

TI. inversion time (time following inversion of spins with 180° inversion pulse).

TPH. tryptophan hydroxylase

TPP. tegmental pedunculopontine nucleus

TR. time for repetition

Turbo FLASH. turbo fast low angle shot

UTR. untranslated region

VNTR. variable number of tandem repeats

VS. ventral striatum

VTA. ventral tegmental area

WAY. N-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-N-(2-pyridinyl)-cyclohexane carboxamide

Chapter 1.

INTRODUCTION

> **A custom loathsome to the eye, hateful to the nose, harmful to the brain, dangerous to the lungs, and in the black stinking fume thereof nearest resembling the horrible stygian smoke of the pit that is bottomless.**
>
> ***King James I of England (1604) referring to tobacco smoking***[5]

1.1 Tobacco Smoking and Public Health

1.1.1 Mortality and Morbidity

In the more than 50 years since Sir Richard Doll and Sir Austin Bradford Hill published preliminary results from the British physicians study,[6] the scientific and public communities have become well aware of the detrimental effects of tobacco use.[7] Over these five decades it has been established that tobacco use has been causally related to cancer of the lung, kidney, larynx, oral cavity, bladder, pancreas and other neoplasms, atherosclerosis (ischemic heart disease, cerebrovasular disease, aortic aneurism), chronic obstructive pulmonary disease, peptic ulcer disease, and a wide range of other chronic diseases and disabilities.[8] Yet, despite dramatic declines in smoking prevalence and resultant mortality,[9] tobacco use remains the leading cause of preventable death in the United Kingdom,[10] and in developing countries where more than three million premature tobacco-related deaths occur each year.[11] The

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[5] James I, king of England (1604). *A Counterblaste to Tobacco by King James I* <http://www.tobacco.org/resources/history/Tobacco_Historynotes.html#aa4> [accessed 27 December 2004].

[6] Doll, R., Hill, A. B. (1954). 'The Mortality of Doctors in Relation to their Smoking Habits; a Preliminary Report', *Br Med J.* **4877**: pp. 1451-5.

[7] Koop, C.E. (2003). 'Tobacco Addiction: Accomplishments and Challenges in Science, Health, and Policy', *Nicotine Tob Res.* **5**(5): pp. 613-9; Bradbury, J. (1998). 'UK "White Paper on Tobacco" Welcomed', *Lancet*, **352**(9145): p. 1991.

[8] Colditz, G.A. (2000). 'Illnesses Caused by Smoking Cigarettes', *Cancer Causes Control*, **11**(1): pp. 93-7; Doll, R. (2000). 'Fifty Years of Research on Tobacco', *J Epidemiol Biostat.* **5**(6): pp. 321-9.

[9] Peto, R., Darby, S., Deo, H., Silcocks, P., Whitley, E. and Doll, R. (2000). 'Smoking, Smoking Cessation, and Lung Cancer in the UK since 1950: Combination of National Statistics with Two Case-Control Studies', *BMJ.* **321**(7257): pp. 323-9.

[10] Twigg, L., Moon, G., Walker, S. (2004). *The Smoking Epidemic in England* (National Health Service: The Health Development Agency).

[11] Colditz, G.A. (2000); Peto, R., Lopez, A. D., Boreham, J., Thun, M., Heath, C., Jr. (1992). 'Mortality from Tobacco in Developed Countries: Indirect Estimation from National Vital Statistics', *Lancet*, **339**(8804): pp. 1268-78; Peto, R., Lopez, A. D., Boreham, J., Thun, M., Heath, C., Jr., Doll, R. (1996). 'Mortality from

burden of death and disability is matched by tremendous economic costs to society.[12] Indeed, according to the World Health Organisation, it is estimated that about half of the people who smoke today (approximately 650 million) will die a tobacco-related death and that tobacco use results in an annual global net loss of approximately £400, 000 million.[13]

Whilst nearly one third of these premature deaths would be prevented by reducing tobacco consumption in half,[14] current trends, particularly in developing countries, suggest that dramatic declines in smoking prevalence are unlikely in the coming decades. Even with established evidence-based smoking cessation therapies available in Western countries such as the UK, fewer than ten percent of smokers who attempt to stop smoking with nicotine skin patches remain abstinent from tobacco at eight years of follow-up.[15]

Achieving meaningful reductions in smoking prevalence is a formidable challenge for physicians, public health practitioners, and other biomedical scientists because, due to the addictive nature of nicotine and other constituents of tobacco smoke, tobacco cessation is an extremely difficult task for patients to achieve. As I will describe in this thesis, the pharmacology of nicotine combined with the behavioural conditioning that occurs with repetitive tobacco use, results in neuroadaptations that produce dependence. Despite having learned much about the neurotransmitter systems implicated in nicotine dependence from animal studies and, more recently, from *in vivo* functional neuroimaging, a more

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Smoking Worldwide', *Br Med Bull.* **52**(1): pp. 12-21; Doll, R., Peto, R., Boreham, J., Sutherland, I. (2004). 'Mortality in Relation to Smoking: 50 Years' Observations on Male British Doctors', *BMJ.* **328**(7455): pp. 1519.

[12] World Bank, The (1999). *Curbing the Epidemic: Governments and the Economics of Tobacco Control* (Washington, D.C., USA: The World Bank).

[13] WHO. (2005). 'Why is Tobacco a Public Health Priority?' <http://www.who.int/tobacco/health_priority/en/> [accessed 20 May 2005].

[14] Peto, R., Darby, S., Deo, H., Silcocks, P., Whitley, E. and Doll, R. (2000).

[15] Yudkin, P., Hey, K., Roberts, S., Welch, S., Murphy, M., Walton, R. (2003). 'Abstinence from Smoking Eight Years after Participation in Randomised Controlled Trial of Nicotine Patch', *BMJ.* **327**(7405): pp. 28-9.

comprehensive understanding is needed to advance the effectiveness of smoking cessation therapy.

1.1.2 Natural History of Nicotine Dependence

Smoking behaviour can be characterised by a number of stages, each stage with unique neuropsychological processes influenced by genetic and environmental factors.[16] Typically, the process of nicotine dependence begins with experimentation during the adolescent years. Many of the individuals who experiment with smoking during adolescence go on to smoke on a regular basis. In time, conditioned learning and neuroadaptive changes in the brain of smokers who are biologically vulnerable ultimately results in nicotine dependence. As with the addiction process observed for other drugs, chronic nicotine consumption leads to a dysregulation of reward systems resulting in compulsive tobacco use and loss of control over tobacco consumption.[17] Furthermore, when individuals become nicotine dependent, they develop tolerance, and may experience craving and withdrawal symptoms in the abstinent state, which predisposes individuals to relapse during attempts to quit.[18]

Approximately 80% of adult smokers in the UK became regular smokers during adolescence and approximately 450 children start smoking every day.[19] The process of developing nicotine dependence is complex and highly variable but usually requires about two to three years to progress from experimentation to daily use and then to dependence. Once dependent on nicotine, approximately 70% of smokers wish to quit smoking each year

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[16] Lerman, C., Berrettini, W. (2003). 'Elucidating the Role of Genetic Factors in Smoking Behavior and Nicotine Dependence', *Am J Med Genet B Neuropsychiatr Genet.* **118**(1): pp. 48-54.
[17] Koob, G.F., Le Moal, M. (1997). 'Drug Abuse: Hedonic Homeostatic Dysregulation', *Science*, **278**(5335): pp. 52-8; Koob, G.F., Le Moal, M. (2001). 'Drug Addiction, Dysregulation of Reward, and Allostasis', *Neuropsychopharmacology*, **24**(2): pp. 97-129.
[18] Niaura, R., Shadel, W. G., Abrams, D. B., Monti, P. M., Rohsenow, D. J., Sirota, A. (1998). 'Individual Differences in Cue Reactivity Among Smokers Trying to Quit: Effects of Gender and Cue Type', *Addict Behav.* **23**(2): pp. 209-24; Benowitz, N.L. (1999). 'Nicotine addiction', *Prim Care*, **26**(3): pp. 611-31.
[19] ASH-UK (2000). 'Basic Facts', <http://www.ash.org.uk/> [accessed 20 May 2005].

and about 50% try to quit each year;[20] but only about 10% are successful.[21] One of the hallmarks of failure to remain abstinent from smoking is the development of craving during periods of brief or prolonged abstinence.[22] Substance dependence has been defined by the American Psychological Association in their Diagnostic and Statistical Manual of Mental Disorders (DSM-IV)[23] as involving three or more of the following symptoms: tolerance, withdrawal, unsuccessful efforts to cut down on use of the drug, or excessive time spent in obtaining the drug at the expense of other activities. As will be made clear in the sections to come, such symptoms are characteristic of the behaviour of individuals who make the transition from occasional, casual tobacco use to regular, daily use.

There is evidence that the adolescent brain is especially vulnerable because adolescent neurodevelopment occurs in brain regions involved in motivation and impulsivity. The impulsivity and novelty-seeking characteristic of adolescence are transitional traits that can be explained by neurodevelopment of frontal cortical and subcortical monoamine (dopamine, serotonin, norepinephrine) neurotransmitter systems.[24] Thus, adolescents may be vulnerable to addiction as a result of greater participation in experimentation as well as greater susceptibility to developing motivational drives and operant conditioning favouring tobacco use upon exposure to nicotine.[25] Once addiction to nicotine and regular use of tobacco develops, smokers find it difficult to abstain from tobacco as a result of

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[20] Fiore, M.C., Bailey, W.C., Cohen, S.J., et al. (2000). *Treating Tobacco Use and Dependence: Clinical Practice Guideline* (Rockville, MD, USA: US Department of Health and Human Services, Public Health Service).

[21] Yudkin, P., Hey, K., Roberts, S., Welch, S., Murphy, M., Walton, R. (2003).

[22] Lerman, C., Berrettini, W. (2003); Niaura, R., Shadel, W. G., Abrams, D. B., Monti, P. M., Rohsenow, D. J., Sirota, A. (1998).

[23] APA (2002). *Diagnostic and Statistical Manual of Mental Disorders [DSM-IV-TR (text revision 2002)]*, 4th edn (Washington, D.C: American Psychiatric Association, American Psychiatric Press).

[24] Chambers, R.A., Taylor, J. R., Potenza, M. N. (2003). 'Developmental Neurocircuitry of Motivation in Adolescence: A Critical Period of Addiction Vulnerability', *Am J Psychiatry*, **160**(6): pp. 1041-52.

[25] Ibid.

neuroadaptations, which result in tolerance, loss of control over tobacco use, withdrawal symptoms, and craving.[26] Prevailing theoretical models and converging evidence from animal and human studies suggest biologically plausible mechanisms underlying the development of nicotine dependence, as will be discussed in details in Sections 1.2.1.2 (*Pharmacodynamics*) and 1.2.1.3.5 (*Models of nicotine dependence*). From a clinical perspective, the end result of such complex neuroadaptations for many patients is a chronic syndrome with smokers cycling through a series of stages of preparedness to quit smoking, resulting in multiple quit attempts, relapse, and ambivalence about quitting smoking.[27]

1.1.3 Current Smoking Cessation Therapies

State-of-the-art of smoking cessation therapy comprises behavioural and pharmacological therapies which have demonstrated efficacy in systematic reviews and meta-analyses.[28] Whilst there have been reports of successful community-wide smoking prevention and cessation programmes,[29] a recent meta-analysis did not find a significant effect of such interventions on reducing smoking prevalence.[30]

1.1.3.1 Behavioural

Behavioural therapies range from minimal contact interventions by health professionals to telephone counselling to intensive individual or group-level counselling. The basic components of smoking cessation counselling include encouragement to quit, planning a quit

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[26] Robinson, T.E., Berridge, K. C. (2001). 'Incentive-Sensitization and Addiction', *Addiction*, **96**(1): pp. 103-14.
[27] Prochaska, J.O., DiClemente, C. C. (1983). 'Stages and Processes of Self-Change of Smoking: Toward an Integrative Model of Change', *J Consult Clin Psychol.* **51**(3): pp. 390-5.
[28] Fiore, M.C., Bailey, W.C., Cohen, S.J., et al., 2000; Hughes, J.R., Stead, L. F., Lancaster, T. (2004). 'Antidepressants for Smoking Cessation'. Cochrane Database Syst Rev. (4): p. CD000031.pub2; Silagy, C., Lancaster, T., Stead, L., Mant, D., Fowler, G. (2004). 'Nicotine Replacement Therapy for Smoking Cessation'. Cochrane Database Syst Rev. (3): p. CD000146.
[29] Zucker, D., Hopkins, R. S., Sly, D. F., Urich, J., Kershaw, J. M., Solari, S. (2000). 'Florida's "Truth" Campaign: A Counter-Marketing, Anti-Tobacco Media Campaign', *J Public Health Manag Pract.* **6**(3): pp. 1-6; Fichtenberg, C.M. and Glantz, S.A. (2000). 'Association of the California Tobacco Control Program with Declines in Cigarette Consumption and Mortality from Heart Disease', *N Engl J Med.* **343**(24): pp. 1772-7.
[30] Secker-Walker, R.H., Gnich, W., Platt, S., Lancaster, T. (2002). 'Community Interventions for Reducing Smoking among Adults'. Cochrane Database Syst Rev. (3): p. CD001745.

date, acquiring emotional support, avoiding relapse triggers, and following up with health care providers.[31] Meta-analyses of behavioural interventions including individual-level counselling,[32] group-level counselling,[33] and advice from health care providers[34] have all demonstrated efficacy in smoking cessation. However, behavioural counselling without pharmacological therapy rarely results in success rates of greater than 15%.[35]

1.1.3.2 Pharmacological

The most commonly used pharmacological methods for smoking cessation include nicotine replacement therapy or non-nicotine replacement therapies such as the antidepressants bupropion and nortriptyline and the antihypertensive agent clonidine. Bupropion and nicotine replacement therapies are considered first-line with other therapies considered adjuncts or second-line.[36]

Sustained-release bupropion hydrochloride (Zyban™, GlaxoSmithKline, GlaxoSmithKline plc Brentford, Middlesex, UK) has demonstrated the highest efficacy of any NHS-approved medication for smoking cessation, with six-month abstinence rates ranging from 25%–35%, as compared with 15%–20% for placebo,[37] and a recent meta-

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[31] David, S.P. (2001b). 'Smoking Cessation for the Primary Care Physician', *Primary Care Reports*, 7(24): pp. 211-221.

[32] Lancaster, T., Stead, L. (2005). 'Individual Behavioural Counselling for Smoking Cessation'. Cochrane Database Syst Rev. (2): p. CD001292.

[33] Stead, L., Lancaster, T. (2005). 'Group Behaviour Therapy Programmes for Smoking Cessation'. Cochrane Database Syst Rev. (2): p. CD001007.

[34] Lancaster, T., Stead, L., 2004; Rice, V.H., Stead, L. F. (2001). 'Nursing Interventions for Smoking Cessation'. Cochrane Database Syst Rev. (3): p. CD001188.

[35] Fiore, M.C. (2000). 'US Public Health Service Clinical Practice Guideline: Treating Tobacco Use and Dependence', *Respir Care*. **45**(10): pp. 1200-62.

[36] USPHS (2000). Chapter 4, 'Management of Nicotine Addiction', in *Reducing Tobacco Use: A Report of the Surgeon General* (Bethesda, MD, U.S.A United States Department of Health and Human Services, Public Health Service).

[37] Jorenby, D.E., Leischow, S. J., Nides, M. A., Rennard, S. I., Johnston, J. A., Hughes, A. R., Smith, S. S., Muramoto, M. L., Daughton, D. M., Doan, K., Fiore, M. C., Baker, T. B. (1999). 'A Controlled Trial of Sustained-Release Bupropion, a Nicotine Patch, or Both for Smoking Cessation', *N Engl J Med*. **340**(9): pp. 685-91; Hurt, R. D., Sachs, D. P., Glover, E.D., Offord, K.P., Johnstone, J.A., Dale, L.C. et al. (1997). 'A Comparison of Sustained-Release Bupropion and Placebo for Smoking Cessation', *N Engl J Med*. 337(17): pp. 1195-1202.

analysis of 16 clinical trials indicated that smokers using bupropion hydrochloride are twice as likely to remain abstinent from smoking for six months following a quit attempt relative to placebo [Odds ratio (OR) for quitting at six months = 2.06 (95% Confidence Interval (CI) 1.77 to 2.40)].[38] Bupropion is an antidepressant medication with somewhat complex pharmacodynamic and pharmacokinetic properties. Bupropion is a weak inhibitor of dopamine (DA) and norepinephrine reuptake[39] and may serve as a non-competitive inhibitor of nicotinic acetylcholine receptors.[40] These pharmacodynamic properties are consistent with known effects of bupropion on nicotine withdrawal,[41] which appear to be moderated by genetic variation in the DA D_2 receptor gene.[42] With regard to its pharmacokinetic properties, bupropion is metabolised to a primary metabolite, hydroxybupropion, by the cytochrome P450 enzyme 2B6 (CYP2B6).[43]

Another antidepressant with different pharmacological properties, nortriptyline, has also demonstrated efficacy for smoking cessation when evaluated in a recent meta-analysis (OR 2.79, 95% CI 1.70 to 4.59).[44] At three months of follow-up the mean abstinence rate of smokers attempting to quit using nortriptyline in published clinical trials for smoking cessation is 30.1% (95% CI 18.1, 41.6).[45] Other antidepressants such as selective serotonin

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[38] Hughes, J.R., Stead, L. F., Lancaster, T. (2004).
[39] Ascher, J.A., Cole, J.O, Collin, J.N., Feighner, J.P., Ferris, R.M., Fibiger, H.C. et al. (1995). 'Bupropion: A Review of its Mechanism of Antidepressant Activity', *J Clin Psychiatry*, 56(9): 395-401.
[40] Fryer, J.D., Lukas, R.J. (1999). 'Non-Competitive Functional Inhibition at Diverse, Human Nicotinic Acetylcholine Receptor Subtypes by Bupropion, Phencyclidine, and Ibogaine', *J Pharmacol Exp Ther.* 288(1): pp. 88-92.
[41] Lerman, C., Roth, D., Kaufman, V., Audrian, J., Hawk, L., Liu, A., et al. (2002). 'Mediating Mechanisms for the Impact of Bupropion in Smoking Cessation Treatment', *Drug Alcohol Depend.* **67**(2): pp. 219-23.
[42] David, S.P., Niaura, R., Papandonatos, G., Shadel, W., Burkholder, G., Britt, D., et al. (2003). 'Does the DRD2-Taq1 A Polymorphism Influence Treatment Response to Bupropion Hydrochloride for Reduction of the Nicotine Withdrawal Syndrome?' *Nicotine Tob Res.* **5**(6): 935-42.
[43] Faucette, S.R., Hawke, R.L., Lecluyse, E.L., Shord, S.S., Yan, B., Laethem, R.M., et al. (2000). 'Validation of Bupropion Hydroxylation as a Selective Marker of Human Cytochrome P450 2B6 Catalytic Activity', *Drug Metab Dipos.* **28**(10): 1222-30.
[44] Hughes, J.R., Stead, L. F., Lancaster, T. (2004).
[45] USPHS, (2000).

reuptake inhibitors have not demonstrated consistent results with regard to smoking cessation efficacy.[46]

Nicotine replacement therapy (NRT) is believed to be effective by reducing or eliminating the psychomotor and physiological symptoms of nicotine withdrawal. NRT is available as chewing gum, transdermal skin patch, nasal spray, oral inhaler, and lozenges.[47] A recent meta-analysis determined that all forms of NRT are effective with odds ratios of cessation between 1.5 and 2.0.[48] The mean abstinence rate of NRT in published clinical trials ranges from 17.7% (95% CI 16.0, 19.5) for transdermal patch to 30.5% (95% CI 21.8, 39.2) for nasal spray at three month follow-up evaluation.[49] Although gender differences in NRT efficacy have been reported, a recent meta-analysis did not find a significant effect of gender on NRT efficacy.[50]

Clonidine is a α_2-adrenergic receptor agonist with indications for the treatment of hypertension and perimenopausal symptoms. A recent meta-analysis of clonidine demonstrated that it is effective for smoking cessation [OR = 1.89 (95% CI 1.30 to 2.74)], but that there is a high side-effect profile including dry mouth, sedation, dizziness, and postural hypotension.[51] The mean abstinence rate for clonidine across clinical trials at three month follow-up is 25.6% (95%CI 17.7, 33.6).[52]

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[46] Hughes, J.R., Stead, L. F., Lancaster, T. (2004).
[47] David, S.P. (2001a). 'Should We Recommend Nicotine Replacement Therapy?' *Am Fam Physician*, **63**(11): pp. 2245-7.
[48] Silagy, C., Lancaster, T., Stead, L., Mant, D., Fowler, G. (2004).
[49] USPHS, (2000).
[50] Munafo', M., Bradburn, M., Bowes, L., David, S. (2004).
[51] Gourlay, S.G., Stead, L. F., Benowitz, N. L. (2004). 'Clonidine for Smoking Cessation'. Cochrane Database Syst Rev. (3): p. CD000058.
[52] USPHS, (2000).

1.1.3.3 Combined Therapies

The most widely used combination therapies for smoking cessation have combined bupropion with NRT and NRT and/or bupropion with intensive counselling. Even so, only two clinical trials have been conducted comparing bupropion plus NRT to NRT alone or to placebo, and whilst one trial found that bupropion plus NRT had greater efficacy than NRT alone,[53] a second study found no difference.[54] Given that most clinical trials combine counselling of some level with pharmacological therapy, and placebo, in part for logical ethical reasons, sufficient data are not available to compare any therapy plus counselling to placebo without counselling. That being stated, the highest reported abstinence rates in the literature have combined bupropion with NRT and intensive counselling.[55]

1.1.3.4 Limitations of Current Therapies

The highest sustained smoking cessation rates with the best available therapies do not exceed 35%.[56] Yudkin and colleagues followed up smokers who participated in a clinical trial of nicotine patches eight years after the trial. Less than 10% of those who attempted to quit on the nicotine patch or placebo patch remained abstinent.[57] Studies of bupropion and other pharmacological therapies with more than one year of follow-up have not been reported in the published literature. Even so, the long-term efficacy of the state-of-the-art therapies is quite limited when one compares them to therapies for depression,[58]

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[53] Jorenby, D.E., Leischow, S. J., Nides, M. A., Rennard, S. I., Johnston, J. A., Hughes, A. R., Smith, S. S., Muramoto, M. L., Daughton, D. M., Doan, K., Fiore, M. C., Baker, T. B. (1999).

[54] Simon, J.A., Duncan, C., Carmody, T.P., Hudes, E.S. (2002). 'Bupropion for Smoking Cessation: A randomized Trial', paper presented at the National Conference on Tobacco or Health in San Francisco, CA, U.S.A.

[55] Jorenby, D.E., Leischow, S. J., Nides, M. A., Rennard, S. I., Johnston, J. A., Hughes, A. R., Smith, S. S., Muramoto, M. L., Daughton, D. M., Doan, K., Fiore, M. C., Baker, T. B. (1999).

[56] USPH, (2000).

[57] Yudkin, P., Hey, K., Roberts, S., Welch, S., Murphy, M., Walton, R. (2003).

[58] Geddes, J.R., Freemantle, N., Mason, J., Eccles, M. P., Boynton, J. (2000). 'SSRIs Versus Other Antidepressants for Depressive Disorder'. Cochrane Database Syst Rev. (2): p. CD001851.

hypertension,[59] hypercholesterolemia,[60] or other chronic diseases or behaviours. Given that tobacco use is the leading cause of preventable death and results in millions of premature deaths each year, quite clearly it is imperative that the scientific community advances its knowledge base of the complex neurological pharmacology of nicotine dependence.

1.2 Neuropharmacology of Nicotine Addiction

1.2.1 Nicotine Pharmacokinetics and Pharmacodynamics

The empirical study of the pharmacokinetics and pharmacodynamics of nicotine dates back to at least 1876 when Lautenbach observed that the toxic effects of nicotine on dogs were reduced after passage through the liver.[61] However, the systematic study of nicotine pharmacology began in earnest in the 1940s with the work of Larson and Haag[62] and Werle and Uschold[63] who conducted the seminal studies in nicotine metabolism and toxicology. Over the next six decades, the pharmacology of nicotine has been comprehensively studied and described. Whilst it is plausible that other constituents of tobacco and tobacco smoke contribute to nicotine dependence,[64] nicotine is considered the primary psychoactive substance in tobacco. Therefore, this discussion will focus primarily on nicotine and its metabolites.

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[59] Doulton, T.W., He, F. J., MacGregor, G. A. (2005). 'Systematic Review of Combined Angiotensin-Converting Enzyme Inhibition and Angiotensin Receptor Blockade in Hypertension', *Hypertension*, **45**(5): pp. 880-6.

[60] Edwards, J.E., Moore, R. A. (2003). 'Statins in Hypercholesterolaemia: A Dose-Specific Meta-Analysis of Lipid Changes in Randomised, Double Blind Trials', *BMC Fam Pract.* **4**(1): p. 18.

[61] Lautenbach, B.F. (1876). 'On a New Function of the Liver', *Philad Med Times*, 7(7): pp. 387-39.

[62] Larson, P.S., Haag, H.B. (1943). 'Studies on the Fate of Nicotine in the Body III. On the Pharmacology of Some Methylated and Demethylated Derivatives of Nicotine', *J Pharmacol Exp Ther.* **77:** pp. 343-349.

[63] Werle, E., Uschold, E. (1948). 'Ueber Fermentative Nicotinentgiftung durch Tierisches Gewebe', *Biochem Z.* **318**: pp. 531-537.

[64] USPHS (1998). *The Health Consequences of Smoking: Nicotine Addiction: A Report of the Surgeon General* (Rockville, MD, USA: United States Department of Health and Human Services: Public Health Service).

1.2.1.1 Pharmacokinetics

1.2.1.1.1 Route of Administration and Bioavailability

Nicotine is a racemic tertiary amine with a pyridine and a pyrrolidine ring. Tobacco contains only (S)-nicotine, which is the most pharmacologically active isomer and tobacco smoke also contains the less potent (R)-nicotine comprising only approximately 10% of the total nicotine delivered.[65] In addition to nicotine, there are other pharmacologically active alkaloids that make up about 8 to 12% of the total alkaloid content of tobacco products. These alkaloids include nornicotine, anabasine, myosmine, nicotyrine, and antababine. As nicotine is a constituent of a plant, there are variations in nicotine concentrations in different strains of tobacco and tobacco products. The concentrations of nicotine in manufactured cigarettes, moist snuff, and chewing tobacco have been extensively tested and are well described and summarised in Table 1.1 below. Benowitz and colleagues tested 15 brands of cigarettes[66] and determined that, on average, nicotine composed 1.5% of the tobacco in cigarettes by weight, the concentration of nicotine was 15.7 mg/g, and the typical dose (one cigarette) was 8.4 mg. The route of administration of nicotine is most commonly through inhalation of tobacco smoke but is also through mucous membranes of the oral and nasal mucosa of chewing tobacco and snuff users. In tobacco smoke nicotine is distilled and carried principally on tar droplets (mass median diameter 0.3 to 0.5 μm)[67] and in the vapour phase, which are inhaled.

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[65] Pool, W.F., Godin, C.S., Crooks, P.A. (1985). 'Nicotine Racemization During Cigarette Smoking (Abstract)', *Toxicologist*, **5**: p. 232.
[66] Benowitz, N.L., Hall, S. M., Herning, R. I., Jacob, P., 3rd, Jones, R. T., Osman, A. L. (1983). 'Smokers of Low-Yield Cigarettes Do Not Consume Less Nicotine', *N Engl J Med*, **309**(3): pp. 139-42.
[67] USPHS, (1988).

Table 1.1

Nicotine Content of Tobacco Products

Product	Number of brands tested	Concentration of nicotine (mg/g tobacco)	Typical single dose (g tobacco)	Nicotine in single dose (mg)	Nicotine in dose typically consumed/day
Cigarettes[68]	15	15.7 (13.3-26.9)	0.54	8.4	168 mg/ 20 cigs
Moist snuff[69]	8	10.5 (6.1-16.6)	1.4	14.5	157 mg/15 g
Chewing tobacco[70]	2	16.8 (9.1-24.5)	7.9	133	1,176 mg/70 g

Legend: Single dose refers to a cigarette or an amount of smokeless tobacco placed in the mouth.[71]

1.2.1.1.2 Absorption and Systemic Availability

The absorption of nicotine across mucous membranes in the oropharynx and lungs is highly dependent on pH. Nicotine is a weak base (pKa = 8.0) and therefore in physiological environments with a pH of 8.0, 50% of the nicotine is ionised. The nicotine in typical puffs of flue-cured tobacco smoke ranges from 6.0 to 5.5[72] and therefore, at these pHs, the nicotine is almost entirely ionised. Thus, there is little absorption across the buccal mucosa when cigarette smoke is held in the mouth.[73] When tobacco smoke from flue-cured cigarettes reaches the small airways and alveoli the nicotine is rapidly absorbed because of the large

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[68] Ibid.

[69] Gritz, E.R., Baer-Weiss, V., Benowitz, N. L., Van Vunakis, H., Jarvik, M. E. (1981). 'Plasma Nicotine and Cotinine Concentrations in Habitual Smokeless Tobacco Users', *Clin Pharmacol Ther.* **30**(2): pp. 201-9; Kozlowski, L.T. (1981). 'The Determinants of Tobacco Use: Cigarette Smoking in the Context of Other Forms of Tobacco Use', *Can J Public Health*, **72**(6): pp. 396-401.

[70] Gritz, E.R., Baer-Weiss, V., Benowitz, N. L., Van Vunakis, H., Jarvik, M. E. (1981); Benowitz, N.L., Porchet, H., Sheiner, L., Jacob, P., 3rd, (1988). 'Nicotine Absorption and Cardiovascular Effects with Smokeless Tobacco Use: Comparison with Cigarettes and Nicotine Gum', *Clin Pharmacol Ther.* **44**(1): pp. 23-8.

[71] Adapted from Table 2 in Chapter 2 of USPHS (1988).

[72] Brunnemann, K.D., Hoffmann, D. (1974). 'The pH of Tobacco Smoke', *Food Cosmet Toxicol.* **12**(1): pp. 115-24.

[73] Gori, G.B., Benowitz, N. L., Lynch, C. J. (1986). 'Mouth Versus Deep Airways Absorption of Nicotine in Cigarette Smokers', *Pharmacol Biochem Behav.* **25**(6): pp. 1181-4.

surface area and the dissolution of nicotine at a pH of 7.4.[74] However, with air-cured tobaccos used in cigars and pipes the pH is higher with progressive inhalations and, in alkaline environments, most of the nicotine is non-ionised and is readily absorbed by the mucous membranes. When nicotine is absorbed across biological membranes, plasma concentrations of nicotine rise rapidly, reaching peak concentrations in minutes. The brain/blood ratio as demonstrated in rats injected with [^{14}C] nicotine is about 4.6 ± 0.07, suggesting a preferential partitioning of nicotine from peripheral circulation to the brain.[75] As seen in figure (1.1), the absorption of nicotine with oral snuff, chewing tobacco, and nicotine gum is more gradual. The higher pH of nicotine in these preparations permits absorption not only in the oral mucosa but also in the small intestine.

1.2.1.1.3 Distribution

Once nicotine is absorbed into the blood it is rapidly distributed through the arterial circulation with a steady state volume of distribution of 180 litres. Nicotine has a relatively high affinity for brain, kidney, lung, heart, and liver and low affinity for muscle and adipose tissue. Uptake of nicotine into the brain is rapid, reaching peak levels in 1 to 2 minutes as demonstrated with [^{11}C or ^{14}C] nicotine in mice,[76] monkeys,[77] and humans.[78]

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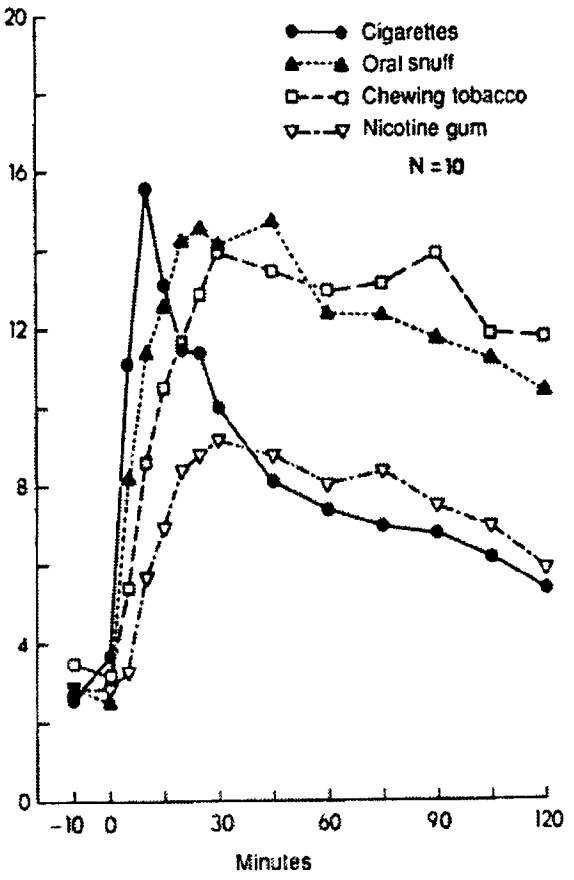
[74] USPHS, (1988).
[75] Ghosheh, O.A., Dwoskin, L. P., Miller, D. K., Crooks, P. A. (2001).
[76] Stalhandske, T. (1970). 'Effects of Increased Liver Metabolism of Nicotine on its Uptake, Elimination and Toxicity in Mice', *Acta Physiol Scand.* **80**(2): pp. 222-34.
[77] Maziere, M., Comar, D., Marazano, C., Berger, G. (1976). 'Nicotine-11C: Synthesis and Distribution Kinetics in Animals', *Eur J Nucl Med.* **1**(4): pp. 255-8.
[78] Halldin, C., Nagren, K., Swahn, C. G., Langstrom, B., Nyback, H. (1992). '(S)- and (R)-[11C]Nicotine and the Metabolite (R/S)-[11C]Cotinine. Preparation, Metabolite Studies and *In Vivo* Distribution in the Human Brain Using PET', *Int J Rad Appl Instrum B.* **19**(8): pp. 871-80.

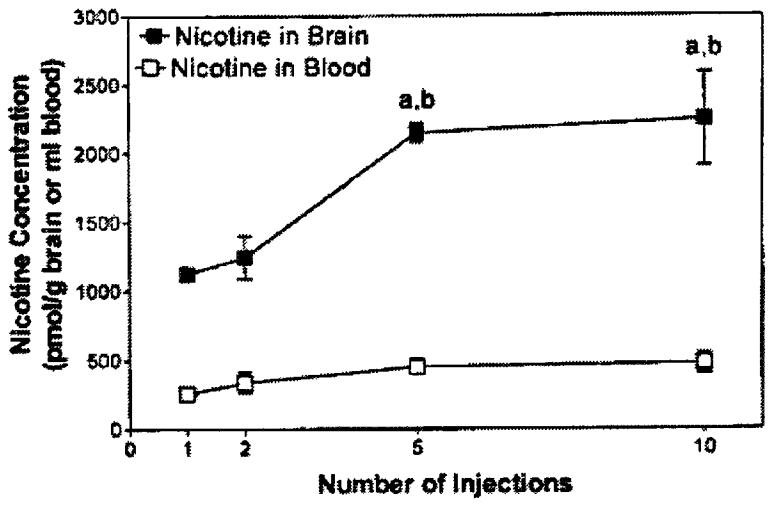
Figure 1.1

Bioavailability of Nicotine in Blood and Brain

A. *Blood nicotine concentrations (venous)*



B. *Nicotine concentrations in brain compared to venous blood during and after nicotine administration*



LEGEND: (A) Venous blood concentrations during and after smoking cigarettes (1 1/3 cigs), using oral snuff (2.5 g), using chewing tobacco (ave. 7.9 g), and nicotine gum (two 2 mg pieces) in humans. (B) Nicotine concentrations in brain and venous blood after 1-10 peripheral injections of nicotine in rats. [79]

The nicotine inhaled in tobacco smoke reaches the brain rapidly as it is absorbed instantly into the arterial circulation through the lung and readily crosses the blood-brain barrier. The distribution half-life of nicotine is 9 minutes. Thus, as nicotine reaches peak concentrations in the brain rapidly it is also re-distributed in a relatively brief period of time to other tissue

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[79] Benowitz, N.L., Porchet, H., Sheiner, L., Jacob, P., 3rd. 'Nicotine absorption and cardiovascular effects with smokeless tobacco use: comparison with cigarettes and nicotine gum' *Clin Pharmacol Ther.* **44**(1): p. 23-8; Ghosheh, O.A., Dwoskin, L. P., Miller, D. K., Crooks, P. A. (2001). 'Accumulation of nicotine and its metabolites in rat brain after intermittent or continuous peripheral administration of [2'-(14)C]nicotine' *Drug Metab Dispos.* 2001. **29**(5): p. 645-51.

compartments. Moreover, nicotine is secreted into saliva, providing a route for recirculation through the portal circulation.

1.2.1.1.4 Metabolism

Nicotine is extensively and rapidly metabolised by the liver but also in the lung and kidney to a lesser extent.[80] Approximately 85-90% of nicotine is hepatically metabolised with about 70% of nicotine being extracted from the blood with each pass through the liver.[81] Six primary metabolites of nicotine have been identified. The most important metabolite of nicotine is cotinine; 70-80% of nicotine is converted to cotinine in a two-step process.[82] First, mediated by the CYP450 system (predominantly by CYP2A6),[83] nicotine is converted to nicotine-$\Delta^{1'(5')}$-iminium ion, which is in equilibrium with 5'-hydroxynicotine.[84] In the second step, the nicotine-$\Delta^{1'(5')}$-iminium ion is converted to cotinine by a cytoplasmic aldehyde oxidase.[85] About 4-7% of nicotine is metabolised to nicotine *N'*-oxide,[86] which is almost entirely excreted in the liver but may be recycled back to nicotine by bacteria in the large intestine. Nicotine and its primary metabolites are shown in figure 1.2.

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[80] Gorrod, J.W., Jenner, P. (1975). 'The Metabolism of Tobacco Alkaloids', in *Essays in Toxicology* (New York: Academic), pp. 35-78.

[81] Benowitz, N.L., Jacob, P., 3rd, Jones, R. T., Rosenberg, J. (1982). 'Interindividual Variability in the Metabolism and Cardiovascular Effects of Nicotine in Man', *J Pharmacol Exp Ther.* **221**(2): pp. 368-72.

[82] Benowitz, N.L., Jacob, P., 3rd, (1994). 'Metabolism of Nicotine to Cotinine Studied by a Dual Stable Isotope Method', *Clin Pharmacol Ther.* **56**(5): pp. 483-93.

[83] Cashman, J.R., Park, S. B., Yang, Z. C., Wrighton, S. A., Jacob, P., 3rd, Benowitz, N. L. (1992). 'Metabolism of Nicotine by Human Liver Microsomes: Stereoselective Formation of Trans-Nicotine N'-Oxide', *Chem Res Toxicol.* **5**(5): pp. 639-46; Berkman, C.E., Park, S. B., Wrighton, S. A., Cashman, J. R. (1995). '*In Vitro-In Vivo* Correlations of Human (S)-Nicotine Metabolism', *Biochem Pharmacol.* **50**(4): pp. 565-70; Nakajima, M., Yamamoto, T., Nunoya, K., Yokoi, T., Nagashima, K., Inoue, K., Funae, Y., Shimada, N., Kamataki, T., Kuroiwa, Y. (1996). 'Role of Human Cytochrome P4502A6 in C-Oxidation of Nicotine', *Drug Metab Dispos.* **24**(11): pp. 1212-7.

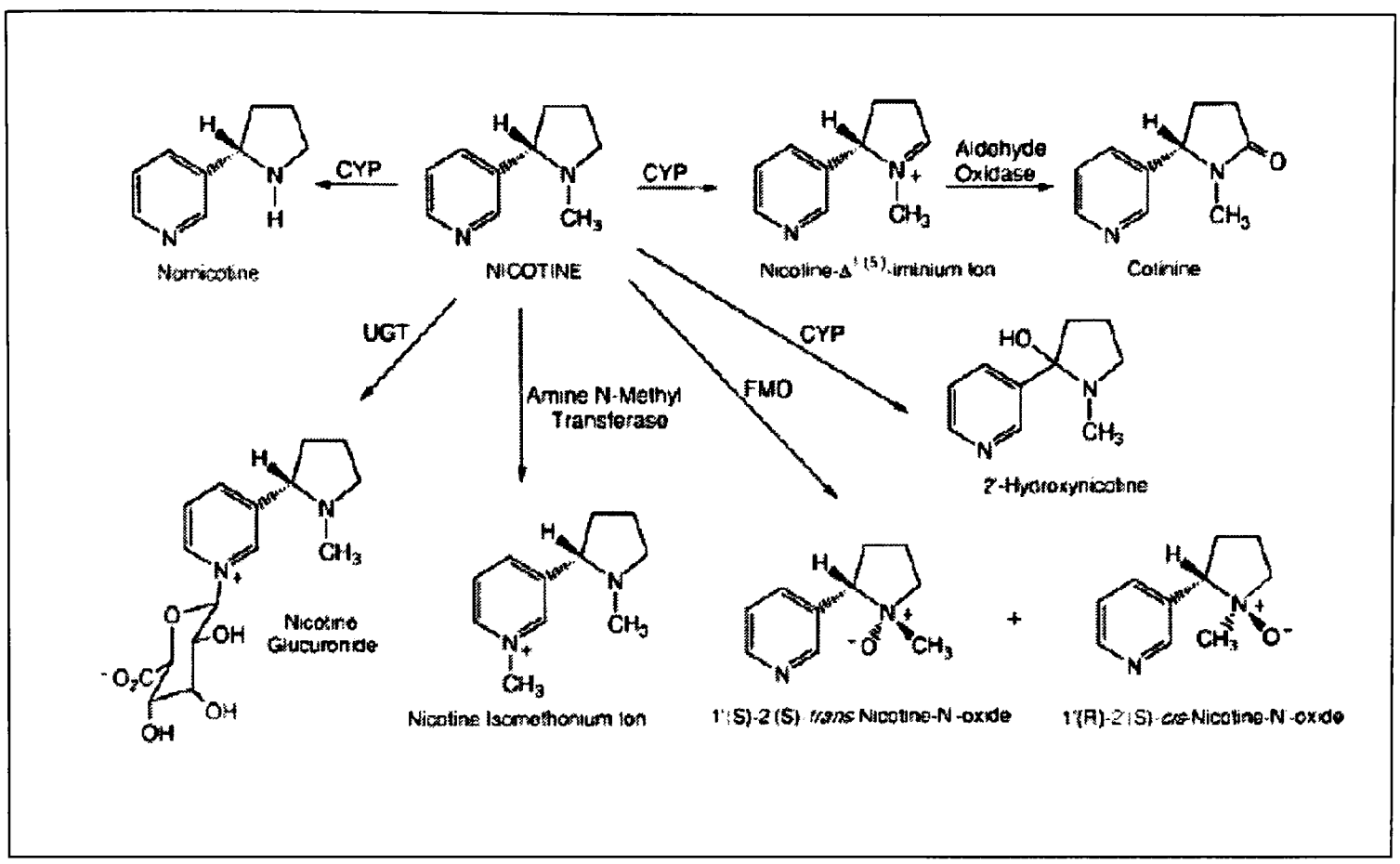
[84] Murphy, P.J. (1973). 'Enzymatic Oxidation of Nicotine to Nicotine 1'(5') Iminium Ion. A Newly Discovered Intermediate in the Metabolism of Nicotine', *J Biol Chem.* **248**(8): pp. 2796-800; Brandange, S., Lindblom, L. (1979). 'The Enzyme "Aldehyde Oxidase" is an Iminium Oxidase. Reaction with Nicotine Delta 1'(5') Iminium Ion', *Biochem Biophys Res Commun.* **91**(3): pp. 991-6; Gorrod, J.W., Hibberd, A. R. (1982). 'The Metabolism of Nicotine-Delta 1'(5')-Iminium Ion, *In Vivo* and *In Vitro*', *Eur J Drug Metab Pharmacokinet.* **7**(4): pp. 293-8.

[85] Brandange, S., Lindblom, L. (1979); Gorrod, J.W., Hibberd, A. R. (1982).

[86] Benowitz, N.L., Jacob, P., 3rd, (1994); Byrd, G.D., Chang, K. M., Greene, J. M., deBethizy, J. D. (1992). 'Evidence for Urinary Excretion of Glucuronide Conjugates of Nicotine, Cotinine, and Trans-3'-Hydroxycotinine in Smokers', *Drug Metab Dispos.* **20**(2): pp. 192-7.

Figure 1.2

Nicotine and Primary Metabolites



LEGEND: Primary routes of nicotine metabolism with illustrations of the chemical composition of major metabolites of nicotine.[87] CYP = Cytochrome P450. FMO = flavin-containing monooxygenase. UGT = uridine diphosphate-glucuronosyltransferase.

In addition, there are two non-oxidative pathways involved in nicotine metabolism. These pathways include the methylation of the pyridine nitrogen to produce nicotine isomethonium ion (also known as *N*-methylnicotinium ion) catalysed by *N*-methyltransferase and glucuronidation catalysed by uridine diphosphate-glucuronosyltransferase (UGT)[88] to an *N*-quaternary glucuronide (*S*)-nicotine-*N*-*β*-glucuronide.[89] The rate of nicotine metabolism is

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[87] Illustrations from Figure 6 in: Hukkanen, J., Jacob, P., 3rd, Benowitz, N. L. (2005). 'Metabolism and Disposition Kinetics of Nicotine', Pharmacol Rev. **57**(1): pp. 79-115.

[88] Byrd, G.D., Chang, K. M., Greene, J. M., deBethizy, J. D. (1992); Nwosu, C.G., Crooks, P. A. (1988). 'Species Variation and Stereoselectivity in the Metabolism of Nicotine Enantiomers', *Xenobiotica*, **18**(12): pp. 1361-72

[89] Seaton, M.J., Vesell, E. S., Luo, H., Hawes, E. M. (1993). 'Identification of Radiolabeled Metabolites of Nicotine in Rat Bile. Synthesis of S-(-)-Nicotine N-Glucuronide and Direct Separation of Nicotine-Derived Conjugates Using High-Performance Liquid Chromatography', *J Chromatogr.* **621**(1): pp. 49-53

highly variable between populations[90] and under the influence of genetic[91] and environmental factors.[92] Nicotine is also oxidated to form nornicotine by the cytochrome P450 system and to 2' hyroxycotinine as an intermediary step to the production of 4-oxo-4-(3-pyridyl) butanoic acid and 4-hydroxy-4-(3-pyridyl) butanoic acid.

1.2.1.1.5 Excretion

Between 10 and 15% of the nicotine absorbed by smokers is excreted as unchanged cotinine in the urine. Cotinine is further metabolised to six different metabolites, which are all excreted in the urine. The most common cotinine metabolites excreted in the urine are 3'hydroxycotinine and its glucoronide conjugate, accounting for 40-60% of urinary excretion.[93] The remaining metabolites of nicotine and cotinine and un-metabolised nicotine (~10%) account for the remaining fractions of urinary excretion. Renal excretion of nicotine and its metabolites is influenced by the pH of the urine and the urine flow rates. However, as only about 10% of nicotine is renally excreted, variations in urinary nicotine levels due to perturbations in urine pH and flow rates do not correlate well with nicotine intake. Rather, urinary cotinine concentration, which is less influenced by urine flow rate and pH correlates well with blood cotinine levels.[94] Because of the much longer half-life of cotinine, blood,

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[90] Benowitz, N.L., Perez-Stable, E. J., Fong, I., Modin, G., Herrera, B., Jacob, P., 3rd, (1999). 'Ethnic Differences in N-Glucuronidation of Nicotine and Cotinine', *J Pharmacol Exp Ther.* **291**(3): pp. 1196-203

[91] Oscarson, M., McLellan, R. A., Asp, V., Ledesma, M. and M.L. Ruiz, Sinues, B., Rautio, A., Ingelman-Sundberg, M. (2002). 'Characterization of a Novel CYP2A7/CYP2A6 Hybrid Allele (CYP2A6*12) that Causes Reduced CYP2A6 Activity', *Hum Mutat.* **20**(4): pp. 275-83; Pianezza, M.L, Sellers, E. M, Tyndale, R.F. (1998). 'A Common Genetic Defect in Nicotine Metabolism Defect Reduces Smoking', *Nature* **393**(6687): p. 750; Swan, G.E., Benowitz, N. L., Lessov, C. N., Jacob, P., 3rd, Tyndale, R. F., Wilhelmsen, K. (2005). 'Nicotine Metabolism: The Impact of CYP2A6 on Estimates of Additive Genetic Influence', *Pharmacogenet Genomics*, **15**(2): pp. 115-25

[92] Schoedel, K.A., Sellers, E. M., Palmour, R., Tyndale, R. F. (2003). 'Down-Regulation of Hepatic Nicotine Metabolism and a CYP2A6-Like Enzyme in African Green Monkeys after Long-Term Nicotine Administration', *Mol Pharmacol.* **63**(1): pp. 96-104

[93] Ibid.

[94] Haley, N.J., Axelrad, C. M., Tilton, K. A. (1983). 'Validation of Self-Reported Smoking Behavior: Biochemical Analyses of Cotinine and Thiocyanate', *Am J Public Health*, **73**(10): pp. 1204-7; Jarvis, M., Tunstall-Pedoe, H., Feyerabend, C., Vesey, C., Salloojee, Y. (1984). 'Biochemical Markers of Smoke

urine, or salivary cotinine are useful indices of average nicotine intake over several days or weeks and are commonly utilised in longitudinal studies of cigarette smoking. Table 1.2 provides a recent overview of the pharmacokinetics of nicotine and cotinine.

Table 1.2

Pharmacokinetic Properties of (S)-Nicotine and (S)-Cotinine

Pharmacological Property	Nicotine	Cotinine
Half-life	100-150 min	770-1130 min
Volume of distribution	2.2-3.3 L/kg	0.69-0.93 L/kg
Total clearance	1,110-1,500 mL/min	42-55 mL/min
Renal clearance	35-200 mL/min	3-12 mL/min
Non-renal clearance	1,050-1,460 mL/min	36-60 mL/min

LEGEND: Published estimates of pharmacokinetic properties of the pharmacologically active isomer of nicotine (S-nicotine) and its major metabolite S-cotinine in humans.[95] Note the relatively short half-life of nicotine compared to cotinine.

Nicotine has a half-life of approximately two hours (range: 100 to 150 minutes) and brain nicotine levels rise and fall rapidly. Thus, frequent dosing throughout the day is required to maintain a level of nicotine blood concentration that is satisfying for dependent smokers. Benowitz and colleagues have determined that blood nicotine levels accumulate over six to eight hours to peak, steady-state plasma levels, and if a smoker smokes until bedtime, significant levels persist throughout the night.[96]

Absorption and Self Reported Exposure to Passive Smoking', *J Epidemiol Community Health*, **38**(4): pp. 335-9.

[95] USPHS, (1998); Hukkanen, J., Jacob, P., 3rd, Benowitz, N. L. (2005); Benowitz, N.L., Kuyt, F., Jacob, P., 3rd, (1982). 'Circadian Blood Nicotine Concentrations During Cigarette Smoking', *Clin Pharmacol Ther.* **32**(6): pp. 758-64; Benowitz, N.L., Kuyt, F., Jacob, P., 3rd, Jones, R. T., Osman, A. L. (1983). 'Cotinine Disposition and Effects', *Clin Pharmacol Ther.* **34**(5): pp. 604-11.

[96] Benowitz, N.L., Kuyt, F., Jacob, P., 3rd, (1982).

1.2.1.2 Pharmacodynamics

1.2.1.2.1 Pharmacological Actions of Nicotine

The pharmacological effects of cigarette smoking, both centrally and peripherally, are complex and involve multiple organ systems and interactions between numerous neurotransmitter systems and brain regions. Whilst I will focus primarily on central effects of nicotine (the primary pharmacologically active chemical in tobacco smoke), many of the peripheral effects are centrally mediated and will be discussed briefly. As previously described, nicotine is rapidly absorbed into the arterial system from the lungs and crosses the blood-brain barriers within seconds, reaching peak levels in one to two minutes.

Nicotine binds to nicotinic acetylcholine receptors, which are distributed ubiquitously throughout the brain. Nicotinic acetylcholine receptors (nAChRs) are pentameric receptor complexes that serve ligand-gated ion channels. To date, 12 different subunits of central nAChRs have been identified consisting of α2-α10 and β2-β4.[97] However, centrally, nAChRs are predominantly composed of α4β2 and α7 homomeric subunits located predominantly in the mesocorticolimbic system, cortex, and hippocampus.[98] The structure and function of central nAChRs are described in more detail in Section 1.2.1.3.1.

The pharmacodynamic effects of nicotine in the central nervous system can be widely grouped into acute effects and chronic effects resulting in tolerance. *In vitro* studies have examined nicotine's acute and chronic effects on multiple brain regions and indicate that mesocorticolimbic dopaminergic pathways originating from the midbrain ventral tegmental

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[97] McGehee, D.S., Role, L. W. (1995). 'Physiological Diversity of Nicotinic Acetylcholine Receptors Expressed by Vertebrate Neurons', *Annu Rev Physiol.* **57**: pp. 521-46; Jones, S., Sudweeks, S., Yakel, J. L. (1999). 'Nicotinic Receptors in the Brain: Correlating Physiology with Function', *Trends Neurosci.* **22**(12): pp. 555-61; Colquhoun, L.M., Patrick, J. W. (1997), 'Pharmacology of Neuronal Nicotinic Acetylcholine Receptor Subtypes', *Adv Pharmacol.* **39**: pp. 191-220.

[98] Jones, S., Sudweeks, S., Yakel, J. L. (1999).

area (VTA) are predominantly important in reward signalling for nicotine[99] and other drugs of abuse including cocaine,[100] alcohol,[101] and opiates.[102] In addition to dopaminergic pathways, multiple neurotransmitter systems contribute to and interact with the mesolimbic DA network in the pharmacological actions of acute and chronic nicotine administration.[103]

A series of rodent studies with experimenter-administered nicotine have demonstrated that nicotine alters the mesolimbic DA system. Di Chiara and Imperato demonstrated that subcutaneous nicotine administration in freely moving rats increased synaptic DA concentrations by 100% in nucleus accumbens dialysates and stimulated increased locomotion, rearing, and grooming.[104] Mifsud and colleagues observed that nicotine administration into the nucleus accumbens (NAc) itself through microdialysis tubes increased synaptic DA in a dose-dependent manner within the NAc and that this pharmacodynamic effect was blocked by administration of the nAChR antagonist

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[99] Corrigall, W.A., Franklin, K. B., Coen, K. M., Clarke, P. B. (1992). 'The Mesolimbic Dopaminergic System is Implicated in the Reinforcing Effects of Nicotine', *Psychopharmacology* (Berl), **107**(2-3): pp. 285-9; Corrigall, W.A., Coen, K. M., Adamson, K. L., Chow, B. L., Zhang, J. (2000). 'Response of Nicotine Self-Administration in the Rat to Manipulations of Mu-Opioid and Gamma-Aminobutyric Acid Receptors in the Ventral Tegmental Area', *Psychopharmacology* (Berl), **149**(2): pp. 107-14; Robinson, T.E., Berridge, K. C. (1993). 'The Neural Basis of Drug Craving: An Incentive-Sensitization Theory of Addiction', *Brain Res Brain Res Rev.* **18**(3): pp. 247-91; Kalivas, P.W. and Stewart, J. (1991). 'Dopamine Transmission in the Initiation and Expression of Drug- and Stress-Induced Sensitization of Motor Activity', *Brain Res Brain Res Rev.* **16**(3): pp. 223-44; Benwell, M.E., Balfour, D. J. (1992). 'The Effects of Acute and Repeated Nicotine Treatment on Nucleus Accumbens Dopamine and Locomotor Activity', *Br J Pharmacol.* **105**(4): pp. 849-56.

[100] Phillips, P.E., Stuber, G. D., Heien, M. L., Wightman, R. M., Carelli, R. M. (2003). 'Subsecond Dopamine Release Promotes Cocaine Seeking', *Nature*, **422**(6932): pp. 614-8.

[101] Diana, M., Brodie, M., Muntoni, A., Puddu, M. C., Pillolla, G., Steffensen, S., Spiga, S., Little, H. J. (2003). 'Enduring Effects of Chronic Ethanol in the CNS: Basis for Alcoholism', *Alcohol Clin Exp Res.* **27**(2): pp. 354-61.

[102] Nader, K., van der Kooy, D. (1997). 'Deprivation State Switches the Neurobiological Substrates Mediating Opiate Reward in the Ventral Tegmental Area', *J Neurosci.* **17**(1): pp. 383-90; Carlezon, W.A., Jr., Haile, C. N., Coppersmith, R., Hayashi, Y., Malinow, R., Neve, R. L., Nestler, E. J. (2000). 'Distinct Sites of Opiate Reward and Aversion within the Midbrain Identified Using a Herpes Simplex Virus Vector Expressing GluR1', *J Neurosci.* **20**(5): pp. RC62.

[103] Robinson, T.E., Berridge, K. C. (2001).

[104] Di Chiara, G., Imperato, A. (1988). 'Drugs Abused by Humans Preferentially Increase Synaptic Dopamine Concentrations in the Mesolimbic System of Freely Moving Rats', *Proc Natl Acad Sci U S A*, **85**(14): pp. 5274-8; Imperato, A., Mulas, A., Di Chiara, G. (1986). 'Nicotine Preferentially Stimulates Dopamine Release in the Limbic System of Freely Moving Rats', *Eur J Pharmacol.* **132**(2-3): pp. 337-8.

mecamylamine.[105] Furthermore, Benwell and Balfour demonstrated that acute administration of nicotine subcutaneously resulted in dose-dependent increases in locomotor activity and in concentrations of the metabolites of dopamine dihyrdroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) in the NAc and that pre-treatment with nicotine resulted in significant increases in basal levels of DA in the NAc 24 hours later. Moreover, pre-treatment with nicotine for five days enhanced the effect of nicotine on spontaneous locomotor activity and significantly increased extracellular DA in the NAc.[106]

Additional studies in rodents have suggested that nicotine self-administration alters the mesolimbic DA system, in part, through stimulation of nAChRs in the ventral tegmental area. First, Corrigall and colleagues produced lesions in the mesolimbic DA system via microinfusion of 6-hydroxydopamine into the NAc of rats who were trained to self-administer nicotine.[107] Self-administration of nicotine was dramatically reduced during the three-week follow-up period. Postmortem brain analyses indicated that there was a decrease in DA content of the NAc, other regions of the striatum, and the olfactory tubercle. Next, these investigators applied microinfusions of the nAChR antagonist dihyrdo-beta-erythroidine (DH-β-E) into the ventral tegmental area (VTA) of rats prior to initiation of intravenous (IV) nicotine self-administration. [108] Nicotine self-administration was significantly reduced. However, DH-β-E infusions into the NAc did not affect nicotine self-administration or spontaneous locomotor activity. Furthermore, lesions applied to the

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[105] Mifsud, J.C., Hernandez, L., Hoebel, B. G. (1989). 'Nicotine Infused into the Nucleus Accumbens Increases Synaptic Dopamine as Measured by In Vivo Microdialysis', *Brain Res.* **478**(2): pp. 365-7.
[106] Benwell, M.E., Balfour, D. J. (1992).
[107] Corrigall, W.A., Franklin, K. B., Coen, K. M., Clarke, P. B. (1992).
[108] Corrigall, W.A., Coen, K. M., Adamson, K. L. (1994). 'Self-Administered Nicotine Activates the Mesolimbic Dopamine System through the Ventral Tegmental Area', *Brain Res.* **653**(1-2): pp. 278-84.

tegmental pedunculopontine nucleus (TPP), which supplies afferent cholinergic fibres to the VTA, had no effect on nicotine self-administration.

The conclusions from these and other investigator-administered and nicotine self-administration studies were that nicotine binds to nAChRs in the VTA specifically and the effect of nicotine in the VTA is the activation of dopaminergic neurones[109] which mediate DA release in the NAc[110] and stimulate locomotor activity in rats.[111] The A10 mesolimbic DA system also consists of ascending projections to the prefrontal cortex, a region that is important in reward signalling[112] and will be discussed in Section 1.2.1.3.2. In addition to dopaminergic (DA) neurones, which express mainly α2-α7 and β2-β4 subunits,[113] GABA (γ-aminobutyric acid) neurones express nAChRs consisting primarily of α3, α5, α6 and β4 subunits.[114] GABA neurones within the VTA provide inhibitory input to A10 DA neurones and send descending fibres to the TPP. There is limited evidence that nicotine binding to

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[109] Calabresi, P., Lacey, M. G., North, R. A. (1989). 'Nicotinic Excitation of Rat Ventral Tegmental Neurones In Vitro Studied by Intracellular Recording', *Br J Pharmacol.* **98**(1): pp. 135-40; Pidoplichko, V.I., DeBiasi, M., Williams, J. T., Dani, J. A. (1997). 'Nicotine Activates and Desensitizes Midbrain Dopamine Neurons', *Nature*, **390**(6658): pp. 401-4.

[110] Nisell, M., Nomikos, G. G., Svensson, T. H. (1994). 'Systemic Nicotine-Induced Dopamine Release in the Rat Nucleus Accumbens is Regulated by Nicotinic Receptors in the Ventral Tegmental Area', *Synapse.* **16**(1): pp. 36-44.

[111] Reavill, C., Stolerman, I. P. (1990). 'Locomotor Activity in Rats after Administration of Nicotinic Agonists Intracerebrally', *Br J Pharmacol.* **99**(2): pp. 273-8.

[112] Vezina, P., Blanc, G., Glowinski, J., Tassin, J. P. (1992). 'Nicotine and Morphine Differentially Activate Brain Dopamine in Prefrontocortical and Subcortical Terminal Fields: Effects of Acute and Repeated Injections', *J Pharmacol Exp Ther.* **261**(2): pp. 484-90; Volkow, N.D., Fowler, J. S., Ding, Y. S., Wang, G. J., Gatley, S. J. (1999). 'Imaging the Neurochemistry of Nicotine Actions: Studies with Positron Emission Tomography', *Nicotine Tob Res.* **1 Suppl 2**: pp. S127-32; discussion S139-40; Rolls, E.T. (2004). 'Convergence of Sensory Systems in the Orbitofrontal Cortex in Primates and Brain Design for Emotion', *Anat Rec A Discov Mol Cell Evol Biol.* **281**(1): pp. 1212-25.

[113] Charpantier, E., Barneoud, P., Moser, P., Besnard, F., Sgard, F. (1998). 'Nicotinic Acetylcholine Subunit mRNA Expression in Dopaminergic Neurons of the Rat Substantia Nigra and Ventral Tegmental Area', *Neuroreport*, **9**(13): pp. 3097-101; Klink, R., de Kerchove d'Exaerde, A., Zoli, M., Changeux, J. P. (2001). 'Molecular and Physiological Diversity of Nicotinic Acetylcholine Receptors in the Midbrain Dopaminergic Nuclei', *J Neurosci.* **21**(5): pp. 1452-63.

[114] Klink, R., de Kerchove d'Exaerde, A., Zoli, M., Changeux, J. P. (2001).

GABA neurones in the VTA modifies and possibly inhibits the activity of DA neurones.[115] The role of GABA neurones in reward signalling in the VTA and mesopontine region is complex and not completely understood. Ascending excitatory glutamateric and cholinergic neurones in the TPP and laterodorsal tegmental nucleus are sent to the VTA as well as inhibitory GABA neurones. In addition, the VTA receives multiple excitatory glutamatergic inputs from cortical and subcortical brain regions, which synapse on DA and GABA neurones.[116] There is also evidence that NMDA (*N*-methyl-D-aspartate)[117] and mu-opioid receptors[118] contribute to the complex mediation of nicotine reward signalling in the VTA. Furthermore, there is evidence that serotonin (5-HT) inhibits electrophysiological activity via stimulation of 5-HT_2 receptors[119] and may influence locomotor and neurochemical sensitisation to nicotine.[120] Thus, a large corpus of research implicates the VTA as a central locus of stimulation of nAChRs, and that nicotine reward signalling is mediated by a complex interplay of neurotransmitter systems. Nicotine also stimulates central release of serotonin, acetylcholine (ACh) and norepinephrine, which will be described in section 1.2.1.3.

I have primarily discussed the central acute pharmacodynamic effects of nicotine on the VTA. The effects of repetitive nicotine administration lead to a number of neuroadaptions to

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[115] Horvitz, J.C. (2000). 'Mesolimbocortical and Nigrostriatal Dopamine Responses to Salient Non-Reward Events', *Neuroscience*, **96**(4): pp. 651-6.

[116] Kalivas, P.W. (1993). 'Neurotransmitter Regulation of Dopamine Neurons in the Ventral Tegmental Area', *Brain Res Brain Res Rev.* **18**(1): pp. 75-113; Garzon, M., Vaughan, R. A., Uhl, G. R., Kuhar, M. J., Pickel, V. M. (1999). 'Cholinergic Axon Terminals in the Ventral Tegmental Area Target a Subpopulation of Neurons Expressing Low Levels of the Dopamine Transporter', *J Comp Neurol.* **410**(2): pp. 197-210.

[117] Charpantier, E., Barneoud, P., Moser, P., Besnard, F., Sgard, F. (1998).

[118] Corrigall, W.A., Coen, K. M., Adamson, K. L., Chow, B. L., Zhang, J. (2000).

[119] North, R.A., Uchimura, N. (1989). '5-Hydroxytryptamine Acts at 5-HT2 Receptors to Decrease Potassium Conductance in Rat Nucleus Accumbens Neurones', *J Physiol.* **417**: pp. 1-12; Ugedo, L., Grenhoff, J., Svensson, T. H. (1989). 'Ritanserin, a 5-HT2 Receptor Antagonist, Activates Midbrain Dopamine Neurons by Blocking Serotonergic Inhibition', *Psychopharmacology* (Berl), **98**(1): pp. 45-50.

[120] Olausson, P., Engel, J. A., Soderpalm, B. (1999). 'Behavioral Sensitization to Nicotine is Associated with Behavioral Disinhibition; Counteraction by Citalopram', *Psychopharmacology* (Berl), **142**(2): pp. 111-9.

dopaminergic, serotonergic, and other neurotransmitter systems resulting in tolerance. These effects will be discussed in Section 1.2.1.2.3. Furthermore, in the next sections I will provide examples of dose-response relationships that further build the case that nicotine is pharmacologically active via activation of mesolimbic DA systems and interactions with other neurotransmitters.

Whilst not the focus of this thesis, I shall briefly summarise the wide range of systemic effects of nicotine administration because many of the systemic pharmacodynamics are centrally mediated and may contribute to the development of nicotine dependence. Systemically, nAChRs are present primarily in sympathetic and parasympathetic ganglia consisting of the $(\alpha 3)_2(\beta 2)_3$ type, and skeletal neuromuscular junctions consisting of the $(\alpha 1)_2\beta 1\delta\varepsilon$ type.[121] However, many of the systemic effects of low-dose acute nicotine administration result from stimulation of the hypothalamic-pituitary-adrenal (HPA) axis. There is evidence that nicotine activates corticotrophin releasing hormone (CRH)[122] and that nicotine injected intraperitoneally or in the cerebral ventricles of rodents stimulates the rapid release of adrenocorticotropin hormone (ACTH)[123] and these effects are blocked by mecamylamine.[124] As a result of nicotine administration it has been shown in man that, not only does ACTH increase, but that, as expected, cortisol and epinephrine are subsequently

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[121] Cordero-Erausquin, M., Marubio, L. M., Klink, R., Changeux, J. P. (2000). 'Nicotinic Receptor Function: New Perspectives from Knockout Mice', *Trends Pharmacol Sci.* **21**(6): pp. 211-7.

[122] Matta, S.G., Foster, C. A., Sharp, B. M. (1993a). 'Nicotine Stimulates the Expression of cFos Protein in the Parvocellular Paraventricular Nucleus and Brainstem Catecholaminergic Regions', *Endocrinology*, **132**(5): pp. 2149-56; Matta, S.G., Valentine, J. D., Sharp, B. M. (1997). 'Nicotinic Activation of CRH Neurons in Extrahypothalamic Regions of the Rat Brain', *Endocrine*. **7**(2): pp. 245-53.

[123] Matta, S.G., Valentine, J. D., Sharp, B. M. (1997); Matta, S.G., Foster, C. A., Sharp, B. M. (1993b). 'Selective Administration of Nicotine into Catecholaminergic Regions of Rat Brainstem Stimulates Adrenocorticotropin Secretion', *Endocrinology*, **133**(6): pp. 2935-42; Matta, S.G., McAllen, K. M., Sharp, B. M. (1990). 'Role of the Fourth Cerebroventricle in Mediating Rat Plasma ACTH Responses to Intravenous Nicotine', *J Pharmacol Exp Ther*. **252**(2): pp. 623-30; Matta, S.G., Fu, Y., Valentine, J. D., Sharp, B. M. (1998). 'Response of the Hypothalamo-Pituitary-Adrenal Axis to Nicotine', *Psychoneuroendocrinology*, **23**(2): pp. 103-13.

[124] Matta, S.G., Fu, Y., Valentine, J. D., Sharp, B. M. (1998).

and rapidly released from the adrenal cortex.[125] Stimulation of the HPA axis and subsequent systemic release of epinephrine and cortisol are thought to explain the main systemic effects of low-dose nicotine, which include tachycardia, elevated blood pressure and cardiac output, sweating, decreased lower oesophageal pressure resulting in gastroesoesophageal reflux, and a generalised stress response.[126] In addition, nicotine stimulates release of vasopressin[127] and antidiuretic hormone,[128] resulting in decreased renal perfusion and decreased urine flow.

At high doses nicotine may act directly on the peripheral nervous system producing ganglionic stimulation and the release of catecholamines from the adrenral cortex. With high doses or rapid administration nicotine may produce a parasympathomimetic response including bradycardia and hypotension either by vagal stimulation or through centrally mediated actions. As a result, nicotine has been considered a drug with biphasic pharmacodynamics.[129]

1.2.1.2.2 Dose-Response Relationships

As mentioned above, in many cases, the pharmacodynamics of nicotine is biphasic, with different effects at low and high dosages. Moreover, the sensitivity of pharmacodynamic responses can either increase or decrease with repetitive nicotine use and may contribute to

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[125] Mendelson, J.H., Sholar, M. B., Goletiani, N., Siegel, A. J., Mello, N. K. (2005). 'Effects of Low- and High-Nicotine Cigarette Smoking on Mood States and the HPA Axis in Men', *Neuropsychopharmacology* (manuscript in press).

[126] Comroe, J.H., Jr. (1960). 'The Pharmacological Actions of Nicotine', *Ann N Y Acad Sci.* **90**: pp. 48-51; Su, C. (1982). 'Actions of Nicotine and Smoking on Circulation', *Pharmacol Ther.* **17**(1): pp. 129-41; Tugay, M., Yildiz, F., Utkan, Z., Utkan, T., Sarioglu, Y. (2003). 'Impaired Gastric Motility in the Gastroesophageal Reflux Rat Model: An *In Vitro* Study', *J Surg Res.* **115**(2): pp. 272-8; Kadakia, S.C., Kikendall, J. W., Maydonovitch, C., Johnson, L. F. (1995). 'Effect of Cigarette Smoking on Gastroesophageal Reflux Measured by 24-h Ambulatory Esophageal pH Monitoring', *Am J Gastroenterol.* **90**(10): pp. 1785-90; Kadakia, S.C., De La Baume, H. R., Shaffer, R. T. (1996). 'Effects of Transdermal Nicotine on Lower Esophageal Sphincter and Esophageal Motility', *Dig Dis Sci.* **41**(11): pp. 2130-4.

[127] Stalke, J., Hader, O., Bahr, V., Hensen, J., Scherer, G., Oelkers, W. (1992). 'The Role of Vasopressin in the Nicotine-Induced Stimulation of ACTH and Cortisol in Men', *Clin Investig.* **70**(3-4): pp. 218-23.

[128] Legros, J.J., Conte-Devolx, B., Rougon-Rapuzzi, G., Millet, Y., Franchimont, P. (1977). '[Simultaneous Liberation of Vasopressin (ADH) and of Neurophysins During Nicotine Perfusion in Man]', *C R Seances Soc Biol Fil.* **171**(2): pp. 478-83.

[129] Comroe, J.H., Jr. (1960).

tolerance. However, there are some examples of pharmacodynamic responses directly associated with nicotine dose. Mifsud and colleagues observed that nicotine infusion into the NAc produced a dose-dependent increase in NAc extracellular DA.[130] Benwell and Balfour observed that acute nicotine administration led to a dose-dependent increase in the spontaneous activity of rats (infrared beam crossings measured in an activity box) and that nicotine administered 24 hours after the latest dose of nicotine resulted in greater DA concentrations in the NAc with the higher nicotine dose tested (0.4 mg/kg vs. 0.1 mg/kg).[131]

Stolerman and colleagues demonstrated that nicotine produced a dose-dependent increase in locomotor activity in rats pre-treated with nicotine but the opposite effect (dose-dependent decrease) in nicotine-naive rats.[132] Shim and colleagues also observed a dose-dependent increase in NAc DA release and, like Benwell and Balfour, noted evidence of sensitisation, as the increase in NAc release was greater following nicotine pre-treatment.[133] In a study that demonstrated both the rewarding and aversive effects of VTA signalling following nicotine administration, Laviolette and colleagues administered a range of doses of nicotine into the VTA and observed the effect on conditioned place preference (CPP) in trained rats.[134] Using a CPP model, these investigators observed a biphasic pharmacodynamic response to intra-VTA injection of nicotine such that at lower dosages nicotine was reinforcing and produced motivational effects whilst at higher doses nicotine was aversive. Pre-treatment intra-NAc injection of the D_1 and D_2 receptor antagonist α-flupenthixol resulted in a change in valence

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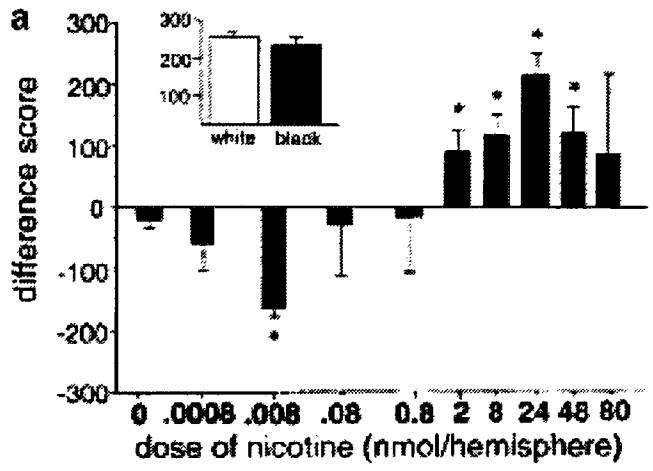
[130] Mifsud, J.C., Hernandez, L., Hoebel, B. G. (1989).
[131] Benwell, M.E., Balfour, D. J. (1992).
[132] Stolerman, I.P., Garcha, H. S., Mirza, N. R. (1995). 'Dissociations Between the Locomotor Stimulant and Depressant Effects of Nicotinic Agonists in Rats', *Psychopharmacology* (Berl), **117**(4): pp. 430-7.
[133] Shim, I., Javaid, J. I., Wirtshafter, D., Jang, S. Y., Shin, K. H., Lee, H. J., Chung, Y. C., Chun, B. G. (2001). 'Nicotine-Induced Behavioral Sensitization is Associated with Extracellular Dopamine Release and Expression of c-Fos in the Striatum and Nucleus Accumbens of the Rat', *Behav Brain Res*. **121**(1-2): pp. 137-47.
[134] Laviolette, S.R., van der Kooy, D. (2003). 'Blockade of Mesolimbic Dopamine Transmission Dramatically Increases Sensitivity to the Rewarding Effects of Nicotine in the Ventral Tegmental Area', *Mol Psychiatry*, **8**(1): pp. 50-9.

of motivational effects such that lower dose intra-VTA nicotine was switched from aversive to rewarding and did not block the reinforcing effects of higher doses of nicotine (Figure 1.3).

As has been mentioned in this section and will be discussed further in subsequent sections, many of the central and peripheral effects of nicotine differ in intensity and valence with different dosages, and pharmacodynamic effects are altered in complex ways ranging from sensitisation to desensitisation.

Figure 1.3

Biphasic Motivational Effects of Nicotine in the Ventral Tegmental Area



LEGEND: Results of conditioned place preference task in trained rats given intra-VTA infusions of nicotine. Scores above the x-axis represent rewarding effects and below represent aversive effects. Intra-NAc infusion of the neuroleptic D_1/D_2 receptor antagonist α-flupenthixol reversed the valence of the pharmacodynamic response at lower doses of nicotine from aversive to reinforcing but did not block the rewarding effects of higher doses of nicotine.

Source of figure.[135]

1.2.1.2.3 Neuroadaptations to Chronic Nicotine Use

There is abundant evidence that tolerance to the reinforcing effects of nicotine develops after days of repetitive use. Functional or pharmacodynamic tolerance has been defined as "where a particular drug concentration at a receptor site produces less effect than it did after a prior exposure".[136] Studies in rodents demonstrate evidence of acute tolerance or

[135] Source of image in Figure 1.3 is: Laviolette, S.R., van der Kooy, D., (2003).
[136] USPHS (1998).

"tachyphylaxis" as well as chronic tolerance resulting from long-term drug administration and subsequent neuroadaptations.

1.2.1.2.3.1 Acute Tolerance

Tachyphylaxis to nicotine has been demonstrated in multiple animal and human studies. Balfour and colleagues observed acute tolerance to the corticosterone response to nicotine in rats[137] and Sharp and colleagues demonstrated acute tolerance to the increased ACTH and prolactin response to nicotine also in rats.[138] Our understanding of the precise mechanisms underlying acute tolerance has evolved dramatically over the last 50 years since Katz and Thesleff observed desensitisation of nAChRs at the motor endplate.[139] Later animal studies have suggested mechanisms by which tolerance develops at the level of central nAChRs and other neurotransmitter receptors.

Central nAChR desensitisation has been studied extensively in multiple species for α4β2 and α7 receptor complexes.[140] Desensitisation as a general term has been described as attenuation or loss of biological response following prolonged or repetitive stimulation.[141] According to the original model proposed by Katz and Thesleff,[142] nAChRs exist in two conformational states, 'R' (resting or activatable) and 'D' (desensitised). Unwin and colleagues, using electron microscopy, have confirmed the model proposed by Katz and

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[137] Benwell, M.E., Balfour, D. J. (1979). 'Effects of Nicotine Administration and Its Withdrawal on Plasma Corticosterone and Brain 5-hHydroxyindoles', *Psychopharmacology* (Berl). **63**(1): pp. 7-11.
[138] Sharp, B.M., Beyer, H. S. (1986). 'Rapid Desensitization of the Acute Stimulatory Effects of Nicotine on Rat Plasma Adrenocorticotropin and Prolactin', *J Pharmacol Exp Ther*. **238**(2): pp. 486-91.
[139] Katz, B., Thesleff, S. (1957). 'A Study of the Desensitization Produced by Acetylcholine at the Motor End-Plate', *J Physiol*. **138**(1): pp. 63-80.
[140] Quick, M.W., Lester, R. A. (2002). 'Desensitization of Neuronal Nicotinic Receptors', *J Neurobiol*. **53**(4): pp. 457-78.
[141] Ochoa, E.L., Chattopadhyay, A., McNamee, M. G. (1989). 'Desensitization of the Nicotinic Acetylcholine Receptor: Molecular Mechanisms and Effect of Modulators', *Cell Mol Neurobiol*. **9**(2): pp. 141-78.
[142] Katz, B., Thesleff, S. (1957).

Thesleff.[143] Furthermore, Lester and Dani, using patch-clamp procedures, observed that with continuous application of nicotine agonists there was an exponential decrease in the number of open nAChRs[144] and with continuous application of nicotine demonstrated progressively decreased current across the nAChRs.[145] Pipoplichko and colleagues[146] and Dani and colleagues[147] observed that the nAChRs present on VTA DA neurones desensitise in seconds to minutes. Finally, Mansvelder and colleagues demonstrated that nAChRs on excitatory glutamatergic neurones desensitise less rapidly than nAChRs on GABA neurones in the VTA.[148] They posed that differential desensitisation of GABA neurones leads to a net shift toward excitation of the mesolimbic DA reward system. Thus, in summary, there is abundant evidence from animal studies that acute tolerance to nicotine occurs centrally.

The work of Mansvelder and colleagues converges with earlier studies in rats in that it provides a mechanism whereby pre-treatment with nicotine would increase mesolimbic DA neurones resulting in DA overflow in the nucleus accumbens shell and promotion of reinforcing effects of tobacco use early in the addiction cycle. In addition, there is evidence from animal studies of tolerance to systemic effects of nicotine. For example, Barrass and colleagues found that administration of even a single intravenous (IV) dose of nicotine increased the LD_{50} (dose lethal to 50% of animals) of nicotine in mice.[149] Moreover, Stolerman and colleagues observed that pre-treatment of rats with nicotine decreased the

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[143] Unwin, N. (2005). 'Refined Structure of the Nicotinic Acetylcholine Receptor at 4A Resolution', *J Mol Biol.* **346**(4): pp. 967-89.
[144] Lester, R.A., Dani, J. A. (1995). 'Acetylcholine Receptor Desensitization Induced by Nicotine in Rat Medial Habenula Neurons', *J Neurophysiol.* **74**(1): pp. 195-206.
[145] Ibid.
[146] Pidoplichko, V.I., DeBiasi, M., Williams, J. T., Dani, J. A. (1997).
[147] Dani, J.A., Radcliffe, K. A., Pidoplichko, V. I. (2000). 'Variations in Desensitization of Nicotinic Acetylcholine Receptors from Hippocampus and Midbrain Dopamine Areas', *Eur J Pharmacol.* **393**(1-3): pp. 31-8.
[148] Mansvelder, H.D., Keath, J. R., McGehee, D. S. (2002). 'Synaptic Mechanisms Underlie Nicotine-Induced Excitability of Brain Reward Areas', *Neuron.* **33**(6): pp 905-19.
[149] Barrass, B.C., Blackburn, J. W., Brimblecombe, R. W., Rich, P. (1969). 'Modification of Nicotine Toxicity by Pretreatment with Different Drugs', *Biochem Pharmacol.* **18**(9): pp. 2145-52.

ED_{50} (effective dose in 50% of animals tested) values – effectively shifting the dose-response curve to the right – for locomotor activity with subsequent doses of nicotine.[150] In addition, Wenzel and colleagues found that pre-treatment with nicotine led to tachyphylaxis of blood pressure elevation in rats.[151]

There is evidence from human studies of subjective, physiological, and behavioural acute tolerance to nicotine,[152] and many investigators have consistently noted wide inter-individual variability in acute sensitivity to nicotine.[153] Moreover, some investigators suggest that individual differences in acute sensitivity to nicotine may affect vulnerability to nicotine dependence.[154] Perkins and colleagues investigated the consistency of acute subjective and physiological responses to nicotine.[155] The subjects were not naive to nicotine but were regular daily smokers who were abstinent overnight. Nicotine was administered via nasal spray at 20 g/kg (three sessions) or placebo (one session) every 30 minutes for four consecutive days. These investigators found that the internal consistency to nicotine's acute pharmacodynamic effects was generally high between subjects for systolic blood pressure,

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[150] Stolerman, I.P., Bunker, P., Jarvik, M. E. (1974). 'Nicotine Tolerance in Rats; Role of Dose and Dose Interval', *Psychopharmacologia*, **34**(4): pp. 317-24.

[151] Wenzel, D.G., Azmeh, N., Clark, I. J. (1971). 'Studies on the Acute and Chronic Depressor Actions of Nicotine in the Rat', *Arch Int Pharmacodyn Ther.* **193**(1): pp. 23-36.

[152] Heishman, S.J., Henningfield, J. E. (2000). 'Tolerance to repeated nicotine administration on performance, subjective, and.physiological responses in nonsmokers', *Psychopharmacology* (Berl), **152**(3): pp. 321-33; Kalman, D. (2002). 'The Subjective Effects of Nicotine: Methodological Issues, a Review of Experimental Studies, and Recommendations for Future Research', *Nicotine Tob Res.* **4**(1): pp. 25-70.

[153] Benowitz, N.L., Kuyt, F., Jacob, P., 3rd, (1982); Nesbitt, P.D. (1973). 'Smoking, Physiological Arousal, and Emotional Response', *J Pers Soc Psychol.* **25**(1): pp. 137-44; Jones, R.A. (1986). 'Individual Differences in Nicotine Sensitivity', *Addict Behav.* **11**(4): pp. 435-8; Perkins, K.A., Gerlach, D., Broge, M., Grobe, J. E., Wilson, A. (2000). 'Greater Sensitivity to Subjective Effects of Nicotine in Nonsmokers High in Sensation Seeking', *Exp Clin Psychopharmacol.* **8**(4): pp. 462-71; Niaura, R., Shadel, W. G., Abrams, D. B., Monti, P. M., Rohsenow, D. J., Sirota, A. (1998).

[154] Pomerleau, O.F. (1995). 'Individual Differences in Sensitivity to Nicotine: Implications for Genetic Research on Nicotine Dependence', *Behav Genet.* **25**(2): pp 161-77; Benowitz, N.L., Pomerleau, O. F., Pomerleau, C. S., Jacob, P., 3rd, (2003). 'Nicotine metabolite ratio as a predictor of cigarette consumption', *Nicotine Tob Res.* **5**(5): pp. 621-4.

[155] Perkins, K.A., Jetton, C., Stolinski, A., Fonte, C., Conklin, C. A. (2003). 'The Consistency of Acute Responses to Nicotine in Humans', *Nicotine Tob Res.* **5**(6): pp. 877-84.

heart rate, head rush, and the Profile of Mood States (POMS)[156] scores including "tension", "confusion", "fatigue" and "vigour". Similar to the Perkins study, many human studies of acute nicotine tolerance include only subjects classified as smokers and do not provide information about sensitisation and desensitisation in individuals naïve to nicotine.[157] Thus, most of our knowledge about acute nicotine tolerance in nicotine-naïve subjects is derived from animal studies.

1.2.1.2.3.2 Chronic Tolerance and Neuroadaptations

The transition from experimentation to nicotine dependence is extremely complex pharmacologically and is the subject of much debate with many competing models. Some of these models will be discussed in detail in Section 1.2.1.3.5. Prior to discussion of these models I will describe some of the animal research and neurotransmitter systems supporting prevalent dependence models. Laviolette and van der Kooy suggest that progressive differences in the balance of α7 and β2-containing nAChRs over time with chronic nicotine use may result in sensitisation of the mesolimbic DA system to nicotine.[158] α7-containing nAChRs may be preferentially involved in the regulation of presynaptic effects of nicotine on glutamate release. α7-containing nAChRs desensitise less rapidly than non α7-containing nAChRs. Glutamate receptors present on A10 DA neurones are excitatory when activated. Moreover, pharmacological or genetic blockade of α7-containing nAChRs has been shown

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[156] McNair, D.M., Loor, J., Droppelman, L.F. (1971). *Profile of Mood States* (San Diego, CA: Educational Testing Service).

[157] Niaura, R., Shadel, W. G., Goldstein, M. G., Hutchinson, K. E., Abrams, D. B. (2001). 'Individual Differences in Responses to the First Cigarette Following Overnight Abstinence in Regular Smokers', *Nicotine Tob Res.* **3**(1): pp. 37-44; Houlihan, M., Pritchard, W., Robinson, J. (2001). 'EEG effects of smoking: Is there tachyphylaxis?' *Neuropsychobiology*, **44**(1): pp. 54-8; Houlihan, M.E., Pritchard, W. S., Robinson, J. H. (2001). Effects of Smoking/Nicotine on Performance and Event-Related Potentials During a Short-Term Memory Scanning Task', *Psychopharmacology* (Berl), **156**(4): pp. 388-96; Perkins, K.A., Fonte, C., Meeker, J., White, W., Wilson, A. (2001). 'The Discriminative Stimulus and Reinforcing Effects of Nicotine in Humans Following Nicotine Pretreatment', *Behav Pharmacol.* **12**(1): pp. 35-44.

[158] Laviolette, S.R., van der Kooy, D. (2004). 'The Neurobiology of Nicotine Addiction: Bridging the Gap from Molecules to Behaviour', *Nat Rev Neurosci.* **5**(1): pp. 55-65.

to block the reinforcing psychomotor stimulant effects of nicotine in the VTA. It has also been demonstrated that β2-containing nAChRs desensitise more rapidly and as they are present on inhibitory GABA neurones in the VTA, this process effectively inhibits GABA neurones. As a result, there would be less inhibition of DA neurones projecting to the NAc, which are predominantly of the α4β2 composition. In fact, there is evidence that stimulation of α7 nicotinic subunits may regulate DA biosynthesis through promotion of expression of tyrosine hydroxylase (the rate-limiting enzyme in the biosynthesis of dopamine) in the VTA. Serova and Sabban observed that systemic administration of the alpha7 nAChR agonist 3-[2,4-dimethoxybenzilidene] anabaseine in rats stimulated increased expression of tyrosine hyroxylase in the substantia nigra.[159] Systemic administration of nicotine itself led to a dose-dependent increase in VTA TH mRNA as well as an increase in expression of GTP cyclohydrolase I, which is the rate limiting step in the biosynthesis of tetrahyrdrobiopterin (an essential cofactor for TH synthesis). Thus, at the transcriptional level in the VTA, there is evidence of interactions between nAChR and DA-related neuroadaptations.

However, studies of knockout (KO) mice suggest that this model may be oversimplified. Changeux and colleagues observed that β2-containing nAChRs are required for nicotine to affect the firing rate of DA neurones *in vitro*[160] and to stimulate striatal DA release *in vitro*[161] and *in vivo*. [162] There is additional *in vivo* evidence in α4 nAChR KO mice that α4 or α6-

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[159] Serova, L., Sabban, E. L. (2002). 'Involvement of Alpha 7 Nicotinic Acetylcholine Receptors in Gene Expression of Dopamine Biosynthetic Enzymes in Rat Brain', *J Pharmacol Exp Ther.* **303**(3): pp. 896-903.
[160] Picciotto, M.R., Zoli, M., Rimondini, R., Lena, C., Marubio, L. M., Pich, E. M., Fuxe, K., Changeux, J. P. (1998). 'Acetylcholine Receptors Containing the Beta2 Subunit Are Involved in the Reinforcing Properties of Nicotine', *Nature*, **391**(6663): pp. 173-7.
[161] Grady, S.R., Meinerz, N. M., Cao, J., Reynolds, A. M., Picciotto, M. R., Changeux, J. P., McIntosh, J. M., Marks, M. J., Collins, A. C. (2001). 'Nicotinic Agonists Stimulate Acetylcholine Release from Mouse Interpeduncular Nucleus: A Function Mediated by a Different nAChR than Dopamine Release from Striatum', *J Neurochem.* **76**(1): pp. 258-68.
[162] Picciotto, M.R., Zoli, M., Rimondini, R., Lena, C., Marubio, L. M., Pich, E. M., Fuxe, K., Changeux, J. P. (1998).

containing nAChRs are required to stimulate burst firing of DA in the NAc.[163] Thus, the finding that α4 and β2 KO mice fail to stimulate DA release in the NAc calls into question whether α7-containing nAChRs play a predominant role in stimulating NAc DA release through indirect glutamatergic activation.[164] Furthermore, Ryan and Loiacono used *in situ* hybridisation to examine the distribution of α7 nAChR subunit mRNA in the brains of rats and found decreased expression of subunit transcript in the VTA following only seven days of chronic nicotine treatment.[165]

However, the role of α7nAChRs appears to be complex as pharmacological blockade with methyllycaconitine (a selective inhibitor of r α7-containing nAChRs) injected into the VTA attenuates the effects of nicotine on DA overflow in the NAc.[166] Thus, whilst the precise mechanism (direct nAChR activation on VTA DA neurones or indirect stimulation via nAChRs on excitatory glutamatergic projections to VTA DA neurones) is not entirely understood it is clear that the presence of nicotine in the VTA stimulates VTA DA neurones that project to the NAc resulting in DA overflow in the nucleus accumbens.[167]

Zahm and Heimer demonstrated, in a series of experiments, that the NAc is composed of subterritories distinct immunohistochemically, an inner core and a surrounding shell, giving

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[163] Role, L.W., Berg, D. K. (1996) 'Nicotinic Receptors in the Development and Modulation of CNS Synapses', *Neuron*. **16**(6): pp. 1077-85; Champtiaux, N., Han, Z. Y., Bessis, A., Rossi, F. M., Zoli, M., Marubio, L., McIntosh, J. M., Changeux, J. P. (2002). 'Distribution and Pharmacology of Alpha 6-Containing Nicotinic Acetylcholine Receptors Analyzed with Mutant Mice', *J Neurosci.* **22**(4): pp. 1208-17; Whiteaker, P., Peterson, C. G., Xu, W., McIntosh, J. M., Paylor, R., Beaudet, A. L., Collins, A. C., Marks, M. J. (2002). 'Involvement of the Alpha3 Subunit in Central Nicotinic Binding Populations', *J Neurosci.* **22**(7): pp. 2522-9.

[164] Champtiaux, N., Changeux, J. P. (2002). 'Knock-Out and Knock-In Mice to Investigate the Role of Nicotinic Receptors in the Central Nervous System', *Curr Drug Targets CNS Neurol Disord*, **1**(4): pp. 319-30.

[165] Ryan, R.E., Loiacono, R. E. (2001). 'Nicotine Regulates Alpha7 Nicotinic Receptor Subunit mRNA: Implications for Nicotine Dependence', *Neuroreport*, **12**(3): pp. 569-72.

[166] Schilstrom, B., Svensson, H. M., Svensson, T. H., Nomikos, G. G. (1998). 'Nicotine and Food Induced Dopamine Release in the Nucleus Accumbens of the Rat: Putative Role of Alpha7 Nicotinic Receptors in the Ventral Tegmental Area', *Neuroscience*, **85**(4): pp. 1005-9.

[167] Di Chiara, G., Imperato, A. (1988).

rise to dichotomous projection systems.[168] The shell is considered part of the extended amygdala and mesolimbic system with projections to the amygdala and lateral hypothalamus.[169] However, the core remembles its adjoining striatopallidal components of the caudate and putamen and sends projections to brain regions involved in sensorimotor function such as the substantia nigra.[170] Furthermore, the shell is implicated in mediation of the rewarding or reinforcing properties of drugs of abuse[171] whilst the core is involved with psychomotor sensitisation and motivation.[172]

There is growing evidence that the NAc shell and core contribute to the development of nicotine dependence through different mechanisms. [173] Whilst nicotine administration stimulates release of DA in the nucleus accumbens shell in naïve rats,[174] only rats who have been pre-treated with nicotine appear to release DA in the accumbal core.[175] Nicotine stimulates the degree of burst firing (high frequency firing of action potentials resulting in an increase in neurotransmitter efflux) of DA neurones, which results in a much greater increase in DA in the extrasynaptic space than spike firing (tonic, low-frequency action potentials).[176]

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[168] Zahm, D.S., Heimer, L., (1988). Ventral Striatopallidal Parts of the Basal Ganglia in the Rat:I. Neurochemical Compartmentation as Reflected by the Distributions of Neurotensin and Substance P Immunoreactivity', *J Comp Neurol.*, **272**(4):516-35; Zahm, D.S., (1989). 'The Ventral Striatopallidal Parts of the Basal Ganglia in the Rat: II. Compartmentation of Ventral Pallidal Efferents', *Neuroscience*, **30**(1):33-50; Zahm, D.S., Heimer, L., (1990). 'Two Transpallidal Pathways Originating in the Rat Nucleus Accumbens', *J Comp Neurol*, **302**(3):437-46.

[169] Heimer, L., Zahm, D.S., Churchill, L., Kalivas, P.W., Wohltmann, C. (1991). 'Specificity in the Projection Patterns of Accumbal Core and Shell in the Rat', *Neuroscience*, **41**(1):89-125.

[170] Zahm, D.S., Heimer, L., (1990).

[171] Corrigall, W.A., Franklin, K. B., Coen, K. M., Clarke, P. B. (1992).

[172] Li, Y., Acerbo, M.J., Robinson, T.E. (2004). 'The Induction of Behavioural Sensitisatiopn is associated with Cocaine-Induced Structural Plasticity in the Core (but not Shell) of the Nucleus Accumbens' *Eur J Neurosci.* **20**(6):1647-54.

[173] Balfour, D.J. (2002). 'Neuroplasticity Within the Mesoaccumbens Dopamine System and Its Role in Tobacco Dependence', *Curr Drug Targets CNS Neurol Disord.* **1**(4): pp. 413-21.

[174] Iyaniwura, T.T., Wright, A.E., Balfour, D.J. (2001). 'Evidence that Mesoaccumbens Dopamine and Locomotor Responses to Nicotine in the Rat are Influenced by Pretreatment Dose and Strain', *Psychopharmacology (Berl)*, **158**(1):73-9.

[175] Benwell, M.E., Balfour, D. J. (1992).

[176] Grenhoff, J., Aston-Jones, G., Svensson, T. H. (1986). 'Nicotinic Effects on the Firing Pattern of Midbrain Dopamine Neurons', *Acta Physiol Scand.* **128**(3): pp. 351-8; Sammut, S., Dec, A., Mitchell, D., Linardakis, J., Ortiguela, M., West, A. R. (2005). 'Phasic Dopaminergic Transmission Increases NO Efflux in the Rat Dorsal

In addition, the distribution of the majority of DA transporters in the NAc are arrayed along the DA fibres innervating the NAc often not facing the synaptic cleft directly[177] suggesting that DA transporters play an important role in regulating the DA concentration in the extracellular space as well as in the synaptic cleft.[178] Thus, with increased burst firing the cells within the NAc core and shell are essentially "bathed" in large concentrations of DA.[179] Whilst the reinforcing effect of DA release into the NAc shell is known to desensitise, the NAc core appears to become more sensitised to DA with chronic nicotine exposure.[180] As the effects of DA stimulation of NAc shell are believed to be reinforcing and essential in promoting behaviours involved in acquiring the drug early in the process of dependence, evidence suggests that DA overflow to the accumbens core may be important in the development of Pavlovian conditioned learning.[181]

Thus, consistent with the addiction model of Robinson and Berridge,[182] a potential effect of DA overflow into the accumbens core is to sensitise individuals to sensory cues associated with tobacco use.[183] The long-term behavioural result of NAc core sensitisation would be an increase in drug wanting or craving. At the transcriptional level, there is evidence to support the incentive sensitisation model proposed by Robinson and Berridge and Balfour and Benwell. Le Foll and colleagues noted increased expression of DA D_3 receptors in the accumbens shell during behavioural sensitisation to nicotine over one

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Striatum via a Neuronal NOS and a Dopamine D(1/5) Receptor-Dependent Mechanism', *Neuropsychopharmacology* (electronic publication in press).

[177] Nirenberg, M.J., Chan, J., Pohorille, A., Vaughan, R.A., Uhl, G.R., Kuhar, M.J., Pickel, V.M. (1997). 'The Dopamine Transporter: Comparitive Ultrastructure of Dopaminergic Axons in Limbic and Motor Compartments of the Nucleus Accumbens', *J Neurosci.*, **17**(18):6899-907.

[178] Ibid.

[179] Balfour, D.J. (2002).

[180] Robinson, T.E., Berridge, K. C. (1993).

[181] Ito, R., Dalley, J.W., Howes, S.R., Robbins, T.W., Everitt, B.J. (2000). 'Dissociation in Conditioned Dopamine Release in the Nucleus Accumbens Core and Shell in Response to Cocaine Cues and During Cocaine-Seeking Behavior in Rats', *J Neurosci.* **20**(19):7489-95.

[182] Robinson, T.E., Berridge, K.C. (2001).

[183] Ibid.

week.[184] Furthermore, Bahk and colleagues observed that rats exposed to passive cigarette smoke or systemic nicotine administration increased D_1 and D_2 receptor expression in the NAc, caudate, putamen, and olfactory tubercle.[185] In addition, expression of the proto-oncogene c-fos and immunohistochemical detection of the c-fos protein has been used to track the neural circuitry affected by a variety of behavioural and pharmacological manipulations. Nicotine administration has been shown to increase c-fos expression in DA terminal fields including the NAc, other regions of the striatum, and the prefrontal cortex.[186]

Balfour has proposed a mechanism whereby, even with the desensitisation of VTA nAChRs, conditioned cues are repetitively reinforced. Every time an addicted smoker smokes a cigarette following a period of abstinence long enough for blood nicotine concentrations to fall and nAChRs to re-sensitise, they again experience reinforcing pleasurable effects of DA release in the NAc shell and sensitisation to sensory stimuli associated with smoking in the accumbens core.[187] As noted in Section 1.2.1.1.1, the half-life of nicotine is approximately two hours[188] and thus smokers experience multiple peaks and troughs in blood nicotine levels throughout the day with levels lowest upon awakening.[189] Therefore, during periods of low systemic nicotine the nAChRs theoretically have sufficient time to re-sensitise. Upon awakening, therefore, addicted smokers experience the reinforcing

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[184] Le Foll, B., Diaz, J., Sokoloff, P. (2003). 'Increased Dopamine D3 Receptor Expression Accompanying Behavioral Sensitization to Nicotine in Rats', *Synapse*, **47**(3): pp. 176-83.

[185] Bahk, J.Y., Li, S., Park, M. S., Kim, M. O. (2002). 'Dopamine D1 and D2 Receptor mRNA Up-Regulation in the Caudate-Putamen and Nucleus Accumbens of Rat Brains by Smoking', *Prog Neuropsychopharmacol Biol Psychiatry*, **26**(6): pp. 1095-104.

[186] Shim, I., Javaid, J. I., Wirtshafter, D., Jang, S. Y., Shin, K. H., Lee, H. J., Chung, Y. C., Chun, B. G. (2001); Kiba, H., Jayaraman, A. (1994). 'Nicotine Induced c-fos Expression in the Striatum is Mediated Mostly by Dopamine D1 Receptor and Is Dependent on NMDA Stimulation', *Brain Res Mol Brain Res.* **23**(1-2): pp. 1-13; Mathieu-Kia, A.M., Pages, C., Besson, M. J. (1998) 'Inducibility of c-Fos Protein in Visuo-Motor System and Limbic Structures after Acute and Repeated Administration of Nicotine in the Rat', *Synapse*, **29**(4): pp. 343-54.

[187] Balfour, D.J. (2002).

[188] Hukkanen, J., Jacob, P., 3rd, Benowitz, N. L. (2005).

[189] Benowitz, N.L., Jacob, P., 3rd, (1984). 'Daily Intake of Nicotine During Cigarette Smoking', *Clin Pharmacol Ther*, **35**(4): pp. 499-504.

effects of nicotine with the first cigarette in the morning and thereafter at times of low systemic nicotine during the day.

The neuroadaptations that accompany the development of nicotine withdrawal are equally as complex as those implicated in development of acute and chronic tolerance. In addition to DA systems, serotonin neurotransmission appears to play a particularly important role. In studies of rats, nicotine withdrawal is indicated when rats demonstrate facial fasciculations, increased eyeblinks, abdominal constrictions, increased acoustic startle response, ptosis, changes in locomotor activity, and other manifestations.[190]

Neuroadaptations in striatal DA appear to contribute to the nicotine withdrawal syndrome in rats. Hildebrand and colleagues infused rats with nicotine for seven days and then administered the nAChR antagonist mecamylamine or saline.[191] The mecamylamine-treated rats displayed a greater intensity of abstinence signs than controls and the levels of DA and its metabolites HVA and DOPAC were significantly reduced in the NAc dialysate when compared to rats receiving saline injection. In addition, mecamylamine-treated rats[192] and rats who experience spontaneous nicotine withdrawal without mecamylamine administration[193] demonstrate increased reward thresholds in intracranial self-stimulation (ICCS) tests. These animal studies as well as *in vivo* studies of other drugs of abuse

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[190] Malin, D.H., Lake, J. R., Newlin-Maultsby, P., Roberts, L. K., Lanier, J. G., Carter, V. A., Cunningham, J. S., Wilson, O. B. (1992). 'Rodent Model of Nicotine Abstinence Syndrome', *Pharmacol Biochem Behav.* **43**(3): pp. 779-84; Hildebrand, B.E., Nomikos, G. G., Bondjers, C., Nisell, M., Svensson, T. H. (1997). 'Behavioral Manifestations of the Nicotine Abstinence Syndrome in the Rat: Peripheral Versus Central Mechanisms', *Psychopharmacology* (Berl), **129**(4): pp. 348-56; Epping-Jordan, M.P., Watkins, S. S., Koob, G. F., Markou, A. (1998). 'Dramatic Decreases in Brain Reward Function During Nicotine Withdrawal', *Nature*, **393**(6680): pp. 76-9.

[191] Hildebrand, B.E., Nomikos, G. G., Hertel, P., Schilstrom, B., Svensson, T. H. (1998). 'Reduced Dopamine Output in the Nucleus Accumbens but not in the Medial Prefrontal Cortex in Rats Displaying a Mecamylamine-Precipitated Nicotine Withdrawal Syndrome', *Brain Res.* **779**(1-2): pp. 214-25.

[192] Watkins, S.S., Stinus, L., Koob, G. F., Markou, A. (2000). 'Reward and Somatic Changes During Precipitated Nicotine Withdrawal in Rats: Centrally and Peripherally Mediated Effects', *J Pharmacol Exp Ther.* **292**(3): pp. 1053-64.

[193] Ibid.

including alcohol[194] and nicotine[195] indicate that drug withdrawal is associated with DA dysfunction as well as nAChR desensitisation.

Neuroadaptations in serotonin (5-HT) neurotransmission appear to be particularly important with regard to the anxiogenic component of nicotine withdrawal. Rats undergoing spontaneous nicotine withdrawal demonstrate increased acoustic startle, [196] anxiogenic responses in the social interaction test and elevated plus-maze test,[197] and the light-dark crossing test.[198] The anxiogenic response to the light-dark cross test is reversed by systemic administration of 5-HT_3 receptor antagonists and the enhanced acoustic startle is reversed by the 5-HT_{1A} receptor agonist (+/-)-8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT).[199] The dorsal hippocampus appears to be a particularly important locus for 5-HT-related mediation of the anxiogenic component of nicotine withdrawal as it has been shown that a low dose of nicotine into this region reversed the anxiogenic withdrawal response in the elevated maze test.[200] Indeed, chronic administration of nicotine increases hippocampal 5-

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[194] Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004). 'Correlation between Dopamine D(2) Receptors in the Ventral Striatum and Central Processing of Alcohol Cues and Craving', *Am J Psychiatry*, **161**(10): pp. 1783-9.

[195] Brody, A.L., Olmstead, R. E., London, E. D., Farahi, J., Meyer, J. H., Grossman, P., Lee, G. S., Huang, J., Hahn, E. L., Mandelkern, M. A. (2004). 'Smoking-Induced Ventral Striatum Dopamine Release', *Am J Psychiatry*, **161**(7): pp. 1211-8.

[196] Rasmussen, K., Kallman, M. J., Helton, D. R. (1997). 'Serotonin-1A Antagonists Attenuate the Effects of Nicotine Withdrawal on the Auditory Startle Response', *Synapse*, **27**(2): pp. 145-52.

[197] Irvine, E.E., Cheeta, S., File, S. E. (1999). 'Time-Course of Changes in the Social Interaction Test of Anxiety Following Acute and Chronic Administration of Nicotine', *Behav Pharmacol.* **10**(6-7): pp. 691-7; Irvine, E.E., Cheeta, S., File, S. E. (2001). 'Development of Tolerance to Nicotine's Anxiogenic Effect in the Social Interaction Test', *Brain Res.* **894**(1): pp. 95-100; Cheeta, S., Irvine, E., File, S. E. (2001). 'Social Isolation Modifies Nicotine's Effects in Animal Tests of Anxiety', *Br J Pharmacol.* **132**(7): pp. 1389-95.

[198] Barnes, N.M., Costall, B., Kelly, M. E., Onaivi, E. S., Naylor, R. J. (1990). 'Ketotifen and Its Analogues Reduce Aversive Responding in the Rodent', *Pharmacol Biochem Behav.* **37**(4): pp. 785-93; Costall, B., Jones, B. J., Kelly, M. E., Naylor, R. J., Onaivi, E. S., Tyers, M. B. (1990). 'Sites of Action of Ondansetron to Inhibit Withdrawal from Drugs of Abuse', *Pharmacol Biochem Behav.* **36**(1): pp. 97-104.

[199] Rasmussen, K., Kallman, M. J., Helton, D. R. (1997).

[200] Irvine, E.E., Cheeta, S., File, S. E. (2001).

HT_{1A} density in humans[201] and expression in rodents.[202] Moreover, repetitive nicotine administration results in a regionally selective decrease in the formation of, concentration of, and release of 5-HT in the hippocampus.[203] As will be discussed in more detail in Section 1.2.1.3.3, emerging evidence from animal and human *in vivo* studies strongly suggests that 5-HT neurotransmission is an important component of the complex neuroadaptive changes contributing to nicotine dependence.

Section 1.2.1.3.5 provides an overview of predominant models based on animal and *in vivo* studies that address how the biological processes of acute and chronic tolerance explain the behavioural patterns observed in humans who are addicted to nicotine. In the preceding sections I have touched upon many interacting neurotransmitter systems in an explanation of the pharmacokinetics and particularly the pharmacodynamics of nicotine. In the next section, I will describe in greater detail the neurotransmitter systems implicated in nicotine dependence.

1.2.1.3 Neurotransmitter Systems Implicated in and Models of Nicotine Dependence

1.2.1.3.1 Nicotinic Acetylcholine

In the preceding sections I have discussed briefly the role of nAChRs in the pharmacodynamics of acute and chronic tolerance to nicotine. In this section I will describe in more detail the structure and function of central nAChRs as they pertain to nicotine dependence. Given the ubiquitous distribution of nAChRs centrally and somatically, this

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[201] Benwell, M.E., Balfour, D. J. (1979); Benwell, M.E., Balfour, D. J., Anderson, J. M. (1990). 'Smoking-Associated Changes in the Serotonergic Systems of Discrete Regions of Human Brain', *Psychopharmacology* (Berl). **102**(1): pp. 68-72.

[202] Kenny, P.J., File, S. E., Rattray, M. (2001). 'Nicotine Regulates 5-HT(1A) Receptor Gene Expression in the Cerebral Cortex and Dorsal Hippocampus', *Eur J Neurosci.* **13**(6): pp. 1267-71.

[203] Benwell, M.E., Balfour, D. J. (1979); Benwell, M.E., Balfour, D. J., Anderson, J. M. (1990); Benwell, M.E., Balfour, D. J. (1982). 'The Effects of Nicotine Administration on 5-HT Uptake and Biosynthesis in Rat Brain', *Eur J Pharmacol.* **84**(1-2): pp. 71-7; Balfour, D.J., Ridley, D. L. (2000). 'The Effects of Nicotine on Neural Pathways Implicated in Depression: A Factor in Nicotine Addiction?' *Pharmacol Biochem Behav.* **66**(1): pp. 79-85.

discussion is necessarily confined the nAChR-related pathways within a distributed reward signalling network. NAChRs are pentameric receptors which function as ligand-gated ion channels. To date, 16 different subunits have been identified and cloned in vertebrates and the pentameric receptors are broadly classified as skeletal, ganglionic, and central nAChRs.[204] Known nAChR receptor subunits currently identified include α (1-9 types), β (1-4 types), ε (1 type), δ (1 type), and γ (1 type).[205] Twelve different neuronal nAChRs have been identified.[206] However, subunit compositions implicated in nicotine dependence have predominantly included the following compositions: (α4β2, α7 homomeric, $\alpha4\beta6\alpha5(\beta2)_2$, and $(\alpha4)_2\alpha5(\beta2)_2$).[207] Neuronal nAChRs consist of five subunits. Each subunit is a protein with four transmembrane domains and an amino terminal domain containing the ACh binding site. The M2 domain of each subunit faces the inside of the central pore, is negatively charged, and determines the ion selectivity of the receptor (Figure 1.4). Neuronal nAChRs consist of three conformational states, open, closed, and desensitised.[208] When a nicotinic agonist binds to the amino terminal of an nAChR, the receptor complex undergoes a conformational change, allowing the channel pore to open and permitting the passage of particular cations [specifically Ca^{2+} for $(\alpha7)_5$ and K^+ and Na^+ for $(\alpha4)_2(\beta2)_3$] from the

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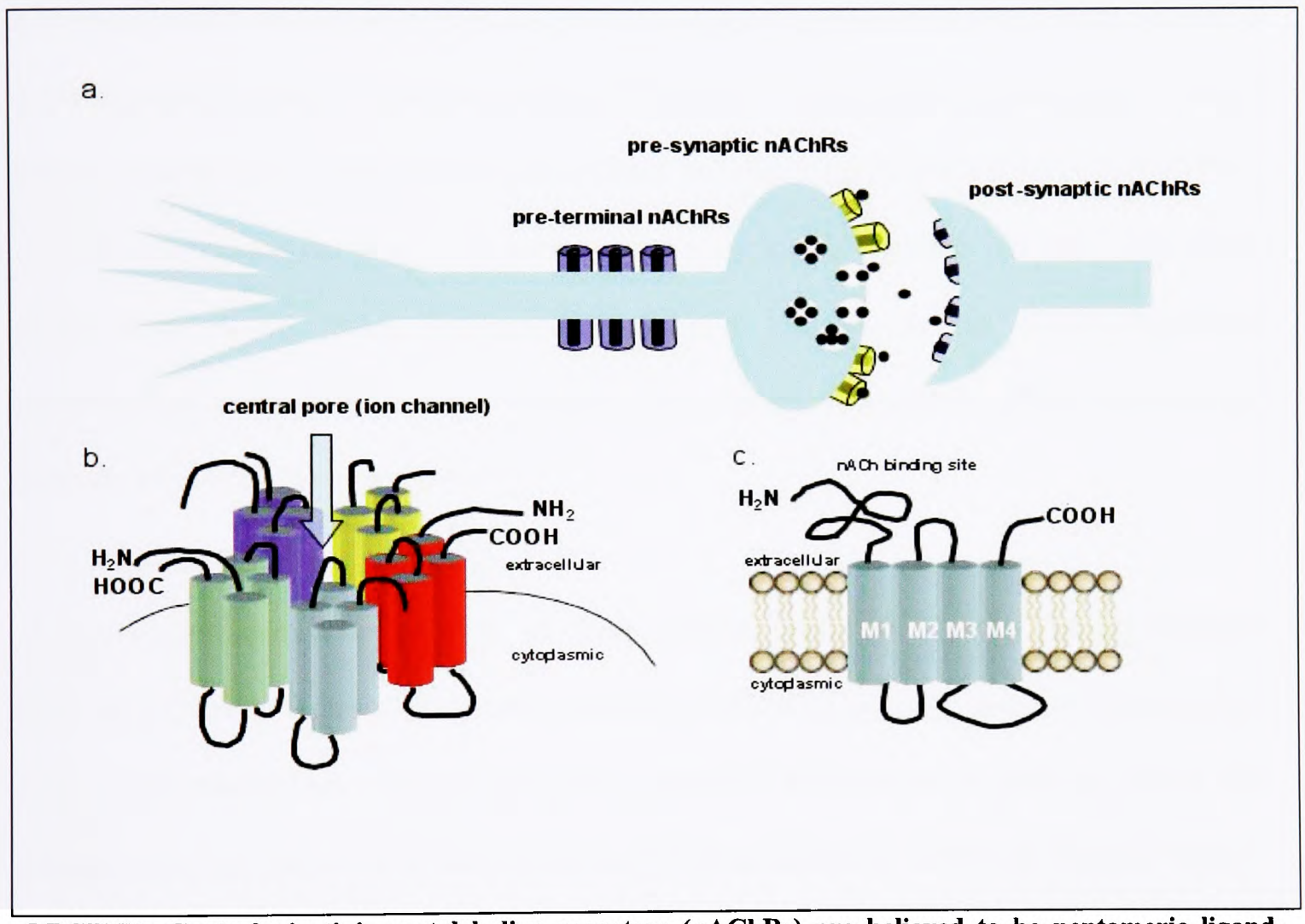
[204] Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K. (ed.) (2003). *Pharmacology*, 5th edn. (London: Churchill Livingstone).
[205] Ibid.
[206] McGehee, D.S., Role, L. W. (1995); Jones, S., Sudweeks, S., Yakel, J. L. (1999); Laviolette, S.R., van der Kooy, D. (2004); Role, L.W., Berg, D. K. (1996); Salamone, F.N., Zhou, M., Auerbach, A. (1999). 'A Re-Examination of Adult Mouse Nicotinic Acetylcholine Receptor Channel Activation Kinetics', *J Physiol.* **516 (Pt 2)**: pp. 315-30; Gotti, C., Fornasari, D., Clementi, F. (1997). 'Human Neuronal Nicotinic Receptors', Prog *Neurobiol.* **53**(2): pp. 199-237.
[207] Klink, R., de Kerchove d'Exaerde, A., Zoli, M., Changeux, J. P. (2001); Laviolette, S.R., van der Kooy, D. (2004).
[208] Unwin, N. (2005); Salamone, F.N., Zhou, M., Auerbach, A. (1999); Unwin, N. (1995). 'Acetylcholine Receptor Channel Imaged in the Open State', *Nature*, **373**(6509): pp. 37-43.

extracellular to the intracellular space.[209] This influx of cations depolarises the cell and increases the probability of firing an action potential (AP).

Figure 1.4

Putative Structure and Function of Neuronal Nicotinic Acetylcholine Receptors



LEGEND: Central nicotinic acetylcholine receptors (nAChRs) are believed to be pentameric ligand-gated ion channels made up of 5 subunits, which are predominantly of the alpha and beta sub-types. A. Demonstration of nAChRs located on the soma, on presynaptic terminals, and on post-synaptic boutons. B. nAChR composed of 5 subunits, which are arranged either heteromerically or homomerically and containing a central pore. C. nAChR subunit putative topology with 4 transmembrane domains (M1-4). The larger amino terminal contains the ACh binding site whilst the M2 domain confers ion selectivity.[210]

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[209] Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K. (ed.) (2003).

[210] Figure 1.4 was recreated and adapted from Figure I in: Laviolette, S.R., van der Kooy, D. (2004) by SD for this thesis.

Neuronal nAChRs are located either on the soma and/or neuronal processes such that nicotine can bind pre-synaptically, post-synaptically, and at the cell body.[211] In the VTA, nAChRs are present somatodendritically on GABA and DA neurones and pre-synaptically on glutamatergic neurones.[212] As discussed in the previous section, the differential rate of desensitisation of different nAChR receptors may explain, in part, the complex neuroadaptations associated with a shift from a GABA-dependent nicotine reward system to a DA-dependent system. However, such an hypothesis is speculative and the topic of much debate. In addition to being present on the soma and dendrites of DA neurones in the VTA, nAChRs are present and play an important role pre-synaptically on DA neurones in terminal fields such as the NAc. Furthermore, nAChRs are present on GABA-dependent interneurones in the NAc and are thought to have pharmacodynamic effects downstream from the release of DA in the NAc.[213]

1.2.1.3.2 Dopamine

DA neurotransmission consists of three primary systems: (1) the A9 nigrostriatal pathway, (2) the A10 mesocorticolimbic pathway, and the (3) neurohypophyseal system. The A10 mesocorticolimbic pathway has been associated with reward signalling and is the primary area of research into the reinforcing pharmacodynamic effects of drugs of abuse. The mesocorticolimbic system, as referred to previously in this chapter, consists of cell

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[211] Laviolette, S.R., van der Kooy, D. (2004).

[212] Kalivas, P.W. (1993); Girod, R., Barazangi, N., McGehee, D., Role, L. W. (2000). 'Facilitation of Glutamatergic Neurotransmission by Presynaptic Nicotinic Acetylcholine Receptors', *Neuropharmacology*, **39**(13): pp. 2715-25; McGehee, D.S., Heath, M. J., Gelber, S., Devay, P., Role, L. W. (1995). 'Nicotine Enhancement of Fast Excitatory Synaptic Transmission in CNS by Presynaptic Receptors', *Science*, **269**(5231): pp. 1692-6.

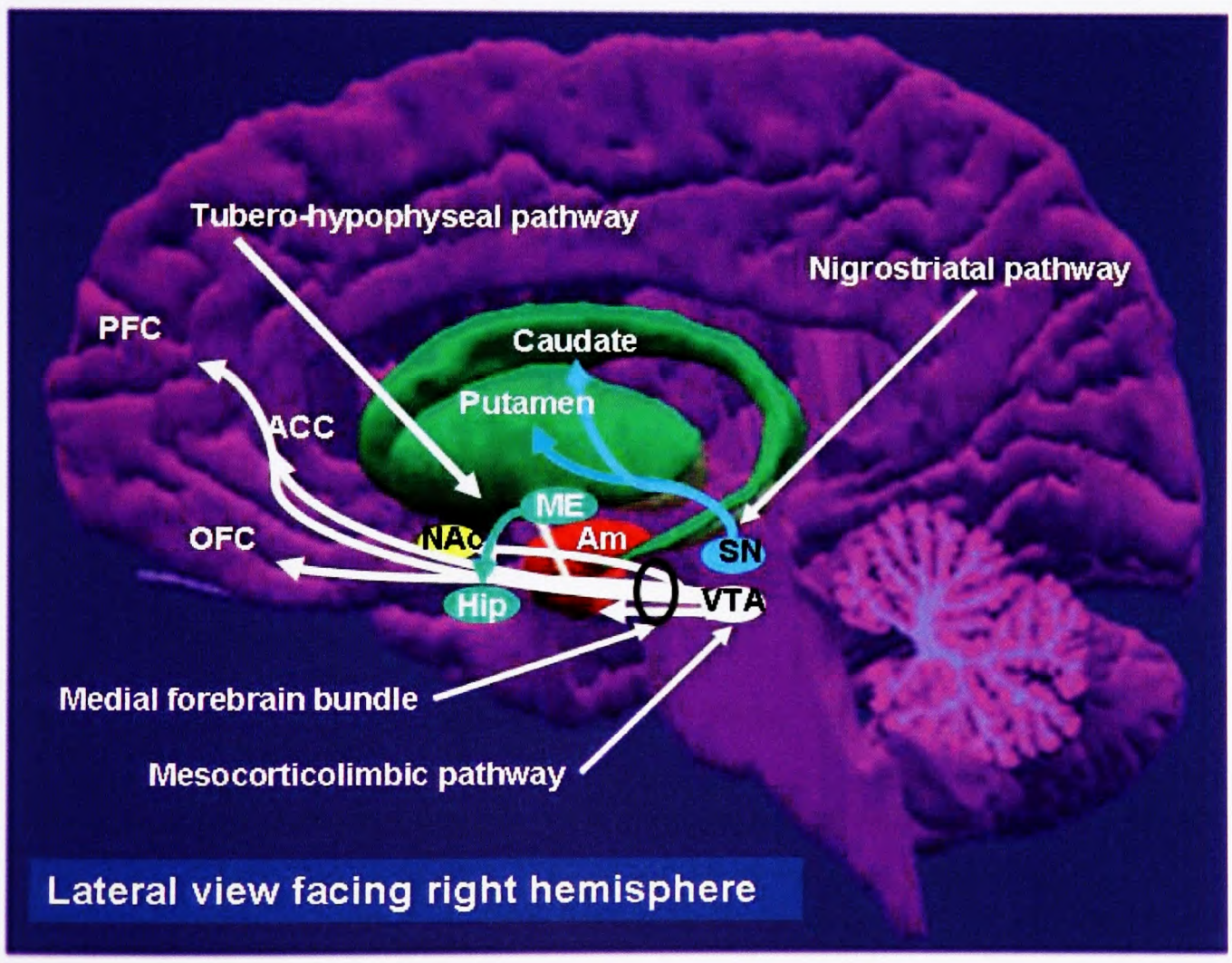
[213] Mansvelder, H.D., De Rover, M., McGehee, D. S., Brussaard, A. B. (2003). 'Cholinergic Modulation of Dopaminergic Reward Areas: Upstream and Downstream Targets of Nicotine Addiction', *Eur J Pharmacol.* **480**(1-3): pp. 117-23.

bodies in the midbrain, which project to the amygdala (Am), NAc, and prefrontal cortex (the primary DA systems are illustrated in figure 1.5).

DA is synthesised in a pathway similar to noradrenaline. The rate-limiting step in the synthesis of DA is the conversion of tyrosine to dihydroxyphenylalanine (DOPA) catalysed by tyrosine hydroxylase (TH). DOPA is then converted to DA by DOPA decarboxylase.

Figure 1.5

Central Dopaminergic Pathways



LEGEND: Lateral view of right hemisphere indicating major dopaminergic pathways. Abbreviations are as follows: Am = amygdala; Hip = hippocampus; MFB = medial forebrain bundle; ME = median eminence; OFC = orbitofrontal cortex; PFC = prefrontal cortex; SN = substantia nigra pars compacta; VTA = ventral tegmental area. Three-dimensional image from the Digital Anatomist Interactive Brain Atlas, Copyright © 1994 University of Washington. All Rights Reserved. Illustration of pathways and labels added by SD for this thesis.

DA is essentially recycled when transferred from the extracellular space to the synaptic terminal or elsewhere along the neurone when it is then metabolised either to noradrenaline by DA β hydroxylase or through monoamine oxidase and/or catechol-O-methyltransferase (COMT) and aldehyde dehydrogenase to the primary metabolites dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA).[214] As will be discussed in Section 1.3, genetic variation in enzymes involved in DA synthesis and degradation may have implications for some characteristics of nicotine dependence.

There are five subgroups of DA receptor classified as D_1-D_5 and functionally further classified as 'D_1-type' (D_1 and D_5) or 'D_2-type' (D_2, D_3, D_4) according to whether activation leads to increased cAMP or decreased cAMP and/or increased IP_3, respectively.[215] DA receptors are part of a large family of G-protein-coupled transmembrane receptors. All DA receptor subtypes have been identified in the NAc (D_1-D_2,[216] D_3,[217] D_4,[218] D_5[219]). However, the D_2 or 'DRD2' receptor, not exclusively but more than any other subtype, has been implicated in the reward signalling of many drugs of abuse including alcohol, cocaine, opiates, and nicotine.[220] In addition VTA-mediated DA release in other terminal fields has

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[214] Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K. (ed.) (2003).
[215] Ibid.
[216] Charara, A., Grace, A. A. (2003). 'Dopamine Receptor Subtypes Selectively Modulate Excitatory Afferents from the Hippocampus and Amygdala to Rat Nucleus Accumbens Neurons', *Neuropsychopharmacology*, **28**(8): pp. 1412-21.
[217] Champagne, F.A., Chretien, P., Stevenson, C. W., Zhang, T. Y., Gratton, A., Meaney, M. J. (2004). 'Variations in Nucleus Accumbens Dopamine Associated with Individual Differences in Maternal Behavior in the Rat', *J Neurosci.* **24**(17): pp. 4113-23.
[218] Rivera, A., Cuellar, B., Giron, F. J.,Grandy, D. K., de la Calle, A., Moratalla, R. (2002). 'Dopamine D4 Receptors are Heterogeneously Distributed in the Striosomes/Matrix Compartments of the Striatum', *J Neurochem.* **80**(2): pp. 219-29.
[219] Khan, Z.U., Gutierrez, A., Martin, R., Penafiel, A., Rivera, A., de la Calle, A. (2000). 'Dopamine D5 Receptors of Rat and Human Brain', *Neuroscience*, **100**(4): pp. 689-99.
[220] Blum, K., Braverman, E. R., Holder, J. M., Lubar, J. F., Monastra, V. J., Miller, D., Lubar, J. O., Chen, T. J., Comings, D. E. (2000). 'Reward Deficiency Syndrome: A Biogenetic Model for the Diagnosis and Treatment of Impulsive, Addictive, and Compulsive Behaviors', *J Psychoactive Drugs*, **32 Suppl**: pp. i-iv, 1-112; Maldonado, R., Saiardi, A., Valverde, O., Samad, T. A., Roques, B. P., Borrelli, E. (1997). 'Absence of Opiate Rewarding Effects in Mice Lacking Dopamine D2 Receptors', *Nature*, **388**(6642): pp. 586-9.

been postulated to contribute to the reinforcing properties, conditioned cue reactivity, and ultimate loss of control in nicotine dependence.[221]

1.2.1.3.3 Serotonin

Most research into neurotransmitter systems implicated in nicotine dependence has focused on DA neurotransmission. However, it is clear that nicotine administration results in the release of serotonin and other neurotransmitters in terminal fields and, furthermore, there is an emerging corpus of evidence suggesting that serotonin neurotransmission plays an important role in nicotine dependence.[222] It has been well established that serotonin neurotransmission regulates mood, and dysfunction within this system is associated with mood disorders, anxiety disorders, and depression, all of which have major comorbidity with nicotine dependence. Evidence from animal models and from genetic association studies presented later suggests mechanisms through which nicotine-serotonin interactions might influence the development of nicotine dependence.

This discussion focuses on central serotonin neurotransmission. However, brain serotonin accounts for only approximately 1% of total body content and serotonin receptors are located ubiquitously throughout the body, particularly in the wall of the intestine and in blood platelets. The synthesis of serotonin (5-hydroxytryptamine or '5-HT') is similar to that of noradrenaline. The precursor for 5-HT, however, is tryptophan rather than tyrosine, which is converted to 5-hydroxytryptophan in neurones by tryptophan hydroxylase. Next, 5-

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[221] Koob, G.F. (1999). 'The Role of the Striatopallidal and Extended Amygdala Systems in Drug Addiction', *Ann N Y Acad Sci.* **877**: pp. 445-60; Volkow, N.D., Fowler, J. S., Wang, G. J., Goldstein, R. Z. (2002). 'Role of Dopamine, the Frontal Cortex and Memory Circuits in Drug Addiction: Insight from Imaging Studies', *Neurobiol Learn Mem.* **78**(3): pp. 610-24.

[222] Seth, P., Cheeta, S., Tucci, S., File, S. E. (2002). 'Nicotinic-Serotonergic Interactions in Brain and Behaviour', *Pharmacol Biochem Behav.* **71**(4): pp. 795-805.

hydroxytryptophan is decarboxylated to 5-HT by the L-Aromatic acid decarboxylase (also known as 'dopa decarboxylase') enzyme. Degradation of central 5-HT involves the reuptake of 5-HT from the synaptic cleft by the serotonin transporter and subsequent deamination by monoamine oxidase (MAO) followed by oxidation to 5-hydroxyindoleacetic acid (5-HIAA)[223] by aldehyde dehydrogenase. 5-HT is also taken up into astrocytes by serotonin transporters where again degradation involves deamination by monoamine oxidase followed by oxidation to 5-HIAA by aldehyde dehydrogenase.[224] MAO exists in two forms (MAO-A and MAO-B) and there is evidence that the predominant form of MAO in neurones is MAO-A and in astrocytes is MAO-B,[225] whilst detectable levels of both MAO subtypes are present in neurones and astrocytes.

5-HT pathways in the brain are extensively distributed throughout the cortex, subcortical and limbic regions, and cerebellum as shown in Figure 1.6. The cell bodies of 5-HT neurones are present in the pons and midbrain in the raphe nuclei. The rostrally located raphe nuclei project through the medial forebrain bundle to the cortex, striatum, Am, hippocampus, and hypothalamus whilst the caudally situated cells project directly to the cerebellum, medulla, and spinal cord.

To date, no fewer than 14 subtypes of 5-HT receptors have been identified. However, there is speculation on the existence of an additional 5-HT receptor subtype that has not as

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[223] Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K. (ed.) (2003).

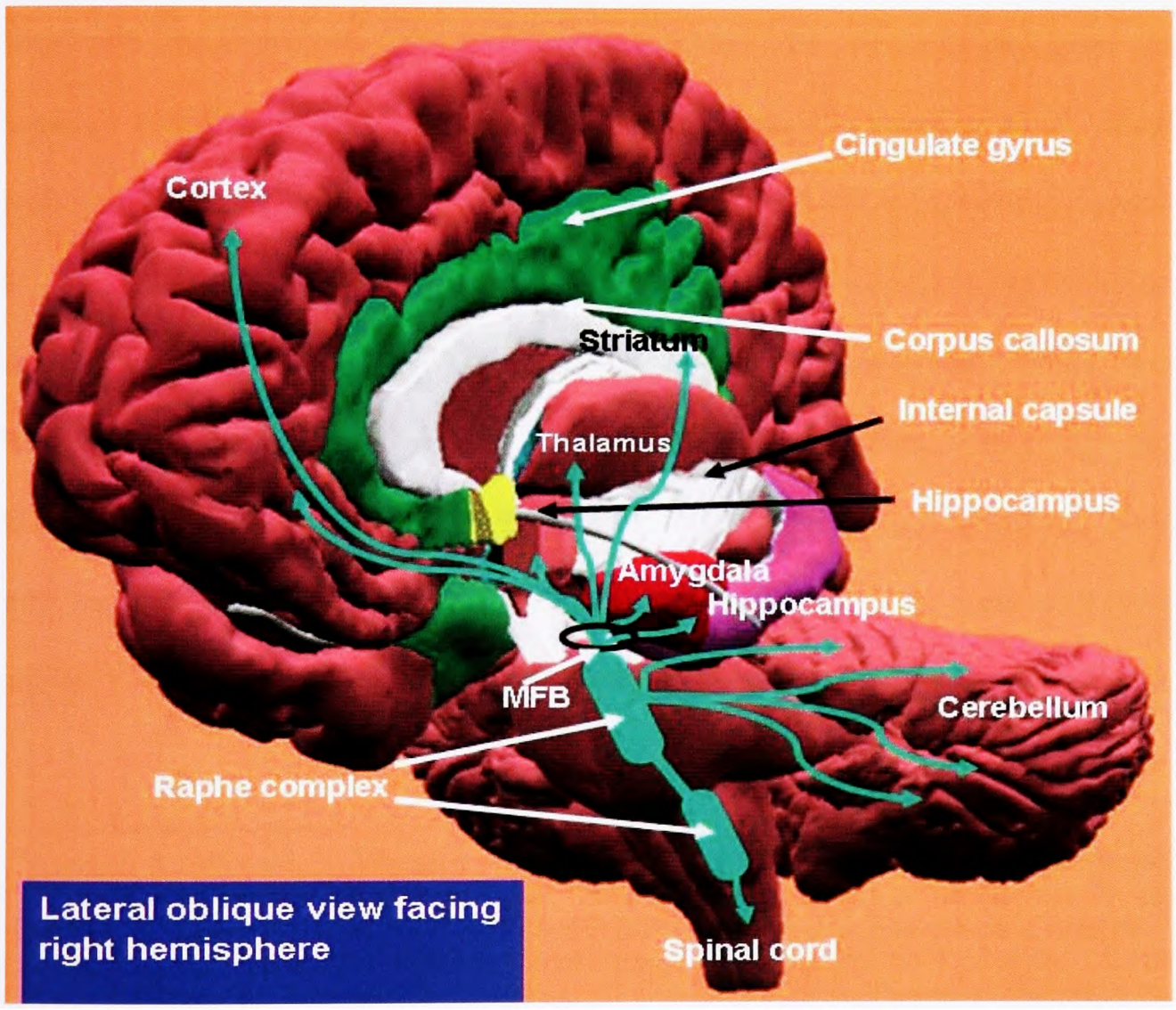
[224] Fitzgerald, L.W., Kaplinsky, L., Kimelberg, H. K. (1990). 'Serotonin Metabolism by Monoamine Oxidase in Rat Primary Astrocyte Cultures', *J Neurochem.* **55**(6): pp. 2008-14.

[225] Levitt, P., Pintar, J. E., Breakefield, X. O. (1982). 'Immunocytochemical Demonstration of Monoamine Oxidase B in Brain Astrocytes and Serotonergic Neurons', *Proc Natl Acad Sci U S A*, **79**(20): pp. 6385-9; Riederer, P., Konradi, C., Schay, V., Kienzl, E., Birkmayer, G., Danielczyk, W., Sofic, E., Youdim, M. B. (1987). 'Localization of MAO-A and MAO-B in Human Brain: A Step in Understanding the Therapeutic Action of L-Deprenyl', *Adv Neurol.* **45**: pp. 111-8.

yet been fully characterised.[226] These receptors have been classified into seven families (5-HT_{1-7}). All 5-HT receptor subtypes have been identified as having seven transmembrane domains coupled to G-protein signal transduction with the exception of the 5-HT_3 receptor, which is a ligand-gated ion channel.[227]

Figure 1.6

Central Serotonergic Pathways



LEGEND: Lateral oblique view of right hemisphere indicating major serotonegic pathways. MFB = medial forebrain bundle. Three-dimensional image from the Digital Anatomist Interactive Brain Atlas, Copyright © 1994 University of Washington. All Rights Reserved. Illustration of pathways and labels added by SD for this thesis.

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[226] Castro, E., Romon, T., Castillo, J. (1997). 'Identification and Characterisation of a New Serotonergic Recognition with High Affinity for 5-Carboxamidotryptamine in Mammalian Brain', *J neurochem*. **69**: pp. 2123-2131.

[227] Riederer, P., Konradi, C., Schay, V., Kienzl, E., Birkmayer, G., Danielczyk, W., Sofic, E., Youdim, M. B. (1987).

The receptor subtypes currently identified are 5-HT (1A, 1B, 1D, 1E, 1F, 2A, 2B, 2C, 3, 4, 5A, 5B, 6, and 7). The 5-HT_1 receptor subtypes are predominantly inhibitory. However, the 5-HT_2 subtypes, all of which are coupled via G-proteins positively to phospholipase C and stimulation results in release of intracellular Ca^{2+} and depolarisation, are excitatory.

The most extensively studied 5-HT receptor subtype is 5-HT_{1A}. In most brain regions studied, with the possible exception of the hippocampus,[228] 5-HT_{1A} receptors couple negatively via G-proteins (α_i) to adenylate cyclase. The electrophysiological response of negative coupling is hyperpolarisation resulting from the opening of K^+ channels and without the involvement of cAMP. The structure of the 5-HT_{1A} receptor is shown in Figure 1.7. Central 5-HT_{1A} receptors are located on the soma and dendrites of raphe nuclei and post-synaptic to 5-HT neurones in multiple cortical and subcortical terminal fields. According to both *in vitro* autoradiography and *in vivo* positron emission tomography (PET) studies,[229] 5-HT_{1A} receptors are distributed densely in the hippocampus, lateral septum, cortical areas with highest density in cingulate and entorhinal cortex, and in the dorsal and median raphe nuclei.[230] The 5-HT_{1A} gene is located on chromosome 5 (5q11.2-q13), is intronless, consists

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[228] Shenker, A., Maayani, S., Weinstein, H., Green, J. P. (1983). 'Enhanced Serotonin-Stimulated Adenylate Cyclase Activity in Membranes from Adult Guinea Pig Hippocampus', *Life Sci.* **32**(20): pp. 2335-42; Markstein, R., Hoyer, D., Engel, G. (1986). '5-HT1A-Receptors Mediate Stimulation of Adenylate Cyclase in Rat Hippocampus', *Naunyn Schmiedebergs Arch Pharmacol.* **333**(4): pp. 335-41; Fayolle, C., Fillion, M. P., Barone, P., Oudar, P., Rousselle, J. C., Fillion, G. (1988). '5-Hydroxytryptamine Stimulates Two Distinct Adenylate Cyclase Activities in Rat Brain: High-Affinity Activation is Related to a 5-HT1 Subtype Different from 5-HT1A, 5-HT1B, and 5-HT1C', *Fundam Clin Pharmacol.* **2**(3): pp. 195-214.

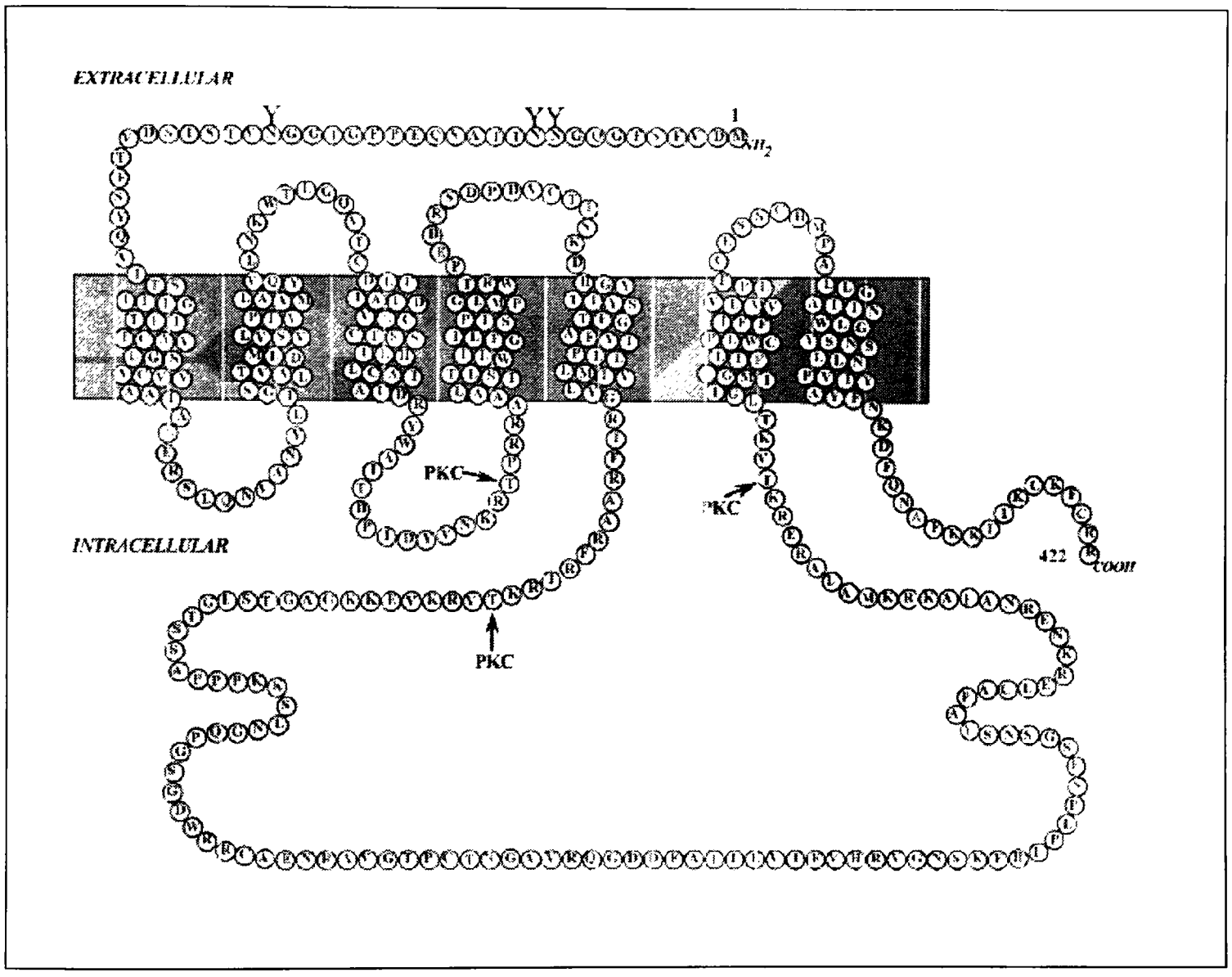
[229] Rabiner, E.A., Messa, C., Sargent, P. A., Husted-Kjaer, K., Montgomery, A., Lawrence, A. D., Bench, C. J., Gunn, R. N., Cowen, P., Grasby, P. M. (2002). 'A Database of [(11)C]WAY-100635 Binding to 5-HT(1A) Receptors in Normal Male Volunteers: Normative Data and Relationship to Methodological, Demographic, Physiological, and Behavioral Variables', Neuroimage, **15**(3): pp. 620-32; Sargent, P.A., Kjaer, K. H., Bench, C. J., Rabiner, E. A., Messa, C., Meyer, J., Gunn, R. N., Grasby, P. M., Cowen, P. J. (2000). 'Brain Serotonin1A Receptor Binding Measured by Positron Emission Tomography with [11C]WAY-100635: Effects of Depression and Antidepressant Treatment', *Arch Gen Psychiatry*, **57**(2): pp. 174-80.

[230] Barnes, N.M., Sharp, T. (1999). 'A Review of Central 5-HT Receptors and their Function', *Neuropharmacology*, **38**(8): pp. 1083-152.

of 1,309 base-pairs (bp) and encodes a protein of 422 amino acids.[231] The 5-HT_{1A} gene and known polymorphisms within the gene are introduced in Section 1.5 and described in depth in Chapter 3 (Section 3.2).

Figure 1.7

Putative Structure of the 5-HT_{1A} Receptor



LEGEND: Shown above is the putative molecular organisation of the 5-HT_{1A} receptor. It has the typical structure of a G-protein-coupled receptor with amino glycosylation sites and consensus sites for protein kinase C (PKC) present in the second and third cytoplasmic loops.[232]

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[231] Kobilka, B.K., Frielle, T., Collins, S., Yang-Feng, T., Kobilka, T. S., Francke, U., Lefkowitz, R. J., Caron, M. G. (1987). 'An Intronless Gene Encoding a Potential Member of the Family of Receptors Coupled to Guanine Nucleotide Regulatory Proteins', *Nature*, **329**(6134): pp. 75-9.

[232] Lanfumey, L., Hamon, M., (2000). 'Central 5-HT(1A) Receptors: Regional Distribution and Functional Characteristics', Nucl Med Biol. **27**(5): pp. 429-35.

A series of animal studies over the last three decades build a plausible case suggesting that acute and chronic nicotine administration may influence the development of nicotine dependence via mediation of anxiety, particularly during nicotine withdrawal, affected in large measure by the pharmacology of 5-HT as influenced by the 5-HT_{1A} receptor and 5-HT transporter. Balfour and colleagues noted that sustained administration of nicotine (0.4 mg/kg) for periods up to 40 days to rats resulted in decreased concentrations of 5-HT and its major metabolite 5-HIAA in the hippocampus[233] but did not affect 5-HT in other brain regions except the hippocampus in rats withdrawn from nicotine after five days of nicotine administration. In a separate study, these same investigators again found that acute and chronic nicotine treatment resulted in exclusively lower 5-HT and 5-HIAA in the hippocampus but also found that these effects were partially reversed after 24 hours of nicotine withdrawal.[234] Chronic nicotine treatment also resulted in decreased hippocampal uptake of L-tryptophan, which appeared pharmacokinetically to be the result of decreased numbers of L-tryptophan carrier molecules.[235]

Furthermore, the same investigators observed significant reductions in 5-HT and 5-HIAA in the hippocampus compared to non-smokers based on [^{3}H]-8-hydroxy-(di-n-propylamino)-tetralin ([^{3}H]-9-DPAT) binding in post-mortem human brains suggesting an increase in 5-HT_{1A} receptors in the hippocampus.[236] Interestingly, 5-HIAA concentrations were also reduced in the median raphe – the brain region known to project 5-HT neurones to the

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[233] Benwell, M.E., Balfour, D. J. (1979).
[234] Benwell, M.E., Balfour, D. J. (1982).
[235] Ibid.
[236] Benwell, M.E., Balfour, D. J., Anderson, J. M. (1990).

hippocampus. Subsequently, it has been demonstrated that nicotine appears to mediate the expression of 5-HT_{1A} mRNA.[237]

Whilst higher doses of nicotine have been shown to be anxiogenic,[238] low doses of nicotine have demonstrated anxiolytic properties in some[239] but not all studies.[240] Thus, given the putative role of 5-HT neurones emanating from the midbrain raphe to the dorsal hippocampus in anxiety and the effect of reduced 5-HT biosynthesis and release in this region, it is plausible that nicotine may mediate anxiety vis-à-vis inhibition of 5-HT biosynthesis and release in the hippocampus.

The pharmacodynamic properties of nicotine are somewhat more straightforward for the dorsal raphe nucleus (DRN) than the hippocampus. For example, Cheeta and colleagues observed that lower doses of nicotine (2.5-10 ng) administered directly into the DRN induced an anxiolytic effect in the social interaction test of anxiety.[241] Interestingly, the anxiolytic effect of nicotine administered in the DRN was similar to the effect of the 5-HT_{1A} agonist 8-OH-DPAT administered to the same region.[242] Moreover, the anxiolytic effects of nicotine were completely antagonised by the coadministration of WAY-100635 – a specific

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[237] Kenny, P.J., File, S. E., Rattray, M. (2001).

[238] File, S.E., Kenny, P. J., Ouagazzal, A. M. (1998). 'Bimodal Modulation by Nicotine of Anxiety in the Social Interaction Test: Role of the Dorsal Hippocampus', *Behav Neurosci.* **112**(6): pp. 1423-9.

[239] Ibid; Cheeta, S., Irvine, E. E., Kenny, P. J., File, S. E. (2001). 'The Dorsal Raphe Nucleus is a Crucial Structure Mediating Nicotine's Anxiolytic Effects and the Development of Tolerance and Withdrawal Responses', *Psychopharmacology* (Berl), **155**(1): pp. 78-85.

[240] Balfour, D.J., Graham, C. A., Vale, A. L. (1986). 'Studies on the Possible Role of Brain 5-HT Systems and Adrenocortical Activity in Behavioural Responses to Nicotine and Diazepam in an Elevated X-maze', *Psychopharmacology* (Berl), **90**(4): pp. 528-32; Ouagazzal, A.M., Kenny, P. J., File, S. E. (1999). 'Modulation of Behaviour on Trials 1 and 2 in the Elevated Plus-Maze Test of Anxiety after Systemic and Hippocampal Administration of Nicotine', *Psychopharmacology* (Berl), **144**(1): pp. 54-60.

[241] Cheeta, S., Irvine, E. E., Kenny, P. J., File, S. E. (2001).

[242] Higgins, G.A., Bradbury, A. J., Jones, B. J., Oakley, N. R. (1988). 'Behavioural and Biochemical Consequences Following Activation of 5HT1-Like and GABA Receptors in the Dorsal Raphe Nucleus of the Rat', *Neuropharmacology*, **27**(10): pp. 993-1001; Hogg, S., Andrews, N., File, S. E. (1994). 'Contrasting Behavioural Effects of 8-OH DPAT in the Dorsal Raphe Nucleus and Ventral Hippocampus', *Neuropharmacology*, **33**(3-4): pp. 343-8; Picazo, O., Lopez-Rubalcava, C., Fernandez-Guasti, A. (1995). 'Anxiolytic Effect of the 5-HT1A Compounds 8-Hydroxy-2-(di-n-propylamino) Tetralin and Ipsapirone in the Social Interaction Paradigm: Evidence of a Presynaptic Action', *Brain Res Bull.* **37**(2): pp. 169-75.

5-HT_{1A} receptor antagonist.[243] These observations have led several investigators to conclude that nicotine exerts its anxiolytic effects via stimulation of 5-HT_{1A} receptors in the DRN, which, as autoreceptors, inhibit 5-HT release in terminal fields of the limbic system.[244] Furthermore, the anxiolytic effect of nicotine administered into the DRN is completely antagonised by administration of the α4β2 antagonist DhβE.[245] Taken together, these data suggest that the DRN may be an important locus for nicotine to exert its anxiolytic effects by stimulating pre-synaptic nAChRs on cholinergic neurones in the DRN resulting in stimulation of somatodendritic 5-HT_{1A} receptors via an as yet undefined mechanism. Studies of knockout mice suggest that α4β2 nAChRs are involved in this process as the same response could not be elicited in mice lacking either the α4 or β2 subunits.[246]

Nicotine administration has also been shown to lead to increased release of 5-HT in the lateral septum, cortex, hypothalamus, and spinal cord. However, the DRN and the dorsal hippocampus are regions where nicotine-5-HT interactions appear to mediate nicotine withdrawal involving the 5-HT_{1A} receptor. Some of these studies have been discussed briefly in Section 1.2.1.2.3, but I refer to them again from a different angle to highlight the contribution of the 5-HT_{1A} receptor specifically in the nicotine withdrawal syndrome. For example, Cheeta and colleagues observed that low dose nicotine administered into the DRN reversed the anxiogenic withdrawal response in the light-dark crossing test[247] and also noted that the 5-HT_{1A} antagonist WAY-100635 reversed the anxiolytic effect of nicotine.

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[243] Balfour, D.J., Graham, C. A., Vale, A. L. (1986).
[244] Seth, P., Cheeta, S., Tucci, S., File, S. E. (2002); Sprouse, J.S., Aghajanian, G. K. (1987). 'Electrophysiological Responses of Serotoninergic Dorsal Raphe Neurons to 5-HT1A and 5-HT1B Agonists', *Synapse*, **1**(1): pp. 3-9.
[245] Cheeta, S., Tucci, S., File, S. E. (2001).
[246] Cordero-Erausquin, M., Marubio, L. M., Klink, R., Changeux, J. P. (2000).
[247] Cheeta, S., Irvine, E. E., Kenny, P. J., File, S. E. (2001).

Furthermore, after six days of nicotine administration, tolerance developed to the anxiolytic effects of nicotine.

Several studies in rats indicate that the acoustic startle response is mediated, in part, via the hippocampus.[248] As mentioned in Section 1.2.1.3.3, Rasmussen and colleagues observed that rats undergoing nicotine withdrawal have enhanced acoustic startle response[249] and pre-treatment with the agonist 8-OH-DPAT either had no effect or enhanced the acoustic startle response whilst treatment with the 5-HT_{1A} antagonist WAY-100635 reversed the acoustic startle response. In addition, these same investigators observed that another 5-HT_{1A} antagonist LY426965 [(2S)-(+)-1-cyclohexyl-4-[4-(2-methoxyphenyl)-1-piperazinyl]2-methlyl-2-phenyl-1-butanone monohydrochloride] reversed the acoustic startle during nicotine withdrawal in rats.[250] The sum of these observations has important clinical implications because enhanced acoustic startle and anxiety traits are reliable predictors of relapse following smoking cessation[251] and, in humans, treatment with the 5-HT_{1A} agonist buspirone has been shown to attenuate the anxiety experienced by smokers during withdrawal.[252]

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[248] Howland, J.G., MacKenzie, E. M., Yim, T. T., Taepavarapruk, P., Phillips, A. G. (2004). 'Electrical Stimulation of the Hippocampus Disrupts Prepulse Inhibition in Rats: Frequency- and Site-Dependent Effects', *Behav Brain Res.* **152**(2): pp. 187-97; Zhang, W.N., Bast, T., Feldon, J. (2002). 'Prepulse Inhibition in Rats with Temporary Inhibition/Inactivation of Ventral or Dorsal Hippocampus', *Pharmacol Biochem Behav.* **73**(4): pp. 929-40.

[249] Rasmussen, K., Kallman, M. J., Helton, D. R. (1997).

[250] Rasmussen, K., Calligaro, D. O., Czachura, J. F., Dreshfield-Ahmad, L. J., Evans, D. C., Hemrick-Luecke, S. K., Kallman, M. J., Kendrick, W. T., Leander, J. D., Nelson, D. L., Overshiner, C. D., Wainscott, D. B., Wolff, M. C., Wong, D. T., Branchek, T. A., Zgombick, J. M., Xu, Y. C. (2000). 'The Novel 5-Hydroxytryptamine(1A) Antagonist LY426965: Effects on Nicotine Withdrawal and Interactions with Fluoxetine', *J Pharmacol Exp Ther.* **294**(2): pp. 688-700.

[251] Niaura, R., Shadel, W. G., Abrams, D. B., Monti, P. M., Rohsenow, D. J., Sirota, A. (1998); Hutchison, K.E., Niaura, R., Swift, R. (1999). 'Smoking Cues Decrease Prepulse Inhibition of the Startle Response and Increase Subjective Craving in Humans', *Exp Clin Psychopharmacol.* 7(3): pp. 250-6.

[252] West, R., Hajek, P., McNeill, A. (1991). 'Effect of Buspirone on Cigarette Withdrawal Symptoms and Short-Term Abstinence Rates in a Smokers Clinic', *Psychopharmacology* (Berl), **104**(1): pp. 91-6.

In addition to evidence pointing to the influence of serotonin on nicotine withdrawal, there is a convergence of research suggesting that nicotine-serotonin interactions influence conditioned reinforcement and may influence incentive sensitisation to nicotine through effects in the striatum. First, Reuben and Clarke observed that nicotine administration stimulated the release of 5-HT in striatal synaptosomes in a dose-dependent manner, effects not observed in the hippocampus or cortex and suggesting a direct effect of pre-synaptic nAChR stimulation in the striatum.[253] The NAc receives input from 5-HT neurones projecting from the raphe nuclei and the 5-HT terminals form synaptic contacts with DA neurones in the NAc. Olausson and colleagues observed that the selective serotonin reuptake inhibitor citalopram significantly attenuated nicotine-induced behavioural disinhibition in rats.[254] SSRIs, which bind to serotonin transporters, do lead to short-term desensitisation of somatodendritic and terminal 5-HT_{1A} receptors *in vitro*,[255] and some of the effects of SSRIs on mood regulation have been attributed to 5-HT_{1A} desensitisation early in the course of treatment. Thus, it is interesting that Olausson and colleagues also found that administration

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[253] Reuben, M., Clarke, P. B. (2000). 'Nicotine-Evoked [3H]5-Hydroxytryptamine Release from Rat Striatal Synaptosomes', *Neuropharmacology*, **39**(2): pp. 290-9.

[254] Olausson, P., Engel, J. A., Soderpalm, B. (1999).

[255] Blier, P., De Montigny, C. (1983). 'Electrophysiological Investigations on the Effect of Repeated Zimelidine Administration on Serotonergic Neurotransmission in the Rat', *J Neurosci.* **3**(6): pp. 1270-8; Chaput, Y., de Montigny, C., Blier, P. (1986). 'Effects of a Selective 5-HT Reuptake Blocker, Citalopram, on the Sensitivity of 5-HT Autoreceptors: Electrophysiological Studies in the Rat Brain', *Naunyn Schmiedebergs Arch Pharmacol.* **333**(4): pp. 342-8; Blier, P., Chaput, Y., de Montigny, C. (1988). 'Long-Term 5-HT Reuptake Blockade, but not Monoamine Oxidase Inhibition, Decreases the Function of Terminal 5-HT Autoreceptors: An Electrophysiological Study in the Rat Brain', *Naunyn Schmiedebergs Arch Pharmacol.* **337**(3): pp. 246-54; de Montigny, C., Chaput, Y., Blier, P. (1990). 'Modification of Serotonergic Neuron Properties by Long-Term Treatment with Serotonin Reuptake Blockers', *J Clin Psychiatry*, **51 Suppl B**: pp. 4-8;Bergqvist, P.B., Bouchard, C., Blier, P. (1999). 'Effect of Long-Term Administration of Antidepressant Treatments on Serotonin Release in Brain Regions Involved in Obsessive-Compulsive Disorder', *Biol Psychiatry*, **45**(2): pp. 164-74.

of the 5-HT_{1A} agonist 8-OH-DPAT also reduced the expression of nicotine-induced behavioural sensitisation as evaluated with the elevated plus-maze in rats.[256]

Given the putative role of the NAc in the development of sensitisation to smoking-related environmental cues and the documented influence of 5-HT systems in mediation of conditioned behaviour and impulse control, it is plausible that accumbens 5-HT plays a role, either directly or indirectly through mediation of DA neurotransmission, in the development of incentive salience to nicotine. Furthermore, the observation that, in rats, striatal 5-HT_{1A} receptors appear to mediate conditioned sensitisation behaviour and that DRN and hippocampal receptors are implicated in the mediation of nicotine-induced attenuation of anxiety lends support to the notion that such receptor subtypes may be important to the development of nicotine dependence. This is not to say that other 5-HT receptor subtypes have no influence in nicotine dependence. To the contrary, there is *in vitro* evidence that 5-HT_2 receptors inhibit electrophysiological activity in the VTA[257] and administration of 5-HT_3 agonists elevates extracellular DA in the ventral striatum.[258] Thus, there is likely a complex interplay between DA and 5-HT neurotransmission involving multiple receptor subtypes.

The serotonin transporter ('SERT' or '5-HTT') is responsible for regulating extracellular 5-HT concentration and recycling of synaptic 5-HT following neuronal firing throughout the brain. SERTs belong to a family of transport proteins including the norepinephrine

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[256] Olausson, P., Akesson, P., Engel, J. A., Soderpalm, B. (2001). 'Effects of 5-HT1A and 5-HT2 Receptor Agonists on the Behavioral and Neurochemical Consequences of Repeated Nicotine Treatment', *Eur J Pharmacol.* **420**(1): pp. 45-54.

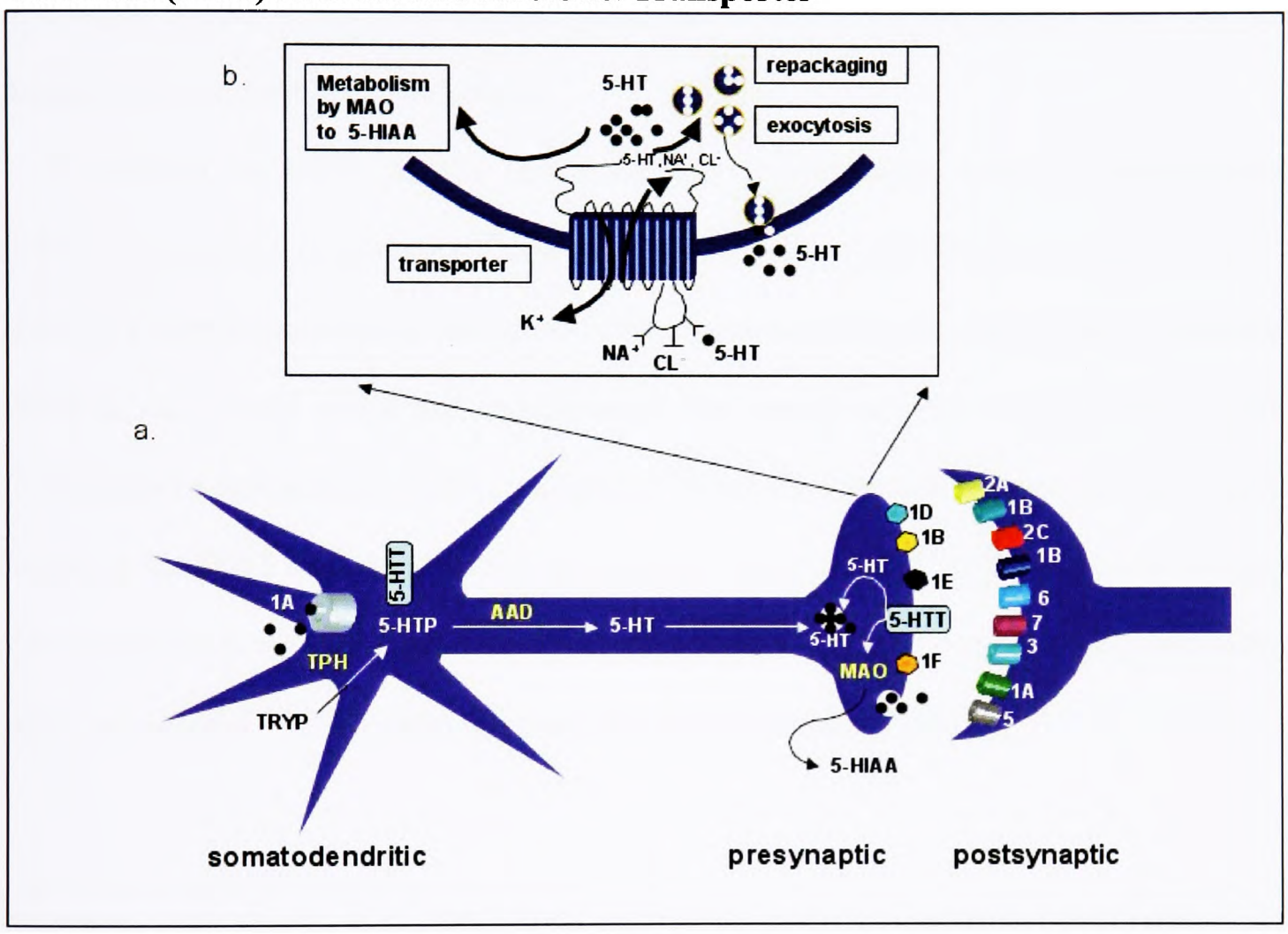
[257] North, R.A., Uchimura, N. (1989); Ugedo, L., Grenhoff, J., Svensson, T. H. (1989).

[258] Benloucif, S., Keegan, M. J., Galloway, M. P. (1993). 'Serotonin-Facilitated Dopamine Release *In Vivo*: Pharmacological Characterization', *J Pharmacol Exp Ther.* **265**(1): pp. 373-7; Jiang, L.H., Ashby, C. R., Jr., Kasser, R. J., Wang, R. Y. (1990). 'The Effect of Intraventricular Administration of the 5-HT3 Receptor Agonist 2-Methylserotonin on the Release of Dopamine in the Nucleus Accumbens: An In Vivo Chronocoulometric Study', *Brain Res.* **513**(1): pp. 156-60.

transporter (NET), and dopamine transporter (DAT), all of which contain 12 trans-membrane helices.[259] The role of the 5-HTT as part of a functional unit within a 5-HT neurone is presented in Figure 1.8.

Figure 1.8

Serotonin (5-HT) Neurone and Serotonin Transporter



LEGEND: Illustration created by SD for this thesis. A. Representation of a 5-HT neurone. Tryptophan (TRYP) is converted to 5-hydroxytryptaphan (5-HTTP) in a rate-limiting step by tryptophan hydroxylase (TPH). 5-HTP is decarboxylated to 5-hydroxytryptamine (5-HT) by L-aromatic acid decarboxylase (AAD) (also known as 'dopa decarboxylase'). 5-HT_{1A} receptors are present as somatodendritic autoreceptors in the raphe complex and on postsynaptic boutons in terminal fields. 5-HT_{1A} is one of at least 14 subtypes of 5-HT receptors, which are differentially located on presynaptic terminals or postsynaptic boutons. All 5-HT receptors, with the exception of the 5-HT_3 subtype, are coupled to G-proteins and have a similar structure of seven transmembrane domains. The 5-HT_3 receptor is a ligand-gated ion channel. B. Schematic of the serotonin transporter (5-HTT) at a presynaptic terminal. 5-HT binds to its binding site on the 5-HTT and is taken into the terminal via a translocation of the 5-HTT coupled with transport of sodium and chloride into the intracellular space. Once inside the terminal 5-HT is either repackaged into synaptosomes for subsequent release via exocytosis or is metabolised in a two-step process by monoamine oxidase (MAO) and aldehyde dehydrogenase (AD) to 5-hydroxyindoleacetic acid (5-HIAA).

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[259] Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K. (ed.) (2003).

SERTs operate as co-transporters of NA^+, Cl^-, and 5-HT such that one of each molecule must bind to the transporter in order to stimulate a conformational change involving transport of all three molecules into the intracellular space. Following transport of NA^+, Cl^-, and 5-HT into the cytoplasm, K^+ binds to the same binding site – now facing the cytoplasm – resulting in transport of K^+ to the extracellular space and a conformational change such that the binding site is accessible extracellularly.

In addition to 5-HT, SERTs also transports several drugs such as amphetamine derivatives and some neurotoxins.[260] Moreover, SERTs have some ability to transport DA.[261] The SERT may be particularly relevant to 5-HT_{1A} neurotransmission, and perhaps to nicotine addiction, as Li and colleagues demonstrated that knockout mice for the 5-HTT gene demonstrated a reduction in 5-HT_{1A} receptors.[262] Over the last decade there has been major interest in the SERT because of its role as a target of many antidepressant drugs and because polymorphisms in the SERT gene have been associated with mood disorders and personality traits, and reduced 5-HTT availability has been associated with depression.[263]

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[260] Andrews, A.M., Murphy, D. L. (1993). 'Sustained Depletion of Cortical and Hippocampal Serotonin and Norepinephrine but not Striatal Dopamine by 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2'-NH2-MPTP): A Comparative Study with 2'-CH3-MPTP and MPTP', *J Neurochem.* **60**(3): pp. 1167-70; Murphy, D.L., Lerner, A., Rudnick, G., Lesch, K. P. (2004). 'Serotonin Transporter: Gene, Genetic Disorders, and Pharmacogenetics', *Mol Interv.* **4**(2): pp. 109-23; Andrews, A.M., Murphy, D. L. (1993). 'Fluoxetine and Desipramine Selectively Attenuate 2'-NH2-MPTP-Induced Depletions in Serotonin and Norepinephrine', *Eur J Pharmacol.* **250**(2): pp. 215-21; Luellen, B.A., Miller, D. B., Chisnell, A. C., Murphy, D. L., O'Callaghan, J. P., Andrews, A. M. (2003). 'Neuronal and Astroglial Responses to the Serotonin and Norepinephrine Neurotoxin: 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine', *J Pharmacol Exp Ther.* **307**(3): pp. 923-31; Schuldiner, S., Steiner-Mordoch, S., Yelin, R., Wall, S. C., Rudnick, G. (1993). 'Amphetamine Derivatives Interact with Both Plasma Membrane and Secretory Vesicle Biogenic Amine Transporters', *Mol Pharmacol.* **44**(6): pp. 1227-31.

[261] Murphy, D.L., Lerner, A., Rudnick, G., Lesch, K. P. (2004).

[262] Li, Q., Wichems, C., Heils, A., Lesch, K. P., Murphy, D. L. (2000). 'Reduction in the Density and Expression, but not G-Protein Coupling, of Serotonin Receptors (5-HT1A) in 5-HT Transporter Knock-Out Mice: Gender and Brain Region Differences', *J Neurosci.* **20**(21): pp. 7888-95.

[263] Lesch, K.P., Gutknecht, L. (2004). 'Focus on the 5-HT1A Receptor: Emerging Role of a Gene Regulatory Variant in Psychopathology and Pharmacogenetics', *Int J Neuropsychopharmacol*, 7(4): pp. 381-5.

The human SERT gene is located at chromosome 17 (17q11.2) and is 37.8 kilobases (kb), composed of 14 exons, and encodes a protein of 630 amino acids and is illustrated in Figure 1.9.[264] Heils and colleagues first described a deletion in the promoter region of the SERT gene (at approximately 1.4 k.b. from the transcription initiation site) that results in either "short" (484 bp) or "long" (528 bp) sequences with a variable number of tandem repeats (VNTR) polymorphism named the 5-HTT gene-linked polymorphic region (5-HTTLPR).[265] Lesch and colleagues demonstrated that the 5-HTTLPR S allele is associated with a three to four-fold decrease in transcription of the 5-HTT gene *in vitro* and was significantly associated with anxiety disorders in humans.[266]

The 5-HTTLPR S allele has been associated with abnormal mood states/emotional behaviours,[267] depressive illness,[268] severity of depressive symptoms in patients with Parkinson's disease,[269] suicide,[270] neuroticism,[271] and nicotine dependence.[272]

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[264] Lesch, K.P., Balling, U., Gross, J., Strauss, K., Wolozin, B. L., Murphy, D. L., Riederer, P. (1994). 'Organization of the Human Serotonin Transporter Gene', *J Neural Transm Gen Sect.* **95**(2): pp. 157-62.

[265] Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., Lesch, K. P. (1996). 'Allelic Variation of Human Serotonin Transporter Gene Expression', *J Neurochem*, **66**(6): pp. 2621-4.

[266] Lesch, K.P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., Murphy, D. L. (1996). 'Association of Anxiety-Related Traits with a Polymorphism in the Serotonin Transporter Gene Regulatory Region', *Science*, **274**(5292): pp. 1527-31.

[267] Ibid; Mazzanti, C.M., Lappalainen, J., Long, J. C., Bengel, D., Naukkarinen, H., Eggert, M., Virkkunen, M., Linnoila, M., Goldman, D. (1998). 'Role of the Serotonin Transporter Promoter Polymorphism in Anxiety-Related Traits', *Arch Gen Psychiatry*, **55**(10): pp. 936-40; Munafo, M.R., Clark, T. G., Moore, L. R., Payne, E., Walton, R., Flint, J. (2003). 'Genetic Polymorphisms and Personality in Healthy Adults: A Systematic Review and Meta-Analysis', *Mol Psychiatry*, **8**(5): pp. 471-84.

[268] Willeit, M., Praschak-Rieder, N., Neumeister, A., Zill, P., Leisch, F., Stastny, J., Hilger, E., Thierry, N., Konstantinidis, A., Winkler, D., Fuchs, K., Sieghart, W., Aschauer, H., Ackenheil, M., Bondy, B., Kasper, S. (2003). 'A Polymorphism (5-HTTLPR) in the Serotonin Transporter Promoter Gene is Associated with DSM-IV Depression Subtypes in Seasonal Affective Disorder', *Mol Psychiatry*, **8**(11): pp. 942-6.

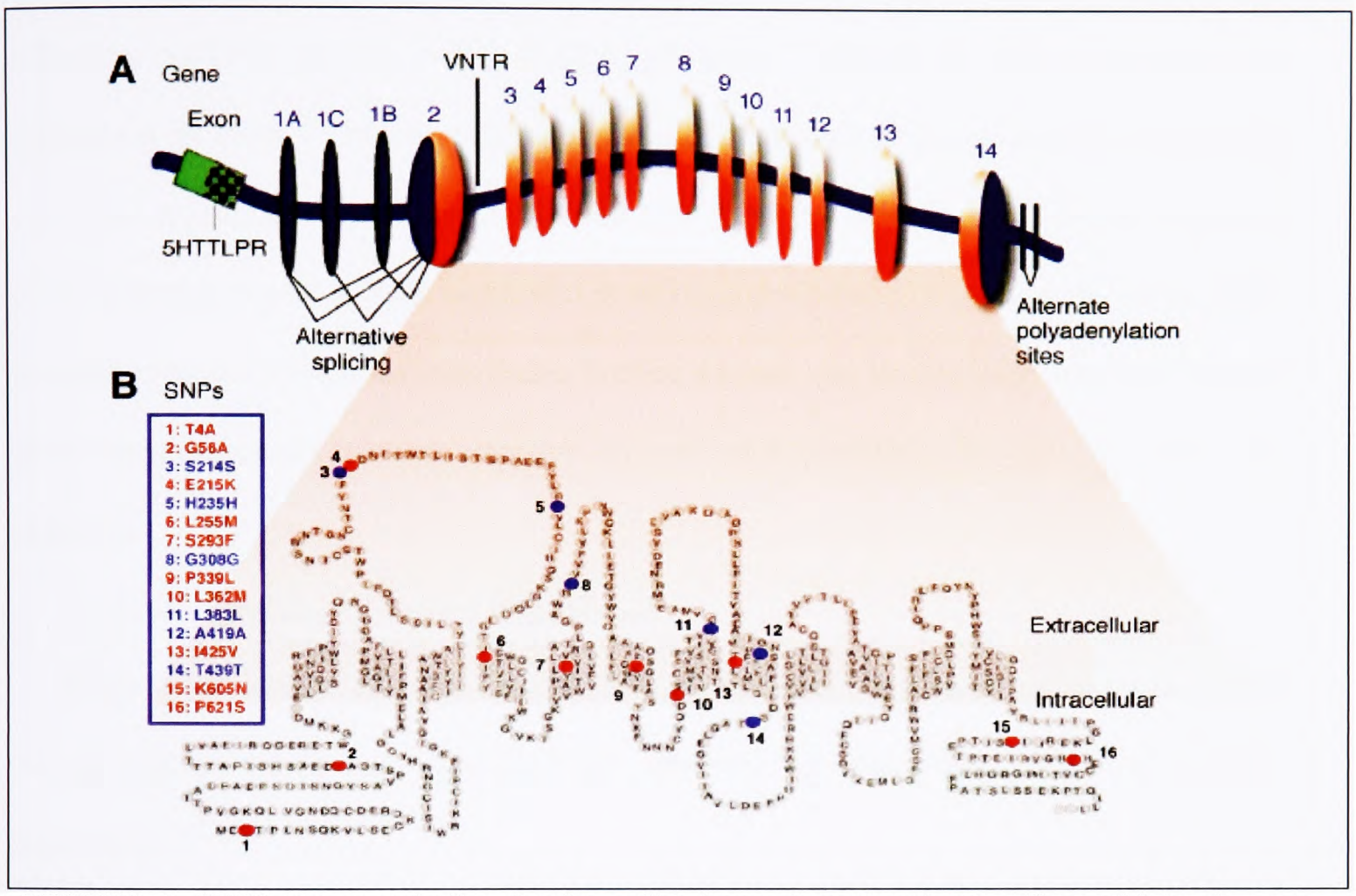
[269] Mossner, R., Henneberg, A., Schmitt, A., Syagailo, Y. V., Grassle, M., Hennig, T., Simantov, R., Gerlach, M., Riederer, P., Lesch, K. P. (2001). 'Allelic Variation of Serotonin Transporter Expression is Associated with Depression in Parkinson's Disease', *Mol Psychiatry*, **6**(3): pp. 350-2.

[270] Anguelova, M., Benkelfat, C., Turecki, G. (2003). 'A Systematic Review of Association Studies Investigating Genes Coding for Serotonin Receptors and the Serotonin Transporter: II. Suicidal behavior', *Mol Psychiatry*, **8**(7): pp. 646-53.

[271] Munafo', M.R., Clark, T. G., Moore, L. R., Payne, E., Walton, R., Flint, J. (2003); Sen, S., Burmeister, M., Ghosh, D. (2004). 'Meta-Analysis of the Association Between a Serotonin Transporter Promoter Polymorphism (5-HTTLPR) and Anxiety-Related Personality Traits', *Am J Med Genet B Neuropsychiatr Genet.* **127**(1): pp. 85-9

Figure 1.9

Putative Structure of the Serotonin Transporter Protein and Gene



LEGEND: A. SERT gene organisation showing coding (orange) exons and non-coding and intronic areas (blue), locations of the 5HTTLPR polymorphism ~1.4 kb upstream and location of the intronic VNTR near exon 2. B. Translated (red) and non-translated (blue) SNP sites are annotated in the 630 amino acids of the 12-TM-containing SERT protein.[273]

In addition to the 5-HTTLPR, there is a 380 bp deletion/mutation polymorphism between the 5-HTTLPR and the transcription initiation site, a VNTR in intron 2, and a G to T polymorphism in the 3' untranslated region (UTR).[274]

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[272] Lerman, C., Caporaso, N. E., Audrain, J., Main, D., Boyd, N. R., Shields, P. G. (2000). 'Interacting Effects of the Serotonin Transporter Gene and Neuroticism in Smoking Practices and Nicotine Dependence', *Mol Psychiatry*, **5**(2): pp. 189-92; Munafo', M., Clark, T., Johnstone, E., Murphy, M., Walton, R. (2004). 'The Genetic Basis for Smoking Behavior: A Systematic Review and Meta-Analysis', *Nicotine Tob Res*. **6**(4): pp. 583-97; Lerman, C., Shields, P. G., Audrain, J., Main, D., Cobb, B., Boyd, N. R., Caporaso, N. (1998). 'The Role of the Serotonin Transporter Gene in Cigarette Smoking', *Cancer Epidemiol Biomarkers Prev*. **7**(3): pp. 253-5.

[273] Source of illustration is Figure 2 in: Murphy, D.L., Lerner, A., Rudnick, G., Lesch, K. P. (2004). 'Serotonin Transporter: Gene, Genetic Disorders, and Pharmacogenetics', *Mol Interv*. **4**(2): pp. 109-23.

[274] Battersby, S., Ogilvie, A. D., Blackwood, D. H. Shen, S., Muqit, M. M., Muir, W. J., Teague, P., Goodwin, G. M., Harmar, A. J. (1999). 'Presence of Multiple Functional Polyadenylation Signals and a Single Nucleotide

1.2.1.3.4 Other Neurotransmitters

As described above, animal and human studies have demonstrated that nicotine stimulates nAChRs on DA, 5-HT, GABA, glutamate, cannabinoid, and opioid neurones throughout the brain.[275] Nicotine also stimulates of the release of norepinephrine (NE), ACh, CRF, and vasopressin as reviewed in Section 1.2.1.2.1.[276] A detailed discussion of the role of every neurotransmitter system implicated in nicotine dependence is beyond the scope of the research presented herein and this thesis. Suffice it to say that the complex interplay of all of these neurotransmitter systems remains an area of exploration that has not been fully explored.

1.2.1.3.5 Models of Nicotine Dependence

Much of our current understanding of nicotine dependence is based on decades of elegant animal studies, which have provided the substrate for theoretical models of nicotine dependence.[277]

Robinson and Berridge hypothesise that drug-seeking behaviour is the manifestation of neuroadaptations that ultimately perpetuate a sub-component of reward which they term drug "wanting".[278] Such neuroadaptations result in sensitisation of brain reward systems to

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Polymorphism in the 3' Untranslated Region of the Human Serotonin Transporter Gene', *J Neurochem.* **72**(4): pp.1384-8; Flattem, N. L., Blakely, R. D. (2000). 'Modified Structure of the Human Serotonin Transporter Promoter', *Mol Psychiatry*, **5**(1): pp.110-5; Mortensen, O. V., Thomassen, M., Larsen, M. B., Whittemore, S. R., Wiborg, O. (1999). 'Functional Analysis of a Novel Human Serotonin Transporter Gene Promoter in Immortalized Raphe Cells', *Brain Res Mol Brain Res.* **68**(1-2): pp.141-8.

[275] Benowitz, N.L. (1999); Corrigall, W.A., Coen, K. M., Adamson, K. L., Chow, B. L., Zhang, J. (2000); Matsuda, L.A., Lolait, S. J., Brownstein, M. J., Young, A. C., Bonner, T. I. (1990). 'Structure of a Cannabinoid Receptor and Functional Expression of the Cloned cDNA', *Nature*, **346**(6284): pp. 561-4; Fu, Y., Matta, S. G., Brower, V. G., Sharp, B. M. (2001). 'Norepinephrine Secretion in the Hypothalamic Paraventricular Nucleus of Rats during Unlimited Access to Self-Administered Nicotine: An *In Vivo* Microdialysis Study', *J Neurosci.* **21**(22): pp. 8979-89.

[276] Benowitz, N.L. (1999)

[277] Corrigall, W.A. (1999). 'Nicotine Self-Administration in Animals as a Dependence Model', *Nicotine Tob Res.* **1**(1): pp. 11-20.

[278] Robinson, T.E., Berridge, K. C. (1993).

drugs and drug-associated stimuli.[279] They cite animal studies whereby chronic consumption of nicotine, methylphenidate, ethanol, and other drugs of abuse result in sensitisation of DA systems and accumbens-related circuitry to psychomotor stimulation, which may persist for months or years. They refer to this process as "neural sensitisation".[280] In contrast, "behavioural sensitisation" signifies the notion that sensitisation to psychomotor effects of drugs such as nicotine is modulated by learning and the circumstances surrounding drug use, as supported by CPP studies.[281] As such, the interactions between neural sensitisation and behavioural sensitisation result in associative learning. Thus, classical conditioning to the environmental context associated with drug use results in sensitisation to drug-associated stimuli, which trigger craving and wanting of a drug. Even as individuals become desensitised to the pleasurable or hedonic effects of drugs such as nicotine, they become

[279] Badiani, A., Oates, M. M., Day, H. E., Watson, S. J., Akil, H., Robinson, T. E. (1998). 'Amphetamine-Induced Behavior, Dopamine Release, and c-fos mRNA Expression: Modulation by Environmental Novelty', *J Neurosci*, **18**(24): pp. 10579-93.

[280] Woolverton, W.L., Goldberg, L. I., Ginos, J. Z. (1984). 'Intravenous Self-Administration of Dopamine Receptor Agonists by Rhesus Monkeys', *J Pharmacol Exp Ther*p. **230**(3): p. 678-83; Piazza, P.V., Deminiere, J. M., Le Moal, M., Simon, H. (1989). 'Factors that Predict Individual Vulnerability to Amphetamine Self-Administration', *Science*, **245**(4925): pp. 1511-3; Piazza, P.V., Deminiere, J. M., Le Moal, M., Simon, H. (1990). 'Stress- and Pharmacologically-Induced Behavioral Sensitization Increases Vulnerability to Acquisition of Amphetamine Self-Administration', *Brain Res.* **514**(1): pp. 22-6; Horger, B.A., Giles, M. K., Schenk, S. (1992). 'Preexposure to Amphetamine and Nicotine Predisposes Rats to self-administer a low dose of cocaine', *Psychopharmacology* (Berl), **107**(2-3): pp. 271-6; Horger, B.A., Shelton, K., Schenk, S. (1990). 'Preexposure Sensitizes Rats to the Rewarding Effects of Cocaine', *Pharmacol Biochem Behav.* **37**(4): pp. 707-11; Valadez, A., Schenk, S. (1994). 'Persistence of the Ability of Amphetamine Preexposure to Facilitate Acquisition of Cocaine Self-Administration', *Pharmacol Biochem Behav.* **47**(1): pp. 203-5; Pierre, P.J., Vezina, P. (1998). 'D1 Dopamine Receptor Blockade Prevents the Facilitation of Amphetamine Self-Administration Induced by Prior Exposure to the Drug', *Psychopharmacology* (Berl), **138**(2): pp. 159-66; Pierre, P.J., Vezina, P. (1997). 'Predisposition to Self-Administer Amphetamine: The Contribution of Response to Novelty and Prior Exposure to the Drug', *Psychopharmacology* (Berl), **129**(3): pp. 277-84.

[281] Lett, B.T. (1989). 'Repeated Exposures Intensify Rather than Diminish the Rewarding Effects of Amphetamine, Morphine, and Cocaine', *Psychopharmacology* (Berl), **98**(3): pp. 357-62; Gaiardi, M., Bartoletti, M., Bacchi, A., Gubellini, C., Costa, M., Babbini, M. (1991). 'Role of Repeated Exposure to Morphine in Determining its Affective Properties: Place and Taste Conditioning Studies in Rats', *Psychopharmacology* (Berl), **103**(2): pp. 183-6; Shippenberg, T.S., Heidbreder, C. (1995). 'Sensitization to the Conditioned Rewarding Effects of Cocaine: Pharmacological and Temporal Characteristics', *J Pharmacol Exp Ther.* **273**(2): pp. 808-15; Shippenberg, T.S., Heidbreder, C., Lefevour, A. (1996). 'Sensitization to the Conditioned Rewarding Effects of Morphine: Pharmacology and Temporal Characteristics', *Eur J Pharmacol.* **299**(1-3): pp. 33-9; Shippenberg, T.S., LeFevour, A., Heidbreder, C. (1996). 'kappa-Opioid Receptor Agonists Prevent Sensitization to the Conditioned Rewarding Effects of Cocaine', *J Pharmacol Exp Ther.* **276**(2): pp. 545-54.

more sensitive to the aversive effects of abstinence (i.e., craving and wanting), which often results in relapse during attempts at drug cessation. Robinson and Berridge have named the phenomenon of sensitisation to drug-associated stimuli and consequent drug-seeking behaviour "incentive-sensitisation".

Koob and Le Moal[282] hypothesise that drug addiction results from a failure of reward circuits to return to normal functioning after prolonged stimulation with a drug. A new equilibrium develops, which they call "allostasis", representing a chronic deviation of reward set points caused by dysregulation of reward circuits and activation of brain and hormonal stress responses through stimulation of the HPA axis. Koob and Le Moal posit that chronic activation of the cortico-striatal-thalamic loop results in an allostatic state using brain circuits, which evolved for the reinforcement of natural rewards. The manifestation of such processes is that positive reinforcement from stimulation of the mesolimbic reward circuitry (particularly, the NAc, anterior cingulate cortex, orbitofrontal cortex, and dorsolateral prefrontal cortex) and the HPA axis results in bingeing or use of drugs for intoxication whilst, negative reinforcement expressed through nicotine withdrawal – resulting from neuroadaptations in the cortico-striatal-thalamic loop – discourages prolonged abstinence. Such conditioned positive and negative reinforcement leads to a progressively downward spiral with loss of control over drug consumption.

Volkow and colleagues proposed an "Impaired Response Inhibition and Salience Attribution (I-RISA)" model connoting addiction as emotional and cognitive processes resulting in the overvaluing of drug reinforcers, the undervaluing of alternative reinforcers,

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[282] Koob, G.F., Le Moal, M. (1997).

and deficits in inhibitory control of drug use.[283] Their model focuses on the interaction between frontal cortical circuits involved in drive and perseverative behaviours and mesolimbic DA circuits involved in drug salience and reward. Specific brain regions identified in their model include the basal ganglia (caudate, putamen, globus pallidus) and the orbitofrontal cortex (OFC). Linked to the limbic system, these regions are intimately involved in behavioural regulation, impulse control and compulsive behaviours.[284] They also have extensive connections with the Am and the hippocampus, regions of the brain responsible for emotional processing and memory consolidation, respectively. Volkow's model posits that repeated exposure to drugs of abuse results in disruption along the striato-thalamo-orbitofrontal pathway, a subcortical brain circuit that links the basal ganglia with the OFC.

These models are not mutually exclusive and, in fact, together begin to craft a more complete understanding of how the complex interplay of multiple neurotransmitter systems and brain regions integrate as circuits as smokers progress from casual use to chronic tolerance and dependence.

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[283] Goldstein, R.Z., Volkow, N. D. (2002). 'Drug Addiction and its Underlying Neurobiological Basis: Neuroimaging Evidence for the Involvement of the Frontal Cortex', *Am J Psychiatry*, **159**(10): pp. 1642-52.

[284] Lewis, S.J., et al. (2003). 'Cognitive Iimpairments in Early Parkinson's Disease are Accompanied by Reductions in Activity in Frontostriatal Neural Circuitry', *J Neurosci.* **23**(15): pp. 6351-6; Afifi, A.K. (2003). 'The Basal Ganglia: A Neural Network with More than Motor Function', *Semin Pediatr Neurol*, **10**(1): pp. 3-10; Koechlin, E., Ody, C., and Kouneiher, F. (2003). 'The Architecture of Cognitive Control in the Human Prefrontal Cortex', *Science*, **302**(5648): pp. 1181-5; Stuss, D.T. and Alexander, M.P. (2000). 'Executive Functions and the Frontal Lobes: A Conceptual View', *Psychol Res.* **63**(3-4): pp. 289-98.

1.3 Genetic Influences on Nicotine Dependence

A confluence of evidence from research in human twin, linkage, and case-control studies strongly suggests that genetic factors contribute substantially to the development of nicotine dependence. As referenced in the Preface, I have published major portions of Section 1.3 as a book chapter as part of the process of reviewing the literature in preparation for my thesis.[285]

1.3.1 Twin Studies

Family studies are designed to examine whether the chance of having a particular characteristic is increased in the relatives of those who have the characteristic, compared to the relatives of those who do not. An increased incidence in relatives of affected individuals indicates that the trait is heritable. Twin studies rely on the "natural experiment" of twinning, whereby monozygotic (MZ) twins come from the same fertilised egg and are genetically identical (share 100% of their variegated genes), while non-identical or dizygotic (DZ) twins, like other siblings, share, on average, 50% of their variegated genes. A greater similarity or correlation between MZ twins than DZ twins indicates a genetic influence on variability in the observed phenotype. Twin study designs go beyond family study designs in allowing the differentiation of genetic and environmental sources of phenotypic variance.

Over the last few decades several extant large-scale twin registries have been established to determine the relative influence of heritability or (h^2), shared environmental (c^2) and unique environmental (e^2) influences on smoking behaviour. The overall conclusion is that

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[285] David, S.P., Munafo', M.R., Walton, R.T. (2005). 'Chapter 21. Pharmacogenomics Research in Nicotine Addiction and Smoking Cessation', in *Clinical Pharmacogenomics and Introduction to PharmacoProteomics*, ed. by Wong, S.H.Y., Linder, M., Valdes, R. (Washington, D.C., U.S.A: American Association for Clinical Chemistry (AACC) Press).

genetic and environmental factors contribute approximately equally to the risk of becoming a smoker and subsequently progressing to long-term use.

Li and colleagues performed a recent meta-analysis[286] summarised in Tables 1.3 and 1.4, which combined the estimates of genetic and environmental influences from twin studies conducted to date, distinguishing between smoking initiation and smoking persistence. For initiation, the mean heritability h^2 was 0.46 – 0.50 for men and women combined, with the mean h^2 being higher for women than for men. For persistence, mean h^2 was 0.52 – 0.59 for men and women combined, with the mean h^2 being higher for men than for women in this case. The authors conclusion was that these results suggested that genetic factors may contribute differently to smoking initiation and smoking persistence in male and female adults.

Heath and Madden[287] suggest a two-process model of smoking initiation and smoking persistence, based on data from several twin registries, as outlined in Figure 1.10, below. This includes a genetic contribution to smoking initiation which is mediated in part by personality traits such as extraversion and neuroticism, with this mediating influence accounting for between one-third and one-half of the heritability of smoking initiation. The progression to smoking persistence is partly under the influence of genetic factors that also influence smoking initiation and other genetic factors that are unique to persistence (influencing, among other things, amount smoked). The data reported by Heath and Madden do not suggest an important role for personality in the development of persistence.

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[286] Li, M.D., Cheng, R., Ma, J. Z., Swan, G. E. (2003). 'A Meta-Analysis of Estimated Genetic and Environmental Effects on Smoking Behavior in Male and Female Adult Twins', *Addiction*, **98**(1): pp. 23-31.
[287] Heath, A.C., Madden, P.A.F. (1995). 'Genetic Influences on Smoking Behavior', in *Behavior Genetics Approaches in Behavioral Medicine*, ed. by Turner, J.R., Cardon, L.R., Hewitt, J.K. (New York, NY, USA: Plenum Press), pp. 45-66.

Table 1. 3

Meta-Analysis of Smoking Initiation in Twin Studies

Country	Sex	MZ	DZ	h^2	c^2	e^2	References
Australia	M	567	352	0.33 (0.15)	0.39 (0.14)	0.28	Heath et al., 1993[288]
US, 1	M	305	354	0.64 (0.16)	0.19 (0.15)	0.17	Heath et al., 1993[289]
US, 2	M	478	232	0.54 (0.19)	0.28 (0.18)	0.18	Heath et al., 1993[290]
US	M	2204	1793	0.39 (0.23-0.56)	0.49 (0.32-0.64)	0.12 (0.09-0.16)	True et al., 1997[291]
Finland	M	1496	3440	0.31 (0.19-0.43)	0.58 (0.47-0.69)	0.11 (0.08-0.15)	Heath et al., 1998[292]
Australia	M	567	350	0.40 (0.04-0.76)	0.51 (0.15-0.85)	0.09 (0.03-0.17)	Heath et al., 1998[293]
Australia	M	274	206	0.49	0.31	0.21	Heath & Martin, 1993[294]
Australia	M	293	146	0.11	0.53	0.36	Heath et al., 1993[295]
US	F	255	179	0.77	0	0.23	Edwards et al., 1995[296]
Australia	F	1232	751	0.67 (0.11)	0.15 (0.10)	0.18	Heath et al., 1993[297]
US, 1	F	459	383	0.58 (0.14)	0.26 (0.13)	0.16	Heath et al., 1993[298]
US, 2	F	1397	799	0.49 (0.10)	0.29 (0.09)	0.22	Heath et al., 1993[299]
Australia	F	570	351	0.56	0.30	0.14	Heath & Martin, 1993[300]
Australia	F	663	400	0.74	0.03	0.23	Heath & Martin, 1993[301]
Australia	F	1232	747	0.70 (0.46-0.92)	0.18 (0-0.41)	0.12 (0-0.17)	Heath et al., 1998[302]
Finland	F	1842	3703	0.32 (0.21-0.42)	0.59 (0.50-0.69)	0.09 (0.06-0.12)	Heath et al., 1998[303]
US	F	497	354	0.78	0.07	0.15	Kendler et al., 1999[304]

LEGEND: h^2, c^2 and e^2 estimate heritability (h^2), shared environmental (c^2) and unique environmental (e^2) effects. Values within parentheses represent 95% CI or SE. Two cohorts (USA, 1 & 2) in same study.[1]

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[288] Heath, A.C., Cates, R., Martin, N. G., Meyer, J., Hewitt, J. K., Neale, M. C., Eaves, L. J. (1993). 'Genetic Contribution to Risk of Smoking Initiation: Comparisons Across Birth Cohorts and Across Cultures', *J Subst Abuse*, **5**(3): pp. 221-46

[289] Ibid.

[290] Ibid.

[291] True, W.R., Heath, A. C., Scherrer, J. F., Waterman, B., Goldberg, J., Lin, N., Eisen, S. A., Lyons, M. J., Tsuang, M. T. (1997). 'Genetic and Environmental Contributions to Smoking', *Addiction*, **92**(10): pp. 1277-87

[292] Heath, A.C., Madden, P. A., Martin, N. G. (1998). 'Statistical Methods in Genetic Research on Smoking', *Stat Methods Med Res*. 7(2): pp. 165-86

[293] Ibid.

[294] Heath, A.C. and Martin, N.G. (1993). 'Genetic Models for the Natural History of Smoking: Evidence for a Genetic Influence on Smoking Persistence', *Addict Behav*. **18**(1): pp. 19-34

[295] Heath, A.C., Cates, R., Martin, N. G., Meyer, J., Hewitt, J. K., Neale, M. C., Eaves, L. J. (1993)

[296] Edwards, K.L., Austin, M. A., Jarvik, G. P. (1995). 'Evidence for Genetic Influences on Smoking in Adult Women Twins', *Clin Genet*. **47**(5): pp. 236-44

[297] Heath, A.C., Cates, R., Martin, N. G., Meyer, J., Hewitt, J. K., Neale, M. C., Eaves, L. J. (1993)

[298] Ibid.

[299] Ibid.

[300] Heath, A.C. and Martin, N.G. (1993)

[301] Ibid.

[302] Heath, A.C., Madden, P. A., Martin, N. G. (1998)

[303] Ibid.

Table 1.4

Meta-Analysis of Smoking Persistence in Twin Studies

Country	Sex	MZ	DZ	h^2	c^2	e^2	References
US	M	2390	2570	0.53 [a]	-	-	Carmelli et al.,1990[305]
US	M	2204	1793	0.68 (0.45-0.74)	0.01 (0-0.21)	0.31 (0.26-0.38)	True et al.,1997[306]
Finland	M	1496	3440	0.50 (0.27-0.71)	0.18(0.01-0.35)	0.33 (0.25-0.42)	Heath et al.,1998[307]
Australia	M	274	206	0.48	0.31	0.21	Heath et al.,1999[308]
Australia	M	293	146	0.11	0.53	0.36	Heath et al., 1999[309]
Australia	M	567	350	0.71 (0.31-0.84)	0 (0-0.36)	0.29 (0.16-0.45)	Heath et al., 1998[310]
US	M	163	166	0.52[a]	-	-	Swan et al., 1990[311]
US	M	2220	2373	0.49	0	0.51	Swan et al., 1997[312]
US	M	173	183	0.56	0	0.44	Swan et al., 1996[313]
US	M	1722	1484	0.60 (0.55-0.65)	0 (0.35-0.45)	0.40	True et al., 1999[314]
Sweden	M	127	191	0.61 (0.36-0.86)	0.20 (0-0.45)	0.19 (0.02-0.36)	Kendler et al., 2000[315]
Australia	F	1232	747	0.04 (0-0.58)	0.57 (0.07-0.72)	0.39 (0.26-0.53)	Heath et al., 1998[316]
Sweden	F	83[c]		0.64	0	0.27	Kendler et al., 2000[317]
US	F	497	354	0.72	0	0.28	Kendler et al., 1999[318]

Continued overleaf.

[304] Kendler, K.S., Neale, M. C., Sullivan, P., Corey, L. A., Gardner, C. O., Prescott, C. A. (1999). 'A Population-Based Twin Study in Women of Smoking Initiation and Nicotine Dependence', *Psychol Med.* **29**(2): pp. 299-308

[305] Carmelli, D., Swan, G. E., Robinette, D., Fabsitz, R. R. (1990). 'Heritability of Substance Use in the NAS-NRC Twin Registry', *Acta Genet Med Gemellol* (Roma), **39**(1): pp. 91-8.

[306] True, W.R., Heath, A. C., Scherrer, J. F., Waterman, B., Goldberg, J., Lin, N., Eisen, S. A., Lyons, M. J., Tsuang, M. T. (1997).

[307] Heath, A.C., Madden, P. A., Martin, N. G. (1998).

[308] Heath, A.C., Kirk, K. M., Meyer, J. M., Martin, N. G. (1999). 'Genetic and Social Determinants of Initiation and Age at Onset of Smoking in Australian Twins', *Behav Genet.* **29**(6): pp. 395-407.

[309] Ibid.

[310] Heath, A.C., Madden, P. A., Martin, N. G. (1998).

[311] Swan, G.E., Carmelli, D., Rosenman, R. H., Fabsitz, R. R., Christian, J. C. (1990). 'Smoking and Alcohol Consumption in Adult Male Twins: Genetic Heritability and Shared Environmental Influences', *J Subst Abuse*, **2**(1): pp. 39-50.

[312] Swan, G.E., Carmelli, D., Cardon, L. R. (1997). 'Heavy Consumption of Cigarettes, Alcohol and Coffee in Male Twins', *J Stud Alcohol.* **58**(2): pp. 182-90.

[313] Swan, G.E., Carmelli, D., Cardon, L. R. (1996). 'The Consumption of Tobacco, Alcohol, and Coffee in Caucasian Male Twins: A Multivariate Genetic Analysis', *J Subst Abuse*, **8**(1): pp. 19-31.

[314] True, W.R., Xian, H., Scherrer, J. F., Madden, P. A., Bucholz, K. K., Heath, A. C., Eisen, S. A., Lyons, M. J., Goldberg, J., Tsuang, M. (1999). 'Common Genetic Vulnerability for Nicotine and Alcohol Dependence in Men', *Arch Gen Psychiatry*, **56**(7): pp. 655-61.

[315] Kendler, K.S., Thornton, L. M., Pedersen, N. L. (2000). 'Tobacco Consumption in Swedish Twins Reared Apart and Reared Together', *Arch Gen Psychiatry*, **57**(9): pp. 886-92.

[316] Heath, A.C., Madden, P. A., Martin, N. G. (1998).

[317] Kendler, K.S., Thornton, L. M., Pedersen, N. L. (2000).

[318] Kendler, K.S., Neale, M. C., Sullivan, P., Corey, L. A., Gardner, C. O., Prescott, C. A. (1999).

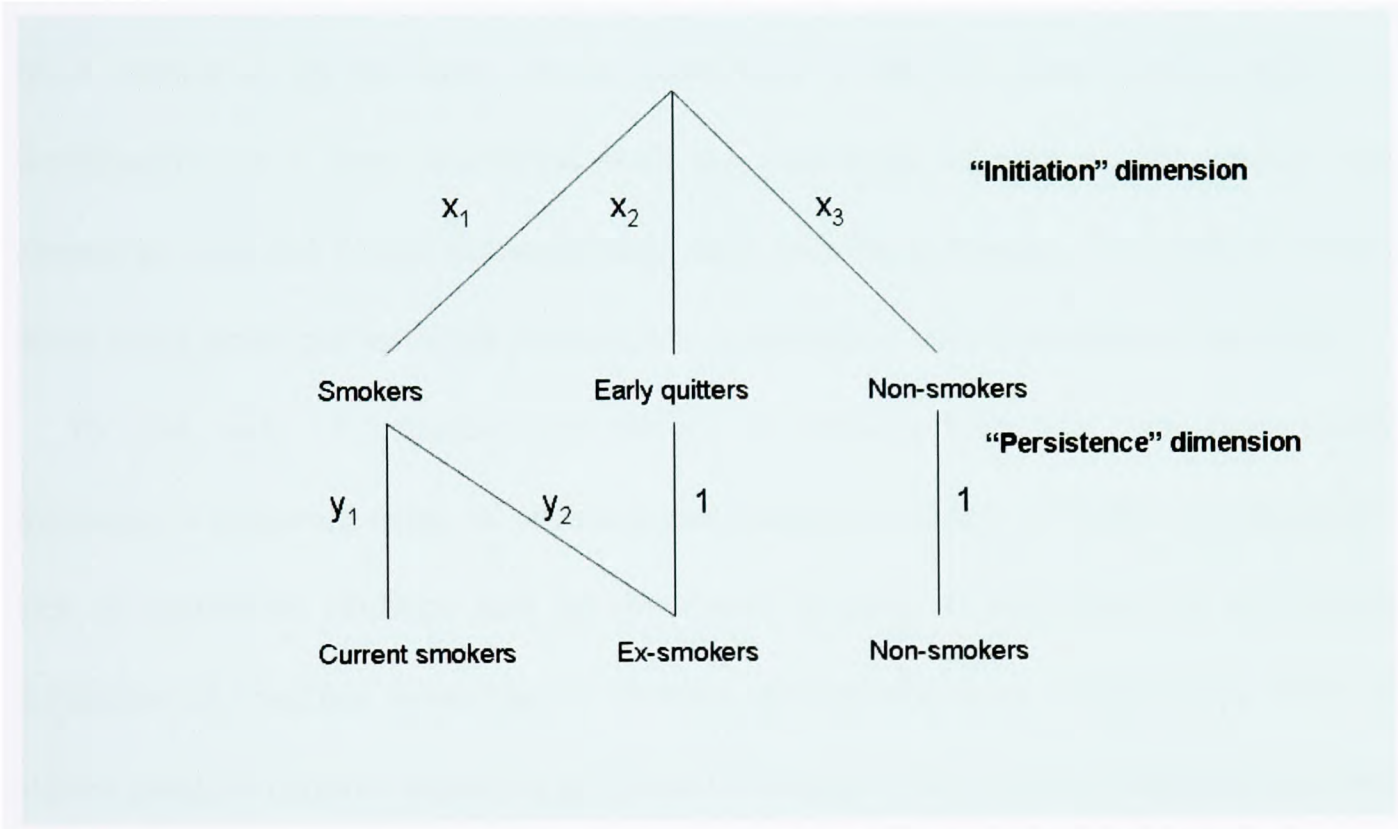
Table 1.4 continued. Meta-Analysis of Smoking Persistence in Twin Studies

Country	Sex	MZ	DZ	h^2	c^2	e^2	References
Australia	F	570	351	0.56	0.29	0.15	Heath et al., 1999[319]
Australia	F	663	400	0.74	0.03	0.23	True et al., 1999[320]
Finland	F	1842	3703	0.49 (0.16-0.80)	0.23 (0-0.47)	0.28 (0.18-0.42)	Heath et al., 1998[321]

LEGEND: h^2, c^2 and e^2 estimate heritability (h^2), shared environmental (c^2) and unique environmental (e^2) effects. Values within parentheses represent 95% CI or SE.

Figure 1.10

Combined Two-Step Process for Smoking Initiation and Persistence



LEGEND: The two-process model posited by Heath and Madden,[322] as described in the text, combines a "multiple threshold model", in which successful quitters are intermediate in liability between persistent smokers and never smokers, with a "two-process model", whereby independent determinants of initiation and persistence, with genetic and environmental influences on persistence, are observable only in individuals who become smokers. In this combined "two-process" model some smokers are "early quitters" who are low in liability on the "initiation" dimension and others are successful quitters who have become regular smokers, but are low in liability in the "persistence" dimension. Adapted from Heath and Madden, 1995 and reprinted with permission from the publisher in David et al., 2005a.[323]

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[319] Heath, A.C., Kirk, K. M., Meyer, J. M., Martin, N. G. (1999).
[320] True, W.R., Xian, H., Scherrer, J. F., Madden, P. A., Bucholz, K. K., Heath, A. C., Eisen, S. A., Lyons, M. J., Goldberg, J., Tsuang, M. (1999).
[321] Heath, A.C., Madden, P. A., Martin, N. G. (1998).
[322] Heath, A.C., Madden, P.A.F. (1995).
[323] David, S.P., Munafo', M.R., Walton, R.T. (2005).

Data such as these illustrate the continuing importance of family and twin designs in the investigation of smoking behaviour, and particularly for the identification of possible moderating and mediating influence of variables such as sex and personality.

1.3.2 Linkage Studies

Candidate gene studies offer a conceptually simple and powerful approach to the identification of genetic variants associated with phenotypes of interest. However, this requires an *a priori* hypothesis regarding which genes to investigate. In contrast, genetic linkage study designs can be used to scan the entire genome with a few hundred markers. If a DNA marker is on the same chromosome, and within the same general region of the chromosome as a gene associated with the phenotype of interest, the marker and the phenotype will not assort independently, thus indicating linkage. This can be done even when many genes are involved for complex, quantitative traits (quantitative trait loci).

To date, only 11 genomic scan studies of smoking behaviour have been published, indicating a disparate range of chromosomal locations worthy of further investigation. The lack of consistent findings may be the result, in part, of differences in the phenotypic definition of smoking behaviour or nicotine dependence used. Furthermore, three of the eleven published studies reporting evidence for linkage with smoking behaviour are based on analyses of data from the same cohort of multiplex families recruited as part of a study of alcoholism,[324] and three of the linkage studies reported data from the same cohort of families

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[324] Bergen, A.W., Korczak, J. F., Weissbecker, K. A., Goldstein, A. M. (1999). 'A Genome-Wide Search for Loci Contributing to Smoking and Alcoholism', *Genet Epidemiol.* **17 Suppl 1**: pp. S55-60; Duggirala, R., Almasy, L., Blangero, J. (1999). 'Smoking Behavior is Under the Influence of a Major Quantitative Trait Locus on Human Chromosome 5q', *Genet Epidemiol.* **17 Suppl 1**: pp. S139-44; Bierut, L.J., Rice, J. P., Goate, A., Hinrichs, A. L., Saccone, N. L., Foroud, T., Edenberg, H. J., Cloninger, C. R., Begleiter, H., Conneally, P. M., Crowe, R. R., Hesselbrock, V., Li, T. K., Nurnberger, J. I., Jr., Porjesz, B., Schuckit, M. A., Reich, T. (2004). 'A Genomic Scan for Habitual Smoking in Families of Alcoholics: Common and Specific Genetic Factors in Substance Dependence', *Am J Med Genet A.* **124**(1): pp. 19-27.

from a study of heart disease prevention[325]. The characteristics of existing genome scan studies of smoking behaviour are described in Table 1.5, below.

Table 1.5

Genome-Wide Investigations of Smoking Behaviour

Study (Country)	Participants	Dominant Ancestry	Number of Families	Number of Markers	Primary Phenotype	Markers Indicating Significant Linkage and Chromosome
Bergen et al., 1999[326] (USA)	Collaborative study on the genetics of alcoholism	European	105 extended families	296	Ever-smoking vs. never-smoking	D1S548 Ch.1 D2S379 Ch.2 D6S474 Ch.6 D9S64 Ch.9 D14S302 Ch.14 D17S968 Ch.17 D18S391 Ch.18 D21S120 Ch.2
Duggirala et al., 1999[327] (USA)	Collaborative study on the genetics of alcoholism	European	105 extended families	296	Pack-years of smoking	D4S244 Ch.4 D5S1354 Ch.5 GATA193 Ch.17
Straub et al., 1999[328] (USA and New Zealand)	Convenience sample	European	130 and 91 nuclear families	451	Nicotine dependence	D2S1326 Ch.2 D10S2469 Ch.10
Goode et al., 2003[329] (USA)	Framingham Heart Study	European	313 extended families	401	Cigarettes per day (maximum)	ATA4F03 Ch.2 GATA151F03 Ch.15 GATA25A04 Ch.17 GATA47F05 Ch.20 321xd1 Ch.20

Continued overleaf.

[325] Goode, E.L., Badzioch, M. D., Kim, H., Gagnon, F., Rozek, L. S., Edwards, K. L., Jarvik, G. P. (2003). 'Multiple Genome-Wide Analyses of Smoking Behavior in the Framingham Heart Study', *BMC Genet.* **4 Suppl 1**: p. S102; Saccone, N.L., Neuman, R. J., Saccone, S. F., Rice, J. P. (2003). 'Genetic Analysis of Maximum Cigarette-Use Phenotypes', *BMC Genet.* **4 Suppl 1**: p. S105; Wang, D., Ma, J. Z., Li, M. D. (2005). 'Mapping and Verification of Susceptibility Loci for Smoking Quantity Using Permutation Linkage Analysis', *Pharmacogenomics J.* **5**(3): pp. 215-6.

[326] Bergen, A.W., Korczak, J. F., Weissbecker, K. A., Goldstein, A. M. (1999).

[327] Duggirala, R., Almasy, L., Blangero, J. (1999).

[328] Straub, R.E., Sullivan, P. F., Ma, Y., Myakishev, M. V., Harris-Kerr, C., Wormley, B., Kadambi, B., Sadek, H., Silverman, M. A., Webb, B. T., Neale, M. C., Bulik, C. M., Joyce, P. R., Kendler, K. S. (1999). 'Susceptibility Genes for Nicotine Dependence: A Genome Scan and Followup in an Independent Sample Suggest that Regions on Chromosomes 2, 4, 10, 16, 17 and 18 Merit Further Study', *Mol Psychiatry*, **4**(2): pp. 129-44.

[329] Goode, E.L., Badzioch, M. D., Kim, H., Gagnon, F., Rozek, L. S., Edwards, K. L., Jarvik, G. P. (2003).

Study (Country)	Participants	Dominant Ancestry	Number of Families	Number of Markers	Primary Phenotype	Markers Indicating Significant Linkage and Chromosome
Li et al., 2003[330] (USA)	Framingham Heart Study	European	313 extended families	401	Cigarettes per day	D9S257 Ch.9 D9S910 Ch.9 D11S1985 Ch.11 D11S2371 Ch.11 ATA78D02 Ch.17 D17S2196 Ch.17
Saccone et al., 2003[331] (USA)	Framingham Heart Study	European	313 extended families	401	Cigarettes per day (maximum)	1648xb8 Ch.5 ATA59H06 Ch.9 GATA6B07 Ch.13 Mfd190 Ch.14 217xf4 Ch.22
Bierut et al., 2004[332] (USA)	Collaborative Study on the Genetic of Alcoholism	European	97 nuclear families	366	Habitual smoking vs. non-habitual smoking	D5S815 Ch.5 D9S1120 Ch.9 D9A261 Ch.9 D9S904 Ch.9 D11S1354 Ch.11 D21S210 Ch.21
Gelernter et al., 2004[333] (USA)	Probands originally identified for panic disorder, Yale CT, USA	European	12 extended families	416	Habitual smoking vs. non-habitual smoking	D9S283 Ch.9 D9S1677 Ch.9 D11S4046 Ch.11
Sullivan et al., 2004[334] (USA)	Convenience sample, Christchurch, New Zealand	European	130 nuclear families	458	Nicotine dependence	D2S1326 Ch.2 D10S2469 Ch.10 CYP17 Ch.10
Continued overleaf.						

[330] Li, M.D., Ma, J. Z., Cheng, R., Dupont, R. T., Williams, N. J., Crews, K. M., Payne, T. J., Elston, R. C. (2003). 'A Genome-Wide Scan to Identify Loci for Smoking Rate in the Framingham Heart Study Population', *BMC Genet.* **4 Suppl 1**: p. S103.

[331] Saccone, N.L., Neuman, R. J., Saccone, S. F., Rice, J. P. (2003).

[332] Bierut, L.J., Rice, J. P., Goate, A., Hinrichs, A. L., Saccone, N. L., Foroud, T., Edenberg, H. J., Cloninger, C. R., Begleiter, H., Conneally, P. M., Crowe, R. R., Hesselbrock, V., Li, T. K., Nurnberger, J. 1., Jr., Porjesz, B., Schuckit, M. A., Reich, T. (2004).

[333] Gelernter, J., Liu, X., Hesselbrock, V., Page, G. P., Goddard, A., Zhang, H. (2004). 'Results of a Genomewide Linkage Scan: Support for Chromosomes 9 and 11 Loci Increasing Risk for Cigarette Smoking', *Am J Med Genet B Neuropsychiatr Genet.* **128**(1): pp. 94-101.

[334] Sullivan, P.F., Neale, B. M., van den Oord, E., Miles, M. F., Neale, M. C., Bulik, C. M., Joyce, P. R., Straub, R. E., Kendler, K. S. (2004). 'Candidate Genes for Nicotine Dependence Via Linkage, Epistasis, and Bioinformatics', *Am J Med Genet B Neuropsychiatr Genet.* **126**(1): pp. 23-36.

Study (Country)	Participants	Dominant Ancestry	Number of Families	Number of Markers	Primary Phenotype	Markers Indicating Significant Linkage and Chromosome
Vink et al., 2004[335] (Holland)	Netherlands Twin Register	European	192 nuclear families	379	Ever-smoking vs. never-smoking and cigarettes per day	<u>Ever</u> D6S2410 Ch.6 D6S1053 Ch.6 Unk283 Ch.14 D14S617 Ch.14 <u>Cig/day</u> D3S3050 Ch.3 Cig/day D3S4545 Ch.3 <u>Both</u> D10S1412 Ch.10 D10S1430 Ch.10
Wang et al., 2005[336] (USA)	Framingham Heart Study	European	430 nuclear families	401	Cigarettes per day	ATA4E02 Ch.1 GATA6G12 Ch.3 GATA5B02 Ch.4 GATA24D12 Ch.7 GATA6B02 Ch.8 GATA12C06 Ch.9 GATA48E02 Ch.11 290vc9 Ch.16 GATA185H04 Ch.17 ATA4E02 Ch.20

LEGEND: Table depicts genome-wide linkage studies pertaining to nicotine dependence. Far right column displays chromosomal markers and the chromosomes (Ch) demonstrating linkage with nicotine dependence.[337].

Studies published to date suggest that regions of interest may exist on chromosomes 9, 10, 14 and 17, all of which have been reported to be of potential importance in relation to some aspect of smoking behaviour in at least two published studies. The 5-HTT gene, for example, is located on chromosome 17 and in the next section (Section 1.3.3), a recent meta-analysis suggests that the 5-HTTLPR is associated with smoking cessation.[338]

Nevertheless, there remains considerable scope for further study, in particular employing combined linkage and association techniques designed to identify chromosomal regions of

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[335] Vink, J.M., Beem, A. L., Posthuma, D., Neale, M. C., Willemsen, G., Kendler, K. S., Slagboom, P. E., Boomsma, D. I. (2004) 'Linkage Analysis of Smoking Initiation and Quantity in Dutch Sibling Pairs', *Pharmacogenomics J.* **4**(4): pp. 274-82.

[336] Wang, D., Ma, J. Z., Li, M. D. (2005).

[337] Originally published in: David, S.P., Munafo', M.R., Walton, R.T. (2005); and substantially updated in June 2005 in collaboration with MM.

[338] Munafo', M., Clark, T., Johnstone, E., Murphy, M., Walton, R. (2004).

potential interest with a higher degree of resolution, perhaps with a view to subsequently targeting candidate genes that may lie within these regions. The relative paucity of research in this area carried out to date is likely to be rectified as high-density genome scans become increasingly affordable. Once again, however, the issue of phenotype definition is likely to be of paramount importance, and biometric genetic analyses employing existing twin registry data, such as those described above, may serve to inform this by suggesting the possible pathways through which genetic factors may exert their influence.

1.3.3 Candidate Gene Association Studies

Candidate gene studies require a population case-control design, with an increased frequency of a specific allele in the case group (e.g. smokers) compared to the control group (e.g. non-smokers) indicating the influence of the polymorphism under investigation on the phenotype of interest. Such studies are relatively simple in concept, and increasingly cost-effective to perform as the cost of genotyping decreases. However, when performing candidate gene case-control studies factors such as study design, methods for recruitment of cases and controls, selection of candidate genes, functional significance of polymorphisms chosen for study and statistical analysis require close attention to ensure that only genuine associations are detected.

In recent years there has been an increasing trend towards candidate gene studies, particularly in relation to those which may play a role in neurobiological pathways, that are of theoretical interest in relation to smoking behaviour specifically and addictive behaviours more generally. The number of published studies has steadily increased over the last ten

years since the first study in this area,[339] so that there are now over 40 studies that report data on the association between specific candidate genes and some aspect of smoking behaviour or nicotine dependence.[340] As with data from twin studies (described above), this volume of published studies lends itself well to meta-analytic techniques. Unfortunately, although certain candidate genes have generated substantial interest (e.g. dopamine receptor DRD2 Taq1A polymorphism), a recent review[341], Munafò and colleagues highlighted the broad range of candidate genes that have been investigated, and the difficulty in drawing any firm conclusions from the literature. In addition, due to substantial (and statistically significant) between-study heterogeneity, there was at best only modest evidence for an association between the specific candidate genes most widely studied (DRD2 Taq1A, DAT VNTR, CYP2A6 and 5HTTLPR) and smoking behaviour. The results of this meta-analysis are presented in Table 1.6.

In particular, the significant between-study heterogeneity reported by Munafò and colleagues[342] suggests the need for extreme care in the design of candidate gene studies, due to the potential problems of population stratification and given the case-control design employed in such studies. Even if the study sample is carefully selected to control for ethnicity, age distribution, gender and other relevant variables, such confounding may still occur. Just as it is so with genome-wide linkage studies, it is extremely important to define smoking-related phenotypes as precisely as possible in association studies. Nevertheless, the potential of the candidate gene association studies approach remains substantial and informs

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[339] Noble, E.P., St Jeor, S. T., Ritchie, T., Syndulko, K., St Jeor, S. C., Fitch, R. J., Brunner, R. L., Sparkes, R. S. (1994). 'D2 Dopamine Receptor Gene and Cigarette Smoking: A Reward Gene?' *Med Hypotheses*. **42**(4): pp. 257-60.
[340] Munafo', M., Clark, T., Johnstone, E., Murphy, M., Walton, R. (2004).
[341] Ibid.
[342] Ibid.

the process of identifying plausible candidate genes for pharmacogenetic studies of smoking cessation as discussed in the next section.

Table 1.6

Meta-Analysis of Smoking Initiation, Persistence, and Cessation in Candidate Gene Association Studies

Comparison	Fixed Effects Models		Random Effects Model		Heterogeneity
	Pooled odds ratio (95% CI)	*P* value	Pooled odds ratio (95% CI)	*P* value	*P* value
Smoking Initiation					
DRD2 Taq1A	0.75 (0.65 – 0.85)	< 0.01	0.84 (0.57 – 1.23)	0.36	< 0.01
DAT VNTR	1.04 (0.90 – 1.20)	0.59	1.03 (0.80 – 1.33)	0.80	0.03
5-HTTLPR	1.06 (0.85 – 1.32)	0.60	1.06 (0.84 – 1.35)	0.61	0.33
CYP2A6 null	0.90 (0.67 – 1.19)	0.46	0.90 (0.67 – 1.19)	0.46	0.52
Adopting and Persisting Smoking					
DRD2 Taq1A	0.73 (0.63 – 0.84)	< 0.01	0.84 (0.58 – 1.23)	0.37	< 0.01
DAT VNTR	0.94 (0.80 – 1.12)	0.52	0.99 (0.72 – 1.35)	0.93	0.02
5-HTTLPR	1.11 (0.87 – 1.41)	0.40	1.15 (0.81 – 1.63)	0.44	0.14
CYP2A6 null	0.68 (0.42 – 1.11)	0.12	0.68 (0.42 – 1.11)	0.12	0.57
Persistent Smoking					
DRD2 Taq1A	0.80 (0.72 – 0.90)	< 0.01	0.87 (0.66 – 1.16)	0.35	< 0.01
DAT VNTR	0.90 (0.77 – 1.07)	0.23	0.94 (0.70 – 1.25)	0.66	0.03
5-HTTLPR	1.19 (0.95 – 1.49)	0.14	1.22 (0.80 – 1.87)	0.35	0.03
CYP2A6 null	0.81 (0.61 – 1.08)	0.15	0.82 (0.59 – 1.14)	0.24	0.28
Smoking Cessation					
DRD2 Taq1A	1.17 (0.89 – 1.55)	0.26	1.17 (0.89 – 1.55)	0.33	0.76
DAT VNTR	0.85 (0.68 – 1.08)	0.18	0.89 (0.63 – 1.28)	0.54	0.14
5-HTTLPR	1.48 (1.03 – 2.14)	0.04	1.49 (0.97 – 2.30)	0.07	0.30
CYP2A6 null	0.67 (0.48 – 0.95)	0.03	0.67 (0.48 – 0.95)	0.03	0.45
Cigarette Consumption					
DRD2Taq1A	2.53 (0.45 – 4.60)	0.02	1.47(-3.88 – 6.81)	0.59	0.02
CYP2A6null	-4.30 (-5.30 – -3.29)	< 0.01	-1.71 (-6.76 – 3.33)	0.51	< 0.01

LEGEND: Results from a meta-analytic study of 5 dimensions of smoking. Pooled odds ratios, 95% confidence intervals (CI), and statistical significance are shown for fixed-effects and random-effects analyses.

1.3.4 Pharmacogenetic Studies

The most effective smoking cessation therapies currently available are antidepressant drugs and nicotine replacement therapy. As reviewed earlier, converging evidence from the

animal model to human candidate gene association studies implicates genetic variation in DA, serotonin, noradrenaline, and cholinergic systems. Thus it is logical to assume that genetic variation that affects the function and availability of proteins (receptors, transporters, and enzymes) in these neurotransmitter systems would influence the efficacy of drugs with sites of action in catecholaminergic and nicotinic acetylcholine receptors. There are very few published pharmacogenetic studies of smoking cessation and there is marked heterogeneity in methods. Thus, at this time, meta-analyses would not as yet be informative. Therefore, rather a summary of relevant individual trials is described below.

1.3.4.1 Nicotine Replacement Therapy

Yudkin and colleagues[343] conducted a randomised trial of the nicotine transdermal patch on 1,865 smokers in 1991-1992 (The PATCJ Trial). In 1999 and 2000, 1,550 of the subjects were contacted and 767 agreed to undergo blood collection for DNA analysis and were genotyped for the DA D_2 receptor (DRD2 32806 C/T, A1 RFLP), dopamine beta hydroxylase (DBH 1368 G/A), and monoamine oxidase (MAO-A 1460 T/C). At 12 weeks of follow-up the rate ratio (quit rate in nicotine group/quit rate in placebo group) was 1.9 (95% CI 1.0, 3.5) for subjects with the DRD2 CT/TT genotyped compared with 1.2 (0.7, 2.1) for subjects with CC; 1.8 (1.1, 2.9) for subjects with the DBH GA/AA genotype vs. 1.1 (0.6, 2.2) for subjects with GG; and 1.7 (1.1, 2.8) for subjects with the MAO TT vs. 1.1 (0.6, 2.3) for subjects with CT/CC. These data suggest the presence of gene x treatment interactions for transdermal nicotine patch therapy and that the presence of one or more of the SNPs studied for DRD2, DBH, or MAO improved the likelihood of quitting on the nicotine patch compared to subjects with wildtype genotypes. In another study of the same clinical trial

[343] Yudkin, P., Walton, R.T., Hey, K., Roberts, S.J., Welch, S.J., Johnstone, E.C., Griffiths, S.E., Jones L.J., Murphy, M.F. (2001). 'Is There an Inherited Basis for Smoking Behaviour?' paper preented at the Society for Research on Nicotine and Tobacco's 7th annual conference in Seattle, Washington.

population and working with mentors in the Departments of Clinical Pharmacology and Primary Health Care, I evaluated the influence of the 5-HTTLPR on smoking cessation outcomes with the nicotine patch.[344] A study population of 750 subjects who participated in the PATCH Trial described above, were genotyped for the 5-HTTLPR. At one week, the odds ratios (OR) for CO-verified abstinence were 1.94 (1.30, 2.88) (35.6% active vs. 22.2% placebo) for SS/SL genotypes compared with 1.81 (1.08, 3.02) (42.2% active vs. 28.8% placebo) for LL. At 1 and 4 weeks and 1 and 12 weeks, the ORs were SS/SL [2.32 (1.46, 3.69)] vs. LL [1.45 (0.82, 2.57)], respectively. There was no relative benefit of nicotine patch according to the 5-HTTLPR (e.g., subjects with S alleles did not demonstrate greater efficacy than those with LL genotypes or vice versa) at 1, 4, or 12 weeks following a quit attempt [Breslow-Day Test of Homogeneity of ORs: 1 week ($X^2 = 0.045$, $df = 1$, $p = 0.83$); 1 and 4 weeks ($X^2 = 1.57$, $df = 1$, $p = 0.21$); 1, 4, and 12 weeks ($X^2 = 0.82$, $df = 1$, $p = 0.365$)]. Thus, whilst smokers with the S allele did not demonstrate relatively higher nicotine patch effectiveness when compared to those with LL genotypes, subgroup analysis demonstrated that only those smokers with the S allele showed a significant improvement in smoking cessation rates on the nicotine patch when compared to placebo, persisting to 4 weeks following a quit attempt.

1.3.4.2 Antidepressant Therapy

To date, the only published pharmacogenetic studies of antidepressants for smoking cessation have been those examining bupropion. Bupropion is metabolised almost exclusively by the enzyme cytochrome P450 2B6 (CYP2B6) and this enzyme also

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[344] David, S.P., Murthy, N.V., Rabiner, E.A., Munafo', M., Jacob, R., Johnstone, E., Grasby, P.M. (2004). 'Serotonin Transporter Polymorphism Linked to Personality Traits Affects Serotonin (5-HT1A) Receptor Function in Positron Emission Tomography Study', paper presented at the North American Primary Care Research Group's (NAPCRG) 32nd annual meeting in Orlando, FL, USA.

contributes to the metabolism of nicotine to cotinine and there is *in vitro* evidence that nicotine induces the CYP2B6 gene.[345] Therefore, Lerman and colleagues conducted a randomised, placebo-controlled trial of sustained release (SR) bupropion or placebo for smoking cessation in an open-label randomised trial to evaluate the potential effects of two established candidate genes (DRD2-Taq1A and SLC6A3 VNTR) and a polymorphism in the CYP2B6 gene (CYP2B6 1459 C/T).[346]

The results of this trial, published in two separate papers, indicated that there was a significant gene x gene interaction between the DRD2 Taq1A and SLC6A3 VNTR polymorphisms such that a significant effect of the SLC6A3 VNRT on abstinence at the end of treatment was found only among participants with the DRD2 Taq1-A2/A2 genotype (OR = 1.74; 95% CI =1.03, 2.93; p = 0.03) but not among those with A1 alleles. There was no significant gene by treatment interactions for either polymorphism. Furthermore, there was a gene x treatment x sex interaction such that, at the end of ten weeks of treatment, women with the low activity alleles (CYP2B6 1459 CT/TT) had the most benefit from treatment with abstinence rates of 19% in the placebo group and 54% in the bupropion group (p = 0.003). However, there was no significant difference in treatment outcome in the CYP2B6 CC group. In addition, subjects in the placebo group but not the bupropion group demonstrated worsening cravings over time only if they carried the low activity CYP2B6 CT/TT genotype. The gene x treatment x gender interaction did not persist beyond ten weeks but demonstrated a persistent and slow decline over 12 months. The trial indicates that

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[345] Hesse, L.M., Venkatakrishnan, K., Court, M. H., von Moltke, L. L., Duan, S. X., Shader, R. I., Greenblatt, D. J. (2000). 'CYP2B6 Mediates the In Vitro Hydroxylation of Bupropion: Potential Drug Interactions with Other Antidepressants', *Drug Metab Dispos*. **28**(10): pp. 1176-83.

[346] Lerman, C., Shields, P. G., Wileyto, E. P., Audrain, J., Pinto, A., Hawk, L., Krishnan, S., Niaura, R., Epstein, L. (2002). 'Pharmacogenetic Investigation of Smoking Cessation Treatment', *Pharmacogenetics*, **12**(8): pp. 627-634; Lerman, C., Niaura, R., Collins, B.N., Wileyto, P., Audrain-McGovern, J., Pinto, A., Hawk, L., Epstein, L.H. (2003) 'Effects of Dopamine Transporter and Receptor Polymorphisms on Smoking Cessation in a Bupropion Clinical Trial', *Health Psychol*. **22**(5):541-8.

bupropion is effective in preventing relapse even among women at higher genetic risk for relapse who carry the CYP2B6 CT/TT alleles. The benefit in relapse prevention may be mediated through attenuation of craving in a subset of smokers. The results of this study lend promise to the prospect of using genotype and gender to individually tailor smoking cessation therapies in the future.

In a similar study I conducted with mentors, smokers of European ancestry (N = 283) provided blood samples for genetic analysis and received bupropion SR or placebo (12 weeks) plus counselling.[347] Assessments included the DA D_2 receptor (DRD2 Taq1A), DA transporter (SLC6A*3* 3'VNTR), and cytochrome P450 2B6 (CYP2B6 1459 C->T) genotypes, and cotinine-verified seven-day point prevalence. Univariate association models with six-month intent-to-treat analyses of smoking abstinence were evaluated using logistic regression. Of the potential interaction effects examined, only that for DRD2 reached statistical significance (p = 0.01). Among smokers with the DRD2 Taq1-A2/A2 genotype there was a 23% difference [35%, 95% CI = (0.25, 0.47) for bupropion group vs. 12%, 95% CI = (0.06, 0.21) for the placebo group] in six-month biochemically-verified abstinence, whereas there was absolutely no response to bupropion therapy among A1/A1 or A1/A2 subjects (21% abstinence rate for bupropion group vs. 24% for placebo group). These data require replication, but also lend credence to the promise of personalised smoking cessation therapies.

Swan and colleagues used a different study design to examine the influence of the DRD2-Taq1A polymorphism on effectiveness of bupropion SR for smoking cessation in an open-

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[347] David, S.P., Brown, R.A., Papandonatos, G.D., Lloyd-Richardson, E.L., Munafo', M.R., Shields, P.G., Lerman, C., Strong, D.R., McCaffery, J.M., Niaura, R. (2005). 'Pharmacogenetic Clinical Trial of Sustained-Release Bupropion for Smoking Cessation', *Pharmacogenomics J.* (manuscript under review).

label trial. Smokers (N = 496) were randomised to receive one of four combinations of bupropion SR and counselling: 150 mg bupropion SR plus either less intensive counselling or more intensive counselling; 300 mg bupropion with less intensive counselling or more intensive counselling. These investigators observed that smokers with DRD2-Taq1A2/A2 were more likely to be abstinent at 12 months than A1 allele carriers (OR for smoking at 12 months = 0.76, 95% CI = 0.56-1.03; P<0.076). In addition, A1 carriers were more likely to drop out of the study due to side effects of bupropion (OR = 1.91, 95% CI = 1.01, 3.60; p = 0.04). Both of these effects were observed only in women.

Whilst each of these studies used different methods the one common finding was that smokers with DRD2-Taq1A A2/A2 genotypes demonstrated greater efficacy of bupropion for smoking cessation than A1 allele carriers. Whether or not there are major effects of sex on bupropion effectiveness will require further study.

1.4 Application of Functional Neuroimaging to Examination of Nicotine Dependence

1.4.1 Overview

There has been a trend of markedly increasing numbers of functional neuroimaging studies of nicotine dependence over the last decade. McClernon and Gilbert conducted a systematic literature review and determined that the number of such studies have steadily increased since 1994 and were the most frequently reported methods in 2003.[348] I will address each of the major functional neuroimaging approaches in this section, however I will focus primarily on functional magnetic resonance imaging (fMRI) and positron emission tomography (PET) and discuss the advantages and disadvantages of fMRI compared with

[348] McClernon, F.J., Gilbert, D. G. (2004). 'Human Functional Neuroimaging in Nicotine and Tobacco Research: Basics, Background, and Beyond', *Nicotine Tob Res.* **6**(6): pp 941-59.

other functional methods. Functional neuroimaging methods have provided cognitive neuroscientists with a way of studying brain activity associated with cognitive and behavioural processes. The reasons for focusing primarily on fMRI in this review are that fMRI has become a widely used technology for neurophysiological nicotine addiction studies because of its high spatial and temporal resolution—relative to some (i.e., positron emission tomography and single photon emission tomography) but not all of the other functional neuroimaging methods, its non-invasiveness and lack of radioactive tracers, its ability to be used for repeated measures, and, when one includes other magnetic resonance imaging techniques, its versatility in measuring multiple physiological phenomenon (i.e., fMRI BOLD, H_20 diffusion, cerebral perfusion, and connectivity). Other approaches contribute different types of data that may be complementary to fMRI and each approach has unique advantages and disadvantages based on the research questions posed as discussed below.

1.4.2 Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging (fMRI) permits the non-invasive study of localised, intrinsic changes in Blood-Oxygen-Level-Dependent (BOLD) signal driven by changes in neuronal activity. FMRI has excellent temporal and spatial resolution, permitting the investigator to examine the temporal relationship between stimuli and neuronal response in localised regions within the resolution of millimetres. The image intensity observed with fMRI is produced by haemodynamic responses to neuronal demand. Two general mechanisms influence fMRI signal: the relaxation times of protons within water molecules known as T1, T2, and T2*; and the magnetic properties of haemoglobin during haemodynamic responses to stimuli driven by neuronal oxygen demand. Both mechanisms

are dependent on oxygenation within a specified volume (voxel) in the brain. During activation of a brain region, there is an excess of arterial (oxygenated) blood delivered into the area with concomitant changes in the ratio of oxyhaemoglobin (HBO_2) to deoxyhaemoglobin (Hbr), which in turn is a function of local arterial autoregulation or vasodilation. The change in HBO_2/Hbr alters the magnetic properties of the region, which affects the spin properties of H_2O (T2*).[349] For example, when a visual stimulus is presented, neuronal activation occurs rapidly in the primary visual cortex, which in turn stimulates increases in cerebral blood flow (CBF), cerebral blood volume (CBV), and oxygen delivery. As CBF increases more than CBV, the oxygen delivery exceeds slight increases in local oxygen demand. The increases in local CBF in the arterioles and small arteries are said to be uncoupled from local metabolism. The net result is a surplus of oxygenated haemoglobin delivered to a specified voxel and increased signal in T2*-weighted images.[350] Neuronal activation occurs in the millisecond range whilst haemodynamic changes are slower (6-9 seconds). Thus, one of the limitations of fMRI BOLD is the difficulty in sequencing the serial or parallel patterns of activation during an experiment.

Considering the large and growing number of functional imaging studies using one or more approaches published over the last decade, as yet relatively few fMRI studies of nicotine dependence have been published. I reviewed the literature with PubMed, Medline and recently published abstracts using key words ("nicotine", "fMRI", "addiction"), and asked researchers within the field of fMRI nicotine addiction about active studies meeting inclusion criteria. Inclusion criteria were: fMRI studies of the brain with (a) participants

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[349] Matthews, P.M. (2001). 'Introduction', in *Functional MRI--An Introduction to Methods*, ed. by Jezzard, P., Matthews, P.M., Smith, S.M. (Oxford, UK: Oxford University Press), pp. 1-34.
[350] Ibid.

identified as smokers or as having DSM-IV-TR[351] diagnosed nicotine dependence, (b) a pharmacological or non-pharmacological nicotine-related stimulus, and (c) a within-subjects or between-subjects control group. Outcomes of interest included (a) significant fMRI-BOLD signal activation, (b) differences in activation between nicotine abstinent and satiated states, (c) differences in activation between smokers and non-smokers, and (d) differences in cerebral perfusion with and without nicotine. Ten studies were identified and met the inclusion criteria.[352] The design characteristics of each of these studies are described in Table 1.7. Of note, there were a broad range of types of fMRI studies that could be classified broadly as "nicotine administration" studies, "cue reactivity" studies, "cognitive task performance" studies, and "cerebral perfusion studies". In fact, substantial overlap exists between studies in these classifications such that some nicotine administration studies may also be cue reactivity, cognitive task performance, or cerebral perfusion investigations. However, for the sake of providing a general overview, such classification may be useful.

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[351] APA (2002).

[352] Kumari, V., Gray, J. A., ffytche, D. H., Mitterschiffthaler, M. T., Das, M., Zachariah, E., Vythelingum, G. N., Williams, S. C., Simmons, A., Sharma, T. (2003). 'Cognitive Effects of Nicotine in Humans: An fMRI Study', *Neuroimage*, **19**(3): pp. 1002-13; Lawrence, N.S., T.J. Ross, and E.A. Stein (2002). 'Cognitive Mechanisms of Nicotine on Visual Attention', *Neuron*. **36**(3): pp. 539-48; Stein, E.A., Pankiewicz, J., Harsch, H. H., Cho, J. K., Fuller, S. A., Hoffmann, R. G., Hawkins, M., Rao, S. M., Bandettini, P. A., Bloom, A. S. (1998). 'Nicotine-Induced Limbic Cortical Activation in the Human Brain: A Functional MRI Study', *Am J Psychiatry*, **155**(8): pp. 1009-15; Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002). 'Activation in Mesolimbic and Visuospatial Neural Circuits Elicited by Smoking Cues: Evidence from Functional Magnetic Resonance Imaging', *Am J Psychiatry*, **159**(6): pp. 954-60; Jacobsen, L.K., Gore, J. C., Skudlarski, P., Lacadie, C. M., Jatlow, P., Krystal, J. H. (2002). 'Impact of Intravenous Nicotine on BOLD Signal Response to Photic Stimulation', *Magn Reson Imaging*. **20**(2): pp. 141-5; Cohen, R., Sweet, L., David, S.P., Niaura, R. (2004). 'Nicotine Satiation and Abstinence Effects on fMRI Brain Activation During Verbal Working Memory', paper presented at 10th annual Society for Research on Nicotine and Tobacco meeting, Scotsdale, AZ; McClernon, F.J., Hiott, F. B., Huettel, S. A., Rose, J. E. (2005). 'Abstinence-Induced Changes in Self-Report Craving Correlate with Event-Related fMRI Responses to Smoking Cues', *Neuropsychopharmacology* (manuscript in press); Thiel, C.M., Zilles, K., Fink, G. R. (2005). 'Nicotine Modulates Reorienting of Visuospatial Attention and Neural Activity in Human Parietal Cortex', *Neuropsychopharmacology*, **30**(4): pp. 810-20; Lim, H.K., Pae, C. U., Joo, R. H., Yoo, S. S., Choi, B. G., Kim, D. J., Lee, C., Lee, C. U. (2005). 'fMRI Investigation on Cue-Induced Smoking Craving', *J Psychiatr Res*. **39**(3): pp. 333-5; Tregellas, J.R., Tanabe, J. L., Martin, L. F., Freedman, R. (2005). 'FMRI of Response to Nicotine During a Smooth Pursuit Eye Movement Task in Schizophrenia', *Am J Psychiatry*, **162**(2): pp. 391-3.

There are a wide variety of designs employed with fMRI ranging from simple "on-off" designs to more complicated designs using randomised presentation of multiple stimulus types. Two broad categories of stimulus presentations are block and event-related designs. Block designs incorporate a series of trials in one condition presented during a discrete epoch of time. The haemodynamic response observed in one condition is contrasted with other blocks involving different task conditions or different stimuli. Event-related paradigms differ from block designs in that individual trial 'events' are measured, rather than a single epoch or temporally-related signal. Event-related studies can incorporate multiple types of stimuli (explanatory variables), which are often presented in random succession with each stimulus duration of several seconds. The haemodynamic responses to each stimulus type can be contrasted with each other or with a resting condition. The advantages and disadvantages of block and event-related designs are discussed in detail in Chapter 2 (Section 2.3.2). In general terms, the major advantages of the two types of experiments are that event-related designs are more flexible in permitting randomisation of stimuli and the measurement of discrete events, whilst block designs are simpler and generally have greater power to detect activation to stimuli of interest.

1.4.2.1 Nicotine Administration Studies

Stein and colleagues[353] conducted a study of 16 active cigarette smokers using fMRI to identify sites of action of nicotine. Nicotine was administered in three, successive, intravenous (IV) dosages (0.75, 1.50, 2.25 mg/70 kg) following IV saline injection in separate, 20-minute trials. Echo planar imaging (EPI) scans of the whole brain (volumes) were obtained every six seconds. Dose-dependent increases in activation were observed in

[353] Stein, E.A., Pankiewicz, J., Harsch, H. H., Cho, J. K., Fuller, S. A., Hoffmann, R. G., Hawkins, M., Rao, S. M., Bandettini, P. A., Bloom, A. S. (1998).

the nucleus accumbens, Am, cingulate, and frontal lobes. Dose-dependent relationships were also seen for several behavioural parameters including "drug liking", "rush", and "high". Of note, the Stein study did not include non-pharmacological stimuli (e.g., smoking-related visual cues, cognitive tasks, etc.). Other studies[354] have examined BOLD signal activation to smoking-related cues, in relation to performance on different cognitive tasks or cerebral perfusion following either cigarette smoking or nicotine replacement therapies (e.g., gum, patch), or nicotine i.v. infusion and are discussed in separate categories below.

1.4.2.2 Cue Reactivity Studies

Due and colleagues[355] conducted an fMRI study of 12 smokers and 6 never-smokers to determine the effects of nicotine-related visual cues on fMRI BOLD signal activation in reward and visual spatial pathways. Nicotine-deprived smokers were exposed to a pseudo-random, event-related sequence of smoking images, neutral non-smoking images, and rare target images. In smokers, the fMRI signal was greater after exposure to smoking-related images than after exposure to neutral images in right posterior Am, posterior hippocampus, ventral tegmental area, and medial thalamus (mesolimbic reward circuit) as well as in bilateral prefrontal and parietal cortex and right fusiform gyrus (visual spatial attention centres). No significant activation was seen in non-smokers. Within the regions of interest (ROI) studied, there was greater activation following target images than neutral images in smokers. The authors concluded that mesolimbic and extrastriate visual brain regions work in concert to process reward signalling to visual smoking-related stimuli that are more salient

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[354] Cohen, R., Sweet, L., David, S.P., Niaura, R. (2004); McClernon, F.J., Hiott, F. B., Huettel, S. A., Rose, J. E. (2005); Thiel, C.M., Zilles, K., Fink, G. R. (2005); Lim, H.K., Pae, C. U., Joo, R. H., Yoo, S. S., Choi, B. G., Kim, D. J., Lee, C., Lee, C. U. (2005); Tregellas, J.R., Tanabe, J. L., Martin, L. F., Freedman, R. (2005); Kumari, V., Gray, J. A., ffytche, D. H., Mitterschiffthaler, M. T., Das, M., Zachariah, E., Vythelingum, G. N., Williams, S. C., Simmons, A., Sharma, T. (2003); Lawrence, N.S., T.J. Ross, and E.A. Stein (2002).

[355] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002).

to smokers than non-smoking related stimuli. McClernon and colleagues, using the same pictorial stimulus presentation as Due and colleagues, conducted a within-subjects fMRI cue reactivity study comparing abstinent and 'satiated' sessions.[356] The satiated sessions were proceeded by 10 minutes of *ad libitum* cigarette smoking and the abstinent sessions following overnight abstinence from smoking. These investigators also observed anterior cingulate cortex activation to smoking-related pictorial cues as well as activation of the superior frontal gyrus and a statistical trend suggesting activation of the ventral striatum. They did not find a significant effect of session (satiated vs. abstinent) on global activation. Whilst BOLD contrast appeared to be greater in the ventral striatum during the satiated sessions compared with the abstinent sessions, there were no significant differences in BOLD contrast between satiated and abstinent sessions in the ventral striatum or in any of the other ROIs analysed.[357]

These investigators analysed potential correlations between cigarette craving and BOLD contrast for 13 ROIs. A significant correlation between craving and BOLD signal was significant only in the right middle frontal gyrus after correction for multiple comparisons. Cigarette craving and ventral striatum activation was not significantly correlated during the abstinent sessions. Analysis of correlations between craving and BOLD response were not reported for any of the ROIs during the smoking sessions. Thus, we do not know whether or not significant correlations were observed following cigarette smoking.

In an fMRI study of one smoker and one non-smoker, Lim and colleagues presented film scenes of smoking situations and neutral stimuli not related to smoking. Both subjects

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[356] McClernon, F.J., Hiott, F. B., Huettel, S. A., Rose, J. E. (2005).
[357] McClernon, F.J. (2005). 'Effect Sizes for Smoking and Neutral Pictorial Cues in the Ventral Striatum Using fMRI', [from private correspondence].

refrained from smoking for 24 hours prior to the scans. Anterior cingulate cortex (ACC) and medial frontal lobe activation was observed only in the abstinent condition. No global activation was seen in either subject following actual or sham smoking.[358]

As further described in Chapter 2, the ACC, prefrontal cortex, and ventral striatum appear to activate to smoking-related pictorial cues in addicted smokers and those addicted to other drugs of abuse. However, across studies and drug types, ventral striatum activation has not been demonstrated consistently nor has the effect of cigarette smoking on cue-elicited BOLD activation been thoroughly investigated.

1.4.2.3 Cognitive Task Performance Studies

Lawrence and colleagues[359] examined the effect of transdermal nicotine on neural activation during a sustained attention (rapid visual information-processing) task. The level of performance was associated with activation in the caudate nucleus, parietal cortex, and thalamus. Independent of task performance, nicotine induced a generalised increase in task-induced occipital cortex activation.

Kumari and colleagues[360] evaluated the effects of IV nicotine administration on behavioural task performance in healthy non-smokers. FMRI was performed using a parametric "N-Back" task after the administration of nicotine (12 μg/kg body weight) or saline. Increased activity relative to the non-nicotine condition was observed in the ACC, superior frontal gyrus, and superior parietal cortex. Nicotine also produced an increased BOLD response to the N-Back task in the midbrain tectum and in the parahippocampal gyrus, cerebellum, and medial occipital lobe during rest.

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[358] Lim, H.K., Pae, C. U., Joo, R. H., Yoo, S. S., Choi, B. G., Kim, D. J., Lee, C., Lee, C. U. (2005). 'fMRI Investigation on Cue-Induced Smoking Craving', *J Psychiatr Res.* **39**(3): pp. 333-5.

[359] Lawrence, N.S., T.J. Ross, and E.A. Stein (2002).

[360] Kumari, V., Gray, J. A., ffytche, D. H., Mitterschiffthaler, M. T., Das, M., Zachariah, E., Vythelingum, G. N., Williams, S. C., Simmons, A., Sharma, T. (2003).

Theil and colleagues examined the effects of nicotine gum administration on reorienting of visuospatial attention in 15 non-smokers. A cued target-detection task was employed such that subjects were presented with target images following valid cues, invalid cues, neutral cues, or no cues. Valid cues indicated the impending presentation of a target image on the correct side of the visual field, whilst invalid cues suggested target image presentation but indicated incorrectly the side of the visual field to which the stimulus would be presented. Neutral cues indicated the subsequent presentation of a target image but provided no spatial information. "Reorientating" was evaluated by comparing validly cued trials with invalidly cued trials whilst "alerting" was evaluated by comparison of neutrally cued trials with no cue trials. Nicotine appeared to modulate alerting-related BOLD contrast in the right angular gyrus and right middle frontal gyrus. Reorienting-related BOLD contrast was observed in the left intraparietal sulcus and precuneous. Furthermore, nicotine appeared to affect alerting by speeding reaction times.

Schizophrenic patients commonly have defects in smooth pursuit eye movements[361] and nicotine has been shown to temporarily normalise such defects in schizophrenics.[362] Tregellas and colleagues evaluated the effect of nicotine gum on smooth eye pursuit in 5 smokers and 4 non-smokers with schizophrenia using fMRI.[363] During the smooth eye

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[361] Levy, D.L., Holzman, P. S., Matthysse, S., Mendell, N. R. (1993). 'Eye Tracking Dysfunction and Schizophrenia: A Critical Perspective', *Schizophr Bull.* **19**(3): pp. 461-536.

[362] Olincy, A., Johnson, L. L., Ross, R. G. (2003). 'Differential Effects of Cigarette Smoking on Performance of a Smooth Pursuit and a Saccadic Eye Movement Task in Schizophrenia', *Psychiatry Res.* **117**(3): pp. 223-36; Avila, M.T., Sherr, J. D., Hong, E., Myers, C. S., Thaker, G. K. (2003). 'Effects of Nicotine on Leading Saccades During Smooth Pursuit Eye Movements in Smokers and Nonsmokers with Schizophrenia', *Neuropsychopharmacology*, **28**(12): pp. 2184-91; Olincy, A., Ross, R. G., Young, D. A., Roath, M., Freedman, R. (1998). 'Improvement in Smooth Pursuit Eye Movements after Cigarette Smoking in Schizophrenic Patients', *Neuropsychopharmacology*, **18**(3): pp. 175-85; Sherr, J.D., Myers, C., Avila, M. T., Elliott, A., Blaxton, T. A., Thaker, G. K. (2002). 'The Effects of Nicotine on Specific Eye Tracking Measures in Schizophrenia', *Biol Psychiatry*, **52**(7): pp. 721-8; Depatie, L., O'Driscoll, G. A., Holahan, A. L., Atkinson, V., Thavundayil, J. X., Kin, N. N., Lal, S. (2002). 'Nicotine and Behavioral Markers of Risk for Schizophrenia: A Double-Blind, Placebo-Controlled, Cross-Over Study', *Neuropsychopharmacology*, **27**(6): pp. 1056-70.

[363] Tregellas, J.R., Tanabe, J. L., Martin, L. F., Freedman, R. (2005).

movement task, greater activation was observed in the anterior and posterior cingulate gyri, precuneus, and medial temporal/medial superior temporal (MT/MST) cortex after nicotine than after placebo administration. Less activation was seen in the hippocampus during nicotine administration than placebo administration. These investigators concluded that nicotine improved perception and attention to moving stimuli given the effects observed in the cingulate gyrus, precuneus, and MT/MST. They also suggested that the decreased hippocampus activation during nicotine administration was consistent with nAChR mediation of inhibitory neuronal dysfunction in schizophrenia.

Cohen and colleagues examined brain activation among smokers during a verbal working task (N-Back paradigm).[364] Activation was observed in brain regions that traditionally respond to this task, including the dorsolateral frontal lobes, supplementary motor area, and left temporal-parietal cortex. Brain activation was contrasted during two different conditions for the same group of smokers after 12 hours of tobacco abstinence and immediately following ingestion of two cigarettes. Smokers showed increased frontal lobe activation during the N-Back task following nicotine intake compared to the nicotine abstinence condition. This finding suggests that nicotine causes increased activation of the frontal cortex among smokers that is apparent when nicotine is in their system, but not when it is withdrawn. This effect on working memory is consistent with nicotine's more general effect on focused and sustained attention.

1.4.2.4 Cerebral Perfusion Studies

Jacobsen and colleagues[365] conducted a study to evaluate whether nicotine-induced activation was independent of the macrovascular effects of nicotine during photic

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[364] Cohen, R., Sweet, L., David, S.P., Niaura, R. (2004).
[365] Jacobsen, L.K., Gore, J. C., Skudlarski, P., Lacadie, C. M., Jatlow, P., Krystal, J. H. (2002).

stimulation. Nicotine dependent healthy smokers were withdrawn from nicotine under controlled conditions and then scanned while receiving photic stimulation and successive intravenous infusions of saline and nicotine. No evidence for an effect of nicotine on BOLD signal response to photic stimulation was detected at the doses studied. This observation suggests that nicotine does not alter the coupling between BOLD signal and neuronal activity in the visual cortex.

The characteristics of each study included in the literature review are listed below in Table 1.7 demonstrating wide variability in the study designs.

Table 1.7

fMRI Studies of Nicotine Dependence

Study	Subjects	Design	Drug Administration	Paradigm	Behavioural Measures
Cohen et al. (2004)[366] USA	Experimental Group: 6 smokers	2 x 2 repeated measures ANOVA Drug (nicotine, placebo) Task (N-Back, control)	Experimental Group: Nicotine (2 cigarettes) Comparison Group: None, 12 hours abstinence	N-back	None described
Due et al. (2002)[367] USA	Experimental Group: 12 smokers Mean 23.5 cig/day 67% male Mean age 22.7 years Comparison Group: 6 never-smokers Never smoked regularly 67% male Mean age 25.0 years	2 x 2 x 4 repeated measures ANOVA Cue (smoking, neutral) Hemisphere (right, left) Time (4, 6, 8, 10 sec) *Post-hoc* comparison of smokers and never-smokers	Experimental Group: 10 hours abstinence Mean CO 13.2 ppm No nicotine Comparison Group: No nicotine	Pictorial cues: Smoking-related Neutral Target (animals) Key press to target cues	Likert scale self-reported mood Likert scale self-reported craving

Continued overleaf.

[366] Cohen, R., Sweet, L., David, S.P., Niaura, R. (2004).

[367] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002). 'Activation in Mesolimbic and Visuospatial Neural Circuits Elicited by Smoking Cues: Evidence from Functional Magnetic Resonance Imaging', *Am J Psychiatry*, **159**(6): pp. 954-60.

Study	Subjects	Design	Drug Administration	Paradigm	Behavioural Measures
Jacobsen et al. (2002)[368] USA	Experimental Group: 9 smokers Mean 25.3 cig/day 44% male Mean age 29.4 years Comparison Group: None	Impact of nicotine infusion on signal change in occipital cortex	Experimental Group: Overnight abstinence Mean CO < 10 ppm 2x saline infusion 3x nicotine infusion Comparison Group: n/a	Photic stimulation: Black and white checked pattern Flashing at 8Hz for 30s alternating with rest	Likert scale self-reported craving Smoking urges Nicotine withdrawal
Kumari et al. (2003)[369] UK	Experimental Group: 11 never-smokers "Never" not defined 100% male Mean age not stated Comparison Group: None	2 x 2 x 4 mixed design ANOVA Drug (nicotine, placebo) Order (1st, 2nd) Load (0, 1, 2, 3-back) Separate analyses for accuracy and latency	Experimental Group: 1x saline infusion 1x nicotine infusion Double-blind crossover 2 weeks apart Comparison Group: n/a	Parametric n-back working memory task	Accuracy (% correct) Latency (ms)
Lawrence et al. (2003)[370] USA	Experimental Group: 15 smokers Mean 21.8 cig/day 47% male Mean age 22.2 years Comparison Group: 14 non-smokers "Non" not defined 50% male Mean age 22.4 years	2 x 2 repeated measures ANOVA Drug (nicotine, placebo) Task (RVIP, control) Group (smoker, non) Task (RVIP, control)	Experimental Group: 1x nicotine patch 1x placebo patch Single-blind crossover Separation not stated Comparison Group: No nicotine	Rapid visual information processing task	Self-reported mood scale Number of targets detected Latency to target stimuli (ms)

Continued overleaf.

[368] Jacobsen, L.K., Gore, J. C., Skudlarski, P., Lacadie, C. M., Jatlow, P., Krystal, J. H. (2002). 'Impact of Intravenous Nicotine on BOLD Signal Response to Photic Stimulation', *Magn Reson Imaging*. **20**(2): pp. 141-5.

[369] Kumari, V., Gray, J. A., ffytche, D. H., Mitterschiffthaler, M. T., Das, M., Zachariah, E., Vythelingum, G. N., Williams, S. C., Simmons, A., Sharma, T. (2003). 'Cognitive Effects of Nicotine in Humans: An fMRI Study', *Neuroimage*, **19**(3): pp. 1002-13.

[370] Lawrence, N.S., T.J. Ross, and E.A. Stein (2002). 'Cognitive Mechanisms of Nicotine on Visual Attention', *Neuron*. **36**(3): pp. 539-48.

Study	Subjects	Design	Drug Administration	Paradigm	Behavioural Measures
Lim et al. (2005)[371] South Korea	Experimental Group: 1 smoker Comparison Group: 1 non-smoker	Case series Within subjects comparison of Cue (smoking, neutral) and Smoking (satiated, abstinent)	Experimental Group: Smoking and sham smoking Control Group: Smoking and sham smoking	Pictorial cues: Smoking-related Neutral	None
McClernon et al. (2005)[372] USA	Experimental Group: 13 smokers Comparison Group: within-subjects comparison	2 x 2 x 4 repeated measures ANOVA Cue (smoking, neutral) Smoking (satiated, abstinent) Time (4, 6, 8, 10 sec)	Experimental Group: Ad libitum smoking Mean CO 26.5 ppm Comparison Group: 10 hours abstinence Mean CO 9.4 ppm	Pictorial cues: Smoking-related Neutral Target (animals) Key press target cues	Likert scale self-reported mood Likert scale self-reported craving
Stein et al. (1998)[373] USA	Experimental Group: 16 smokers Mean cig/day not stated 56% male Mean age 25.9 years Comparison Group: None	Dose-response effect on regional brain activity	Experimental Group: 3x nicotine infusion Comparison Group: n/a	None	Likert scale self-reported feelings
Thiel et al. (2005)[374] Germany	Experimental Group: 15 non-smokers Comparison Group: within-subjects comparison	3 x 2 mixed design ANOVA Drug (nicotine 1mg gum, nicotine 2 mg gum, placebo) Cue (alerting contrast: neutral – no cue, reorienting contrast: invalid – valid cue)	Experimental Group: placebo, 1 mg, 2 mg nicotine gum Comparison Group: n/a	Cued-target attention task	Reaction times for each task Subjective drug effects

Continued overleaf.

[371] Lim, H.K., Pae, C. U., Joo, R. H., Yoo, S. S., Choi, B. G., Kim, D. J., Lee, C., Lee, C. U. (2005).
[372] McClernon, F.J., Hiott, F. B., Huettel, S. A., Rose, J. E. (2005).
[373] Stein, E.A., Pankiewicz, J., Harsch, H. H., Cho, J. K., Fuller, S. A., Hoffmann, R. G., Hawkins, M., Rao, S. M., Bandettini, P. A., Bloom, A. S. (1998). 'Nicotine-Induced Limbic Cortical Activation in the Human Brain: A Functional MRI Study', *Am J Psychiatry*, **155**(8): pp. 1009-15.
[374] Thiel, C.M., Zilles, K., Fink, G. R. (2005). 'Nicotine Modulates Reorienting of Visuospatial Attention and Neural Activity in Human Parietal Cortex', *Neuropsychopharmacology*, **30**(4): pp. 810-20.

Study	Subjects	Design	Drug Administration	Paradigm	Behavioural Measures
Tregellas et al. (2005)[375]	Experimental Group: 5 smokers and 4 non-smokers with schizophrenia Comparison Group: within-subjects comparison	2 x 2 repeated measures ANOVA Drug (nicotine gum, placebo) Task (pursuit task, control)	Experimental Group: placebo, 4 mg (non-smokers) or 6 mg (smokers) gum Comparison Group: n/a* *Smokers and non-smokers were not compared	Smooth pursuit eye movement task	Pursuit task performance

LEGEND: Table 1.7 provides an overview of the breadth of study designs employed for examining multiple cognitive, emotional, motivational, and haemodynamic responses to pharmacological nicotine and smoking-related envrinmental stimuli. Studies included in Table 1.7 were identified in a structured literature review, updated in July 2005, utilising PubMed, Medline and recently published abstracts using key words ("nicotine", "fMRI", "addiction").

1.4.3 Positron Emission Tomography

Positron Emission Tomography (PET) involves the use of intravenously administered radioactive ligands labelled with short-lived positron-emitting isotopes of carbon, oxygen, nitrogen, or fluorine attached to molecules. When ligands are administered systemically they cross the blood-brain barrier and bind to specific molecular targets such as neurotransmitter receptors or transporters. The ligands emit positrons, which collide with electrons and release gamma rays. Serial blood sampling of arterial concentrations of the ligand and the detection of gamma rays indicate the location of the receptor targets. When combined with the established pharmacokinetic properties of each ligand, investigators can estimate the binding potential (BP) of receptors, and affinity for specific endogenous ligands using established mathematical models. The BP is directly proportional to the availability of ligand binding sites and thus provides an estimation of the concentration or density of the receptor of interest in specified brain ROIs. Thus, PET provides the ability to trace the binding and

[375] Tregellas, J.R., Tanabe, J. L., Martin, L. F., Freedman, R. (2005). 'FMRI of Response to Nicotine During a Smooth Pursuit Eye Movement Task in Schizophrenia', *Am J Psychiatry*, **162**(2): pp. 391-3.

distribution of specific ligands such as nicotine (e.g., [^{11}C]-labelled nicotine). PET is therefore useful for the examination of quantity and distribution of specific molecular targets with direct relevance to nicotine dependence such as the DA D_2 receptor ([^{11}C]raclopride)[376] and others. Table 1.8 provides examples of radioactive ligands specific to particular receptor subtypes, which are being used increasingly in studies of nicotine dependence.

Table 1.8

Radioligands commonly used in nicotine dependence research

Ligand	*Molecular Target*	*Permits evaluation of:*
[^{11}C]SCH-23390	D_1	D1 receptor occupancy
[^{11}C]raclopride	D_2	D_2 receptor occupancy
[^{99m}Tx]TRODAT-1 [^{123}I]β-CIT	Dopamine transporter	Dopamine transporter availability
[^{11}C]WAY-100635	5-HT_{1A}	5-HT_{1A} receptor occupancy
[^{18}F]altanserin	5-HT_{2A}	5-HT_{2A} receptor occupancy
[^{11}C]McN-5652 [^{123}I]β-CIT	Serotonin transporter	Serotonin transporter availability
[^{11}C]nicotine 2-[^{18}F]-A-85380	Nicotinic acetylcholine	Nicotinic acetylcholine receptor distribution
[^{11}C]clorgyline	MAO-A	MAO-A activity
[^{11}C]^{L}-deprynyl-D2	MAO-B	MAO-B activity
[^{18}F]DOPA	All DA receptors	Dopamine activity
[^{15}O]H_20	Non-specific	Global and regional cerebral blood flow
[^{18}F]FDG	Non-specific	Glucose metabolism

LEGEND: Table 1.8 lists radioligands used in positron emission tomography studies with relevance to nicotine dependence. Abbreviations: SCH-23390 = ((*R*)-(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride); TRODAT-1 = Tc99m Tropane; McN-5652 = Pyrrolo[2,1-a]isoquinoline, 1,2,3,5,6,10b-hexahydro-6-[4-(methylthio)phenyl]-, (6S,10bR)-, (2R,3R)-2,3-di-(O-4-methylphenyloxy)butanedioate; FDG = 2-fluoro-2-deoxy-D-glucose.

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[376] Tsukada, H., Miyasato, K., Kakiuchi, T., Nishiyama, S., Harada, N., Domino, E. F. (2002). 'Comparative Effects of Methamphetamine and Nicotine on the Striatal [(I1)C]Raclopride Binding in Unanesthetized Monkeys', *Synapse*, **45**(4): pp. 207-12.

As many of the ligands are competitive antagonists of receptors of interest, PET also provides information on endogenous neurotransmitter release in response to pharmacological challenges. Combined with fMRI and other imaging techniques described below, functional imaging can therefore provide a rich array of data *in vivo* once only available in invasive animal studies. A comprehensive list of PET studies with pertaining to nicotine dependence is not feasible as most of the radioligands used in PET studies have potential relevance to the pharmacology of nicotine dependence and the number of associated publications is too large to include here.[377]

However, two recent PET studies have been particularly informative. In a PET study using [^{11}C]raclopride (a specific ligand for DA D_2 receptors), Brody and colleagues examined the effect of cigarette smoking on ventral striatum DA release.[378] Twenty nicotine-dependent smokers underwent [^{11}C]raclopride boluses followed by continuous-infusion. During the PET session ten of the subjects took a ten-minute break during wliich they smoked a cigarette and ten subjects who did not smoke cigarettes were used as controls. The subjects who smoked a cigarette demonstrated greater reductions in [^{11}C]raclopride BP in ventral striatum ROIs than the group that did not smoke. Moreover, significant correlations were found between change from before to after tlie smoking break in craving ratings and change from before to after the break in BP for these two regions.

The study by Brody and colleagues is intriguing for many reasons. First, it demonstrates that, in liumans, nicotine administration is associated with increased release of DA in the ventral striatum and is consistent with the findings of Stein and colleagues who observed

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[377] A Medline search performed on 15 August 2005 with 'nicotine' and 'PET' as key word generated 69 publications. Internet citation: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi.

[378] Brody, A.L., Olmstead, R. E., London, E. D., Farahi, J., Meyer, J. H., Grossman, P., Lee, G. S., Huang, J., Hahn, E. L., Mandelkern, M. A. (2004). 'Smoking-Induced Ventral Striatum Dopamine Release', *Am J Psychiatry*, **161**(7): pp. 1211-8.

increased activation in the NAc following nicotine administration. Secondly, Brody and colleagues observed a significantly positive correlation between D_2 receptor BP and craving amongst those who smoked cigarettes. This finding suggests that the craving observed during nicotine administration is more closely associated with release of DA in the ventral striatum than during the abstinent condition. Whether this observation holds true for smokers exposed to smoking-related cues is an area for further study.

Martin-Soelch and colleagues conducted another PET study relevant to nicotine dependence.[379] Smokers and non-smokers performed a pattern recognition task with delayed response whilst regional cerebral blood flow (rCBF) was measured using $H_2{}^{15}0$ PET. Correct responses to tasks were reinforced with increasing monetary reward. Significant differences were observed in rCBF between smokers and non-smokers in the striatum, with significant correlations between striatal rCBF and increasing monetary reward only in non-smokers. The authors concluded that cognitive responses to less salient rewards are impaired in smokers.

1.4.4 Other Approaches

In addition to fMRI and PET, other functional imaging approaches have utility in nicotine addiction studies.

1.4.4.1 Single Photon Emission Computed Tomography

Similar to PET, Single Photon Emission Computed Tomography (SPECT) utilises radioactive isotypes but with much longer half-lives than those used in PET. The longer half-life of isotopes limits the temporal resolution of SPECT and, as such, SPECT has not been used extensively in nicotine dependence research in recent years.

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[379] Martin-Soelch, C., Missimer, J., Leenders, K. L., Schultz, W. (2003). 'Neural Activity Related to the Processing of Increasing Monetary Reward in Smokers and Nonsmokers', *Eur J Neurosci.* **18**(3): pp. 680-8.

1.4.4.2 Electroencephalography and Event-Related Potentials

Electroencephalography (EEG) measures the difference in voltage between a site located on the scalp and a reference site where no EEG activity is expected. EEG waveforms are thought to represent the summed post-synaptic activity of cortical pyramidal cells. EEG activity in the frequency of different waveforms has been associated with functional outcomes such as working memory and with emotional traits and states and, as such, can be useful in nicotine addition research. Event-related potentials (ERP) are useful in providing information on cortical responses to discrete stimuli and differ from EEG in the high temporal resolution of responses to stimuli (within ms). The spatial resolution of EEG and ERP is relatively low when compared to fMRI. However, spatial resolution is proportional to the number of electrodes and has improved with the development of high-density electrode arrays. A promising feature of EEG and ERP is that they can be used with fMRI and PET and could therefore complement these methods by providing additional information on large distributions of neurones.

1.4.4.3 Magnetoencephalography

Magnetoencephalography (MEG), similar to EEG and ERP, measures electrical changes in electrical activity in the brain, but unlike EEG and ERG, MEG also measures changes in magnetic fields generated by such activity. MEG has better spatial resolution than EEG or ERP but has not been used extensively in nicotine dependence research.

1.4.4.4 Magnetic Resonance Spectroscopy

Although used less frequently, magnetic resonance spectroscopy (MRS) can also be used to study biochemistry of the brain, including measurements of concentrations of some key neurotransmitters such as GABA and glutamate.

1.4.4.5 Advantages and Disadvantages of Different Approaches

fMRI and PET/SPECT each have advantages and disadvantages depending on the research questions posed. If one is examining activation patterns responding to specific stimuli, fMRI is clearly advantageous compared with PET and SPECT because of its (fMRI) higher spatial and temporal resolution. The non-invasive nature of fMRI also permits investigators to maximise statistical power with the employment of multiple measures to evaluate the effect of specific stimuli on neural activation. Furthermore, fMRI permits greater flexibility and versatility in experimental design than *in vivo* molecular imaging approaches. For example, PET requires block designs and repeated-measures studies are difficult given the health risks associated with repetitive exposure to radioactive ligands. PET, SPECT, and MRS have advantages in terms of ability to provide functional data on specific neurochemical substrates. The major disadvantages of PET and SPECT are the radiation exposure and invasiveness involved, limiting the number of scans a subject may have, the cost, the need to prepare appropriate radioligands, and the lower spatial and temporal resolution.[380] As previously mentioned, ERP has very high temporal resolution and thus may have advantages over MRI for measurement of responses to stimuli in the millisecond range. MRS and fMRI have obvious advantages in providing statistical activation maps overlaid upon high-resolution structural brain images and the ability to conveniently acquire additional types of images during the same session – such as clinical scans.

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[380] Volkow, N.D., Rosen, B., Farde, L. (1997). 'Imaging the Living Human Brain: Magnetic Resonance Imaging and Positron Emission Tomography', *Proc Natl Acad Sci U S A*, **94**(7): pp. 2787-8; Talbot, P.S., Laruelle, M. (2002). 'The Role of *In Vivo* Molecular Imaging with PET and SPECT in the Elucidation of Psychiatric Drug Action and New Drug Development', *Eur Neuropsychopharmacol*, **12**(6): pp. 503-11.

In summary, functional neuroimaging methods provide a powerful armamentarium of tools to evaluate physiological indicants related to nicotine dependence in human brains and their relationship to specific behavioural counterparts, particularly in the case of fMRI. In addition, methods such as PET and SPECT provide functional data at the molecular level, more proximal to the gene variant of interest or to the pharmacodynamic response under investigation than subjective or objective evaluations of behaviour without neuroimaging. As such, fMRI and PET, in particular, have paved new avenues of opportunity for scientists to better understand the complex neurophysiology of nicotine dependence. As can be seen above, there are a multiplicity of different approaches for evaluating nicotine addiction endophenotypes with functional neuroimaging and, at present, few replication studies of any one approach or construct have been published. Therefore, the field of functional neuroimaging in nicotine and tobacco research remains in its "infancy" and opportunities abound to begin to connect elegant animal models and genetic hypotheses with *in vivo* human molecular phenotypes.

1.5 Application of Functional Neuroimaging to the Examination of Genetic Influences on Nicotine Dependence

The power of functional neuroimaging to better understand associations between genotype and function provides a potential bridge to connect genetic association studies to behaviour through triangulation with phenotypes more more directly related to the influence of genetic variation on molecular and cellular function underlying the behaviour of interest than less precise behavioural phenotypes typically evaluated in genetic association studies. One of the problems observable in genetic association studies is non-replication of associations between

genotype and behaviour,[381] particularly in complex behaviours such as smoking or personality traits, which are broadly defined constructs under the influence of multiple environmental factors.[382]

Hariri and colleagues have proposed a model through which to evaluate associations between genetic variation in the serotonin system and neurological function using functional neuroimaging.[383] In this model, Hariri proposes that genes do not directly encode behaviour. Rather, genetic variation may encode subtle cellular and molecular changes (proximal), which alter physiology and affect response biases at the systems level (intermediate), which may or may not affect behaviour.

"Imaging genomics" is a type of genetic association study but differs from conventional association studies by defining phenotypes as discrete, physiological responses to behavioural tasks, environmental or pharmacological stimuli. As such, candidate genes ideal for imaging genomic studies are those with well-characterised functional effects as demonstrated *in vitro* and/or *in vivo*. Given the emerging evidence from animal studies implicating serotonin in nicotine dependence, two polymorphisms in particular may be ideal candidates for imaging genomic studies pertaining to tobacco smoking.

The serotonin transporter 5-HTTLPR and the serotonin receptor $5HT_{1A}$ -1018 C>G polymorphisms have well defined physiological phenotypes *in vitro* and have demonstrated

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[381] Munafo', M. (2004). 'Replication Validity of Genetic Association Studies of Smoking Behavior: What Can Meta-Analytic Techniques Offer?' *Nicotine Tob Res.* **6**(2): pp. 381-2; Munafo', M.R., Clark, T. G., Moore, L. R., Payne, E., Walton, R., Flint, J. (2003). 'Genetic Polymorphisms and Personality in Healthy Adults: A Systematic Review and Meta-Analysis', *Mol Psychiatry*, **8**(5): pp. 471-84; Munafo', M., Clark, T., Johnstone, E., Murphy, M., Walton, R. (2004).

[382] Munafo', M. (2004). 'Replication Validity of Genetic Association Studies of Smoking Behavior: What Can Meta-Analytic Techniques Offer?' *Nicotine Tob Res.* **6**(2): pp. 381-2; Munafo', M.R., Clark, T. G., Moore, L. R., Payne, E., Walton, R., Flint, J. (2003). 'Genetic Polymorphisms and Personality in Healthy Adults: A Systematic Review and Meta-Analysis', *Mol Psychiatry*, **8**(5): pp. 471-84; Munafo', M., Clark, T., Johnstone, E., Murphy, M., Walton, R. (2004).

[383] Hariri, A.R., Weinberger, D. R. (2003). 'Functional Neuroimaging of Genetic Variation in Serotonergic Neurotransmission', *Genes Brain Behav.* **2**(6): pp. 341-9.

associations with multiple behavioural traits and states. The 5-HTTLPR polymorphism, as described in Section 1.2.1.3.3, results in either a short "S" or long "L" alleles with 14 or 16 copies of base-pair repeat units, respectively.[384] Not only has the S allele been associated with decreased transcription in cloned fusion cells,[385] but similar reductions in serotonin transporter expression have been observed *in vivo* using SPECT[386] and in post-mortem brains.[387] In addition to associations with neuroticism and harm avoidance robust to meta-analysis,[388] the 5-HTTLPR is associated with persistent smoking,[389] and may interact with the nicotine skin patch to affect pharmacogenetic smoking cessation outcomes. [390] Furthermore, the 5-HTTLPR is common in populations of European ancestry (LL = 0.36, LS = 0.48, SS = 0.16), albeit with substantial variation between populations.[391]

The $5HT_{1A}$ -1018 C>G single nucleotide polymorphism, which will be described further in Chapter 3, is located in the promoter region between 1018 bp upstream from the initiation codon. Lemonde and colleagues demonstrated that the presence of the G allele inhibited the

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[384] Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., Lesch, K. P. (1996). 'Allelic Variation of Human Serotonin Transporter Gene Expression', *J Neurochem*, **66**(6): pp. 2621-4.

[385] Lesch, K.P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., Murphy, D. L. (1996). 'Association of Anxiety-Related Traits with a Polymorphism in the Serotonin Transporter Gene Regulatory Region', *Science*, **274**(5292): pp. 1527-31.

[386] Heinz, A., Jones, D. W., Mazzanti, C., Goldman, D., Ragan, P., Hommer, D., Linnoila, M., Weinberger, D. R. (2000). 'A Relationship Between Serotonin Transporter Genotype and In Vivo Protein Expression and Alcohol Neurotoxicity', *Biol Psychiatry*, **47**(7): pp. 643-9.

[387] Little, K.Y., McLaughlin, D. P., Zhang, L., Livermore, C. S., Dalack, G. W., McFinton, P. R., DelProposto, Z. S., Hill, E., Cassin, B. J., Watson, S. J., Cook, E. H. (1998). 'Cocaine, Ethanol, and Genotype Effects on Human Midbrain Serotonin Transporter Binding Sites and mRNA Levels', *Am J Psychiatry*, **155**(2): pp. 207-13.

[388] Munafo', M.R., Clark, T. G., Moore, L. R., Payne, E., Walton, R., Flint, J. (2003); Sen, S., Burmeister, M., Ghosh, D. (2004). 'Meta-Analysis of the Association Between a Serotonin Transporter Promoter Polymorphism (5-HTTLPR) and Anxiety-Related Personality Traits', *Am J Med Genet B Neuropsychiatr Genet.* **127**(1): pp. 85-9.

[389] Munafo', M., Clark, T., Johnstone, E., Murphy, M., Walton, R. (2004).

[390] David, S.P., Murthy, N.V., Rabiner, E.A., Munafo', M., Jacob, R., Johnstone, E., Grasby, P.M. (2004). 'Serotonin Transporter Polymorphism Linked to Personality Traits Affects Serotonin (5-HT1A) Receptor Function in Positron Emission Tomography Study', paper presented at the North American Primary Care Research Group's (NAPCRG) 32nd annual meeting in Orlando, FL, USA.

[391] Gelernter, J., Kranzler, H., Cubells, J. F. (1997). 'Serotonin Transporter Protein (SLC6A4) Allele and Haplotype Frequencies and Linkage Disequilibria in African- and European-American and Japanese Populations and in Alcohol-Dependent Subjects', *Hum Genet.* **101**(2): pp. 243-6.

repression of transcription by the transcription factor nuclear deformed epidermal autoregulatory factor (NUDR/DEAF-1) *in vitro* and went on to demonstrate associations between the $5HT_{1A}$ -1018 allele and depression and suicide.[392] In addition, the G allele has been associated with schizophrenia,[393] panic disorder,[394] and pharmacogenetic treatment response to fluvoxamine.[395]

A fine example of imaging genomics is the work of Hariri and colleagues who compared subjects with 5-HTTLPR LL and SS genotypes in an fMRI study where the task involved identifying fearful and angry facial expressions.[396] Hariri and colleagues observed that subjects with SS genotypes demonstrated greater Am activation to fearful stimuli when compared to those with LL genotypes. In fact, the magnitude of difference in effect size between genotypic groups was nearly five-fold. These findings have since been replicated with a larger sample size.[397] In these studies by Hariri and colleagues, the deductive process began with an established biological association between the 5-HTTLPR S allele and decreased serotonin transporter expression and 5-HT reuptake. Combined with extant knowledge of the role of serotonergic projections to the Am in mediating anxiety, Hariri and

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[392] Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P. D., Bown, C. D., Sequeira, A., Kushwaha, N., Morris, S. J., Basak, A., Ou, X. M., Albert, P. R. (2003). 'Impaired Repression at a 5-Hydroxytryptamine 1A Receptor Gene Polymorphism Associated with Major Depression and Suicide', *J Neurosci.* **23**(25): pp. 8788-99.

[393] Huang, Y.Y., Battistuzzi, C., Oquendo, M. A., Harkavy-Friedman, J., Greenhill, L., Zalsman, G., Brodsky, B., Arango, V., Brent, D. A., Mann, J. J. (2004). 'Human 5-HT1A Receptor C(-1019)G Polymorphism and Psychopathology', *Int J Neuropsychopharmacol*, **7**(4): pp. 441-51.

[394] Rothe, C., Gutknecht, L., Freitag, C., Tauber, R., Mossner, R., Franke, P., Fritze, J., Wagner, G., Peikert, G., Wenda, B., Sand, P., Jacob, C., Rietschel, M., Nothen, M. M., Garritsen, H., Fimmers, R., Deckert, J., Lesch, K. P. (2004). 'Association of a Functional 1019C>G 5-HT1A Receptor Gene Polymorphism with Panic Disorder with Agoraphobia', *Int J Neuropsychopharmacol*, **7**(2): pp. 189-92.

[395] Serretti, A., Artioli, P., Lorenzi, C., Pirovano, A., Tubazio, V., Zanardi, R. (2004). 'The C(-1019)G Polymorphism of the 5-HT1A Gene Promoter and Antidepressant Response in Mood Disorders: Preliminary Findings', *Int J Neuropsychopharmacol*, **7**(4): pp. 453-60.

[396] Hariri, A.R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M. F., Weinberger, D. R. (2002). 'Serotonin Transporter Genetic Variation and the Response of the Human Amygdala', *Science*, **297**(5580): pp. 400-3.

[397] Hariri, A.R., Drabant, E. M., Munoz, K. E., Kolachana, B. S., Mattay, V. S., Egan, M. F., Weinberger, D. R. (2005). 'A Susceptibility Gene for Affective Disorders and the Response of the Human Amygdala', *Arch Gen Psychiatry*, **62**(2): pp. 146-52.

colleagues identified a ROI with a well-supported *a priori* hypothesis of a genetic association with function. Finally, the functional role of the Am in anxiety-related responses to fearful stimuli having been well established, the observation of Am activation to fear-provoking stimuli suggests a biologically-plausible relationship between 5-HTTLPR and anxiety traits and states.[398]

A similar approach can be applied to the study of genetic influences on nicotine dependence. Converging data from animal and human studies implicate serotonin neurotransmission in the mediation of nicotine withdrawal and conditioned cue reactivity. If an fMRI paradigm that reliably activates the ventral striatum is established, functional polymorphisms such as the 5-HTTLPR and -1018 $5HT_{1A}$ SNP are reasonable candidates for imaging genomic studies of nicotine dependence. However, given the fact that neither polymorphism has, as yet, been evaluated for potential influence on 5-HT_{1A} receptor expression in man, PET evaluations of genetic associations utilising a specific 5-HT_{1A} receptor ligand such as [^{11}C]WAY-100635, would be useful in the establishment of how such polymorphisms affect neurophysiology at the molecular level. Thus, an approach similar to the powerful methods applied by Hariri and colleagues could be applied to nicotine dependence research if and when the functional affects of these polymorphisms are established *in vivo* and fMRI paradigms that reliably activate reward centres associated with nicotine dependence are identified.

1.5.1 Aims of Research

In Section 1.2, I reviewed the pharmacology of nicotine and complex neuroadaptations resulting from chronic use and I described how the cumulative knowledge of decades of

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[398] Munafo', M.R., Clark, T. G., Moore, L. R., Payne, E., Walton, R., Flint, J. (2003); Sen, S., Burmeister, M., Ghosh, D. (2004).

animal research has led to models of nicotine dependence, all of which suggest that neuroadaptations in chronic smokers alter the salience of smoking-related environmental cues and result in loss of control over use of and increasing motivation to acquire nicotine at the expense of other natural reinforcers. A growing body of converging evidence indicates that there is indeed a major genetic contribution to nicotine dependence and that polymorphisms in genes related to DA and 5-HT neurotransmission appear to influence various aspects of smoking behaviour. However, a gap in knowledge remains between how such genetic variants might influence smoking behaviour, and our understanding of the precise neurological mechanisms involved at the molecular, cellular, and systems levels. The advent of functional neuroimaging has provided an opportunity to apply such models to humans *in vivo* and begin to explain at the molecular level the wide variation in ability to quit smoking and hopefully offer clues as to how one might endeavour to develop more effective pharmacological agents to promote smoking cessation. Imaging genomics, as described in the previous section, offers a distinct opportunity to take the next such translational step.

The following evidence supports the notion that 5-HT_{1A} receptors play a critical role in the mediation of nicotine withdrawal and behavioural sensitisation to smoking-related environmental cues. 5-HT_{1A} receptors appear to contribute to the development of incentive sensitisation to smoking-related environmental cues. 5-HT_{1A} receptors inhibit nicotine-induced behavioural sensitisation as demonstrated by administration of the 5-HT_{1A} agonist 8-OH-DPAT in rats[399]. A biologically plausible mechanism for this observation is as follows:

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[399] Olausson, P., Engel, J. A., Soderpalm, B. (1999).

Nicotine-induced 5-HT release in the DRN[400] results in decreased release of 5-HT in terminal fields[401] such as the ventral striatum[402] and hippocampus[403] resulting from stimulation of inhibitory somatodendritic 5-HT_{1A} autoreceptors. 5-HT_3 receptors are present on pre-synaptic DA terminals in the striatum[404] and stimulation of these receptors results in elevated striatal DA levels.[405] Thus, inhibition of 5-HT release in the striatum as a result of nicotine-induced DRN 5-HT_{1A} receptor stimulation would theoretically reduce stimulation of pre-synaptic 5-HT_3 receptors and subsequently reduce the stimulation of striatal DA release.

Given the importance of nicotine-induced DA release in the NAc in the development of incentive sensitisation and the role of 5-HT_{1A} receptors in mediating nicotine withdrawal, 5-HT_{1A} receptors are therefore logical molecular targets for exploration of genetic influences on smoking-related cue reactivity. Furthermore, the NAc is a primary region of interest for examination of the precise neural processes involved in the development of and treatment for nicotine dependence.

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[400] Cheeta, S., Irvine, E. E., Kenny, P. J., File, S. E. (2001). 'The Dorsal Raphe Nucleus is a Crucial Structure Mediating Nicotine's Anxiolytic Effects and the Development of Tolerance and Withdrawal Responses', *Psychopharmacology* (Berl), **155**(1): pp. 78-85.

[401] Cheeta, S., Irvine, E. E., Kenny, P. J., File, S. E. (2001). 'The Dorsal Raphe Nucleus is a Crucial Structure Mediating Nicotine's Anxiolytic Effects and the Development of Tolerance and Withdrawal Responses', *Psychopharmacology* (Berl), **155**(1): pp. 78-85.

[402] Sprouse, J.S., Aghajanian, G. K. (1987). 'Electrophysiological Responses of Serotoninergic Dorsal Raphe Neurons to 5-HT1A and 5-HT1B Agonists', *Synapse*, **1**(1): pp. 3-9.

[403] Benwell, M.E., Balfour, D. J. (1979). 'Effects of Nicotine Administration and Its Withdrawal on Plasma Corticosterone and Brain 5-hHydroxyindoles', *Psychopharmacology* (Berl). **63**(1): pp. 7-11; Benwell, M.E., Balfour, D. J., Anderson, J. M. (1990). 'Smoking-Associated Changes in the Serotonergic Systems of Discrete Regions of Human Brain', *Psychopharmacology* (Berl). **102**(1): pp. 68-72; Benwell, M.E., Balfour, D. J. (1982). 'The Effects of Nicotine Administration on 5-HT Uptake and Biosynthesis in Rat Brain', *Eur J Pharmacol.* **84**(1-2): pp. 71-7; Balfour, D.J., Ridley, D. L. (2000). 'The Effects of Nicotine on Neural Pathways Implicated in Depression: A Factor in Nicotine Addiction?' *Pharmacol Biochem Behav.* **66**(1): pp. 79-85.

[404] Nayak, S.V., Ronde, P., Spier, A. D., Lummis, S. C., Nichols, R. A. (2000). 'Nicotinic Receptors Co-Localize with 5-HT(3) Serotonin Receptors on Striatal Nerve Terminals', *Neuropharmacology*, **39**(13): pp. 2681-90.

[405] Benloucif, S., Keegan, M. J., Galloway, M. P. (1993). 'Serotonin-Facilitated Dopamine Release *In Vivo*: Pharmacological Characterization', *J Pharmacol Exp Ther.* **265**(1): pp. 373-7; Jiang, L.H., Ashby, C. R., Jr., Kasser, R. J., Wang, R. Y. (1990). 'The Effect of Intraventricular Administration of the 5-HT3 Receptor Agonist 2-Methylserotonin on the Release of Dopamine in the Nucleus Accumbens: An In Vivo Chronocoulometric Study', *Brain Res.* **513**(1): pp. 156-60.

Given this background, the overarching aims of my research are to:

1. Identify novel nicotine dependence endophenotypes using fMRI;
2. Examine the functional significance of polymorphisms in the serotonin 5-HT_{1A} receptor and serotonin transporter genes utilising positron emission tomography (PET);
3. Triangulate results from both arms of research in order to examine whether or not a trait variable (5-HTTLPR genotype) or a state variable (smoking condition) would significantly influence cue-elicited activation of the ventral striatum including the nucleus accumbens

1.5.2 Hypotheses

In Chapters 2 to 4 I describe three studies designed to carry out these aims and examine novel hypotheses, intended to advance the understanding of the neuropharmacology of nicotine dependence. In Chapter 2 **(Specific Aim 1)**, I describe an event-related fMRI experiment of smokers and non-smokers to examine the following hypotheses:

1. I hypothesised that in abstaining addicted smokers there would be greater activation in response to smoking-related pictorial cues than non-smoking cues in the ventral striatum including the nucleus accumbens VS/NAc and in a distributed reward network including the ACC and OFC.
2. Furthermore, I hypothesised that activation associated with smoking-related cues would not be observed in the VS/NAc in non-smokers.
3. Finally, I hypothesised that there would be greater activation associated with smoking-related cues in the VS/NAc, ACC, and OFC smokers compared to non-smokers.

Chapter 3 **(Specific Aim 2)** reports an imaging genomics study whereby healthy volunteers who underwent PET with the 5-HT_{1A} receptor ligand [^{11}C]WAY-100635 were genotyped for serotonergic candidate genes implicated in nicotine dependence to examine genetic influences on 5-HT_{1A} receptor binding in order to examine the following hypothesis:

1. I hypothesised that there would be increased 5-HT_{1A}R binding in healthy volunteers with one or more copies of the –1018 5-HT_{1A} G allele (GG/CC) than in individuals with CC genotypes, and that
2. The 5-HTTLPR would interact with the –1018 5-HT_{1A} G allele to influence 5-HT_{1A}R binding.

Chapter 4 **(Specific Aim 3)** describes a study that triangulates the results from studies 1 and 2 to examine whether specific state (genotype) and/or trait (smoking condition) variables influenced cue-elicited reactivity in mesocorticolimbic brain circuitry. The following hypotheses were therefore examined:

1. I hypothesised that there would be greater activation associated with smoking-related pictorial cues in the ventral striatum including the nucleus accumbens (VS/NAc) in the abstinent condition than in the nicotine satiated condition (following *ad libitum* cigarette smoking);
2. I hypothesised that in the abstinent condition, smokers with 5-HTTLPR SS or SL genotypes would demonstrate greater activation in VS/NAc than smokers with LL genotypes, and
3. I hypothesised that in the smoking condition, smokers with LL genotypes would demonstrate equal or greater activation in the VS/NAc than smokers with SS or SL genotypes.

In Chapter 5, I synthesised the results from these three investigations, assert generalisable conclusions, and describe the next logical steps for further research. Moreover, I discuss the strengths and weaknesses of the research and how I will apply lessons learned from my doctoral work to future research endeavours. Finally, I suggest potential translational applications of this research including the development of novel compounds for smoking cessation through further application of imaging genomics.

Chapter 2.

FUNCTIONAL MAGNETIC RESONANCE IMAGING INVESTIGATION OF REWARD NETWORK ACTIVATION TO SMOKING-RELATED PICTORIAL CUES

2.1 Introduction

The overall aim of this pilot study was to:

Identify novel nicotine dependence endophenotypes using functional magnetic resonance imaging (fMRI).

The following study was designed as a pilot project to refine a reliable and robust fMRI protocol, examining reward pathway activation associated with smoking-related pictorial cues. As mentioned in the Preface, I published the main results of this study in a peer-reviewed journal.[406] In this chapter I trace the steps taken to develop and refine the fMRI protocol and present data from a comparison of smokers and non-smokers taken from the Oxfordshire population. More specifically, the sub-aims of this research were to:

1. Establish a reliable fMRI paradigm to examine cue-elicited Blood-Oxygen-Level-Dependent (BOLD) response to smoking-related pictorial cues in addicted smokers.
2. Identify brain regions reactive to smoking-related pictorial cues in smokers.
3. Determine whether smoking-related pictorial cues stimulated greater activation in pre-specified brain reward regions in smokers than non-smokers.

2.2 Background

A major contributor to relapse following cessation of tobacco smoking and use of other drugs of abuse is the development of craving or wanting when presented with environmental stimuli (visual, olfactory, tactile, and imaginary) associated with drug use.[407] Cue reactivity

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[406] David, S.P., Munafo', M., Johansen-Berg, H., Smith, S.M., Rogers, R.D., Matthews, P.M., Walton, R.T. (2005). 'Ventral Striatum/Nucleus Accumbens Activation to Smoking-Related Pictorial Cues in Smokers and Non-Smokers: An fMRI study', *Biol Psychiatry*, **58**(6):488-94.

[407] Niaura, R., Shadel, W. G., Abrams, D. B., Monti, P. M., Rohsenow, D. J., Sirota, A. (1998). 'Individual Differences in Cue Reactivity Among Smokers Trying to Quit: Effects of Gender and Cue Type', *Addict Behav.* **23**(2): pp. 209-24; Niaura, R.S., Rohsenow, D. J., Binkoff, J. A., Monti, P. M., Pedraza, M., Abrams, D. B. (1988). 'Relevance of Cue Reactivity to Understanding Alcohol and Smoking Relapse', *J Abnorm Psychol.*

in individuals dependent on drugs of abuse is demonstrated not only by its affecting drug wanting or craving but also through dopaminergically-mediated reductions in inhibition of the acoustic startle reflex,[408] cardiovascular reactivity,[409] and altered skin conductance.[410]

Functional neuroimaging has demonstrated that the very same types of environmental cues that evoke drug craving activate an integrated network of brain regions involved in the motivational and appetitive processes of addiction to nicotine and other drugs of abuse.[411] Converging data from animal models and *in vivo* studies implicate several overlapping neurological circuits, which, together, provide a model for the development of nicotine addiction. In particular, amongst these circuits there are: (1) a reward network including limbic regions such as the ventral striatum including nucleus accumbens (VS/NAc), amygdala (Am), and anterior cingulate cortex (ACC), (2) a motivational/drive network including the orbitofrontal cortex OFC and ACC, (3) a memory and learning network including the Am and hippocampus, and (4) an inhibitory control network including the medial OFC and ACC.[412] Each of these circuits receives direct dopaminergic innervation and is connected to each other in a network through both excitatory glutamatergic and inhibitory

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97(2): pp. 133-52; Rohsenow, D.J., Niaura, R. S., Childress, A. R., Abrams, D. B., Monti, P. M. (1990). 'Cue Reactivity in Addictive Behaviors: Theoretical and Treatment Implications', *Int J Addict*, **25**(7A-8A): pp. 957-93.

[408] Hutchison, K.E., McGeary, J., Wooden, A., Blumenthal, T., Ito, T. (2003). 'Startle Magnitude and Prepulse Inhibition: Effects of Alcohol and Attention', *Psychopharmacology* (Berl), **167**(3): pp. 235-41; Hutchison, K.E., Niaura, R., Swift, R. (2000). 'The Effects of Smoking High Nicotine Cigarettes on Prepulse Inhibition, Startle Latency, and Subjective Responses', *Psychopharmacology* (Berl), **150**(3): pp. 244-52; Hutchison, K.E., Niaura, R., Swift, R. (1999). 'Smoking Cues Decrease Prepulse Inhibition of the Startle Response and Increase Subjective Craving in Humans', *Exp Clin Psychopharmacol.* 7(3): pp. 250-6.

[409] Niaura, R.S., Rohsenow, D. J., Binkoff, J. A., Monti, P. M., Pedraza, M., Abrams, D. B. (1988). 'Relevance of Cue Reactivity to Understanding Alcohol and Smoking Relapse', *J Abnorm Psychol.* **97**(2): pp. 133-52.

[410] Ibid.

[411] Breiter, H.C., Rosen, B. R. (1999). 'Functional Magnetic Resonance Imaging of Brain Reward Circuitry in the Human', *Ann N Y Acad Sci.* **877**: pp. 523-47; Koob, G.F., Le Moal, M. (2001). 'Drug Addiction, Dysregulation of Reward, and Allostasis', *Neuropsychopharmacology*, **24**(2): pp. 97-129; Volkow, N.D., Fowler, J. S., Wang, G. J. (2003). 'The Addicted Human Brain: Insights from Imaging Studies', *J Clin Invest.* **111**(10): pp. 1444-51.

[412] Breiter, H.C., Rosen, B. R. (1999); Volkow, N.D., Fowler, J. S., Wang, G. J. (2003); O'Doherty, J., Rolls, E.T., Francis, S., Bowtell, R., McGlone, F., Kobal, G., Renner, B., Ahne, G. (2000). 'Sensory-Specific Satiety-Related Olfactory Activation of the Human Orbitofrontal Cortex', *Neuroreport*, **11**(4): pp. 893-7.

GABAergic projections. Furthermore, there is evidence that ventral extrastriate visual pathways (caudate, ventral temporal cortex: inferior temporal gyrus, lateral and medial fusiform gyrus) play a role in nicotine dependence by contributing to the identification of more salient visual objects associated with drug acquisition and consumption.[413]

Of all of the regions implicated in development of nicotine addiction, the ventral striatum including the nucleus accumbens (VS/NAc) is a region of major interest particularly because of its dual role in processing the hedonic effects of nicotine administration in the shell of the nucleus accumbens (NAc) and in signalling the presence of nicotine-related environmental stimuli in the NAc core[414] (reviewed in Chapter 1, Section 1.2.1.2). Studies in rats have demonstrated that addictive drugs stimulate dopaminergic (DA) neurons in the midbrain tegmentum resulting in increased burst firing and release of dopamine (DA) in the shell of the NAc.[415] Dopaminergic projections from the midbrain VTA to the shell signal the presence of a rewarding stimulus, facilitate the acquisition of behaviours—ranging from drug self-administration in animal studies to a repertoire of behaviours aimed at acquiring

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[413] Courtney, S.M., Ungerleider, L. G., Keil, K., Haxby, J. V. (1997). 'Transient and Sustained Activity in a Distributed Neural System for Human Working Memory', *Nature*, **386**(6625): pp. 608-11; Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002). 'Activation in Mesolimbic and Visuospatial Neural Circuits Elicited by Smoking Cues: Evidence from Functional Magnetic Resonance Imaging', *Am J Psychiatry*, **159**(6): pp. 954-60; Ishai, A., Ungerleider, L. G., Martin, A., Schouten, J. L., Haxby, J. V. (1999). 'Distributed Representation of Objects in the Human Ventral Visual Pathway', *Proc Natl Acad Sci U S A*, **96**(16): pp. 9379-84; McClernon, F.J., Hiott, F. B., Huettel, S. A., Rose, J. E. (2005). 'Abstinence-Induced Changes in Self-Report Craving Correlate with Event-Related fMRI Responses to Smoking Cues', *Neuropsychopharmacology* (manuscript in press).

[414] Balfour, D.J. (2002). 'Neuroplasticity Within the Mesoaccumbens Dopamine System and Its Role in Tobacco Dependence', *Curr Drug Targets CNS Neurol Disord*, **1**(4): pp. 413-21; Balfour, D.J., Benwell, M. E., Birrell, C. E., Kelly, R. J., Al-Aloul, M. (1998). 'Sensitization of the Mesoaccumbens Dopamine Response to Nicotine', *Pharmacol Biochem Behav.* **59**(4): pp. 1021-30; Janhunen, S., Ahtee, L. (2004). 'Comparison of the Effects of Nicotine and Epibatidine on the Striatal Extracellular Dopamine', *Eur J Pharmacol.* **494**(2-3): pp. 167-77; Stein, E.A., Pankiewicz, J., Harsch, H. H., Cho, J. K., Fuller, S. A., Hoffmann, R. G., Hawkins, M., Rao, S. M., Bandettini, P. A., Bloom, A. S. (1998). 'Nicotine-Induced Limbic Cortical Activation in the Human Brain: A Functional MRI Study', *Am J Psychiatry*, **155**(8): pp. 1009-15; Benwell, M.E., Balfour, D. J. (1992). 'The Effects of Acute and Repeated Nicotine Treatment on Nucleus Accumbens Dopamine and Locomotor Activity', *Br J Pharmacol.* **105**(4): pp. 849-56.

[415] Benwell, M.E., Balfour, D. J. (1992); Corrigall, W.A., Franklin, K. B., Coen, K. M., Clarke, P. B. (1992). 'The Mesolimbic Dopaminergic System is Implicated in the Reinforcing Effects of Nicotine', *Psychopharmacology* (Berl), **107**(2-3): pp. 285-9.

and consuming the drug in humans—related to obtaining the reward, and become desensitised with repeated drug exposure.[416]

Breiter and colleagues,[417] Koob and Le Moal,[418] Volkow and colleagues,[419] and others have posited that drug-seeking behaviour is a pattern of activity in the four integrated circuits (reward, memory, motivation, and control) that influences how an individual makes choices between behavioural alternatives. The response to a stimulus is influenced by its momentary salience (i.e., expected reward, which is processed in part by DA release in the NAc) in a hierarchical structure where the saliency value of the stimulus changes as a function of the previous experience and memory of the individual. Memories are stored in response to positive and negative experiences and the drug-related stimuli are weighed against the non-drug related stimuli.

The stronger the saliency value of the stimulus, the higher the motivational drive to obtain the stimulus becomes. The cognitive decision to act to obtain the reward is processed in the prefrontal cortex and ACC.[420] With increasing use of a substance, the degree of control over the motivational drive to obtain nicotine or other drugs is diminished in a process termed "hedonic dysregulation".[421] Over time the increasing reward value assigned to the drug leads to a resetting of reward thresholds with decreased sensitivity to naturally occurring stimuli. Thus, drug seeking becomes the primary motivational behaviour. In the view of Koob and Le Moal,[422] with diminishing negative feedback from control circuits, a

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[416] Benwell, M.E., Balfour, D. J., Birrell, C. E. (1995). 'Desensitization of the Nicotine-Induced Mesolimbic Dopamine Responses During Constant Infusion with Nicotine', *Br J Pharmacol.* **114**(2): pp. 454-60.
[417] Breiter, H.C., Rosen, B. R. (1999).
[418] Koob, G.F., Le Moal, M. (1997). 'Drug Abuse: Hedonic Homeostatic Dysregulation', *Science*, **278**(5335): pp. 52-8.
[419] Volkow, N.D., Fowler, J. S., Wang, G. J. (2003).
[420] Ibid.
[421] Koob, G.F., Le Moal, M. (1997).
[422] Koob, G.F., Le Moal, M. (2001).

positive feedback loop is established such that memory, reward, and drive perpetuate chronic drug use. Robinson & Berridge propose that positive and negative feedback are not at play per se but that increased sensitivity to the negative consequences of drug abstinence (i.e., withdrawal symptoms and drug "wanting") are responsible for the observed difficulty in abstaining from drugs of abuse such as nicotine.[423] Despite the complex nature of nicotine addiction and the neurological adaptations that accompany heightened salience to smoking-related environmental cues, the VS/NAc in particular is a brain region that is ubiquitously implicated in both the rewarding elements of nicotine administration and in the development of sensitisation to aversive effects of the drug such as craving and wanting.[424]

In nicotine addiction in humans, environmental smoking-related cues reliably generate craving and trigger withdrawal symptoms.[425] As described in Chapter 1 (Section 1.4), functional neuroimaging studies using PET[426] and fMRI[427] have demonstrated that smoking-related cues activate multiple cortical and subcortical limbic regions associated with DA-dependent incentive sensitisation processes. A recent study by Heinz and colleagues demonstrated a significantly negative correlation between DA D_2 receptor binding potential

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[423] Robinson, T.E., Berridge, K. C. (2001). 'Incentive-Sensitization and Addiction', *Addiction*, **96**(1): pp. 103-14.

[424] Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004). 'Correlation between Dopamine D(2) Receptors in the Ventral Striatum and Central Processing of Alcohol Cues and Craving', *Am J Psychiatry*, **161**(10): pp. 1783-9; Brody, A.L., Olmstead, R. E., London, E. D., Farahi, J., Meyer, J. H., Grossman, P., Lee, G. S., Huang, J., Hahn, E. L., Mandelkern, M. A. (2004). 'Smoking-Induced Ventral Striatum Dopamine Release', *Am J Psychiatry*, **161**(7): pp. 1211-8; Robinson, T.E., Berridge, K. C. (1993). 'The Neural Basis of Drug Craving: An Incentive-Sensitization Theory of Addiction', *Brain Res Brain Res Rev.* **18**(3): pp. 247-91; Balfour, D.J. (2002).

[425] Niaura, R.S., Rohsenow, D. J., Binkoff, J. A., Monti, P. M., Pedraza, M., Abrams, D. B. (1988).

[426] Brody, A.L., Mandelkern, M. A., London, E. D., Childress, A. R., Lee, G. S., Bota, R. G., Ho, M. L., Saxena, S., Baxter, L. R., Jr., Madsen, D., Jarvik, M. E. (2002). 'Brain Metabolic Changes During Cigarette Craving', *Arch Gen Psychiatry*, **59**(12): pp. 1162-72.

[427] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002).

(BP) and alcohol craving in the VS in abstinent alcoholics.[428] These findings are consistent with the incentive-sensitisation model posed by Robinson and Berridge[429] suggesting that low availability of D_2 receptors in the VS mediates excessive attribution of incentive salience to drug-related stimuli, leading to a pathological "wanting" to consume drugs such as alcohol and nicotine.

Therefore, given this background, I sought to explore whether or not there would be greater activation associated with smoking-related pictorial cues than non-smoking-related neutral cues in the VS/NAc of smokers and non-smokers using fMRI. Furthermore, given the putative involvement of the ACC and OFC in cue-elicited tobacco craving, I chose to include these two regions of interest to my analyses. The results of this study would potentially reinforce or refute the hypothesis that the VS/NAc is associated with incentive sensitisation to nicotine in humans, which has been so well described in animal studies.[430]

2.3 Materials and Methods

2.3.1 Protocol Development

The process of protocol development began by meeting with a multidisciplinary group of advisors including Professor Paul Matthews from the Department of Clinical Neurology, Dr. Robert Walton and Dr. Marcus Munafo' from the Department of Clinical Pharmacology, and Dr. Robert Rogers from the Department of Psychiatry. The aim of the group was to develop a pictorial cue paradigm that would elicit Blood-Oxygen-Level-Dependent (BOLD)

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[428] Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004).

[429] Robinson, T.E., Berridge, K. C. (2001); Robinson, T.E., Berridge, K. C. (1993); Berridge, K.C., Robinson, T. E. (1998). 'What is the Role of Dopamine in Reward: Hedonic Impact, Reward Learning, or Incentive Salience?' *Brain Res Brain Res Rev.* **28**(3): pp. 309-69.

[430] Robinson, T.E., Berridge, K.C. (1993); Robinson, T.E., Berridge, K.C. (2001).

activation in the NAc and in associated reward signalling regions (i.e., ACC, OFC) as described in detail in Chapter 1.

2.3.2 Choice of Design

Given the scientific background discussed in section 2.2, the following hypotheses were formed:

1. I hypothesised that in abstaining addicted smokers there would be greater activation in response to smoking-related pictorial cues than non-smoking cues in the ventral striatum including the nucleus accumbens VS/NAc and in a distributed reward network including the ACC and OFC.
2. Furthermore, I hypothesised that activation associated with smoking-related cues would not be observed in the VS/NAc in non-smokers.
3. Finally, I hypothesised that there would be greater activation associated with smoking-related cues in the VS/NAc, ACC, and OFC smokers compared to non-smokers.

In order to test these hypotheses we considered many factors in designing the experimental protocol. The first decision regarded whether to use a block or event-related stimulus design. Each type of design has distinct advantages and disadvantages. The advantages of an event-related design include the ability to detect transient BOLD signal affects from individual events (stimuli), and flexibility in experimental design (e.g. randomisation, odd-ball paradigms).[431] An advantage of block designs is that they have greater sensitivity and statistical power to detect haemodynamic responses to stimuli than event-related designs.[432] However potential disadvantages of block designs are that cognitive sets are studied and not events, subjects may adapt to the stimulus presentations such that

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[431] Donaldson, D.I., Buckner, R.L. (2001). 'Effective Paradigm Design', in *Functional MRI – An Introduction to Methods*, ed. by Jezzard, P., Matthews, P.M., Smith, S.M. (Oxford, UK: Oxford University Press).
[432] Ibid.

they are able to predict the next type of stimulus (expectation), and boredom resulting from repetitive presentations of stimuli.

Given the noted advantages of the event-related design (i.e., greater flexibility in study design and ability to examine individual events), disadvantages of block designs (i.e., less flexibility, inability to examine individual events, expectation, boredom) and demonstration of adequate sensitivity with a smoking-related pictorial event-related design by Due and colleagues, [433] I proceeded with an event-related pictorial experiment. The design incorporated smoking-related and neutral pictures presented in pseudo-random order from a standardised set of smoking-related and non-smoking related pictures, [434] which has demonstrated validity in evoking BOLD signal change in reward centres using fMRI.[435] Pictures included smoking-related scenes (individuals smoking cigarettes) and non-smoking scenes (e.g., individuals holding pencils or eye glasses). The final decision on design specifications was made under advisement from PM, RR, and MM.

The aim of the paradigm was to evaluate the effect of smoking-related visual stimuli compared to non-smoking related "neutral" visual stimuli during a state of overnight abstinence from tobacco. We chose to include a greater frequency of neutral pictures than smoking pictures, as will be described below, in order to provide time for the cognitive and haemodynamic response to return to baseline during neutral and rest periods. This is a standard approach to presenting target images, which has been frequently employed in oddball designs.[436] Oddball designs utilise target stimuli that occur infrequently relative to a

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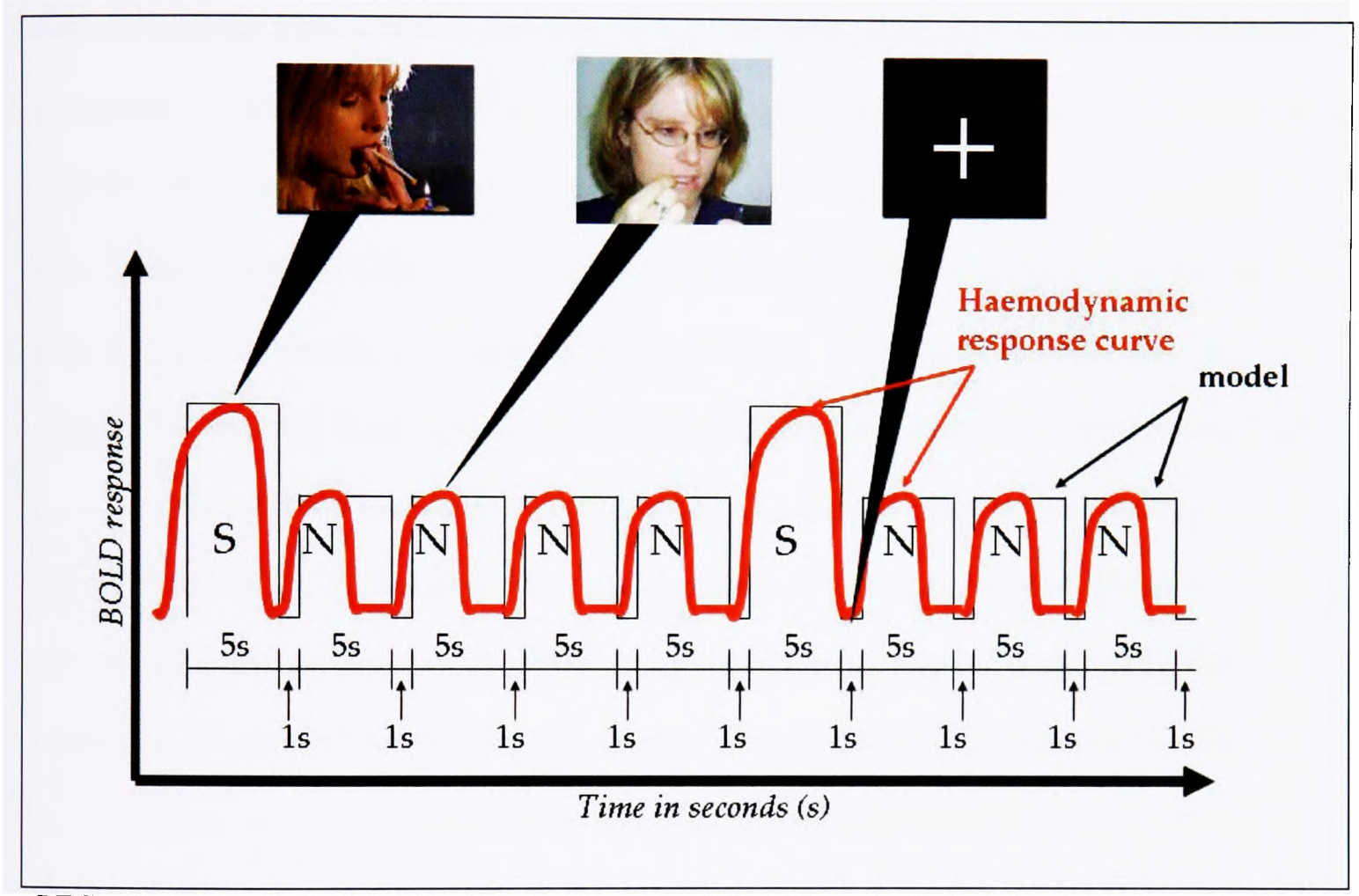
[433] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002).
[434] Gilbert, D.G., Rabinovich, N.E. (1999). *International Smoking Images Series (With Neutral Counterparts).* (Southern Illinois University: Integrative Neuroscience Laboratory, Department of Psychology) [on CD-ROM].
[435] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002).
[436] Garreffa, G., Bianciardi, M., Hagberg, G. E., Macaluso, E., Marciani, M. G., Maraviglia, B., Abbafati, M., Carni, M., Bruni, I., Bianchi, L. (2004). 'Simultaneous EEG-fMRI Acquisition: How Far is it from Being a Standardized Technique?' *Magn Reson Imaging* **22**(10): 1445-55.

series of standard stimuli; a method that reliably stimulates transient neural activity in prefrontal cortical regions.[437]

Figure 2.1

Model of Predicted BOLD Response Associated with Smoking-Related Pictorial Cues



LEGEND: Illustration of predicted blood-oxygen-level-dependent signal during a smoking cue reactivity paradigm. 'S' = smoking-related picture; 'N' = non-smoking/neutral picture; the fixation cross is present during the 1 second interstimulus intervals.

Furthermore, we chose to randomise the sequence of picture cue type (i.e., smoking-related or neutral) to avoid potential bias arising from repetitive patterns of cue sequences. The sequence of images was determined by randomising three sequences of picture blocks consisting of eight neutral (n) and two smoking (s) pictures (nnnnsnnnns) ("A block"), five smoking and five neutral pictures (nsnsnsnsns) ("B block"), and nine neutral pictures and one smoking picture (nnnnnnnnns) ("C block"). Next, the three block types were

[437] Huettal, S.A., McCarthy, G. (2004). 'What is odd in the oddball task? Prefrontal cortex is activated by dynamic changes in response strategy.' *Neuropsychologia* **42**(3):379-386.

randomised. The resulting stimulus sequence was composed of 28% smoking-related and 72% neutral pictures. The model described is illustrated in Figure 2.1.

2.3.3 Pre-Pilot Testing

We first considered including a stimulus paradigm with a stimulus duration of 2 seconds and a 1 second inter-stimulus interval, which has been successful in previous research examining reward processing.[438] However, pre-pilot testing of stimulus duration of two seconds did not produce reliable activation among three smokers. We hypothesised that the short presentation duration did not allow sufficient time between stimuli to allow for cognitive and motivational processing of the pictures. Therefore a decision was made to increase the stimulus duration to five seconds, which is similar in duration to protocols used by other researchers who successfully examined cue-elicited response to smoking-related stimuli using fMRI.[439] (Subjects who participated in the experiment with the two-second stimuli were not included in the final study population analysed in this chapter). We controlled for gender by matching the number of male and female pictures for each cue type.[440] Next, we decided to limit the stimulus presentation to ten minutes because we hypothesised that presentations of longer duration would be unpleasant to subjects undergoing nicotine withdrawal and might result in excessive head motion or decreased attention to pictorial cues as seen in other studies of longer duration in abstinent smokers[441]

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[438] O'Doherty, J., Kringlebach, M.L., Rolls, E.T., Hornak, J., Andrews, C. (2001). 'Abstract Reward and Punishment Representations in the Human Orbitofrontal Cortex', *Nat Neurosci.* **4**(1): 95-102.

[439] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002); McClernon, F.J., Hiott, F.B., Huettel, S.A., Rose, J.E. (2005).

[440] In the first 4 smokers and 1 non-smoker, we controlled for gender by randomly selecting pictures from a sex-balanced picture set. In all other subjects, equal numbers of male and female pictures were presented for each type as a protocol refinement.

[441] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002).

and alcoholics.[442] Thus, the final protocol as described below utilised an event-related, pseudo-randomised design of 100 trials of ten minutes duration composed of 72% neutral and 28% smoking-related cues and a fixed inter-stimulus rest interval of one second.

2.3.4 Procedure

2.3.4.1 Informed Consent and Recruitment

The Oxfordshire Research Ethics Committee approved the study in December 2002. Subjects were recruited through convenience sampling from the greater Oxfordshire area and through adverts in the *Daily Information* local newspaper. The subjects who responded to the adverts were contacted by telephone, where I ascertained interest in the study, screened for contraindications, and made an appointment for the experiment at the Oxford Centre for Functional Magnetic Resonance Imaging of the Brain (FMRIB). On the day of the experiment, written and informed consent was obtained following an explanation and confirmation of no MRI contraindications. Inclusion criteria were right-handed male and female current smokers or non-smokers (smoked less than 100 cigarettes in lifetime), ≥18 years of age. Exclusion criteria included any unstable medical condition, any contraindications to MRI scanning as determined through screening with a standard safety questionnaire, pregnancy, current Axis I psychiatric disorder excluding nicotine dependence, use of psychotropic medications, or current use of nicotine replacement therapy or smokeless tobacco.

Subjects were asked to abstain from smoking overnight, which I verified by measuring exhaled breath carbon monoxide (CO) with a piCO Smokerlyzer® (Bedfont Scientific Ltd, Sittingborne, UK). Overnight abstinence was confirmed by demonstration of exhaled CO of

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[442] Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004).

less than 11 parts per million (p.p.m). I gave instructions on the primary task of identifying a male or female picture with a forced-choice key press on a response box. Subjects were given the opportunity to practice in the preparation room prior to the scan. Each subject was asked to practice responses to male or female pictures until they rapidly achieved 100% accuracy. I reviewed the safety screening criteria with each subject prior to each scan and ensured that all metallic objects were removed before entering the magnet chamber.

Twenty-six right-handed healthy volunteers (smokers n = 14, non-smokers n = 12) underwent the fMRI experiment. One smoker, who suffered a stroke after completing the study, was excluded from the analysis because of concerns about her health at the time of the scanning. One smoker and one non-smoker were excluded because of computer malfunction with paradigm presentation. Three smokers were excluded because of exhaled CO ≥11 p.p.m. indicating cigarette smoking in the previous 12 hours. The final sample for analysis therefore consisted of 9 smokers and 11 non-smokers.

2.3.4.2 Stimulus Paradigm

Subjects were supine within the scanner as per routine. Foam padding was applied to minimise head motion and subjects were given earplugs for protection against the high-decibel noise generated by the scanner. A headset with earphones and a microphone was placed on each subject to permit communication with the researcher (SD) and radiographer, who observed study participants through a window in the console room. An event-related fMRI design was employed as follows. Stimuli were standardised colour photographs, subtending approximately 20° by 16° of visual angle. These images were projected on a screen placed near the subjects' feet, viewed with prism glasses. The pictures depicted scenes with people smoking cigarettes (smoking-related) or engaged in a non-smoking

activity (neutral). Smoking-related and neutral images were drawn from the International Smoking Image Series.[443] The pictures were presented for five seconds at a frequency of one image each six seconds with a one-second inter stimulus interval using Presentation™ software (Neurobehavioral Systems, Inc., San Pablo, CA, USA). Fixation crosses were presented during rest periods. Reaction times for the key press response to the gender discrimination task were recorded on a computer-generated log file.

2.3.4.3 Behavioural and Sociodemographic Measures

Subjects completed a questionnaire including sociodemographic questions, smoking history and the Fagerström Test of Nicotine Dependence (FTND)[444] – a highly reliable measure of the severity of nicotine dependence – prior to entry into the scanner. Before and after each echo planar imaging (EPI) run and whilst in the scanner, subjects were asked by the investigator (located in the console room) to provide verbal ratings of items querying symptoms of nicotine withdrawal and tobacco craving utilising well-validated self-report assessment scales[445] for nicotine craving [Shiffman-Jarvik Craving Scale (5 items rated 0-100)][446] and withdrawal [Minnesota Withdrawal Scale (8 items rated 0-4)].[447]

2.3.4.4 Imaging Procedure

All scans were performed at FMRIB (The John Radcliffe Hospital, Headington, Oxford, UK) with a 3 Tesla Varian Inova MRI system. Following a sagittal localisation scan [2D

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[443] Gilbert, D.G., Rabinovich, N.E. (1999).

[444] Heatherton, T.F., Kozlowski, L. T., Frecker, R. C., Fagerstrom, K. O. (1991). 'The Fagerstrom Test for Nicotine Dependence: A Revision of the Fagerstrom Tolerance Questionnaire', *Br J Addict*, **86**(9): pp. 1119-27.

[445] Shiffman, S., West, R., Gilbert, D. (2004). 'Recommendation for the Assessment of Tobacco Craving and Withdrawal in Smoking Cessation Trials', *Nicotine Tob Res*. **6**(4): pp. 599-614.

[446] Shiffman, S.M., Jarvik, M. E. (1976). 'Smoking Withdrawal Symptoms in Two Weeks of Abstinence', *Psychopharmacology* (Berl), **50**(1): pp. 35-9.

[447] Hughes, J.R., Hatsukami, D. (1986). 'Signs and Symptoms of Tobacco Withdrawal', *Arch Gen Psychiatry*, **43**(3): pp. 289-94.

Turbo Flash (turbo fast low angle shot), TR (time for repetition) = 30 ms, TE (time to echo) = 5 ms, TI (inversion time) = 800 ms, flip angle = 50°)], whole brain functional MRI data were acquired continuously through the period of visual stimulus presentation using a multi-slice gradient EPI sequence [(TR = 3000 ms, TE = 30 ms, flip angle = 90°, field of view (FOV) = 256 x 192, matrix = 64 x 64, 25 x 5mm axial slices)]. Following functional imaging, a T_1-weighted structural image was also acquired [IR (inversion recovery) 3D Turbo Flash TR = 15 ms, TE = 5 ms, flip angle = 12°, FOV = 256 x 256, matrix = 256 x 256, 64 x 3 or 128 x 1.5 mm axial slices)].

2.3.4.5 Individual Level fMRI Analysis

Data from each patient were initially analysed separately. Data pre-processing was conducted using FEAT (FMRI Expert Analysis Tool) Version 5.42 from the FMRIB Software Library (www.fmrib.ox.ac.uk/fsl). Registration to high resolution structural images of each individual subject was carried out using FMRIB's Linear Registration Tool (FLIRT)[448] with 7 degrees of freedom (DOF) and all high-resolution structural images were co-registered to standard Montreal Neurological Institute (MNI) space with 12 DOF. Motion correction was conducted using FMRIB's Linear Registration Tool (MCFLIRT),[449] which works by making serial registrations of each MRI image in a time-series to the half-way time point image. Non-brain exclusion was carried out using FMRIB's Brain Extraction Tool (BET).[450] 3D convolution was conducted with a Gaussian kernel of 5 mm full-width half maximum to improve the reliability of the data. Mean-based intensity normalisation was performed in order to minimise drift of the mean signal intensity. Non-linear high-pass

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[448] Jenkinson, M., Bannister, P., Brady, M., Smith, S. (2002). 'Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images', *Neuroimage*, **17**(2): pp. 825-41.
[449] Jenkinson, M., Smith, S. (2001). 'A Global Optimisation Method for Robust Affine Registration of Brain Images', *Med Image Anal*, **5**(2): pp. 143-56.
[450] Smith, S.M. (2002). 'Fast Robust Automated Brain Extraction', *Hum Brain Mapp*. **17**(3): pp. 143-55.

temporal filtering was conducted to remove low frequency noise such as that caused by respiration with Gaussian-weighted least squares straight line fitting (sigma = 25.0 s). Time series statistical analysis was carried out using FMRIB's Improved Linear Model (FILM) with local autocorrelation correction.[451] Statistical analysis was performed by modelling smoking vs. neutral conditions (boxcar functions convolved with the haemodynamic response function) as explanatory variables (EV) within the context of the general linear model on a voxel-by-voxel basis. *Z* (Gaussianised T/F) statistic images were thresholded at $Z > 2.3$ and significant clusters defined according to extent at $p = 0.05$ (corrected for multiple comparisons and number of clusters) according to random field theory.[452]

2.3.4.6 Higher Level Analysis

All higher-level analyses were carried out using FMRIB's Local Analysis of Mixed Effects (FLAME).[453] Individual summary statistics from each first-level GLM analysis were analysed using FLAME to evaluate the mean group effect of the stimulus and determine whether each group (i.e., smokers and non-smokers) activated as a whole. *Z* (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z > 2.3$ and a corrected cluster significance threshold of $p = 0.05$.[454] Next, the smoking group was compared to the non-smoking group to determine whether there was a significant difference between groups in the mean effect of the stimulus with the GLM using FLAME.

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[451] Woolrich, M.W., Ripley, B. D., Brady, M., Smith, S. M. (2001). 'Temporal Autocorrelation in Univariate Linear Modeling of FMRI Data', *Neuroimage*, **14**(6): pp. 1370-86.

[452] Worsley, K.J., Evans, A. C., Marrett, S., Neelin, P. (1992). 'A Three-Dimensional Statistical Analysis for CBF Activation Studies in Human Brain', *J Cereb Blood Flow Metab.* **12**(6): pp. 900-18; Friston, K.J., Worsley, K.J., Frackowiak, R.S., Mazziotta, J.C., Evans, A.C. (1994). 'Assessing the Significance of Focal Activations Using their Spatial Extent', *Hum Brain Mapp.* **1**: pp. 214–20; Forman, S.D., Cohen, J.D., Fitzgerald, M., Eddy, W.F., Mintun, M.A., Noll, D.C. (1995). 'Improved Assessment of Significant Activation in Functional Magnetic Resonance Imaging (fMRI): Use of a Cluster-Size Threshold', *Magn Reson Med.*, **33**(5): pp. 636–47.

[453] Beckmann, C.F., Jenkinson, M., Smith, S. M. (2003). 'General Multilevel Linear Modeling for Group Analysis in FMRI', *Neuroimage*, **20**(2): pp. 1052-63; Woolrich, M.W., Behrens, T. E., Beckmann, C. F., Jenkinson, M., Smith, S. M. (2004). 'Multilevel Linear Modelling for FMRI Group Analysis Using Bayesian Inference', *Neuroimage*, **21**(4): pp. 1732-47.

[454] Worsley, K.J., Evans, A. C., Marrett, S., Neelin, P. (1992).

2.3.4.7 Region of Interest Analysis

Region of interest (ROI) anatomical masks were created on the group mean anatomical image in standard space for the VS/NAc using FSL View software (www.fmrib.ox.ac.uk). The brain atlas by Duvernoy[455] was used as a guide for defining anatomical landmarks, which ranged from (MNI coordinates X: ±4 to 10; Y: +6 to +18, 0 to -10) consistent with the landmarks of the "limbic-related" striatum as defined by Fudge and Haber [456] and Mawlawi[457] and colleagues, including the NAc proper, the medial tail of the ventral caudate, and the medio-ventral putamen (sharing amygdalo-striatal afferents). Therefore, a binary mask was anatomically defined in standard MNI space such that subsequent ROI-based analyses calculated the mean COPEs across all of the voxels contained within the defined margins of the mask. The COPE is a linear combination of the two parameter estimates (β_1 = 'smoking cue'; β_2 = 'neutral cue') using the GLM described in section 2.3.4.5 (COPE = 1x β_1 + -1x β_2).

The rationale for using a group-averaged mask was that by transforming all images to standard MNI space, we could minimize ascertainment bias with regard to determination of VS/NAc anatomical boundaries according to each individual's statistical maps of functional data. In other words, by incorporating a standardized anatomical template, we were able to "blind" the task of defining the precise voxels for ROI analysis, and thereby eliminate the circularity of defining the anatomical boundaries based upon the statistical results. A disadvantage of using a group mask is that subtle anatomical differences in the size, shape,

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[455]Duvernoy, H. (1999). *The Human Brain*, 2nd ed. (London: Springer-Verlab).

[456] Fudge, J.L., Haber, S. N. (2002). 'Defining the Caudal Ventral Striatum in Primates: Cellular and Histochemical Features', *J Neurosci.* **22**(23): pp. 10078-82.

[457] Mawlawi, O., Martinez, D., Slifstein, M., Broft, A., Chatterjee, R., Hwang, D. R., Huang, Y., Simpson, N., Ngo, K., Van Heertum, R., Laruelle, M. (2001). 'Imaging Human Mesolimbic Dopamine Transmission with Positron Emission Tomography: I. Accuracy and Precision of D(2) Receptor Parameter Measurements in Ventral Striatum', *J Cereb Blood Flow Metab.* **21**(9): pp. 1034-57.

and volume of the VS/NAc would not be taken into account and thus the group-averaged mask would have the potential to over- or under-estimate the full extent of the VS/NAc with an approximate error margin of 1-2 mm. We did, in fact, determine that, in each subject, the VS/NAc mask incorporated the VS/NAc in each high resolution structural scan. The very same rationale was applied in the ROI analysis described in Chapter 4.

ROI analyses were strictly anatomically-based and were not confined to activation clusters above a particular *z*-threshold. Mean COPEs were calculated within the VS/NAc mask from each individual's first-level GLM analysis using Featquery. In addition to the main focus of examination (VS/NAc), anatomically-based binary masks were created for the OFC and subgenual ACC also based on landmarks anatomically-defined by other investigators.[458]

2.4 Results

Smokers did not differ from non-smokers in mean age [smokers: mean = 34.4, standard deviation (SD) = 11.9 years; non-smokers: mean = 28.3, SD = 7.2 years respectively, t [1, 18] = 1.43, p = 0.2) or sex distribution (56% female vs. 73% female respectively, χ^2 [1] = 1.8, p = 0.2). The ethnic composition of the smokers was 78% of European ancestry compared with 100% of non-smokers, and 78% of smokers had a college education compared with 100% of non-smokers (for both comparisons: χ^2 [1] = 2.78, p = 0.1).

2.4.1 Imaging Results

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[458] Pezawas, L., Meyer-Lindenberg, A., Drabant, E. M., Verchinski, B. A., Munoz, K. E., Kolachana, B. S., Egan, M. F., Mattay, V. S., Hariri, A. R., Weinberger, D. R. (2005). '5-HTTLPR Polymorphism Impacts Human Cingulate-Amygdala Interactions: A Genetic Susceptibility Mechanism for Depression', *Nat Neurosci.* **8**(6): pp. 828-34; Rogers, R.D., Ramnani, N., Mackay, C., Wilson, J.L., Jezzard, P., Carter, C.S., Smith, S.M. (2004). 'Distinct Portions of Anterior Cingulate Cortex and Medial Prefrontal Cortex are Activated by Reward Processing in Separable Phases of Decision-Making Cognition', *Biol Psychiatry* 55(6): 594-602.

Analysis of EPI data was conducted for the first 150 volumes (reduced from the original protocol of 200 volumes) as we observed a trend of excessive head motion in both groups of subjects after the first 450 seconds (150 volumes) in the first-level analysis and were concerned that the excessive head motion would artefactually elevate estimates of BOLD response. Whole brain group analysis of activation during smoking-related cues vs. neutral cues using mixed effects and corrected cluster statistics ($z > 2.3$, cluster $p < 0.05$) demonstrated bilateral activation in smokers in three large clusters with centres of gravity in the anterior cingulate cortex (ACC)/orbitofrontal cortex (OFC), superior frontal gyrus (SFG), and occipital cortex (Table 2.1 and Figure 3.1). Within the ACC/OFC cluster were local maxima in the medial orbitofrontal cortex (8, 56, -12; *z*-statistic 3.60), and ACC (-6, 50, 0; *z*-statistic 3.73). The SFG cluster demonstrated several local maxima on the left with peak at (-14, 56, 28; *z*-statistic 3.28). The occipital cluster included local maxima in the posterior fusiform gyrus (32, -86, -20; *z*-statistic 3.23), and lingual gyrus (-6, -82, -12; *z*-statistic 3.06). Significant group activation was not observed in non-smokers.

Table 2.1

Corrected Mixed Effects Cluster Analysis in Smokers

Cluster	COG *x* (mm)	COG *y* (mm)	COG *z* (mm)	Max *Z*-statistic	Mean COPE	*P*
Anterior Cingulate Cortex/Orbitofrontal Cortex	1	44	-5	3.74	44.3	<0.001
Superior Frontal Gyrus	-14	56	28	3.28	45.8	0.008
Occipital Cortex	14	-78	-17	3.23	42.9	<0.001

LEGEND: Group mixed-effects analysis of smoking vs. neutral contrast demonstrated significant activation in three main clusters consisting of multiple local maxima. COG *x*, *y*, *z*, are the MNI coordinates of the centres of gravity (COG) for each cluster. *P* represents the *p* value corresponding to the maximum *z*-statistic within each cluster.

Given our hypotheses, we were particularly interested in activation in VS/NAc and so carried out a secondary analysis restricted to this area in all subjects using an uncorrected *z*-threshold of 2.3. This revealed group activation for smoking vs. neutral cues in smokers (peak voxel coordinates VS: right 8, 14, -2, *z*-statistic = 2.8; left -4, 14, -2, *z*-statistic = 3.2) (Figure 2.2) and no activation in non-smokers.

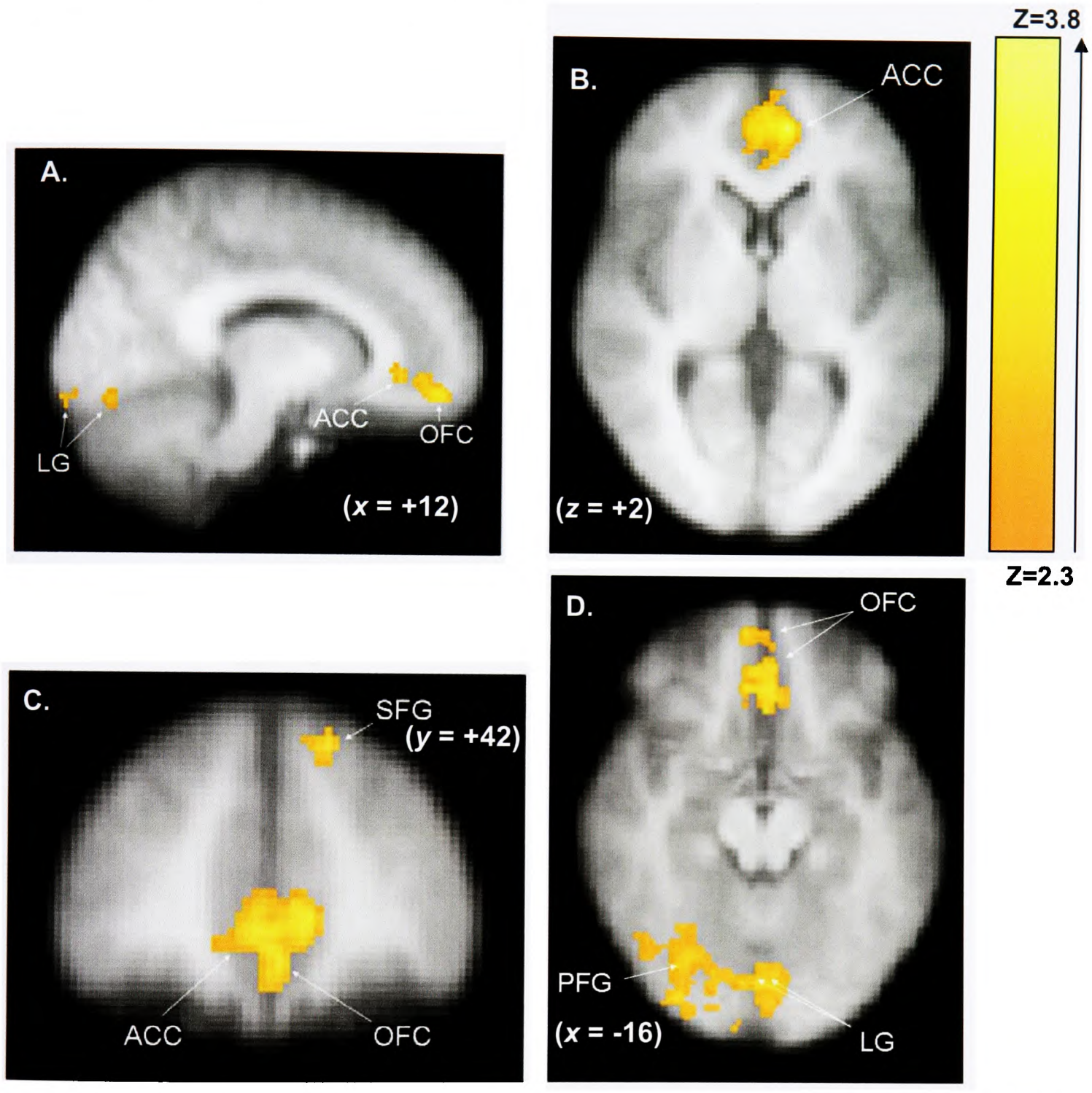
In sum, we observed activation in a distributed reward network, including ACC, prefrontal cortex (orbitofrontal cortex and superior frontal gyrus), visual-spatial attention regions (posterior fusiform and lingual gyri) and no activation with whole-brain analysis in non-smokers.

Next, I sought to determine whether the mean COPE for the smoking vs. neutral contrast would be greater in smokers than non-smokers. Whole-brain voxel-wise mixed-effects comparison of smokers and non-smokers did not reveal significant differences in activation at a *z*-threshold of 2.3 and corrected cluster significance of $p < 0.05$. Potential explanations for why we did not detect significant differences between groups with voxel-wise analysis are covered in the Discussion section.

However, our main hypothesis concerned activity in the VS/NAc. Therefore, the effect size for the contrast between the parameter estimates associated with the haemodynamic response to smoking and neutral cues (COPE) was calculated within the group VS/NAc ROI mask (as described in Section 2.3.4.7) for smokers and non-smokers.

Figure 2.2

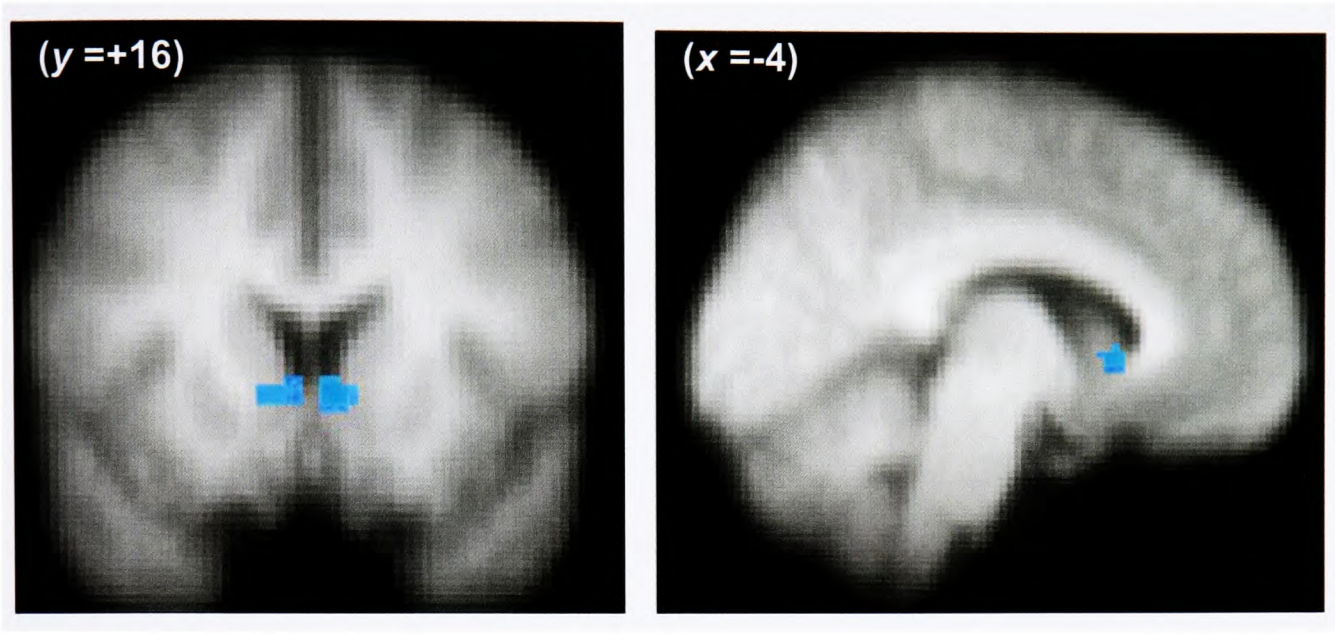
Mixed-Effects Group Analysis in Smokers



LEGEND: Statistical map of activation associated with smoking-related pictorial cues in addicted smokers (n=9). Sagittal (A), axial (B, D), and coronal (C) views of three activation clusters within the mixed-effects group analysis of smokers only. Colour bar (upper right) indicates z-statistics ranging from 2.3 (threshold) to 3.8 and correspondingly from dark to light yellow, respectively. Structural image is a group-averaged T1 structural scan from all subjects in the study (smokers and non-smokers) registered to standard space. ACC = anterior cingulate cortex, LG = lingual gyrus, OFC = orbitofrontal cortex, PFG = posterior fusiform gyrus.

Figure 2.3

Region of Interest Analysis of Ventral Striatum in Smokers



LEGEND: Statistical map for voxel-wise analysis of the ventral striatum including the nucleus accumbens (VS/NAc) in smokers (n = 9) in coronal (left) and sagittal view (right). Colour bar (lower right) indicates *z*-statistics ranging from 2.3 (threshold) to 3.2 (maximum) and correspondingly from darker to light blue, respectively. Analysis was restricted to voxels contained in a bilateral anatomical mask of the VS (below) at an uncorrected *Z*-statistic threshold of 2.3. No activation was seen within this region in non-smokers. VS/NAc mask MNI coordinates (*X*: ±4 to 10; *Y*: +6 to +18, *Z*: 0 to -10).[459]

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[459] Fudge, J.L., Haber, S. N. (2002); Mawlawi, O., Martinez, D., Slifstein, M., Broft, A., Chatterjee, R., Hwang, D. R., Huang, Y., Simpson, N., Ngo, K., Van Heertum, R., Laruelle, M. (2001).

A repeated-measures analysis of variance (ANOVA) was performed with smoking status as a between subjects variable and hemisphere as a within-subjects variable. There was no main effect of hemisphere on mean VS/NAc COPE (F = 0.15, df = 1, p = 0.707). Furthermore, there was no smoking by hemisphere interaction in VS/NAc COPE (F = 0.77; df = 1,18; p = 0.392).

As there were no significant differences in mean COPE by hemisphere in smokers or non-smokers, I restricted between-group comparisons of BOLD contrast to the anatomical region within a bilateral (bi-hemispheric) VS/NAc mask. First, in order to test the null hypothesis that there was no difference in activation between smoking and neutral cues in each group we conducted one-sample t-tests comparing the mean COPE in VS/NAc to zero. Mean VS/NAc COPE was significantly greater than zero in smokers (one-sample t-test, t = 3.41, df = 8, p = 0.009), but not in non-smokers (one-sample t-test, t = -1.08, df = 10, p = 0.308). Next, we directly compared the mean COPE for the VS/NAc, as defined by the anatomical mask described above, between smokers and non-smokers. Bilateral VS/NAc activation was greater in smokers than non-smokers [mean COPE = 33.6 (SD = 29.6) vs. -7.9 (SD = 24.3), student t-test, t = 3.45, df = 18, p = 0.003] (Figure 2.3 and Table 2.2).

As part of the planned hypothesis testing, I was also particularly interested in the ACC and orbitofrontal cortex as two brain regions, which, like the VS/NAc are part of a distributed reward signalling network important in processing emotionally salient perceptual stimuli and are each components of the extended Am.[460] Mean COPE values for the

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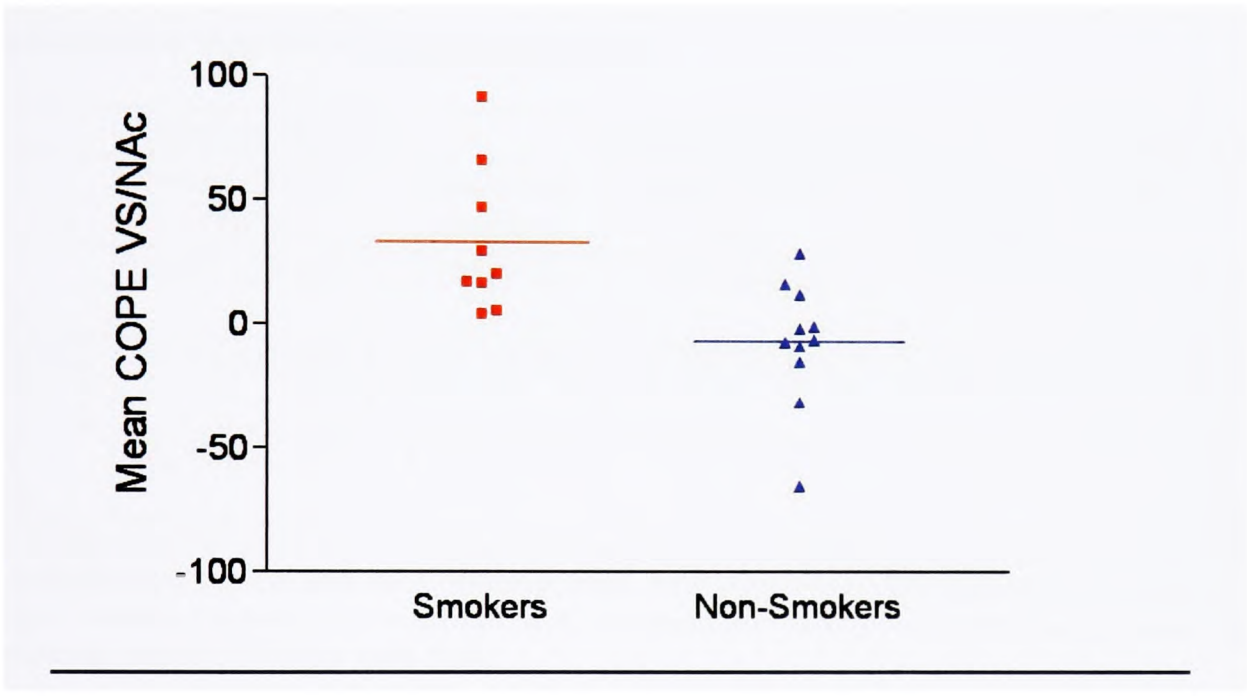
[460] Hariri, A.R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M. F., Weinberger, D. R. (2002). 'Serotonin Transporter Genetic Variation and the Response of the Human Amygdala', *Science*, **297**(5580): pp. 400-3.

smoking-neutral contrast for the anatomical regions within each mask are presented in Table 3.2.

Figure 2.4

Comparison of BOLD Contrast between Smokers and Non-Smokers in Ventral Striatum



LEGEND: Scatter plot comparing smokers and non-smokers in COPE for smoking vs. neutral picture cues. ROI analysis performed was within a bilateral mask of VS/NAc (Figure 2.2). The mean COPE values for each group are represented by the horizontal bars. Mean ROI-wide COPE values for individual smokers are represented in red and for non-smokers in blue.

I performed repeated-measures ANOVA with smoking status as a between-subjects variable and hemisphere as a within-subjects variable for both the ACC and OFC. There were no main effects of hemisphere on mean COPE in the ACC ($F = 0.01$; $df = 1,18$; $p = 0.935$) or the OFC ($F = 1.99$; $df = 1,18$; $p = 0.176$). Furthermore, there were no interactions between smoking status and hemisphere for ACC ($F = 0.02$; $df = 1,18$; $p = 0.887$) or OFC ($F = 0.18$; $df = 1,18$; $p = 0.68$).

Similar to the results presented above comparing VS/NAc COPE between groups, the effect sizes for the smoking vs. neutral contrast was significantly greater in magnitude in the smokers than non-smokers in the orbitofrontal cortex and there was a statistical trend suggesting greater bilateral ACC activation in smokers than non-smokers. Effect sizes for the ROI analyses of VS/NAc, ACC, and OFC are presented in Table 2.2.

Table 2.2

Mesocorticolimbic Region of Interest Analyses

Region	Smokers (n = 9)		Non-Smokers (N = 11)		ANOVA (df = 1)
	Mean COPE (SD)	Maximum Z-statistic	Mean COPE (SD)	Maximum Z-statistic	
Ventral Striatum	33.61 (29.61)	2.09 (0.5)	-7.86 (24.29)	1.43 (0.78)	F = 12.4 p = 0.002
Orbitofrontal Cortex	24.47 (21.88)	2.99 (0.69)	-3.98 (10.81)	2.39 (0.60)	F = 14.4 p = 0.001
Anterior Cingulate	38.98 (32.63)	2.76 (1.23)	3.33 (41.47)	2.19 (1.11)	F = 4.4 p = 0.050

LEGEND: Effect sizes for smoking vs. neutral cue contrast (mean COPE) and standard deviation (SD) for VS/NAc, orbitofrontal cortex, and ACC derived from ROI analyses within anatomically defined group masks. Right column represents *F* statistics and *P* values for ANOVA demonstrating main effect of smoking status on mean COPE for each ROI.

2.4.2 Behavioural Data

Amongst smokers, the mean score of the Fagerström Test of Nicotine Dependence was 4.7 (SD = 1.7) – which is similar to reported population means of regular smokers[461] – the mean number of cigarettes per day was 18.3 (SD = 8.7) and the mean exhaled CO indicated overnight abstinence (mean = 2.9 p.p.m., SD = 2.9).[462] Mean craving scores in the smokers

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[461] Heatherton, T.F., Kozlowski, L. T., Frecker, R. C., Fagerstrom, K. O. (1991); Prokhorov, A.V., et al. (1996). 'Measuring Nicotine Dependence among High-Risk Adolescent Smokers', *Addict Behav.* **21**(1): pp. 117-27; Benowitz, N.L. (1999). 'Nicotine addiction', *Prim Care*, **26**(3): pp. 611-31.

[462] Sato, S., Nishimura, K., Koyama, H.Tsukino, M., Oga, T., Hajiro, T., Mishima, M. (2003). 'Optimal Cutoff Level of Breath Carbon Monoxide for Assessing Smoking Status in Patients with Asthma and COPD', *Chest*, **124**(5): pp. 1749-54; Javors, M.A., Hatch, J. P., Lamb, R. J. (2005). 'Cut-off Levels for Breath Carbon Monoxide as a Marker for Cigarette Smoking', *Addiction*, **100**(2): pp. 159-67.

were [145.2 (SD = 170.3) pre-scan; 250.2 (SD = 205.4) post-scan, paired-sample t-test, t = 0.98, df = 8, p = 0.357]. Mean withdrawal scores in the smokers were [6.8 (SD = 5.4) pre-scan; 9.1 (SD = 6.5) post-scan, paired-sample t-test, t = 1.15, df = 8, p = 0.285].

There were no significant correlations between bilateral VS/NAc mean COPE and cigarette craving or withdrawal pre-scan, post-scan, or with the difference in craving during the experiment (post minus pre scan) when using non-parametric Spearman's correlational analyses. However, there was a significant positive correlation between global ACC mean COPE and the change in craving score during the experiment (Spearman's rho = 0.668, p = 0.049) and there were significant negative correlations between the global mean OFC COPE and craving scores pre-scan, post-scan, and post minus pre scan (Spearman's rho = -0.731, p = 0.025; -815, p = 0.007; -0.695, p = 0.038, respectively).

A 2 x 2 MANOVA of reaction times to smoking-related and neutral cues, with cue type (smoking-related, neutral) as a within-subjects factor and smoking status (smoker, non-smoker) as a between-subjects factor indicated a main effect of smoking status on reaction time (F [1, 18] = 7.78, p = 0.012), with smokers demonstrating slower reactions times to all cues compared to non-smokers. The main effect of cue type and the cue type x smoking status interaction were non-significant ($p \geq 0.18$), suggesting that smokers' reaction times to smoking-related cues compared to neutral cues did not differ from non-smokers.

2.5 Discussion

The observations of activation in a distributed reward network, including VS/NAc, ACC, prefrontal cortex (orbitofrontal cortex and superior frontal gyrus), visual-spatial attention regions (posterior fusiform and lingual gyri), were consistent with our first hypothesis

predicting such activation in smokers. Furthermore, the observation of no activation with voxel-wise random-effects analysis in non-smokers is consistent with our second hypothesis.

Moreover, the observation of activation in VS/NAc, oribitofrontal cortex, ACC, and fusiform gyrus in addicted smokers presented with smoking-related cues vs. neutral cues is consistent with other studies examining drug-related cue reactivity. Due and colleagues employed a similar event-related design and observed activation in prefrontal gyrus and fusiform gyrus and a statistical trend toward activation in ACC, which is consistent with our study.[463] We observed activation in posterior fusiform and lingual gyrus, both extrastriate visual cortical areas and part of a visual-spatial attention circuit including ACC and prefrontal cortex.[464] Despite differences in paradigm design, the convergence of findings amongst smokers in both studies reinforces the notion posed by Due and colleagues that mesocorticolimbic and visual-spatial attention circuits may work in concert to increase attention to stimuli of heightened salience such as the sight of a burning cigarette.

The observation of VS/NAc activation to smoking-related cues is also consistent with the findings of Heinz and colleagues[465] who demonstrated activation in VS/NAc (inclusive of NAc and ventral caudate) to alcohol-related picture cues. Although we observed a main effect of smoking status on global reaction times, which is likely to be the result of nicotine

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[463] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002).

[464] Saito, D.N., Okada, T., Morita, Y., Yonekura, Y., Sadato, N. (2003). 'Tactile-Visual Cross-Modal Shape Matching: A Functional MRI Study', *Brain Res Cogn Brain Res.* **17**(1): pp. 14-25; Kirino, E., Belger, A., Goldman-Rakic, P.l., McCarthy, G. (2000). 'Prefrontal Activation Evoked by Infrequent Target and Novel Stimuli in a Visual Target Detection Task: An Event-Related Functional Magnetic Resonance Imaging Study', *J Neurosci.* **20**(17): pp. 6612-8; McCarthy, G., Luby, M., Gore, J., Goldman-Rakic, P. (1997). 'Infrequent Events Transiently Activate Human Prefrontal and Parietal Cortex as Measured by Functional MRI', *J Neurophysiol.* **77**(3): pp. 1630-4; Yoshiura, T., Zhong, J., Shibata, D. K., Kwok, W. E., Shrier, D. A., Numaguchi, Y. (1999). 'Functional MRI Study of Auditory and Visual Oddball Tasks', *Neuroreport*, **10**(8): pp. 1683-8.

[465] Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004).

deprivation in abstinent smokers,[466] we did not observe any differential effect of cue type on reaction time in smokers compared to non-smokers. However, there was substantial variability in reaction times, and any effect may have been masked by this. Studies demonstrating main effects of cue type on reaction time typically employ a greater number of trials and thus the brevity of our presentation may have reduced sensitivity to detect effects of cue type on tobacco craving.[467]

It is also interesting to note neither Due and colleagues,[468] McClernon and colleagues,[469] or Heinz and colleagues[470] reported significant correlations between fMRI BOLD response in ventral VS/NAc in the abstinent state and craving measures related to cigarettes or alcohol, respectively. While we did see an increase in craving during the experiment in smokers, this increase was not statistically significant. It may be that the time duration of the experiment was not sufficient to induce a long-term increase in tobacco craving. However, regions similar to those activating in our study have demonstrated activation when stimulus duration was sufficient to evoke heightened drug craving.[471] An explanation of why a correlation was not seen with craving may be that the activation of the VS/NAc in our

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[466] Trimmel, M., Wittberger, S. (2004). 'Effects of Transdermally Administered Nicotine on Aspects of Attention, Task Load, and Mood in Women and Men', *Pharmacol Biochem Behav.* **78**(3): pp. 639-45; Havermans, R.C., Debaere, S., Smulders, F. T., Wiers, R. W., Jansen, A. T. (2003). 'Effect of Cue Exposure, Urge to Smoke, and Nicotine Deprivation on Cognitive Performance in Smokers', *Psychol Addict Behav.* **17**(4): pp. 336-9.

[467] Hogarth, L.C., Mogg, K., Bradley, B. P., Duka, T., Dickinson, A. (2003). 'Attentional Orienting towards Smoking-Related Stimuli', *Behav Pharmacol.* **14**(2): pp. 153-60; Bradley, B., Field, M., Mogg, K., De Houwer, J. (2004). 'Attentional and Evaluative Biases for Smoking Cues in Nicotine Dependence: Component Processes of Biases in Visual Orienting', *Behav Pharmacol.* **15**(1): pp. 29-36.

[468] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002).

[469] McClernon, F.J., Hiott, F.B., Huettel, S.A., Rose, J.E. (2005).

[470] Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004).

[471] Garavan, H., Pankiewicz, J., Bloom, A., Cho, J. K., Sperry, L., Ross, T. J., Salmeron, B. J., Risinger, R., Kelley, D., Stein, E. A. (2000). 'Cue-Induced Cocaine Craving: Neuroanatomical Specificity for Drug Users and Drug Stimuli', *Am J Psychiatry*, **157**(11): pp. 1789-98; Brody, A.L., Mandelkern, M. A., Lee, G., Smith, E., Sadeghi, M., Saxena, S., Jarvik, M. E., London, E. D. (2004). 'Attenuation of Cue-Induced Cigarette Craving and Anterior Cingulate Cortex Activation in Bupropion-Treated Smokers: A Preliminary Study', *Psychiatry Res.* **130**(3): pp. 269-81.

experiment represents pre-conscious processes indicative of heightened incentive salience. An alternative explanation might be that our paradigm was not sensitive enough to detect the conscious manifestation of tobacco craving or wanting.

The observation of a correlation between the ACC mean COPE and with the increase in craving during the experiment should be interpreted with caution given the sample size and lack of an *a priori* hypothesis predicting a correlation. It is not clear why the ACC would be more sensitive to subtle increases in tobacco craving than the VS/NAc or the OFC except perhaps as a result of the nature of the task. The rare smoking-related target images may evoke ACC activation as would be seen in error detection tasks and the increase in craving resulting from the stimuli might represent heightened salience to smoking-related cues.

Previous studies have demonstrated positive correlations between drug craving and OFC activation. However, the OFC is important in motivation for goal-directed behaviours such as smoking a cigarette.[472] A negative correlation between OFC activation and craving may indicate inhibition of the OFC by the ACC, a ROI that conversely demonstrated a positive correlation with craving. Thus, the inhibition of the motivation to seek nicotine in the OFC might be inversely proportional to the subjective experience of craving. This hypothesis would be consistent with theories of addiction postulating DA dysfunction in the OFC[473] and the observation by Heinz and colleagues of a negative correlation between cue-induced alcohol craving in the striatum and D_2 receptor BP.[474] Another explanation would be that

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[472] Volkow, N.D., Fowler, J. S., Wang, G. J. (2003).

[473] Koob, G.F., Le Moal, M. (1997); Goldstein, R.Z., Volkow, N. D. (2002). 'Drug Addiction and its Underlying Neurobiological Basis: Neuroimaging Evidence for the Involvement of the Frontal Cortex', *Am J Psychiatry*, **159**(10): pp. 1642-52.

[474] Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004).

OFC is a region prone to susceptibility artefact resulting in signal drop-out, and therefore estimations of effect size may not be as reliable with axial scanning.

The most important finding in this study is the observation that VS/NAc activation to smoking-related (versus neutral) cues was greater in smokers than non-smokers. This is the first time to our knowledge that such an observation has been reported using fMRI. The lack of significant between-group differences in whole-brain analysis may reflect the wide inter-individual variability in the size and precise location of activation changes in both groups but particularly so in the non-smokers as seen in Figure 2.3 and Table 2.2. Despite the wide variability in the COPE for smoking-related vs. neutral cues, VS/NAc activation was significantly greater in smokers than non-smokers using a more sensitive ROI approach. This observation is consistent with many extant models of nicotine addiction discussed above. Consistent with these models, the observation of VS/NAc activation to smoking-related pictures would appear to be consistent with the notion that, as such stimuli can induce craving, the observed activation might be arising from neuroplasticity within the mesoaccumbens DA system with long-term smoking and may mediate conditioned cue reactivity. This hypothesis would be consistent with the observation by Heinz and colleagues of significantly negative correlation between striatal D_2 receptor BP and cue-induced alcohol craving in abstinent alcoholics in withdrawal. As such, the observation of VS/NAc activation may be indicative of dopaminergic dysfunction.

These findings should be interpreted with caution as the VS/NAc and the orbitofrontal cortex border on regions susceptible to signal loss due to susceptibility artefact.[475] We chose

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[475] Rogers, R.D., Ramnani, N., Mackay, C., Wilson, J. L., Jezzard, P., Carter, C. S., Smith, S. M. (2004); Ojemann, J.G., Akbudak, E., Snyder, A. Z., McKinstry, R. C., Raichle, M. E., Conturo, T. E. (1997). 'Anatomic Localization and Quantitative Analysis of Gradient Refocused Echo-Planar fMRI Susceptibility Artifacts', *Neuroimage*, **6**(3): pp. 156-67.

to include the VS/NAc as a principal ROI because of the strength of our *a priori* hypothesis implicating this region and because of the observation in other studies employing similar pulse sequences demonstrating activation. Despite the limitations of our methods, this study does provide a comforting reinforcement of many of the current theories on development of smoking-related cue reactivity in humans. The VS/NAc appears to activate to smoking-related cues appear to activate the VS/NAc in smokers and this appears to be unique to smokers in this study population.

2.6 Future Directions

The process of combining multiple methods in a scientific study required the expertise and mentoring of a multidisciplinary team of investigators in pharmacology, clinical neuroscience, psychiatry, and experimental psychology. The utility of having such a supervisory team became increasingly clear to me in the process of developing this study protocol, carrying out the recruitment and scanning, conducting the analyses, and presenting and publishing the work. Therefore, I have been quite fortunate to have such guidance throughout the execution of this study.

I learned early in the process of pre-pilot testing with the fMRI protocol that it is vitally important to approach changes in experimental protocols with care as such changes may affect the internal validity of a study if subjects are combined in group analyses before and after the protocol change. I was careful to include only the subjects who underwent the 5-second stimulus protocol and were scanned under identical pulse sequences and scan orientations. Refining the process of matching stimuli according to gender could have

potentially affected the results of the study, however, statistically; this refinement does not appear to have significantly affected the main results of the study.[476]

With the data from this pilot study demonstrating activation in pre-specified ROIs, I decided to continue with this experimental protocol for the next fMRI study described in Chapter 4. In so doing, I could seek replication of results from the pilot study. As stated earlier in the chapter, one of our primary aims was to develop a reliable paradigm to examine cue-elicited BOLD signal in pre-defined regions of interest associated with processing reward signalling. The observation of activation in the VS/NAc, ACC, and orbitofrontal cortex reinforced the notion that the experimental model was effective in its stated aims. The wide variability in effect sizes for the smoking vs. neutral contrast was not explained by any of the state or trait variables, which suggests that genetic factors could be important in explaining the observed variance.

In Chapter 1, I described the complex role of the serotonin 5-HT_{1A} receptor in nicotine dependence. Given the putative importance of the 5-HT_{1A} receptor in nicotine dependence and multiple mood and personality states, polymorphisms in genes influencing 5-HT_{1A} binding are reasonable candidates for examination of specific endophenotypes of smoking behaviour such as cue-elicited BOLD activation of the VS/NAc. Therefore, the next logical step in the research was to examine genetic influences on 5-HT_{1A} binding *in vivo* utilising PET through a collaborative effort with the Medical Research Council (MRC) Cyclotron Unit in London.

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[476] We conducted a post-hoc comparison of the mean global VS/NAc COPE in the smokers and found no difference between subjects according to the method of sex-matching (Mann-Whitney U, $Z = -1.23$, $p = 0.286$).

Chapter 3.

POSITRON EMISSION TOMOGRAPHY INVESTIGATION OF THE INFLUENCE OF POLYMORPHISMS IN THE SEROTONIN 5-HT_{1A} RECEPTOR AND SEROTONIN TRANSPORTER GENES ON 5-HT_{1A} RECEPTOR FUNCTION

3.1 Introduction

The overall aim of the research presented in this chapter was to:

Examine the functional significance of polymorphisms in the serotonin $5HT_{1A}$ receptor and serotonin transporter genes utilising positron emission tomography (PET). The results in Chapter 2 demonstrated, in a limited sample of addicted smokers, that smoking-related pictorial cues activated brain reward signalling regions with extensive serotonergic innervation from the amygdala (Am) and raphe complex. Given the major contribution of genetic factors to nicotine dependence (Chapter 1, Section 1.3), it is possible that the wide inter-individual variation in fMRI BOLD response to smoking-related pictures might be explained, in part, by genetic factors. As described in Chapter 1 (Section 1.2.1.3.3), the $5HT_{1A}$ receptor ($5HT_{1A}R$) is an important mediator of behavioural sensitisation to nicotine and may contribute to the development of chronic tolerance through mediation of the anxiolytic and anxiogenic effects of nicotine. In this study I sought to establish whether $5HT_{1A}R$ binding was associated with an established candidate gene for nicotine dependence (serotonin transporter 5-HTTLPR) and whether genetic variation in the $5HT_{1A}$ gene would influence $5HT_{1A}$ binding and therefore be an additional plausible candidate gene for endophenotypes of nicotine dependence. Thus, the specific sub-aims of this study were to:

1. Identify polymorphisms in the $5HT_{1A}$ gene common in a population of healthy Caucasian volunteers.
2. Determine whether genetic variation in the $5HT_{1A}$ gene would influence pre-synaptic and post-synaptic serotonin binding *in vivo* in healthy volunteers who had undergone PET with the specific $5HT_{1A}R$ ligand [^{11}C]WAY 100635.

3. Examine whether $5HT_{1A}R$ binding is influenced by the serotonin transporter 5-HTTLPR polymorphism.

3.2 Background

The neurotransmitter serotonin (5-HT), has been implicated in mood regulation and the pathophysiology and treatment of depression. The 5-HT_{1A} receptor subtype (5-$HT_{1A}R$) appears to contribute to mood disorders and depression, because 5-HT_{1A} receptors have high density in limbic and cortical regions involved in mood regulation, 5-$HT_{1A}R$ agonists are anxiolytic and antidepressant and recent PET studies have reported reduced 5-$HT_{1A}R$ binding in patients with major depressive disorder,[477] panic disorder,[478] and associations with anxiety[479] and aggression traits.[480] Furthermore, increased 5-$HT_{1A}R$ binding and altered gene expression have been observed in schizophrenia.[481] In addition, as described more fully in

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[477] Drevets, W.C., Frank, E., Price, J. C., Kupfer, D. J., Holt, D., Greer, P. J., Huang, Y., Gautier, C., Mathis, C. (1999). 'PET Imaging of Serotonin 1A Receptor Binding in Depression', *Biol Psychiatry*, **46**(10): pp. 1375-87; Bhagwagar, Z., Rabiner, E. A., Sargent, P. A., Grasby, P. M., Cowen, P. J. (2004). 'Persistent Reduction in Brain Serotonin1A Receptor Binding in Recovered Depressed Men Measured by Positron Emission Tomography with [11C]WAY-100635', *Mol Psychiatry*, **9**(4): pp. 386-92; Sargent, P.A., Kjaer, K. H., Bench, C. J., Rabiner, E. A., Messa, C., Meyer, J., Gunn, R. N., Grasby, P. M., Cowen, P. J. (2000). 'Brain Serotonin1A Receptor Binding Measured by Positron Emission Tomography with [11C]WAY-100635: Effects of Depression and Antidepressant Treatment', *Arch Gen Psychiatry*, **57**(2): pp. 174-80.

[478] Neumeister, A., Bain, E., Nugent, A. C., Carson, R. E., Bonne, O., Luckenbaugh, D. A., Eckelman, W., Herscovitch, P., Charney, D. S., Drevets, W. C. (2004). 'Reduced Serotonin Type 1A Receptor Binding in Panic Disorder', *J Neurosci.* **24**(3): pp. 589-91.

[479] Tauscher, J., Bagby, R. M., Javanmard, M., Christensen, B. K., Kasper, S., Kapur, S. (2001). 'Inverse Relationship Between Serotonin 5-HT(1A) Receptor Binding and Anxiety: A [(11)C]WAY-100635 PET Investigation in Healthy Volunteers', *Am J Psychiatry*, **158**(8): pp. 1326-8.

[480] Parsey, R.V., Oquendo, M. A., Simpson, N. R., Ogden, R. T., Van Heertum, R., Arango, V., Mann, J. J. (2002). 'Effects of Sex, Age, and Aggressive Traits in Man on Brain Serotonin 5-HT1A Receptor Binding Potential Measured by PET Using [C-11]WAY-100635', *Brain Res.* **954**(2): pp. 173-82.

[481] Gurevich, E.V., Joyce, J. N. (1997), 'Alterations in the Cortical Serotonergic System in Schizophrenia: A Postmortem Study', *Biol Psychiatry*, **42**(7): pp. 529-45; Burnet, P.W., Eastwood, S. L., Harrison, P. J. (1996). '5-HT1A and 5-HT2A Receptor mRNAs and Binding Site Densities Are Differentially Altered in Schizophrenia', *Neuropsychopharmacology*, **15**(5): pp. 442-55; Burnet, P.W., Eastwood, S. L., Harrison, P. J. (1997). '[3H]WAY-100635 for 5-HT1A Receptor Autoradiography in Human Brain: A Comparison with [3H]8-OH-DPAT and Demonstration of Increased Binding in the Frontal Cortex in Schizophrenia', *Neurochem Int.* **30**(6): pp. 565-74; Hashimoto, T., Nishino, N., Nakai, H., Tanaka, C. (1991). 'Increase in Serotonin 5-HT1A Receptors in Prefrontal and Temporal Cortices of Brains from Patients with Chronic Schizophrenia', *Life Sci.* **48**(4): pp. 355-63; Simpson, M.D., Lubman, D. I., Slater, P., Deakin, J. F. (1996). 'Autoradiography

Chapter 1, there is considerable evidence indicating that 5-HT_{1A}Rs are important in the development of nicotine dependence, particularly from rat studies. These studies demonstrated that the anxiogenic effects of nicotine are antagonised by the 5-HT_{1A}R-specific antagonist WAY-100635 in the dorsal raphe, dorsal hippocampus, and lateral septum.[482] Combined with additional evidence that 5-HT_{1A} agonist 8-OH-DPAT inhibits behavioural sensitisation to nicotine in rats,[483] it is plausible that individuals with reduced binding would be less prone the aversive anxiogenic effects of nicotine in higher doses and more likely to succumb to the process of incentive sensitisation to nicotine. However, this hypothesis is a direct extension from animal studies and requires examination in humans. As described more extensively in Chapter 1 (Section 1.2.1.3.3), 5-HT_{1A}Rs are somatodendritic autoreceptors located on serotonergic raphe neurones and post-synaptically in multiple cortical and sub-cortical terminal fields in the brain. The 5-HT_{1A}R subtype has important clinical applications as, for example, the therapeutic response to selective serotonin reuptake inhibitors is thought to be affected by down-regulation of 5-HT_{1A}Rs resulting from the increased serotonergic

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with [3H]8-OH-DPAT Reveals Increases in 5-HT(1A) Receptors in Ventral Prefrontal Cortex in Schizophrenia', *Biol Psychiatry*, **39**(11): pp. 919-28; Tauscher, J., Kapur, S., Verhoeff, N. P., Hussey, D. F., Daskalakis, Z. J., Tauscher-Wisniewski, S., Wilson, A. A., Houle, S., Kasper, S., Zipursky, R. B. (2002). 'Brain Serotonin 5-HT(1A) Receptor Binding in Schizophrenia Measured by Positron Emission Tomography and [11C]WAY-100635', *Arch Gen Psychiatry*, **59**(6): pp. 514-20.

[482] Tucci, S., Genn, R. F., Marco, E., File, S. E. (2003). 'Do Different Mechanisms Underlie Two Anxiogenic Effects of Systemic Nicotine?' *Behav Pharmacol.* **14**(4): pp. 323-9; Mihailescu, S., Guzman-Marin, R., Drucker-Colin, R. (2001). 'Nicotine Stimulation of Dorsal Raphe Neurons: Effects on Laterodorsal and Pedunculopontine Neurons', *Eur Neuropsychopharmacol*, **11**(5): pp. 359-66; Olausson, P., Akesson, P., Petersson, A., Engel, J. A., Soderpalm, B. (2001). 'Behavioral and Neurochemical Consequences of Repeated Nicotine Treatment in the Serotonin-Depleted Rat', *Psychopharmacology* (Berl), **155**(4): pp. 348-61; Irvine, E.E., Cheeta, S., File, S. E. (2001). 'Development of Tolerance to Nicotine's Anxiogenic Effect in the Social Interaction Test', *Brain Res.* **894**(I): pp. 95-100; Cheeta, S., Irvine, E. E., Kenny, P. J., File, S. E. (2001). 'The Dorsal Raphe Nucleus is a Crucial Structure Mediating Nicotine's Anxiolytic Effects and the Development of Tolerance and Withdrawal Responses', *Psychopharmacology* (Berl), **155**(1): pp. 78-85; Cheeta, S., Kenny, P. J., File, S. E. (2000). 'The Role of 5-HT1A Receptors in Mediating the Anxiogenic Effects of Nicotine Following Lateral Septal Administration', *Eur J Neurosci.* **12**(10): pp. 3797-802; Engberg, G., Erhardt, S., Sharp, T., Hajos, M. (2000). 'Nicotine Inhibits Firing Activity of Dorsal Raphe 5-HT Neurones *In Vivo*', *Naunyn Schmiedebergs Arch Pharmacol.* **362**(1): pp. 41-5.

[483] Olausson, P., Engel, J. A., Soderpalm, B. (1999).

tone generated by serotonin transporter blockade.[484] There are few known physiological regulators of 5-HT_{1A}R expression *in vivo* short of extreme neuroendocrine manipulations (e.g., adrenalectomy). Genetic factors might be important determinants of 5-HT_{1A}R function and thus influence mood states and response to psychotropic medications.

Given the putative role of central 5-HT_{1A}Rs in nicotine addiction, I was interested in examining whether genetic variation in the 5-HT_{1A}R gene would affect 5-HT_{1A}R binding *in vivo*. Of the reported single nucleotide polymorphisms identified in the 5-HT_{1A}R gene, the most extensively characterised, functionally and behaviourally, is a C to G polymorphism in the upstream promoter region of the 5-HT_{1A}R gene.[485] Wu and Comings localised the locus at 1018 base pairs (bp) upstream from the transcription initiation site,[486] but other investigators have described the same polymorphism at position -1019 based on different results from sequencing by different groups.[487] In order to avoid confusion I shall use the original designation of Wu and Comings for the purposes of this thesis.

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[484] Li, Q., Muma, N. A., Battaglia, G., Van de Kar, L. D. (1997). 'A Desensitization of Hypothalamic 5-HT1A Receptors by Repeated Injections of Paroxetine: Reduction in the Levels of G(i) and G(o) Proteins and Neuroendocrine Responses, but not in the Density of 5-HT1A Receptors', *J Pharmacol Exp Ther.* **282**(3): pp. 1581-90; Raap, D.K., Evans, S., Garcia, F., Li, Q., Muma, N. A., Wolf, W. A., Battaglia, G., Van De Kar, L. D. (1999). 'Daily Injections of Fluoxetine Iinduce Dose-Dependent Desensitization of Hypothalamic 5-HT1A Receptors: Reductions in Neuroendocrine Responses to 8-OH-DPAT and in Levels of Gz and Gi Proteins', *J Pharmacol Exp Ther.* **288**(1): pp. 98-106; D'Souza, D.N., Zhang, Y., Garcia, F., Battaglia, G., Van De Kar, L. D. (2002). 'Destruction of Serotonergic Nerve Terminals Prevents Fluoxetine-Induced Desensitization of Hypothalamic 5-HT(1A) Receptors', *Psychopharmacology* (Berl). **164**(4): pp. 392-400.

[485] Lesch, K.P., Gutknecht, L. (2004). 'Focus on the 5-HT1A Receptor: Emerging Role of a Gene Regulatory Variant in Psychopathology and Pharmacogenetics', *Int J Neuropsychopharmacol*, **7**(4): pp. 381-5; Huang, Y.Y., Battistuzzi, C., Oquendo, M. A., Harkavy-Friedman, J., Greenhill, L., Zalsman, G., Brodsky, B., Arango, V., Brent, D. A., Mann, J. J. (2004). 'Human 5-HT1A Receptor C(-1019)G Polymorphism and Psychopathology', *Int J Neuropsychopharmacol*, **7**(4): pp. 441-51; Lemonde, S., Du, L., Bakish, D., Hrdina, P., Albert, P. R. (2004). 'Association of the C(-1019)G 5-HT1A Functional Promoter Polymorphism with Antidepressant Response', *Int J Neuropsychopharmacol*, **7**(4): pp. 501-6; Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P. D., Bown, C. D., Sequeira, A., Kushwaha, N., Morris, S. J., Basak, A., Ou, X. M., Albert, P. R. (2003). 'Impaired Repression at a 5-Hydroxytryptamine 1A Receptor Gene Polymorphism Associated with Major Depression and Suicide', *J Neurosci.* **23**(25): pp. 8788-99.

[486] Wu, S., Comings, D. E. (1999). 'A Common C-1018G Polymorphism in the Human 5-HT1A Receptor Gene', *Psychiatr Genet.* **9**(2): pp. 105-6.

[487] Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P. D., Bown, C. D., Sequeira, A., Kushwaha, N., Morris, S. J., Basak, A., Ou, X. M., Albert, P. R. (2003); Wu, S., Comings, D. E. (1999); Parks, C.L., Shenk, T.

Lemonde and colleagues have suggested a transcriptional model in which a single nucleotide polymorphism of the 5-HT_{1A}R receptor gene de-represses 5-HT_{1A} autoreceptor expression (thus increasing the 5-HT_{1A} autoreceptor density in the raphe nucleus) to reduce serotonergic neurotransmission, predisposing individuals to depression and suicide.[488] These investigators conducted *in vitro* experiments in human raphe cell lines co-transfected with luciferase and a 26 bp palindrome containing either the C or G allele and observed that the 5-HT_{1A} G allele significantly attenuated basal transcription activity of the gene by the transcription factor NUDR (nuclear deformed epidermal autoregulatory factor/DEAF-1). Lemonde and colleagues also demonstrated an association between the 5-HT_{1A}R -1018G allele and treatment response to the antidepressant flibanserin.[489] I was also interested in whether genetic variation in the serotonin transporter (5-HTT) gene would influence 5-HT_{1A}R binding, as Li and colleagues have demonstrated that 5-HTT knockout (KO) mice have reduced mRNA expression and cortical density of 5-HT_{1A}Rs.[490] As described in Section 1.2.1.3.3, transcriptional activity of the 5-HTT gene is modulated, in part, by a repetitive segment of varying length located in the 5' flanking region approximately 1.4 kilobases from the transcription initiation site, which is referred to as the 5-HTT gene-linked polymorphic region or '5-HTTLPR'. An insertion/deletion polymorphism results in either 14 ("short" or "S") or 16 ("long" or "L") repeated segments of 20 to 23 bp.[491] Lesch and colleagues demonstrated that the S allele is associated with three to four-fold reductions in 5-

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(1996). 'The Serotonin 1a Receptor Gene Contains a TATA-less Promoter that Responds to MAZ and Sp1', *J Biol Chem.* **271**(8): pp. 4417-30.

[488] Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P. D., Bown, C. D., Sequeira, A., Kushwaha, N., Morris, S. J., Basak, A., Ou, X. M., Albert, P. R. (2003).

[489] Lemonde, S., Du, L., Bakish, D., Hrdina, P., Albert, P. R. (2004).

[490] Li, Q., Wichems, C., Heils, A., Lesch, K. P., Murphy, D. L. (2000). 'Reduction in the Density and Expression, but not G-Protein Coupling, of Serotonin Receptors (5-HT1A) in 5-HT Transporter Knock-Out Mice: Gender and Brain Region Differences', *J Neurosci.* **20**(21): pp. 7888-95.

[491] Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., Lesch, K. P. (1996). 'Allelic Variation of Human Serotonin Transporter Gene Expression', *J Neurochem*, **66**(6): pp. 2621-4

HTT mRNA expression *in vitro*.[492] The S allele has been associated with depression,[493] harm avoidance,[494] neuroticism,[495] and smoking cessation.[496] Given this background, it appeared plausible that the 5-HTTLPR could theoretically influence 5-HT_{1A}R binding.

Therefore, working in partnership with Professor Paul Grasby and Dr. Venkatesha Murthy at the Medical Research Council (MRC) Hammersmith Unit, we investigated the effects of polymorphisms of the 5-HT_{1A}R and 5-HT transporter (5-HTT) genes on 5-HT_{1A}R binding potential (BP) in healthy volunteers who had undergone PET with [^{11}C]WAY 100635, a radioligand selective for 5-HT_{1A}Rs. Specifically, we studied whether the -1018 C>G single nucleotide polymorphism (SNP) and other SNPs within the 5-HT_{1A} gene and the 5-HTTLPR would affect 5-HT_{1A}R binding in humans. Identification of a genetic association with one or more SNPs in these genes might provide a biologically plausible mechanism explaining the variation in BP observed in healthy volunteers and provide a reasonable candidate gene for studies of nicotine-dependence endophenotypes such as cue-elicited BOLD activation observed in reward signalling brain regions.

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[492] Lesch, K.P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., Murphy, D. L. (1996). 'Association of Anxiety-Related Traits with a Polymorphism in the Serotonin Transporter Gene Regulatory Region', *Science*, **274**(5292): pp. 1527-31.

[493] Willeit, M., Praschak-Rieder, N., Neumeister, A., Zill, P., Leisch, F., Stastny, J., Hilger, E., Thierry, N., Konstantinidis, A., Winkler, D., Fuchs, K., Sieghart, W., Aschauer, H., Ackenheil, M., Bondy, B., Kasper, S. (2003). 'A Polymorphism (5-HTTLPR) in the Serotonin Transporter Promoter Gene is Associated with DSM-IV Depression Subtypes in Seasonal Affective Disorder', *Mol Psychiatry*, **8**(11): pp. 942-6.

[494] Munafo', M.R., Clark, T. G., Moore, L. R., Payne, E., Walton, R., Flint, J. (2003). 'Genetic Polymorphisms and Personality in Healthy Adults: A Systematic Review and Meta-Analysis', *Mol Psychiatry*, **8**(5): pp. 471-84.

[495] Sen, S., Burmeister, M., Ghosh, D. (2004). 'Meta-Analysis of the Association Between a Serotonin Transporter Promoter Polymorphism (5-HTTLPR) and Anxiety-Related Personality Traits', *Am J Med Genet B Neuropsychiatr Genet.* **127**(1): pp. 85-9.

[496] Munafo', M., Clark, T., Johnstone, E., Murphy, M., Walton, R. (2004). 'The Genetic Basis for Smoking Behavior: A Systematic Review and Meta-Analysis', *Nicotine Tob Res.* **6**(4): pp. 583-97.

3.3 Materials and Methods

My contributions to the study were as follows: Specifically, under the guidance of Dr. Walton, I conceived the main study design and hypotheses, wrote the study protocol and research ethics application, contacted research volunteers and general practitioners requesting consent for involvement in the study, arranged for blood collection kits to be sent by post to each consented subject, identified single nucleotide polymorphisms (SNPs) in the gene through an extensive literature search, and analysed the data in collaboration with PG and VM. Mrs. Robyn Jacob and Dr. Elaine Johnstone performed DNA extraction and genotyping. Staff at the MRC Cyclotron Unit performed all PET scans. I published results from this chapter in a peer-reviewed journal.[497] I wrote the first and subsequent drafts of the study manuscript, with later substantial contributions from VM and edited the final published draft in which I was first author with VM as a joint first author.

3.3.1 Study Overview

Two separate cohorts of healthy volunteers were included in this study. The first cohort consisted of 140 healthy British Caucasian subjects [64 males, mean ± standard deviation (SD) age of 51.5 ± 8.8 years; 76 females, mean ± SD age of 52.0 ± 8.8 years; entire group mean ± SD age of 52.0 ± 8.8 years] who were randomly selected from the OXCHECK study. The OXCHECK study was a population-based multiple risk factor reduction trial which took place from 1989 to 1993.[498] This cohort was genotyped to evaluate the population frequencies of all known published single nucleotide polymorphisms (SNPs) in the 5-HT_{1A}R

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[497] David, S.P., Murthy, N.V., Rabiner, E.A., Munafo', M.R., Johnstone, E.C., Jacob, R., Walton, R.T., Grasby, P.M. (2005). 'A Functional Genetic Variation of the Serotonin (5-HT) Transporter Affects 5-HT1A Receptor Binding in Humans', *J Neurosci.* **25**(10): pp. 2586-90.

[498] ICRF (1995). 'Effectiveness of Health Checks Conducted by Nurses in Primary Care: Final Results of the OXCHECK Study. Imperial Cancer Research Fund OXCHECK Study Group', *BMJ.* **310**(6987): pp. 1099-104; ICRF (1994). 'Effectiveness of Health Checks Conducted by Nurses in Primary Care: Results of the OXCHECK Study after One Year. Imperial Cancer Research Fund OXCHECK Study Group. *BMJ.* **308**(6924): pp. 308-12.

receptor gene. The next step was to genotype as many subjects as possible from a cohort of 50 healthy volunteers resulting in a study population of 35 participants (27 males, mean ± SD age of 43.6 ± 13.1 years; eight females, mean ± SD age of 52.6 ± 10.3 years; entire group mean ± SD age of 46.0 ± 13.0) years who had previously undergone PET scans using [^{11}C]WAY-100635,[499] a specific radioligand for 5-HT$_{1A}$R.[500] These participants were genotyped for polymorphisms in the 5-HT$_{1A}$ gene deemed most informative from the analysis of the OXCHECK cohort and also for the 5-HTTLPR.

We were also interested in the 5-HTT gene because of its theoretical potential to affect 5-HT$_{1A}$R binding. Given that genetic variation in this gene has already been extensively studied and is well described in Caucasians[501] and that a functional polymorphism associated with nicotine dependence had been identified (i.e., 5-HTTLPR) (Section 3.2) and also given the limited resources available, we did not explore other SNPs in the 5-HTT gene.

The results of genotyping from the group of healthy Caucasian volunteers for SNPs in the 5-HT$_{1A}$R gene were then used to guide the process of selecting which SNPs or haplotypes would be included in the second cohort of healthy volunteers, who had undergone [^{11}C]WAY-100635 PET, for genotyping. As will be discussed in the next section (Section 3.3.2) and further in the Results section (Section 3.4), only 4 of the 9 known published SNPs in the 5-HT$_{1A}$ gene were polymorphic in the Oxcheck cohort and, of these SNPs, only one SNP at location -1018 in the 5-HT$_{1A}$ gene was common enough to include in genetic

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[499] Rabiner, E.A., Messa, C., Sargent, P. A., Husted-Kjaer, K., Montgomery, A., Lawrence, A. D., Bench, C. J., Gunn, R. N., Cowen, P., Grasby, P. M. (2002). 'A Database of [(11)C]WAY-100635 Binding to 5-HT(1A) Receptors in Normal Male Volunteers: Normative Data and Relationship to Methodological, Demographic, Physiological, and Behavioral Variables', Neuroimage, **15**(3): pp. 620-32.

[500] Pike, V.W., McCarron, J. A., Lammerstma, A. A., Hume, S. P., Poole, K., Grasby, P. M., Malizia, A., Cliffe, I. A., Fletcher, A., Bench, C. J. (1995). 'First Delineation of 5-HT1A Receptors in Human Brain with PET and [11C]WAY-100635', *Eur J Pharmacol*, **283**(1-3): pp. R1-3.

[501] Murphy, D.L., Lerner, A., Rudnick, G., Lesch, K. P. (2004). 'Serotonin Transporter: Gene, Genetic Disorders, and Pharmacogenetics', *Mol Interv.* **4**(2): pp. 109-23.

comparisons in the PET cohort. As these intermediate results were an integral part in the sequence of methodical steps applied in this study, I therefore mention them in general terms in this section to clarify the overall scope of the research described below. All subjects from both populations gave written informed consent for DNA analysis and the local research ethics committees at the University of Oxford approved the genotyping of the Oxcheck subjects and at the Hammersmith Hospital/MRC Cyclotron Unit approved blood collection and genotyping of subjects having undergone PET with [^{11}C]WAY-100635.

Given the background stated above, I formed the following hypotheses:

1. I hypothesised that there would be increased 5-HT_{1A}R binding in healthy volunteers with one or more copies of the –1018 5-HT_{1A} G allele (GG/CC) than in individuals with CC genotypes, and that
2. The 5-HTTLPR would interact with the –1018 5-HT_{1A} G allele to influence 5-HT_{1A}R binding.

3.3.2 Identification of SNPs in the 5-HT_{1A} Receptor Gene

I performed a literature search using 'serotonin', 'serotonin receptor 1A', '5-HT1A', and 'single nucleotide polymorphisms' as key words with the search engines: 'Nucleotide', 'PubMed', and 'Genome' available at (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi), and 'BLAST' at (http://www.ncbi.nlm.nih.gov/BLAST/). I identified nine published SNPs in coding regions (exons) and one SNP described above in the promoter region of the 5-HT_{1A} receptor gene (Table 3.1).

Table 3.1

Reported Population Allele Frequencies of SNPs in 5-HT_{1A} Receptor Gene

Polymorphism	Frequency	Population	Volunteers	Reference
-1018 C>G	50.00% 37.31% 52.32%	United States Canada	Healthy Healthy Depressed	Wu & Comings, 1999[502] Lemonde et al, 2003[503]
+47 C>T (Pro16Leu)	3.42% 2.29%	Japanese Japanese	Healthy Healthy	Kawanishi et al, 1998[504] Harada et al, 1996[505]
+64 A>G (Gly22Ser)	0.20%	Finnish	Healthy	Nakhai et al, 1995[506]
+82 A>G (Ile28Val)	0.55% 1.20% 1.10% 0.54% 0.84%	Finnish German	Healthy Healthy Bipolar Tourette's Schizophrenia	Nakhai et al, 1995[507] Erdmann et al, 1995[508]
+294 G>A	3.42%	Japanese	Healthy	Kawanishi et al 1998[509]
+549 C>T	2.80% 0.35% 0.84%	Japanese German	Healthy Bipolar Schizophrenic	Kawanishi et al 1998[510] Erdmann et al 1995[511]
+656 G>T (Arg219Leu)	2.40% 0.00%	Canada	Tourette's Healthy	Lam et al 1996[512]
+815 G>A (Gly272Asp)	3.73%	Japanese	Healthy	Kawanishi et al 1998[513]
+1254 C>G (Asn417Lys)	2.40% 0.00%	Canada	Tourette's Healthy	Lam et al 1996[514]

LEGEND: Amino acid abbreviations in column 1: 'pro' = proline, 'leu' = leucine, 'gly' = glycine, 'ser' = serine, 'Ile' = isoleucine, 'val' = valine, 'arg' = arginine, 'asp' = aspartic acid, and 'asn' = asparagine.

[502] Wu, S., Comings, D. E. (1999). 'A Common C-1018G Polymorphism in the Human 5-HT1A Receptor Gene', *Psychiatr Genet.* **9**(2): pp. 105-6.

[503] Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P. D., Bown, C. D., Sequeira, A., Kushwaha, N., Morris, S. J., Basak, A., Ou, X. M., Albert, P. R. (2003). 'Impaired Repression at a 5-Hydroxytryptamine 1A Receptor Gene Polymorphism Associated with Major Depression and Suicide', *J Neurosci.* **23**(25): pp. 8788-99.

[504] Kawanishi, Y., Harada, S., Tachikawa, H., Okubo, T., Shiraishi, H. (1998). 'Novel Mutations in the Promoter and Coding Region of the Human 5-HT1A Receptor Gene and Association Analysis in Schizophrenia', *Am J Med Genet.* **81**(5): pp. 434-9.

[505] Harada, S., Okubo, T., Tsutsumi, M., Takase, S., Muramatsu, T. (1996). 'Investigation of Genetic Risk Factors Associated with Alcoholism', *Alcohol Clin Exp Res.* **20**(9 Suppl): pp. 293A-296A.

[506] Nakhai, B., Nielsen, D. A., Linnoila, M., Goldman, D. (1995). 'Two Naturally Occurring Amino Acid Substitutions in the Human 5-HT1A Receptor: Glycine 22 to Serine 22 and Isoleucine 28 to Valine 28', *Biochem Biophys Res Commun.* **210**(2): pp. 530-6.

[507] Ibid.

[508] Erdmann, J., Shimron-Abarbanell, D., Cichon, S., Albus, M., Maier, W., Lichtermann, D., Minges, J., Reuner, U., Franzek, E., Ertl, M. A. and et al. (1995). 'Systematic Screening for Mutations in the Promoter and the Coding Region of the 5-HT1A Gene', *Am J Med Genet.* **60**(5): pp. 393-9.

[509] Kawanishi, Y., Harada, S., Tachikawa, H., Okubo, T., Shiraishi, H. (1998).

[510] Ibid.

[511] Erdmann, J., Shimron-Abarbanell, D., Cichon, S., Albus, M., Maier, W., Lichtermann, D., Minges, J., Reuner, U., Franzek, E., Ertl, M. A. and et al. (1995).

[512] Lam, S., Shen, Y., Nguyen, T., Messier, T. L., Brann, M., Comings, D., George, S. R., O'Dowd, B. F. (1996). 'A Serotonin Receptor Gene (5HT1A) Variant Found in a Tourette's Syndrome Patient', *Biochem Biophys Res Commun.* **219**(3): pp. 853-8.

[513] Kawanishi, Y., Harada, S., Tachikawa, H., Okubo, T., Shiraishi, H. (1998).

[514] Lam, S., Shen, Y., Nguyen, T., Messier, T. L., Brann, M., Comings, D., George, S. R., O'Dowd, B. F. (1996).

Of the SNPs or mutations identified, six were functional (i.e., result in amino acid changes) (47 C>T, 64 A>G, 82 A>G, 656 G>T, 815 G>A, 1254 C>G), and three were structural (i.e., no change in amino acid at that locus) (-1018 C>G, 294 G>A, 549 C>T).

Most of the SNPs identified in the literature search are of such low population frequencies that case-control studies with conventional sample sizes have not had sufficient statistical power to adequately examine associations with disease or behavioural states. However, case-control studies have been performed for some of the less common SNPs and mutations, which thus far have not demonstrated associations with disease or behavioural states. Erdman and colleagues found no association between the 82 A>G or 549 C>T SNPs and schizophrenia, bipolar affective disorder, Tourette's syndrome or suicide. [515] Nonetheless, the identification of all published SNPs within the 5-HT_{1A}R gene permitted us to proceed with a population-based examination of allele frequencies in healthy Caucasians in the Oxfordshire region with potential use in haplotype comparisons in the PET cohort.

3.3.3 Assay Development and Genotyping

For the panel of healthy Caucasians from the OXCHECK population, colleagues RJ and EJ developed assays for each SNP identified and performed genotyping in order to identify informative haplotypes in the 5-HT_{1A}R gene (chromosome 5q11.2-q13). (The primers and assay conditions are included in Table 3.2.) Genomic DNA was extracted from buffy coat lymphocytes using a standard sodium chloride-chloroform technique and stored in sterile distilled water at -20°C. The genotyping assay was carried out as described by Bunce and

[515] Erdmann, J., Shimron-Abarbanell, D., Cichon, S., Albus, M., Maier, W., Lichtermann, D., Minges, J., Reuner, U., Franzek, E., Ertl, M. A. and et al. (1995). 'Systematic Screening for Mutations in the Promoter and the Coding Region of the 5-HT1A Gene', *Am J Med Genet.* **60**(5): pp. 393-9; Nishiguchi, N., Shirakawa, O., Ono, H., Nishimura, A., Nushida, H., Ueno, Y., Maeda, K. (2002). 'Lack of an Association Between 5-HT1A Receptor Gene Structural Polymorphisms and Suicide Victims', *Am J Med Genet.* **114**(4): pp. 423-5.

colleagues, using sequence specific primers.[516] Polymerase chain reactions (PCR) were conducted for both the common and variant alleles and each reaction contained control primers, to detect a conserved sequence in the *adenomatosis polyposis coli* gene thereby eliminating the possibility of false-negative results. In the later part of the study, blood samples from the PET group of 35 subjects were genotyped for the 5-HT_{1A}R gene (5q11.2-q13)[517] SNP at the site (–1018 C>G) using the same PCR based methods described above. In addition, all subjects from the PET cohort were genotyped for 5-HTTLPR (17q11.1-q12) variable number tandem repeat (VNTR) polymorphism using primers and conditions previously described.[518] The 5-HTTLPR assay results in either an inserted long (L) variant of 528 bp or a deleted short (S) variant of 484 bp as described in section 3.1.

Subjects were dichotomised as 5-HTTLPR (SS or SL vs. LL) and 5-HT_{1A} (CC vs. CG or GG) consistent with studies suggesting autosomal dominance for the 5-HTTLPR S allele[519] and 5-HT_{1A} G allele.[520] Evidence supporting a dominant model for the -1018G 5-HT_{1A} allele includes observed associations between an excess of G alleles and schizophrenia[521] and panic disorder[522] and an association between G allele carriers (GC or GG) and treatment

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[516] Bunce, M., Fanning, G. C., Welsh, K. I. (1995). 'Comprehensive, Serologically Equivalent DNA Typing for HLA-B by PCR Using Sequence-Specific Primers (PCR-SSP)', *Tissue Antigens*. **45**(2): pp. 81-90.

[517] Wu, S., Comings, D. E. (1999). 'A Common C-1018G Polymorphism in the Human 5-HT1A Receptor Gene', *Psychiatr Genet*. **9**(2): pp. 105-6.

[518] Lesch, K.P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., Murphy, D. L. (1996). 'Association of Anxiety-Related Traits with a Polymorphism in the Serotonin Transporter Gene Regulatory Region', *Science*, **274**(5292): pp. 1527-31.

[519] Lesch, K.P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., Murphy, D. L. (1996).

[520] Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P. D., Bown, C. D., Sequeira, A., Kushwaha, N., Morris, S. J., Basak, A., Ou, X. M., Albert, P. R. (2003). 'Impaired Repression at a 5-Hydroxytryptamine 1A Receptor Gene Polymorphism Associated with Major Depression and Suicide', *J Neurosci*. **23**(25): pp. 8788-99.

[521] Huang, Y.Y., Battistuzzi, C., Oquendo, M. A., Harkavy-Friedman, J., Greenhill, L., Zalsman, G., Brodsky, B., Arango, V., Brent, D. A., Mann, J. J. (2004). 'Human 5-HT1A Receptor C(-1019)G Polymorphism and Psychopathology', *Int J Neuropsychopharmacol*, 7(4): pp. 441-51.

[522] Rothe, C., Gutknecht, L., Freitag, C., Tauber, R., Mossner, R., Franke, P., Fritze, J., Wagner, G., Peikert, G., Wenda, B., Sand, P., Jacob, C., Rietschel, M., Nothen, M. M., Garritsen, H., Fimmers, R., Deckert, J., Lesch,

response to fluvoxamine.[523] There is also *in vitro,*[524] *in vivo* fMRI,[525] and epidemiological[526] evidence supporting a dominant model for the 5-HTTLPR S allele.[527]

Table 3.2

Primers Used in Genotyping Assay for the 5-HT_{1A} Receptor Gene

Polymorphism	Primer	Primer sequence (5' - 3')	Control	Primer conc. (μM)
-1018C>G	-1018C	GAAGACCGAGTGTGTCTTCC	63/64	2.50
	-1018G	GAAGACCGAGTGTGTCTTCG		2.50
	-1018 control	CTGAGGGAGTAAGGCTGGAC		2.50
47C>T	47C	GCAACAACACCACATCACCAAC	210/211	2.50
	47T	GCAACAACACCACATCACCAAT		2.50
	47 control	GGATAGAGATGAGGAAGCCAAT		2.50
64A>G	64A	ACYGGCTCCCTTTGAGACCA	63/64	2.50
	64G	YGGCTCCCTTTGAGACCG		2.50
	64 control	CCACTTGTTGAGYACCTGATAC		2.50
82A>G	82A	RGCGGCAACACTACTGGTA	63/64	2.50
	82G	RGCGGCAACACTACTGGTG		2.50
	82 control	CCACTTGTTGAGYACCTGATAC		2.50
294G>A	294G	GCCGCGCTGTATCAGGTG	210/211	2.50
	294A	GCCGCGCTGTATCAGGTA		2.50
	294 control	TTTGACCGTCTTGCGGATGC		2.50
549C>T	549C	TGCTAATGGTGCATGCGTCG	210/211	1.25
	549T	TGCTAATGGTGCATGCGTCA		1.25
	549control	GCACGCTCATCTTCTGCG		1.25
656G>T	656G	CTCTATGGGCGCATATTCCG	210/211	2.50
	656T	CTCTATGGGCGCATATTCCT		2.50
	656 control	AGAGGATGAAGGTGCCCATG		2.50
815G>A	815G	CATTGGCGCACAGAGCAC	210/211	2.50
	815A	CCATTGGCGCACAGAGCAT		2.50
	815 control	CTGCGACCTGTTCATCGC		2.50
1254C>G	1254C	CGTCATCACTGGCGGCAG	210/211	2.50
	1254G	CGTCATCACTGGCGGCAC		2.50
	1254 control	GAAGAGTGTGAATGGAGAGTC		2.50
210/211	210	ATGATGTTGACCTTTCCAGGG		2.00
	211	TTCTGTAACTTTTCATCAGTTGC		2.00
63/64	63	TGCCAAGTGGAGCACCCAA		0.10
	64	GCATCTTGCTCTGTGCAGAT		0.10

LEGEND: Primers and primer concentrations (conc.) for wildtype and mutant forms of each single nucleotide polymorphism and a conserved control region listed according to loci along the 5-HT_{1A} gene.

K. P. (2004). 'Association of a Functional 1019C>G 5-HT1A Receptor Gene Polymorphism with Panic Disorder with Agoraphobia', *Int J Neuropsychopharmacol*, **7**(2): pp. 189-92.

[523] Serretti, A., Artioli, P., Lorenzi, C., Pirovano, A., Tubazio, V., Zanardi, R. (2004). 'The C(-1019)G Polymorphism of the 5-HT1A Gene Promoter and Antidepressant Response in Mood Disorders: Preliminary Findings', *Int J Neuropsychopharmacol*, **7**(4): pp. 453-60.

[524] Lesch, K.P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., Murphy, D. L. (1996).

[525] Hariri, A.R., Drabant, E. M., Munoz, K. E., Kolachana, B. S., Mattay, V. S., Egan, M. F., Weinberger, D. R. (2005). 'A Susceptibility Gene for Affective Disorders and the Response of the Human Amygdala', *Arch Gen Psychiatry*, **62**(2): pp. 146-52.

[526] Lesch, K.P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., Murphy, D. L. (1996).

[527] Ibid.

3.3.4 Positron Emission Tomography Imaging Procedures

PET data acquisition and image analysis procedures have been described fully elsewhere.[528] In brief, PET scans were performed on two scanners, a Siemens Electronic Card Assembly Test (ECAT) 953 (N = 29) and ECAT 966 (N = 6) scanners. [^{11}C]WAY-100635 was injected intravenously as a bolus over 30 seconds, the emission data collected over 90 minutes, and quantified via a simplified reference tissue model with the cerebellum as the reference region. Binding Potentials ($BP = f_2 B_{Avail}/K_D$ where f_2 = free fraction of the radioligand in the tissue, B_{Avail} = concentration of available binding sites and K_D = equilibrium dissociation rate constant of the radioligand) were calculated for midbrain and corticolimbic regions of interest (ROIs).

3.3.5 Data Analysis

In order to analyse the effect of the –1018 C>G SNP and 5-HTTLPR polymorphisms on 5-HT_{1A} receptor binding, the general linear model (GLM) was performed with genotype, gender, and scanner as between subjects independent variables and 21 different brain regions and a global measure (mean 5-HT_{1A} BPs of 20 postsynaptic regions) as within-subjects dependent variables using SPSS version 11.0 statistical software (SPSS Inc, Chicago, IL, USA). This statistical approach allowed us to examine global effects of genotype, gender, or scanner on 5-HT_{1A} BP and to correct statistically for repeated measurements of BP.

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[528] Rabiner, E.A., Wilkins, M. R., Turkheimer, F., Gunn, R. N., de Haes, J. U., de Vries, M., Grasby, P. M. (2002). '5-Hydroxytryptamine1A Receptor Occupancy by Novel Full Antagonist 2-[4-[4-(7-chloro-2,3-dihydro-1,4-benzdioxyn-5-yl)-1-piperazinyl]butyl]-1, 2-benzisothiazol-3-(2H)-one-1,1-dioxide: a[11C][O-methyl-3H]-N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-py ridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635) Positron Emission Tomography Study in Humans', *J Pharmacol Exp Ther.* **301**(3): pp. 1144-50; Rabiner, E.A., Messa, C., Sargent, P. A., Husted-Kjaer, K., Montgomery, A., Lawrence, A. D., Bench, C. J., Gunn, R. N., Cowen, P., Grasby, P. M. (2002). 'A Database of [(11)C]WAY-100635 Binding to 5-HT(1A) Receptors in Normal Male Volunteers: Normative Data and Relationship to Methodological, Demographic, Physiological, and Behavioral Variables', Neuroimage, **15**(3): pp. 620-32.

3.4 Results

Results from the genotyping of the healthy OXCHECK Caucasian panel are presented in Table 3.3. Of the nine loci in the 5-HT_{1A}R gene identified from public databases only four were found to be polymorphic in this population (-1018 C>G, +82 A>G, +549 C>T, +656 G>T). Therefore, the allele frequencies for only these four polymorphisms are presented in Table 3.3. The other five polymorphisms (+47 C>T, +64 A>G, +294 G>A, +815 G>A, +1254 C>G) were present only in the homozygous wildtype form in this sample of healthy Caucasian volunteers. Only one SNP at the site (-1018) C>G was sufficiently common to allow group comparisons in the PET group.

Table 3.3

Genotype Frequencies for 5-HT_{1A} SNPs from Population of Healthy Caucasian Volunteers

Healthy Caucasian Volunteers (n = 140)				
Locus	Genotype	Frequency (n = 140)	%	Hardy-Weinberg (*P* value)
-1018 C>G	CC	36	25.7	0.484
	CG	74	52.9	
	GG	30	21.4	
+82 A>G	AA	137	97.9	0.898
	AG	3	2.1	
	GG	0	0	
+549 C>T	CC	138	98.6	0.932
	CT	2	1.4	
	TT	0	0	
+656 G>T	GG	135	96.4	0.830
	GT	5	3.6	
	TT	0	0	

LEGEND: Genotype frequencies and percentages for 140 healthy Caucasian controls from the greater Oxfordshire, UK region are listed. Genetic variation within this population was observed in only four of nine SNPs, for which Hardy-Weinberg equation values are indicated.

Genotype frequencies of the -1018 C>G 5-HT_{1A} and 5-HTTLPR polymorphisms in the PET group of subjects conformed to the Hardy-Weinberg equilibrium (5-HT_{1A}: $X^2 = 0.10$, $p = 0.77$; 5-HTTLPR: $X^2 < 0.01$, p = 0.88).

Results from the GLM analyses, described in Section 3.3.5 examining the effect of the 5-HT_{1A} -1018 SNP on 5-HT_{1A}R BP indicated that there was no significant effect of 5-HT_{1A} – 1018 genotype on 5-HT_{1A}R BP values ($F = 0.001$, df 1,28 $p = 0.973$). Furthermore, there was not a significant effect of scanner ($F = 0.16$, df 1,28 $p = 0.9$) or gender ($F = 2.1$, df 1,28, $p = 0.2$) on 5-HT_{1A}R BP. We also did not detect any interaction effects for allele by scanner ($F = 1.1$, $df = 1,28$, $p = 0.3$), allele by gender ($F = 0.6$, df 1,28 $p = 0.4$) or scanner by gender ($F = 1.7$, df 1,28, $p = 0.2$). However, there was a significant main effect of 5-HTTLPR on 5-HT_{1A}R BP ($F = 4.8$, df 1,27 p = 0.037). Again, there were no significant effects of scanner ($F = 2.3$, df 1,27, $p = 0.1$), or gender ($F = 1.4$, df 1,27, $p = 0.3$), nor were there any significant allele by scanner ($F = 0.3$, df 1,27, $p = 0.6$), allele by gender ($F = 2.5$, df 1,27, p = 0.1), scanner by gender ($F = 0.6$, df 1,27, $p = 0.5$), or allele by scanner by gender interactions ($F = 0.4$, df 1,27, $p = 0.5$). Table 3.4 presents presynaptic (raphe) and global postsynaptic 5-HT_{1A}R BP by 5-HT_{1A}R and 5-HTTLPR genotypes.

Next, *post-hoc* independent t-tests were performed for each ROI comparing subjects with 5-HTTLPR SS or SL to LL genotypes to further evaluate the nature of the differences in 5-HT_{1A}R BP by 5-HTTLPR. 5-HT_{1A}R BP was significantly greater for those with LL genotypes than LS or SS genotypes at 11 of 21 ROIs examined (Table 3.5). However, after adjusting for multiple comparisons, univariate t-tests were robust only at the insula. For illustrative purposes, comparisons of 5-HT_{1A}R BP are depicted graphically for the insula,

which demonstrated the greatest difference in BP by genotype (Figure 3.2) and with an [^{11}C] WAY 100635 BP PET image in Figure 3.3.

Table 3.4

Global Postsynaptic and Presynaptic 5-HT$_{1A}$ Receptor Binding Potential by 5-HTTLPR and 5-HT$_{1A}$ –1018 Genotypes Utilising [^{11}C]WAY-100635 PET

A. Planned Comparisons for 5-HTTLPR and 5-HT$_{1A}$ –1018 Polymorphisms

Polymorphism	Region	SS or SL (N = 27)	LL (N = 8)
5-HTTLPR	Raphe	3.8 (0.9)	4.2 (0.8)
	Postsynaptic†	4.2 (0.5)	4.7 (0.6)
	Region	**GG+CG (N = 24)**	**CC (N = 11)**
5-HT$_{1A}$ (-1018C>G)	Raphe	3.9 (0.9)	3.7 (0.9)
	Postsynaptic†	4.4 (0.5)	4.1 (0.5)

B. Binding Potential by 5-HTTLPR and 5-HT$_{1A}$ –1018 Polymorphisms (all strata)

Polymorphism	Region	SS (N = 10)	SL (N = 17)	LL (N = 8)
5-HTTLPR	Raphe	3.7 (1.0)	3.8 (0.9)	4.2 (0.8)
	Postsynaptic†	4.1 (0.5)	4.3 (0.5)	4.7 (0.6)
	Region	**GG (N = 6)**	**CG (N = 18)**	**CC (N = 11)**
5-HT$_{1A}$ (-1018C>G)	Raphe	4.2(0.7)	3.8(1.0)	3.7(0.9)
	Postsynaptic†	4.4(0.5)	4.4(0.6)	4.1(0.5)

LEGEND: Values shown are mean and standard deviation (SD). (A) 5-HT$_{1A}$R BP by dichotomised -1018 5-HT$_{1A}$ G>C and 5-HTTLPR genotypes. (B) 5-HT$_{1A}$ receptor BP by genotype (not-dichotomised). † Global postsynaptic binding portential (BP) is reported as the mean and standard deviation (SD) BP values of 20 cortical regions of interest. Statistical results from repeated-measures ANOVA in Section 3.4.

Given the sample size, we were not able to formally test for gene x gene interactions. However, I was interested in whether or not there was a suggestion of a gene x gene interaction between 5-HT$_{1A}$ and 5-HTT. There were only two subjects who had both the 5-HTTLPR SS and 5-HT$_{1A}$ –1018 GG genotypes. The mean BP in HTTLPR SS/5-HT$_{1A}$ –1018 GG subjects was 4.1 (SD = 0.5) (n = 2) compared to 3.9 (SD = 0.9) (n = 33) for all other subjects in the midbrain raphe.

Table 3.5

Comparisons of 5-HT_{1A} Receptor Binding Potential by Region and 5-HTTLPR Genotype

Region	5-HTTLPR		*Post-hoc* independent t-tests
	SS + SL (n = 27)	LL (n = 8)	
Hippocampus	5.74 (5.36, 6.12)	6.04 (5.55, 6.53)	0.409
Amygdala	5.16 (4.89, 5.43)	5.57 (4.96, 6.18)	0.152
Anterior medial temporal lobe	4.78 (4.44, 5.12)	5.56 (4.91, 6.21)	0.029*
Anterior lateral temporal lobe	4.83 (4.50, 5.15)	5.57 (4.83, 6.30)	0.035*
Parahippocampus	5.77 (5.50, 6.03)	6.36 (5.92, 6.80)	0.029*
Superior temporal gyrus	4.76 (4.53, 4.99)	5.17 (4.60, 5.75)	0.101
Medial inferior temporal gyrus	4.89 (4.65, 5.12)	5.31 (4.76, 5.87)	0.098
Fusiform gyrus	5.88 (5.53, 6.23)	6.50 (5.89, 7.04)	0.094
Posterior temporal gyrus	4.41 (4.19, 4.63)	4.89 (4.33, 5.44)	0.050
Insula	5.20 (4.95, 5.45)	6.00 (5.52, 6.49)	0.003‡
Anterior cingulate	4.90 (4.64, 5.16)	5.60 (5.06, 6.15)	0.012*
Posterior cingulate	3.83 (3.50, 4.17)	4.23 (3.68, 4.78)	0.233
Parietal lobe	3.95 (3.75, 4.14)	4.45 (3.98, 4.93)	0.020*
Occipital lobe	3.06 (2.87, 3.25)	3.43 (3.04, 3.83)	0.062
Orbitofrontal gyrus	4.25 (3.97, 4.54)	4.92 (4.24, 5.59)	0.032*
Gyrus frontomedialis	4.40 (4.20, 4.60)	4.90 (4.39, 5.41)	0.026
Gyrus precentralis	4.38 (4.16, 4.59)	5.01 (4.52, 5.50)	0.008†
Gyrus frontoinferior	3.72 (3.55, 3.89)	4.27 (3.77, 4.78)	0.007†
Gyrus frontomedius	3.82 (3.62, 4.02)	4.35 (3.89, 4.81)	0.017*
Gyrus frontosuperior	3.73 (3.55, 3.92)	4.21 (3.73, 4.69)	0.022*
Raphe	3.79 (3.41, 4.16)	4.20 (3.54, 4.87)	0.264
Mean postsynaptic	4.23 (4.02, 4.44)	4.67 (4.21, 5.13)	0.048*

Values are mean 5-HT_{1A} BP and 95% confidence intervals (95% CI).
***Significant *post hoc* t tests of healthy volunteers with SS or SL vs. LL genotypes, $p < 0.05$.**
† Significant *post hoc* t tests of healthy volunteers with SS or SL vs. LL genotypes, $p < 0.01$.
‡ Significant *post hoc* t tests of healthy volunteers with SS or SL vs. LL genotypes, $p < 0.005$.

The global postsynaptic BP of HTTLPR SS/5-HT_{1A} –1018GG subjects was 4.7 (SD = 0.3) compared to 4.3 (SD = 0.6) for the remaining subjects. Whilst the higher BP in these subjects is consistent with the known effects of both polymorphisms on transcription, one cannot make any inferences with this sample size.

As some investigators have proposed a recessive model for the 5-HT_{1A} –1018 G>C polymorphism,[529] GLM was performed, as described in Section 3.3.5, using a recessive inheritance model for the G allele (i.e., dichotomised as 5-HT_{1A} –1018 GG vs. CC or CG). Again, there was no main effect of –1018 5-HT_{1A} genotype ($F = 0.002$, df 1,28 $p = 0.962$), scanner ($F = 0.582$, df 1,28 $p = 0.452$), or gender ($F = 2.376$, df 1,28, $p = 0.134$) on 5-HT_{1A}R BP. Furthermore, there were no interaction effects for allele by scanner ($F = 1.192$, df 1,28 $p = 0.284$), allele by gender ($F = 1.22$, df 1,28 $p = 0.279$), or scanner by gender ($F = 2.487$, df 1,28 $p = 0.126$). The 5-HT_{1A}R BP values by–1018 5-HT_{1A} genotype are depicted in Table 3.6 below. Therefore in sum, there were no associations between –1018 5-HT_{1A} genotype using either the dominant or recessive models or 5-HT_{1A}R BP in our sample.

Table 3.6

Global Postsynaptic and Presynaptic 5-HT_{1A} Receptor Binding Potential by 5-HT_{1A} – 1018 Genotype with Recessive Inheritance Model Utilising [^{11}C]WAY-100635 PET

A. CC/CG vs. GG

Polymorphism	Region	GG (N = 6)	CC+CG (N = 29)
5-HT_{1A} (-1018C>G)	Raphe	4.2 (0.7)	3.8 (0.9)
	Postsynaptic†	4.4 (0.5)	4.3 (0.6)

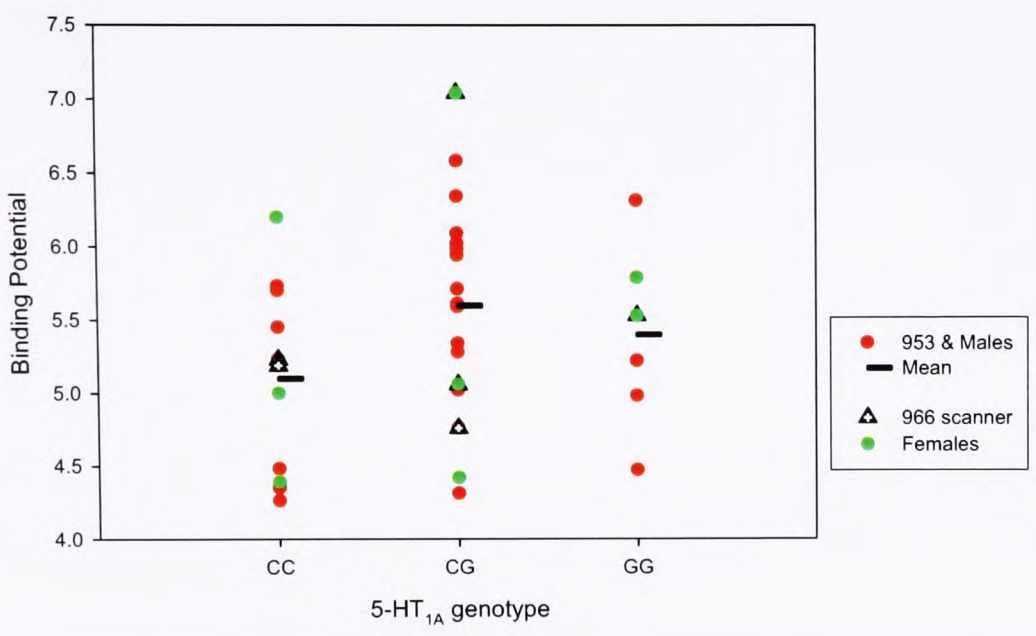
LEGEND: Comparison of 5-HT1A binding potential by 5-HT1A –1018 genotype using alternative model of recessive inheritance. Values shown are mean (SD). † Mean and standard deviation (SD) BP values of 20 corticolimbic ROIs.

[529] Lemonde, S., Du, L., Bakish, D., Hrdina, P., Albert, P. R. (2004).

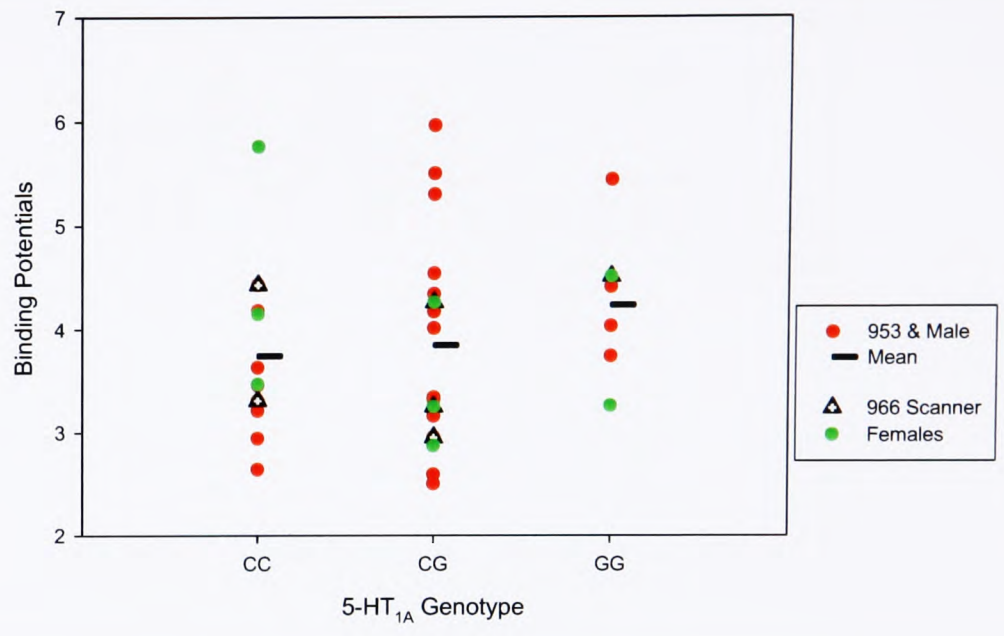
Figure 3.1

Allelic Comparison of [^{11}C] WAY 100635 Binding Potentials by 5-HT$_{1A}$ –1018 Genotype

a. Insula



b. Midbrain Raphe

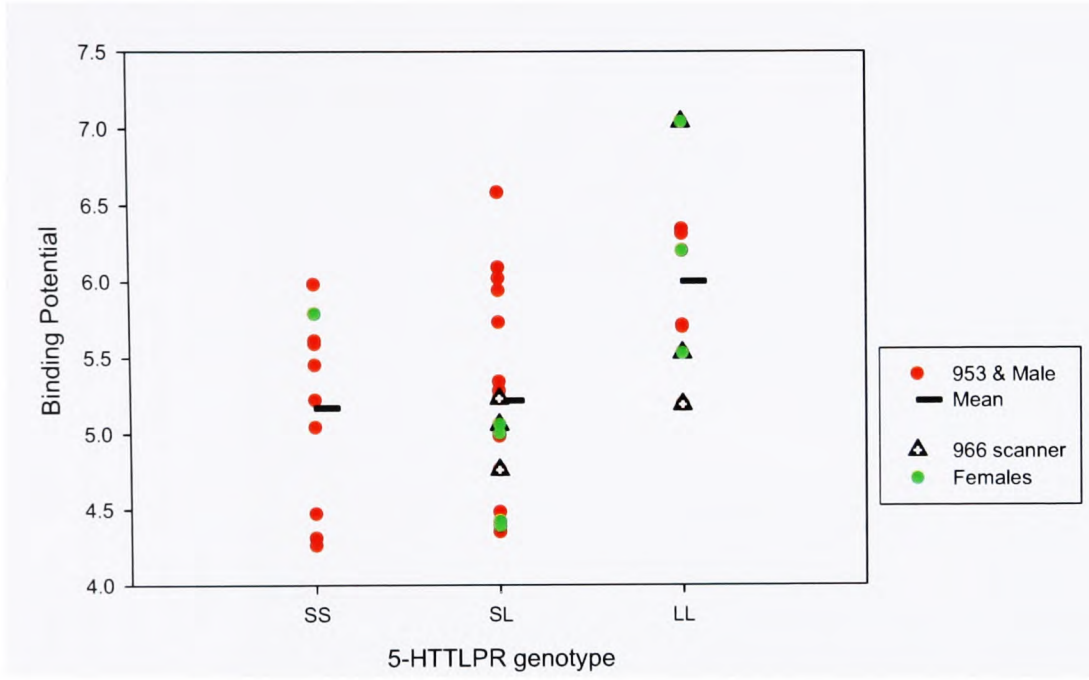


LEGEND: Scatter plot values of [^{11}C] WAY 100635 BP values by 5-HT$_{1A}$R genotype for a post-synaptic ROI (insula) (a) and a pre-synaptic ROI (midbrain raphe) (b). Green circles indicate female and red circles indicate male subjects. Triangles represent ECAT 953 and circles represent ECAT 966 PET scanners, respectively.

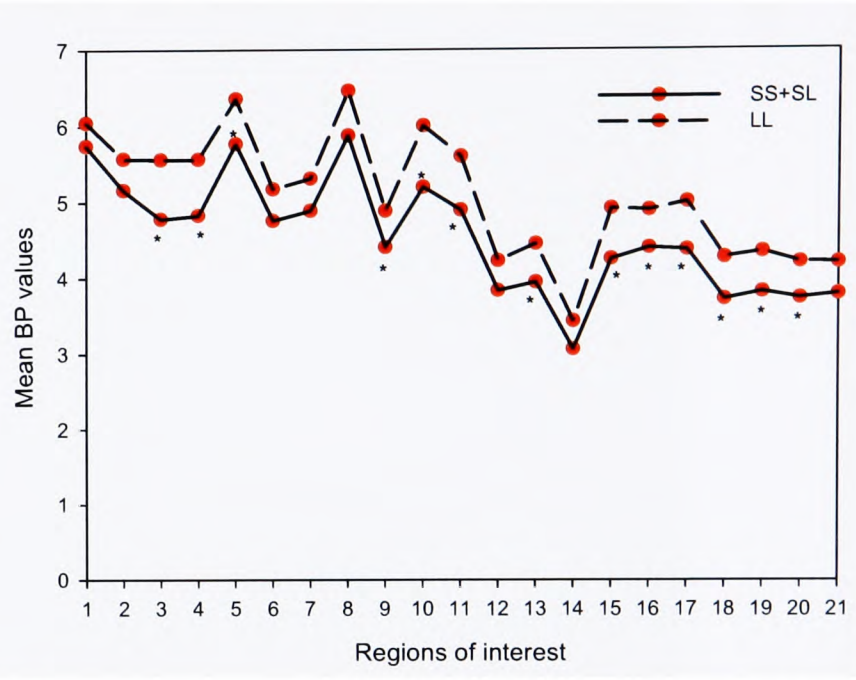
Figure 3.2

Genotypic and Allelic Comparison of [^{11}C]WAY 100635 Binding Potentials by 5-HTTLPR

a. Insula



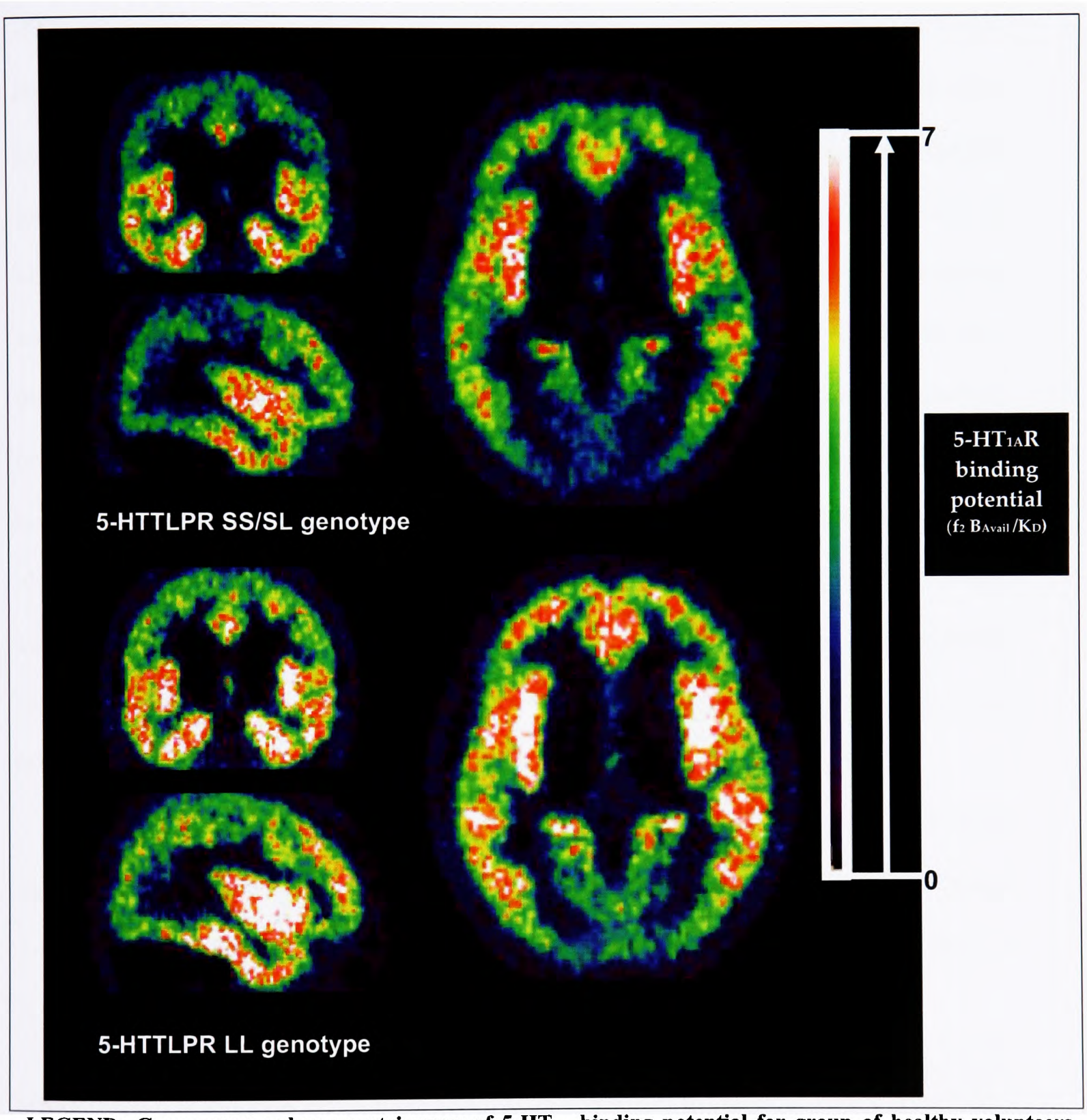
b. All regions of interest



LEGEND: Figure 3.2 (a) is a scatter plot of scatter plot values of [^{11}C] WAY 100635 BP values by 5-HTTLPR alleles. (b) Line graph of $5HT_{1A}R$ BP by 5-HTTLPR alleles (S vs. L), * Significant group difference following *post-hoc* t-test at $p < 0.01$. The numbers in figure 3.2 (b) correspond to the following regions: 1. Hippocampus, 2. Amygdala, 3. Anterior medial temporal lobe, 4. Anterior lateral temporal lobe, 5. Parahippocampus, 6. Superior temporal gyrus, 7. Medial inferior temporal gyrus, 8. Fusiform gyrus, 9. Posterior temporal gyrus, 10. Insula, 11. Anterior cingulate, 12. Posterior cingulate, 13. Parietal lobe, 14. Occipital lobe, 15. Orbitofrontal gyrus, 16. Gyrus frontomedialis, 17. Gyrus precentralis, 18. Gyrus frontoinferior, 19. Gyrus frontomedius, 20. Gyrus frontosuperior, 21. Raphe.

Figure 3.3

[^{11}C]WAY-100635 PET Comparison of 5-HT$_{1A}$ Receptor Binding Potential by 5-HTTLPR Genotype



LEGEND: Group-averaged parametric map of 5-HT$_{1A}$ binding potential for group of healthy volunteers possessing the 5-HTTLPR SS or SL genotypes (top) and LL genotype (bottom) positron emission tomography using [^{11}C]WAY-100635. Coronal, sagitall (right) and axial (left) cortical brain slices normalised to Montreal Neurological Institute (MNI) space. Colour-bar (right) represents binding potential. Orthogonal coordinates in MNI space were axial: (z = +36, sagittal: x = +65 and coronal: y = +60).

3.5 Discussion

This is the first human study to demonstrate effects of the 5-HTTLPR polymorphism on a functionally related but distinct receptor. However, the association between carriers of the 5-HTTLPR S allele and lower 5-HT_{1A}R binding is entirely consistent with the work of Li and colleagues who found a region-specific reduction in 5-HT_{1A}R density in the dorsal raphe nucleus (DRN) in male and female 5-HTT KO mice. In addition, Li found sex-specific reductions in the Am and hypothalamus only in the female HTT KO mice.[530] However, in our study of healthy human volunteers, there was no significant gender effect, although the number of females in the study was small (n = 8). Furthermore, the region-specific effects of the 5-HTTLPR were more widespread in our study than reported in KO mice, particularly in cortical areas. However, 5-HT_{1A}R density measurements were only recorded in a few cortical areas by Li and colleagues in KO mice. The reduction in 5-HT_{1A}R BP in S carriers is also consistent with studies demonstrating that pharmacological blockade of 5-HTTs with selective serotonin reuptake inhibitors[531] or in 5-HTT KO mice[532] leads to desensitisation (decreased physiological responsiveness to stimulation of 5-HT_{1A}Rs) in raphe and hypothalamus.

A biologically plausible mechanism to explain the effect of the 5-HTTLPR on BP might be that the lower transcriptional efficiency associated with the S allele may lead to decreased 5-HTT function which in turn may lead to a lifelong increase in 5-HT tone, which may in

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[530] Li, Q., Wichems, C., Heils, A., Lesch, K. P., Murphy, D. L. (2000).

[531] Li, Q., Muma, N. A., Battaglia, G., Van de Kar, L. D. (1997); Le Poul, E., Laaris, N., Doucet, E., Laporte, A. M., Hamon, M., Lanfumey, L. (1995). 'Early Desensitization of Somato-Dendritic 5-HT1A Autoreceptors in Rats Treated with Fluoxetine or Paroxetine', *Naunyn Schmiedebergs Arch Pharmacol*, **352**(2): pp. 141-8; Li, Q., Muma, N. A., van de Kar, L. D. (1996). 'Chronic Fluoxetine Induces a Gradual Desensitization of 5-HT1A Receptors: Reductions in Hypothalamic and Midbrain Gi and G(o) Proteins and in Neuroendocrine Responses to a 5-HT1A Agonist', *J Pharmacol Exp Ther.* **279**(2): pp. 1035-42; Blier, P., Pineyro, G., el Mansari, M., Bergeron, R., de Montigny, C. (1998). 'Role of Somatodendritic 5-HT Autoreceptors in Modulating 5-HT Neurotransmission', *Ann N Y Acad Sci.* **861**: pp. 204-16.

[532] Li, Q., Wichems, C., Heils, A., Lesch, K. P., Murphy, D. L. (2000).

turn result in desensitisation and downregulation of 5-HT_{1A}Rs. This hypothesis would be consistent with the effects of genetic or pharmacological blockade demonstrated in mice.

In addition to gender, we studied the potential confounding effects of age because two studies[533] have shown an inverse relationship between age and [^{11}C]WAY-100635 binding (the former study showed this effect only in men); however, two other studies[534] have found no such relationship. In this study, when age was included as a covariate in the ANOVA model, there was no significant age effect (F = 2.2; df = 1, 32; p = 0.147), and the allele effect of 5-HTTLPR on [^{11}C] WAY 100635 binding remained significant (F = 5.7; df = 1, 32; p = 0.023).

I had hypothesised that the 5-HT_{1A} –1018 SNP would influence 5-HT_{1A}R BP given the effect of the 5-HT_{1A} –1018 G allele on transcription as demonstrated *in vitro*.[535] The lack of an effect of the allele argues against the recent intriguing hypothesis that depressed patients with this polymorphism would show increased 5-HT_{1A} autoreceptor expression at the raphe nucleus thus mediating increased inhibition of serotonergic neurons.[536] However, given our sample size it would not be reasonable to conclude that the 5-HT_{1A} –1018 G allele has no effect on 5-HT_{1A}R BP. Moreover, it is possible that partial volume effects may have reduced

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[533] Cidis Meltzer, C., Drevets, W. C., Price, J. C., Mathis, C. A., Lopresti, B., Greer, P. J., Villemagne, V. L., Holt, D., Mason, N. S., Houck, P. R., Reynolds, C. F., 3rd, DeKosky, S. T. (2001). 'Gender-Specific Aging Effects on the Serotonin 1A Receptor', *Brain Res*, **895**(1-2): pp. 9-17; Tauscher, J., Verhoeff, N. P., Christensen, B. K., Hussey, D., Meyer, J. H., Kecojevic, A., Javanmard, M., Kasper, S., Kapur, S. (2001). 'Serotonin 5-HT1A Receptor Binding Potential Declines with Age as Measured by [11C]WAY-100635 and PET', *Neuropsychopharmacology*, **24**(5): pp. 522-30.

[534] Parsey, R.V., Oquendo, M. A., Simpson, N. R., Ogden, R. T., Van Heertum, R., Arango, V., Mann, J. J. (2002). 'Effects of Sex, Age, and Aggressive Traits in Man on Brain Serotonin 5-HT1A Receptor Binding Potential Measured by PET Using [C-11]WAY-100635', *Brain Res.* **954**(2): pp. 173-82; Rabiner, E.A., Messa, C., Sargent, P. A., Husted-Kjaer, K., Montgomery, A., Lawrence, A. D., Bench, C. J., Gunn, R. N., Cowen, P., Grasby, P. M. (2002).

[535] Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P. D., Bown, C. D., Sequeira, A., Kushwaha, N., Morris, S. J., Basak, A., Ou, X. M., Albert, P. R. (2003).

[536] Ibid.

the sensitivity of detection of group differences in BP values in the measurement of a small structure such as the raphe nucleus.

Many but not all studies have suggested an association between the S allele and abnormal mood states/emotional behaviours,[537] depressive illness,[538] severity of depressive symptoms in Parkinson's disease,[539] suicidality,[540] neuroticism,[541] and smoking behaviour.[542] Reduced 5-HTT availability (as found in those with S allele) has been demonstrated in living depressed patients.[543] The results of this study suggest that the putative association of the S allele with emotional behaviours and mood disorders may be mediated in part via reductions of 5-HT_{1A}R density.

The limitations of this study include its retrospective nature, small subject numbers, and the use of conventional *p* values. However, the number of subjects who can be scanned using PET, as opposed to those who can be genotyped, is limited because of the cost constraints and radiation exposure. A sample size of 35 subjects is small for a genetic study, but it is a

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[537] Lesch, K.P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., Murphy, D. L. (1996); Munafo', M.R., Clark, T. G., Moore, L. R., Payne, E., Walton, R., Flint, J. (2003); Mazzanti, C.M., Lappalainen, J., Long, J. C., Bengel, D., Naukkarinen, H., Eggert, M., Virkkunen, M., Linnoila, M., Goldman, D. (1998). 'Role of the Serotonin Transporter Promoter Polymorphism in Anxiety-Related Traits', *Arch Gen Psychiatry*, **55**(10): pp. 936-40.

[538] Willeit, M., Praschak-Rieder, N., Neumeister, A., Zill, P., Leisch, F., Stastny, J., Hilger, E., Thierry, N., Konstantinidis, A., Winkler, D., Fuchs, K., Sieghart, W., Aschauer, H., Ackenheil, M., Bondy, B., Kasper, S. (2003)

[539] Mossner, R., Henneberg, A., Schmitt, A., Syagailo, Y. V., Grassle, M., Hennig, T., Simantov, R., Gerlach, M., Riederer, P., Lesch, K. P. (2001). 'Allelic Variation of Serotonin Transporter Expression is Associated with Depression in Parkinson's Disease', *Mol Psychiatry*, **6**(3): pp. 350-2.

[540] Anguelova, M., Benkelfat, C., Turecki, G. (2003). 'A Systematic Review of Association Studies Investigating Genes Coding for Serotonin Receptors and the Serotonin Transporter: II. Suicidal behavior', *Mol Psychiatry*, **8**(7): pp. 646-53.

[541] Sen, S., Burmeister, M., Ghosh, D. (2004). 'Meta-Analysis of the Association Between a Serotonin Transporter Promoter Polymorphism (5-HTTLPR) and Anxiety-Related Personality Traits', *Am J Med Genet B Neuropsychiatr Genet.* **127**(1): pp. 85-9.

[542] Munafo', M., Clark, T., Johnstone, E., Murphy, M., Walton, R. (2004); Lerman, C., Caporaso, N. E., Audrain, J., Main, D., Boyd, N. R., Shields, P. G. (2000). 'Interacting Effects of the Serotonin Transporter Gene and Neuroticism in Smoking Practices and Nicotine Dependence', *Mol Psychiatry*, **5**(2): pp. 189-92; Lerman, C., Shields, P. G., Audrain, J., Main, D., Cobb, B., Boyd, N. R., Caporaso, N. (1998). 'The Role of the Serotonin Transporter Gene in Cigarette Smoking', *Cancer Epidemiol Biomarkers Prev.* **7**(3): pp. 253-5.

[543] Stockmeier, C.A. (2003). 'Involvement of Serotonin in Depression: Evidence from Postmortem and Imaging Studies of Serotonin Receptors and the Serotonin Transporter', *J Psychiatr Res.* **37**(5): pp. 357-73.

large sample for a PET study and is consistent with other recent functional neuroimaging studies showing plausible associations between genotype and other imaging approaches such as the use of functional magnetic resonance imaging (fMRI) BOLD response to emotional face recognition.[544]

Calculations of observed statistical power demonstrated that, for the 5-HT_{1A} -1018 C>G polymorphism, there was a power of 89% to detect a 15% difference of BP between groups with $\alpha = 0.05$. Thus, despite adequate power and a conventional p value we did not find an association between the 5-HT_{1A} -1018 C>G SNP and 5-HT_{1A}R BP. However, for the 5-HTTLPR, we only had a power of 59% to detect the observed 1 SD difference in BP between groups, with $\alpha = 0.05$. Therefore, despite a low statistical power, we found a significant association between 5-HTTLPR and HT_{1A}R binding. These two genetic markers were chosen on the basis of rigorous biological hypotheses derived from elegant *in vitro* studies.[545] In this case, the prior probability of association is likely to be higher for these polymorphisms than in studies without predetermined hypotheses, such as genome-wide scans. As pointed out by Wacholder and colleagues, the likelihood of a false-positive result is a function of the prior probability of the hypothesised association being meaningful, which in this case is based on published data of the functional effects of the specific polymorphisms.[546] Indeed, we integrated animal functional data on the genetic variants into our hypotheses rather than randomly selecting genetic markers.

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[544] Hariri, A.R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M. F., Weinberger, D. R. (2002). 'Serotonin Transporter Genetic Variation and the Response of the Human Amygdala', *Science*, **297**(5580): pp. 400-3.

[545] Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P. D., Bown, C. D., Sequeira, A., Kushwaha, N., Morris, S. J., Basak, A., Ou, X. M., Albert, P. R. (2003); Li, Q., Wichems, C., Heils, A., Lesch, K. P., Murphy, D. L. (2000).

[546] Wacholder, S., Chanock, S., Garcia-Closas, M., El Ghormli, L., Rothman, N. (2004). 'Assessing the Probability that a Positive Report is False: An Approach for Molecular Epidemiology Studies', *J Natl Cancer Inst.* **96**(6): pp. 434-32.

In summary, this *in vivo* human imaging study shows genetic effects can extend beyond the receptor targeted by the gene to functionally related systems and more specifically provides a plausible mechanistic explanation as to how 5-HTTLPR allelic frequencies may influence the expression of dysfunctional moods and personality traits.

3.6 Future Directions

My first hypothesis anticipating a genetic association between the 5-HT_{1A} –1018 C>G polymorphism and 5-HT_{1A}R bp was not supported by the results of this study. My second hypothesis, predicted an interaction between the 5-HTTLPR and the 5-HT_{1A} –1018 C>G polymorphism, however, rather than a demonstrable gene x gene interaction, there was a robust main effect of the 5-HTTLPR on 5-HT_{1A} R BP. This observation provided a useful lesson in the importance of applying rigorous basic research from animal models such as that of Li and colleagues, as the theoretical effect of the 5-HTTLPR on 5-HT_{1A} R BP indeed demonstrated a widespread main effect.[547]

I was particularly interested in the 5-HT_{1A}R given the emerging corpus of evidence implicating somatodendritic 5-HT_{1A}Rs in mediating the acute and chronic effects of nicotine exposure. Whilst we did not have ROI data on the striatum specifically, it is intriguing to observe that some of the largest effects of the 5-HTTLPR on 5-HT_{1A}R binding were in mesocorticolimbic regions such as the anterior cingulate gyrus, orbitofrontal gyrus, and medial prefrontal cortex. As described in Chapter 2, these regions demonstrated the highest BOLD contrast associated with smoking-related pictorial cues in the group of smokers during nicotine withdrawal. Thus, the results of this PET study provide a justification for considering the 5-HTTLPR as a candidate gene with utility in examining genetic influences

[547] Li, Q., Wichems, C., Heils, A., Lesch, K. P., Murphy, D. L. (2000).

on cue-elicited BOLD response to smoking-related pictorial cues. As smoking is a complex behaviour under the influence of a wide range of genetic and environmental determinants, it is unlikely that any single candidate gene would explain more than a small portion of the inter-individual variance in BOLD contrast observed in the fMRI study described in Chapter 2. However, these results provide the basis for studying, in exploratory fashion, the influence of trait variables such as 5-HTTLPR genotype on cue-elicited activation of the VS/NAc. Furthermore, evidence suggesting an important interaction between nicotine and 5-HT in the development of incentive sensitisation, as described in Chapter 1, imply that state variables such as acute nicotine administration vs. abstinence are, in addition to genotype, important moderators of VS/NAc activation.

Chapter 4.

FUNCTIONAL MAGNETIC RESONANCE IMAGING INVESTIGATION OF THE INFLUENCE OF THE SEROTONIN TRANSPORTER 5-HTTLPR POLYMORPHISM ON NUCLEUS ACCUMBENS ACTIVATION TO SMOKING-RELATED PICTORIAL CUES

4.1 Introduction

The overall aim of this pilot study was to:

Triangulate results from both arms of research in order to examine whether or not a trait variable (5-HTTLPR genotype) or a state variable (smoking condition) would significantly influence cue-elicited activation of the ventral striatum including the nucleus accumbens (VS/NAc).

In Chapter 2, I described a functional magnetic resonance imaging (fMRI) study demonstrating that smoking-related picture cues activated a distributed reward network in smokers but not in non-smokers. Furthermore, activation in the VS/NAc was greater in smokers than non-smokers. As discussed in Chapter 1 Sections 1.2 and 1.3, habitual smokers become sensitised to nicotine such that neuroadaptations in the nucleus accumbens (NAc) mediate aversive affects such as craving or wanting of nicotine and that genetic variation in genes encoding receptors and transporters associated with DA and 5-HT neurotransmission are associated with nicotine dependence through, as yet, undefined mechanisms.

In Chapter 3, I demonstrated that a candidate gene polymorphism for nicotine dependence (5-HTTLPR), which influences treatment response to nicotine replacement therapy[548] and is associated with mood disorders and smoking-related personality traits, affects serotonin 5-HT_{1A} receptor binding presynaptically and postsynaptically throughout the brain. As described in Chapter 1 Sections 1.2 through 1.4, nicotine and 5-HT_{1A} receptors interact in numerous ways to mediate anxiety, nicotine withdrawal, and conditioned sensitisation to smoking-related stimuli. Nicotine-5-HT1A receptor interactions have

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[548] David, S.P., Murthy, N.V., Rabiner, E.A., Munafo', M., Jacob, R., Johnstone, E., Grasby, P.M. (2004). 'Serotonin Transporter Polymorphism Linked to Personality Traits Affects Serotonin (5-HT1A) Receptor Function in Positron Emission Tomography Study', paper presented at the North American Primary Care Research Group's (NAPCRG) 32nd annual meeting in Orlando, FL, USA.

potential clinical implications as the 5-HTTLPR, which appears to influence 5-HT_{1A} binding, influences treatment response to the nicotine patch for smoking cessation.[549]

Therefore, given this background and the overall need to develop more efficacious smoking cessation therapies, I chose to explore the influence of a specific state variable (nicotine) and trait variable (5-HTTLPR genotype) on cue-elicited activation of the VS/NAc. More specifically, the sub-aims of this study were to:

1. Examine whether or not there is a difference in the magnitude of cue-elicited BOLD response to smoking-related pictorial stimuli in the VS/NAc between conditions of nicotine abstinence and satiation.

2. To determine whether or not the serotonin transporter 5-HTTLPR polymorphism influences cue-elicited BOLD response to smoking-related picture cues.

4.2 Background

The aetiology of nicotine dependence, as demonstrated by converging evidence from *in vitro* and *in vivo* studies in human and non-human primates, is extremely complex and exhibits marked inter-individual variability. Of paramount importance in the ability to develop more efficacious smoking cessation therapies is the elucidation of the neurobiological processes underlying nicotine craving. This assertion is based on the observation that cue-elicited craving reliably predicts relapse to all drugs of abuse including nicotine[550] and that the low

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[549] David, S.P., Murthy, N.V., Rabiner, E.A., Munafo', M., Jacob, R., Johnstone, E., Grasby, P.M. (2004).

[550] Niaura, R., Shadel, W. G., Abrams, D. B., Monti, P. M., Rohsenow, D. J., Sirota, A. (1998). 'Individual Differences in Cue Reactivity Among Smokers Trying to Quit: Effects of Gender and Cue Type', *Addict Behav.* **23**(2): pp. 209-24; Niaura, R., Abrams, D. B., Monti, P. M., Pedraza, M. (1989). 'Reactivity to High Risk Situations and Smoking Cessation Outcome', *J Subst Abuse*, **1**(4): pp. 393-405; Rohsenow, D.J., Niaura, R. S., Childress, A. R., Abrams, D. B., Monti, P. M. (1990). 'Cue Reactivity in Addictive Behaviors: Theoretical and Treatment Implications', *Int J Addict*, **25**(7A-8A): pp. 957-93; Hutchison, K.E., Niaura, R., Swift, R. (1999). 'Smoking Cues Decrease Prepulse Inhibition of the Startle Response and Increase Subjective Craving in Humans', *Exp Clin Psychopharmacol.* 7(3): pp. 250-6.

efficacy of current smoking cessation therapies is limited not by inability to quit smoking initially, but by the extremely high relapse rates accompanying environmental triggers to smoke.[551]

Animal and human studies have demonstrated that vulnerability to nicotine dependence is highly variable between individuals as is the sub-dimension of incentive sensitisation to smoking-related environmental stimuli. As described in Chapter 1, Section 1.2.1.2.3 *behavioural* and *neural* sensitisation to nicotine are under dopamine (DA) and accumbens-related brain circuitry.[552]

However, a growing chain of evidence supports the hypothesis that 5-HT-DA interactions affect nicotine-induced neural and behavioural sensitisation. Olausson, Engel, and Soderpalm posit that chronic nicotine use results in neuroadaptive changes leading to an "imbalance between DA and 5-HT neurotransmission" in regions involved with inhibitory and stimulatory control of incentive sensitisation (i.e., VS, amygdala (Am), and frontal cortex).[553] Whilst the precise mechanisms underlying such 5-HT-DA interactions have not been clearly elucidated in humans, converging evidence supports this hypothesis. First, the 5-HT_{1A} agonist 8-OH-DPAT inhibits nicotine-induced behavioural sensitisation in rats[554] and nicotine-induced behavioural sensitisation is inhibited by administration of the selective

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[551] Fiore, M.C. (2000). 'US Public Health Service Clinical Practice Guideline: Treating Tobacco Use and Dependence', *Respir Care*. **45**(10): pp. 1200-62.

[552] Robinson, T.E., Berridge, K. C. (2001). 'Incentive-Sensitization and Addiction', *Addiction*, **96**(1): pp. 103-14

[553] Olausson, P., Engel, J. A., Soderpalm, B. (2002). 'Involvement of Serotonin in Nicotine Dependence: Processes Relevant to Positive and Negative Regulation of Drug Intake', *Pharmacol Biochem Behav*. **71**(4): pp. 757-71.

[554] Olausson, P., Akesson, P., Engel, J. A., Soderpalm, B. (2001). 'Effects of 5-HT1A and 5-HT2 Receptor Agonists on the Behavioral and Neurochemical Consequences of Repeated Nicotine Treatment', *Eur J Pharmacol*. **420**(1): pp. 45-54.

serotonin reuptake inhibitor citalopram.[555] Second, administration of the 5-HT-releasing and 5-HTT-inhibiting agent fenfluramine appears to increase DA release in the striatum in humans.[556] Potential mechanisms through which serotonin might mediate nicotine-induced DA release are speculative, but evidence points to activation of somato-dendritic inhibitory 5-HT_{1A} autoreceptors in the dorsal raphe nucleus (DRN) as a locus for serotonin's putative influence on accumbal DA release as discussed in Section 1.2.1.3.3.

Precisely how nicotine stimulates 5-HT release in the DRN is not clear, as there is, as yet, no direct evidence for the presence of nAChRs on 5-HT neurones in the DRN. However, there is *in vitro* evidence suggesting that when nicotine binds to nAChRs on cholinergic terminals, the resulting ACh released may stimulate M_1 muscarinic ACh receptors on 5-HT neurones and increase 5-HT release in the DRN.[557] Another potential locus for 5-HT mediated regulation of behavioural and neural sensitisation is the striatum. Nicotine-induced 5-HT release in the DRN[558] results in decreased release of 5-HT in terminal fields such as the VS[559] and hippocampus.[560] Pre-synaptic 5-HT_3 receptors are present on pre-synaptic DA

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[555] Olausson, P., Engel, J. A., Soderpalm, B. (1999). 'Behavioral Sensitization to Nicotine is Associated with Behavioral Disinhibition; Counteraction by Citalopram', *Psychopharmacology* (Berl), **142**(2): pp. 111-9.

[556] Smith, G.S., Dewey, S. L., Brodie, J. D., Logan, J., Vitkun, S. A., Simkowitz, P., Schloesser, R., Alexoff, D. A., Hurley, A., Cooper, T., Volkow, N. D. (1997). 'Serotonergic Modulation of Dopamine Measured with [11C]raclopride and PET in Normal Human Subjects', *Am J Psychiatry*, **154**(4): pp. 490-6; Shoaib, M., Baumann, M. H., Rothman, R. B., Goldberg, S. R., Schindler, C. W. (1997). 'Behavioural and Neurochemical Characteristics of Phentermine and Fenfluramine Administered Separately and as a Mixture in Rats', *Psychopharmacology* (Berl), **131**(3): pp. 296-306.

[557] File, S.E., Cheeta, S., Kenny, P. J. (2000). 'Neurobiological Mechanisms by which Nicotine Mediates Different Types of Anxiety', *Eur J Pharmacol.* **393**(1-3): pp. 231-6; File, S.E., Kenny, P. J., Cheeta, S. (2000). 'The Role of the Dorsal Hippocampal Serotonergic and Cholinergic Systems in the Modulation of Anxiety', *Pharmacol Biochem Behav.* **66**(1): pp. 65-72; Kenny, P.J., File, S. E., Neal, M. J. (2000). 'Evidence for a Complex Influence of Nicotinic Acetylcholine Receptors on Hippocampal Serotonin Release', *J Neurochem.* **75**(6): pp. 2409-14.

[558] Cheeta, S., Irvine, E. E., Kenny, P. J., File, S. E. (2001). 'The Dorsal Raphe Nucleus is a Crucial Structure Mediating Nicotine's Anxiolytic Effects and the Development of Tolerance and Withdrawal Responses', *Psychopharmacology* (Berl), **155**(1): pp. 78-85.

[559] Sprouse, J.S., Aghajanian, G. K. (1987). 'Electrophysiological Responses of Serotoninergic Dorsal Raphe Neurons to 5-HT1A and 5-HT1B Agonists', *Synapse*, **1**(1): pp. 3-9.

[560] Benwell, M.E., Balfour, D. J. (1979). 'Effects of Nicotine Administration and Its Withdrawal on Plasma Corticosterone and Brain 5-hHydroxyindoles', *Psychopharmacology* (Berl). **63**(1): pp. 7-11; Benwell, M.E., Balfour, D. J., Anderson, J. M. (1990). 'Smoking-Associated Changes in the Serotonergic Systems of Discrete

terminals in the striatum[561] and stimulation of these receptors results in elevated striatal DA levels.[562] Thus, inhibition of 5-HT release in the striatum would be expected to result in reduced stimulation of pre-synaptic 5-HT_3 receptors and subsequently reduce the stimulation of striatal DA release. Post-synaptic 5-HT_{1A} receptors are also present in the NAc but there is, as yet, no evidence that they mediate accumbal DA release directly.[563]

Additional data from pharmacological and pharmacogenetic studies further implicate 5-HT_{1A} receptors as plausible mediators of incentive sensitisation to nicotine. Interventions resulting in decreased 5-HT neurotransmission such as neurotoxic 5-HT depletion or 5-HT synthesis inhibition result in increased consumption of many drugs of abuse in rats.[564] Conversely, interventions that increase 5-HT neurotransmission, such as selective serotonin reuptake inhibitors (SSRIs), have been shown to decrease compulsive drug use in rats, including that of nicotine,[565] alcohol,[566] heroin[567] and psychostimulants.[568] There is

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Regions of Human Brain', *Psychopharmacology* (Berl). **102**(1): pp. 68-72; Benwell, M.E., Balfour, D. J. (1982). 'The Effects of Nicotine Administration on 5-HT Uptake and Biosynthesis in Rat Brain', *Eur J Pharmacol.* **84**(1-2): pp. 71-7; Balfour, D.J., Ridley, D. L. (2000). 'The Effects of Nicotine on Neural Pathways Implicated in Depression: A Factor in Nicotine Addiction?' *Pharmacol Biochem Behav.* **66**(1): pp. 79-85.

[561] Nayak, S.V., Ronde, P., Spier, A. D., Lummis, S. C., Nichols, R. A. (2000). 'Nicotinic Receptors Co-Localize with 5-HT(3) Serotonin Receptors on Striatal Nerve Terminals', *Neuropharmacology*, **39**(13): pp. 2681-90.

[562] Benloucif, S., Keegan, M. J., Galloway, M. P. (1993). 'Serotonin-Facilitated Dopamine Release *In Vivo*: Pharmacological Characterization', *J Pharmacol Exp Ther.* **265**(1): pp. 373-7; Jiang, L.H., Ashby, C. R., Jr., Kasser, R. J., Wang, R. Y. (1990). 'The Effect of Intraventricular Administration of the 5-HT3 Receptor Agonist 2-Methylserotonin on the Release of Dopamine in the Nucleus Accumbens: An *In Vivo* Chronocoulometric Study', *Brain Res.* **513**(1): pp. 156-60.

[563] Rada, P.V., Mark, G. P., Hoebel, B. G. (1993). '*In Vivo* Modulation of Acetylcholine in the Nucleus Accumbens of Freely Moving Rats: I. Inhibition by Rerotonin', *Brain Res.* **619**(1-2): pp. 98-104.

[564] Engel, J.A., Fahlke, C., Hard, E., Johannessen, K., Svensson, L., Soderpalm, B. (1992). 'Serotonergic and Dopaminergic Involvement in Ethanol Intake', *Clin Neuropharmacol.* **15 Suppl 1 Pt A**: pp. 64A-65A; Roberts, D.C., Loh, E. A., Baker, G. B., Vickers, G. (1994). 'Lesions of Central Serotonin Systems Affect Responding on a Progressive Ratio Schedule Reinforced Either by Intravenous Cocaine or by Food', *Pharmacol Biochem Behav.* **49**(1): pp. 177-82.

[565] Opitz, K., Weischer, M. L. (1988). 'Volitional Oral Intake of Nicotine in Tupaias: Drug-Induced Alterations', *Drug Alcohol Depend*, **21**(2): pp. 99-104.

[566] LeMarquand, D., Pihl, R. O., Benkelfat, C. (1994). 'Serotonin and Alcohol Intake, Abuse, and Dependence: Findings of Animal Studies', *Biol Psychiatry*, **36**(6): pp. 395-421.

[567] Higgins, G.A., Wang, Y., Corrigall, W. A., Sellers, E. M. (1994). 'Influence of 5-HT3 Receptor Antagonists and the Indirect 5-HT Agonist, Dexfenfluramine, on Heroin Self-Administration in Rats', *Psychopharmacology* (Berl), **114**(4): pp. 611-9.

substantial evidence that the antidepressant effects of SSRIs and 5-HT_{1A} partial agonists result from desensitisation of 5-HT_{1A} receptors.[569] Interestingly, the efficacy of SSRIs for treatment of depression is reduced in individuals with 5-HTTLPR SS or SL genotypes relative to those with LL genotypes.[570]

In light of the results in Chapter 3, the differential efficacy of SSRIs for depression might be explained by the following mechanism: Individuals with 5-HTTLPR S alleles have increased lifelong 5-HT tone resulting from reduced expression of serotonin transporters (SERT), which might theoretically result in desensitisation of pre-synaptic and post-synaptic 5-HT_{1A} receptors.[571] Therefore, individuals carrying the 5-HTTLPR S allele, whose 5-HT_{1A} receptors are already "desensitised", would not have the same effect of SERT blockade by SSRIs as individuals with LL genotypes.

Following this logic, my working hypothesis of how individuals with 5-HTTLPR S alleles are more prone to nicotine dependence is the following: Individuals who carry the 5-HTTLPR S allele experience less nicotine-induced stimulation of 5-HT_{1A} inhibitory autoreceptors, rendering them less able to counteract nicotine-induced augmentation of

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[568] Porrino, L.J., Ritz, M. C., Goodman, N. L., Sharpe, L. G., Kuhar, M. J., Goldberg, S. R. (1989). 'Differential Effects of the Pharmacological Manipulation of Serotonin Systems on Cocaine and Amphetamine Self-Administration in Rats', *Life Sci.* **45**(17): pp. 1529-35.

[569] Blier, P., Ward, N. M. (2003). 'Is there a Role for 5-HT1A Agonists in the Treatment of Depression?' *Biol Psychiatry*, **53**(3): pp. 193-203; Pineyro, G., Blier, P. (1999). 'Autoregulation of Serotonin Neurons: Role in Antidepressant Drug Action', *Pharmacol Rev.* **51**(3): pp. 533-91; Rueter, L.E., Blier, P. (1999). 'Electrophysiological Examination of the Effects of Sustained Flibanserin Administration on Serotonin Receptors in Rat Brain', *Br J Pharmacol.* **126**(3): pp. 627-38.

[570] Smits, K.M., Smits, L. J., Schouten, J. S., Stelma, F. F., Nelemans, P., Prins, M. H. (2004). 'Influence of SERTPR and STin2 in the Serotonin Transporter Gene on the Effect of Selective Serotonin Reuptake Inhibitors in Depression: A Systematic Review', *Mol Psychiatry*, **9**(5): pp. 433-41.

[571] David, S.P., Murthy, N. V., Rabiner, E. A., Munafo', M. R., Johnstone, E. C., Jacob, R., Walton, R. T., Grasby, P. M. (2005). 'A Functional Genetic Variation of the Serotonin (5-HT) Transporter Affects 5-HT1A Receptor Binding in Humans', *J Neurosci.* **25**(10): pp. 2586-90.

accumbal DA release resulting from decreased striatal 5-HT_3 stimulation[572] and to inhibit behavioural sensitisation to nicotine.[573]

The hypothesis stated above applies to the effect of neuroadaptions on behavioural sensitisation resulting in cue-elicited reactivity to environmental stimuli associated with smoking. In the abstinent condition, in the absence of *nicotine*-induced accumbal DA overflow, there is evidence that drug-related picture cues activate the VS and other orbitofrontal regions in humans.[574] Glutamatergic afferents from the prefrontal cortex, hippocampus, and amygdala (Am) have been shown to converge with DA neurones on medium-sized densely spiny neurones in the NAc,[575] and serotonergic projections from the Am may also activate corticofrontal regions.[576] In the absence of nicotine-induced DA overflow, NAc activation would necessarily rely upon "top-down" processing employing visual-spatial extra-striate attention circuitry integrated with mesolimbic reward signalling circuitry independent of nAChR stimulation in the VTA. This assertion is supported by

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[572] Rada, P.V., Mark, G. P., Hoebel, B. G. (1993).

[573] Olausson, P., Akesson, P., Engel, J. A., Soderpalm, B. (2001).

[574] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002). 'Activation in Mesolimbic and Visuospatial Neural Circuits Elicited by Smoking Cues: Evidence from Functional Magnetic Resonance Imaging', *Am J Psychiatry*, **159**(6): pp. 954-60; Garavan, H., Pankiewicz, J., Bloom, A., Cho, J. K., Sperry, L., Ross, T. J., Salmeron, B. J., Risinger, R., Kelley, D., Stein, E. A. (2000). 'Cue-Induced Cocaine Craving: Neuroanatomical Specificity for Drug Users and Drug Stimuli', *Am J Psychiatry*, **157**(11): pp. 1789-98; Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004). 'Correlation between Dopamine D(2) Receptors in the Ventral Striatum and Central Processing of Alcohol Cues and Craving', *Am J Psychiatry*, **161**(10): pp. 1783-9.

[575] Johnson, L.R., Aylward, R. L., Hussain, Z., Totterdell, S. (1994). 'Input from the Amygdala to the Rat Nucleus Accumbens: Its Relationship with Tyrosine Hydroxylase Immunoreactivity and Identified Neurons', *Neuroscience*, **61**(4): pp. 851-65; Sesack, S.R., Pickel, V. M. (1992). 'Prefrontal Cortical Efferents in the Rat Synapse on Unlabeled Neuronal Targets of Catecholamine Terminals in the Nucleus Accumbens Septi and on Dopamine Neurons in the Ventral Tegmental Area', *J Comp Neurol.* **320**(2): pp. 145-60; Totterdell, S., Smith, A. D. (1989). 'Convergence of Hippocampal and Dopaminergic Input onto Identified Neurons in the Nucleus Accumbens of the Rat', *J Chem Neuroanat.* **2**(5): pp. 285-98.

[576] Pezawas, L., Meyer-Lindenberg, A., Drabant, E. M., Verchinski, B. A., Munoz, K. E., Kolachana, B. S., Egan, M. F., Mattay, V. S., Hariri, A. R., Weinberger, D. R. (2005). '5-HTTLPR Polymorphism Impacts Human Cingulate-Amygdala Interactions: A Genetic Susceptibility Mechanism for Depression', *Nat Neurosci.* **8**(6): pp. 828-34.

limited *in vivo*[577] evidence and is consistent with models proposed by Goldstein and Volkow[578] and Robinson and Berridge.[579]

A currently un-answered question, however, is what effect *acute* nicotine-induced DA overflow has on activation of reward-signalling regions to smoking-related visual stimuli *in vivo*. This question is clinically relevant as nicotine replacement therapy is commonly prescribed for smoking cessation and relapse rates with users of nicotine replacement therapy are as high as 90%.[580] Furthermore, the cumulative evidence to date does not suggest that nicotine replacement therapy relieves tobacco craving.[581] As nicotine craving is reliably higher in smokers undergoing nicotine withdrawal,[582] conventional wisdom dictates that addicted smokers are more susceptible to cue-elicited activation of reward-signalling brain regions when experiencing prolonged abstinence. However, this presumption is not necessarily supported by the natural history of smoking[583] and has not been empirically tested. Indeed, Brody and colleagues performed positron emission tomography (PET) using the DRD2 receptor ligand [^{11}C]raclopride before and after smoking a cigarette in smokers and non-smokers and observed that [^{11}C] raclopride binding potential (BP) decreased in the

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[577] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002); Garavan, H., Pankiewicz, J., Bloom, A., Cho, J. K., Sperry, L., Ross, T. J., Salmeron, B. J., Risinger, R., Kelley, D., Stein, E. A. (2000); Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004).

[578] Goldstein, R.Z., Volkow, N. D. (2002). 'Drug Addiction and its Underlying Neurobiological Basis: Neuroimaging Evidence for the Involvement of the Frontal Cortex', *Am J Psychiatry*, **159**(10): pp. 1642-52.

[579] Robinson, T.E., Berridge, K. C. (2001).

[580] Yudkin, P., Hey, K., Roberts, S., Welch, S., Murphy, M., Walton, R. (2003). 'Abstinence from Smoking Eight Years after Participation in Randomised Controlled Trial of Nicotine Patch', *BMJ*. **327**(7405): pp. 28-9.

[581] West, R., Shiffman, S. (2001). 'Effect of Oral Nicotine Dosing Forms on Cigarette Withdrawal Symptoms and Craving: A Systematic Review', *Psychopharmacology* (Berl), **155**(2): pp. 115-22.

[582] Shiffman, S.M., Jarvik, M. E. (1976). 'Smoking Withdrawal Symptoms in Two Weeks of Abstinence', *Psychopharmacology* (Berl), **50**(1): pp. 35-9.

[583] Benowitz, N.L., Jacob, P., 3rd, (1984). 'Daily Intake of Nicotine During Cigarette Smoking', *Clin Pharmacol Ther*, **35**(4): pp. 499-504.

smokers but not in the non-smokers.[584] These results suggested, consistent with an intravenous nicotine administration study by Stein and colleagues,[585] that smoking stimulated DA release in the VS/NAc. Furthermore, the Brody study demonstrated that the increase in striatal DA release was negatively correlated with smoking urges.[586] However, whilst one could argue that the act of cigarette smoking during the PET study was a form of visual smoking-related stimulation, these investigators did not systematically present smoking-related and control images to subjects. Therefore, it is not clear from these studies how nicotine interacts with environmental cues to influence striatal DA release.

Thus, in summary, converging evidence from animal and human studies suggests that somatodendritic 5-HT_{1A} autoreceptors, when stimulated by nicotine-induced 5-HT release in the DRN, inhibit behavioural sensitisation to smoking-related environmental stimuli and may affect compulsive use of nicotine and other drugs of abuse. The study presented in Chapter 3 of this thesis indicates that 5-HT_{1A} receptor density in the DRN and post-synaptic terminal fields is reduced in individuals who carry the 5-HTTLPR S allele.[587] Individuals with 5-HTTLPR LS or SS genotypes are more likely to become dependent smokers[588] and may achieve higher success rates with nicotine replacement therapy.[589]

Therefore, these data support the following predictions. First, smokers who carry the 5-HTTLPR S allele experience a relative reduction in 5-HT_{1A} receptor mediated inhibition of

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[584] Brody, A.L., Olmstead, R. E., London, E. D., Farahi, J., Meyer, J. H., Grossman, P., Lee, G. S., Huang, J., Hahn, E. L., Mandelkern, M. A. (2004). 'Smoking-Induced Ventral Striatum Dopamine Release', *Am J Psychiatry*, **161**(7): pp. 1211-8.

[585] Stein, E.A., Pankiewicz, J., Harsch, H. H., Cho, J. K., Fuller, S. A., Hoffmann, R. G., Hawkins, M., Rao, S. M., Bandettini, P. A., Bloom, A. S. (1998). 'Nicotine-Induced Limbic Cortical Activation in the Human Brain: A Functional MRI Study', *Am J Psychiatry*, **155**(8): pp. 1009-15.

[586] Ibid.

[587] David, S.P., Murthy, N. V., Rabiner, E. A., Munafo', M. R., Johnstone, E. C., Jacob, R., Walton, R. T., Grasby, P. M. (2005).

[588] Munafo', M., Clark, T., Johnstone, E., Murphy, M., Walton, R. (2004). 'The Genetic Basis for Smoking Behavior: A Systematic Review and Meta-Analysis', *Nicotine Tob Res.* **6**(4): pp. 583-97.

[589] David, S. P., Murthy, N.V., Rabiner, E.A., Munafo', M., Jacob, R., Johnstone, E., Grasby, P.M. (2004).

behavioural sensitisation and are therefore more susceptible to cue-elicited activation of brain reward regions than smokers lacking the S allele. Second, as tobacco craving is reliably greater in the abstinent state than the nicotine-satiated state, I predicted that smoking-related pictorial cues would result in greater activation of the VS/NAc in the abstinent state than in the nicotine-satiated state. As S allele carriers demonstrate significant benefits in smoking cessation and those with LL genotypes do not, I would predict that S allele carriers would demonstrate greater activation of the NAc than those with LL genotypes and conversely, S allele carriers would demonstrate lower VS/NAc activation in the nicotine satiated state.

4.3 Materials and Methods

Given the background outlined in section 4.1, I formed the following hypotheses:

1. I hypothesised that there would be greater activation associated with smoking-related pictorial cues in the ventral striatum including the nucleus accumbens (VS/NAc) in the abstinent condition than in the nicotine satiated condition (following *ad libitum* cigarette smoking);
2. I hypothesised that in the abstinent condition, smokers with 5-HTTLPR SS or SL genotypes would demonstrate greater activation in VS/NAc than smokers with LL genotypes, and
3. I hypothesised that in the smoking condition, smokers with LL genotypes would demonstrate equal or greater activation in the VS/NAc than smokers with SS or SL genotypes.

This study utilised a two (sessions) by two (conditions) within-subjects randomised crossover design to examine the effects of 5-HTTLPR genotype and smoking condition on

activation to smoking-related vs. neutral pictorial cues with subjects randomised to undergo their fMRI scan on a smoking day or abstinent day as described below.

4.3.1 Procedure

4.3.1.1 Informed Consent and Recruitment

The following study was approved by the Central Office for Research Ethics Committee (COREC 04/Q1606/71). In 1991-93, the Cancer Research UK General Practice Research Group (GPRG) performed a randomised controlled trial of the nicotine patch (the PATCH Trial) on 1686 heavy smokers (≥ 15 cigarettes/day) and in 1999-2000 re-contacted 1532 of the 1612 subjects still available (the PATCH II Trial). Of these subjects 767 (50%) completed a questionnaire and gave a blood sample. The population of subjects for this study consisted of a subset of participants in the PATCH II Trial (COREC C99.010) who had undergone genotyping and had indicated willingness to be approached for follow-up studies.

My goal was to recruit equal numbers of smokers who were homozygous for the 5-HTTLPR S and L alleles with target recruitment goals of 15 subjects per group. As we had no benchmark for determining sample size required to observe differences in fMRI BOLD contrast by 5-HTTLPR genotype, sample size incorporated the difference in global post-synaptic 5-HT_{1A} receptor BP by 5-HTTLPR genotype in the PET study described in Chapter 2 with alpha of 0.05 and power of 0.80. However, only 25 subjects with updated contact information met these genetic criteria, thus prompting a change in design to improve statistical power. The design change doubled the number of samples in our repeated-measures design, such that all subjects would be assigned to two scans occurring on *ad libitum* smoking days and two scans on nicotine abstinence days.

Inclusion criteria were similar to those employed in the fMRI pilot study as described in Chapter 2 (Section 2.3.4.1.), but differed in that only current smokers who were enrolled in the PATCH II Trial and had been genotyped for 5-HTTLPR were eligible for inclusion. Specifically, the inclusion criteria were right-handed male and female current smokers who were enrolled in the PATCH II Trial, had been genotyped for 5-HTTLPR, and were ≥ 18 years of age. Exclusion criteria again included any unstable medical condition, any contraindications to MRI scanning as determined through screening with a standard safety questionnaire, pregnancy, current Axis I psychiatric disorder excluding nicotine dependence, use of psychotropic medications, or current use of nicotine replacement therapy.

Participants with the genotypes mentioned above were contacted by telephone. Individuals who indicated interest in study participation were screened for exclusion criteria and contraindications to MRI using the approved Oxford Centre for Functional Magnetic Resonance Imaging (FMRIB) Screening Form. I explained the potential risks and benefits of the study and provided an informational letter describing the study with instructions on how to prepare for each of the four scans and directions to Brain (FMRIB). I then described the risks and benefits of the study at the first appointment and obtained written informed consent. Financial compensation for participation was not provided but travel reimbursement was made for subjects completing a travel reimbursement form.

Smokers were randomized in counterbalanced fashion such that they would either attend a smoking day (S), followed sequentially by an abstinent day (A), a second S, and finally a second A sequence (i.e., SASA) or an alternate sequence (i.e., ASAS) of scanning sessions in order to avoid confounding resulting from different conditions experienced with the first

stimulus paradigm. Once randomized, all participants were asked to agree to attend four sessions on four separate days consisting of two smoking days and two abstinent days.

Prior to each appointment, I phoned each participant in order to reinforce the need to abstain from smoking overnight and the morning of the scan for the abstinent scan days and to smoke according to their own routines on the smoking scan days.

On the morning of the first scan, I reviewed the details of the experiment with each participant, and asked participants if they had any further questions on the study. In addition, as with the pilot study descrived in Chapter 1, I gave instructions on the primary task of identifying a male or female picture with a forced key press. Participants completed the study questionnaire providing details on socioeconomics and smoking habits as described in Chapter 2 (Section 2.3.4.3). Prior to all sessions (smoking or abstinent days) I measured exhaled breath carbon monoxide (CO) with a piCO Smokerlyzer® (Bedfont Scientific Ltd, Sittingborne, UK). An exhaled CO of $\geq$ 11 p.p.m. was considered non-adherence to overnight abstinence, as in the pilot study. Furthermore, I reviewed the screening criteria with each subject prior to each scan and ensured that all metallic objects were removed before entering the magnet chamber. Finally, I collected saliva for cotinine analysis using a buccal swab, which was then processed in the Cancer Research UK General Practice Research Group.

4.3.1.2 Behavioural Measures

All subjects were administered assessment scales[590] for nicotine craving [Shiffman-Jarvik Craving Scale (5 items rated 0-100)][591] and withdrawal [Minnesota Withdrawal Scale

[590] Shiffman, S., West, R., Gilbert, D. (2004). 'Recommendation for the Assessment of Tobacco Craving and Withdrawal in Smoking Cessation Trials', *Nicotine Tob Res.* **6**(4): pp. 599-614.
[591] Shiffman, S.M., Jarvik, M. E. (1976).

(8 items rated 0-4)][592] whilst situated in the scanner before and after the stimulus presentation. In addition, the Fagerström Test of Nicotine Dependence (FTND)[593] was administered as part of the study questionnaire.

4.3.1.3 Imaging Procedure

The imaging procedure and pulse sequence used were identical to those employed in the fMRI pilot study as described in Chapter 2 Section 2.3.4.4 and all scans took place in the Centre for Functional Magnetic Resonance Imaging of the Brain using a 3 Tesla Varian (Palo Alto, CA, USA)/Siemens (Erlangen, Germany) MRI system. Using the same protocol employed in the pilot study described in Chapter 2, 100 picture cues from the International Smoking Image Series (ISIS),[594] each of five seconds' duration, were presented with a one-second inter-stimulus interval during which a fixation cross was presented. The pictures consisted of faces of people in smoking-related or non-smoking related situations pseudo-randomised as also described in Chapter 2. We controlled for gender by incorporating equal numbers of males and females for smoking and neutral pictures (10 male smoking pictures, 10 female smoking pictures, 10 male neutral pictures, 10 female neutral pictures) from the ISIS.

4.3.1.4 Data Pre-Processing

Data pre-processing was conducted using FEAT (FMRI Expert Analysis Tool) Version 5.42 from the FMRIB Software Library (www.fmrib.ox.ac.uk/fsl). Pre-statistical processing

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[592] Hughes, J.R., Hatsukami, D. (1986). 'Signs and Symptoms of Tobacco Withdrawal', *Arch Gen Psychiatry*, **43**(3): pp. 289-94.

[593] Heatherton, T.F., Kozlowski, L. T., Frecker, R. C., Fagerstrom, K. O. (1991). 'The Fagerstrom Test for Nicotine Dependence: A Revision of the Fagerstrom Tolerance Questionnaire', *Br J Addict*, **86**(9): pp. 1119-27.

[594] Gilbert, D.G., Rabinovich, N.E. (1999). *International Smoking Images Series (With Neutral Counterparts)*. (Southern Illinois University: Integrative Neuroscience Laboratory, Department of Psychology) [on CD-ROM].

was as follows: motion correction using FMRIB's Linear Registration Tool (MCFLIRT);[595] exclusion of non-brain areas using FMRIB's Brain Extraction Tool (BET); [596] spatial smoothing with a Gaussian kernel of 5 mm full-width half maximum; mean-based intensity normalisation to remove linear trends; and non-linear high-pass temporal filtering to exclude low frequency confounds such as breathing (Gaussian-weighted least squares straight line fit, with sigma = 25.0 s). Time series statistical analysis was carried out using FMRIB's Improved Linear Model (FILM) with local autocorrelation correction.[597]

4.3.1.5 Multilevel Linear Modelling for fMRI Group Analysis

A multilevel voxel-wise hierarchical analysis was performed with separate general linear model analyses for each of the four levels in the hierarchy (1: within-session level, 2: within-subject level, 3: group level within condition, 4: within-subjects group level). Summary statistics from voxel-wise analyses at each level (contrast of parameter estimates, COPEs; variance of the COPEs, VARCOPEs) were entered as inputs into each higher level analysis, wliich in turn generated summary statistics. This multilevel approach using Bayesian inference provided the means to assess the full uncertainty of the COPEs at the highest level; taking into account the unknown random and fixed variance components within each level in the model:[598]

(1) The first-level voxel-wise GLM analysis examined whether, on average for all trials, the parameter estimate (PE) of the haemodynamic response to smoking-related pictures was

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[595] Jenkinson, M., Smith, S. (2001). 'A Global Optimisation Method for Robust Affine Registration of Brain Images', *Med Image Anal*, **5**(2): pp. 143-56.
[596] Smith, S.M. (2002). 'Fast Robust Automated Brain Extraction', *Hum Brain Mapp*. **17**(3): pp. 143-55.
[597] Woolrich, M.W., Ripley, B. D., Brady, M., Smith, S. M. (2001). 'Temporal Autocorrelation in Univariate Linear Modeling of FMRI Data', *Neuroimage*, **14**(6): pp. 1370-86.
[598] Beckmann, C.F., Jenkinson, M., Smith, S. M. (2003). 'General Multilevel Linear Modeling for Group Analysis in FMRI', *Neuroimage*, **20**(2): pp. 1052-63; Woolrich, M.W., Behrens, T. E., Beckmann, C. F., Jenkinson, M., Smith, S. M. (2004). 'Multilevel Linear Modelling for FMRI Group Analysis Using Bayesian Inference', Neuroimage, **21**(4): pp. 1732-47.

greater than the PE for the haemodynamic response to neutral pictures. In this case, the dependent variable is the COPE for the smoking vs. neutral stimuli contrast. Specifically, smoking vs. neutral conditions were modelled (boxcar functions convolved with the haemodynamic response function) as explanatory variables within the context of the general linear model on a voxel-by-voxel basis. *Z* (Gaussianized T/F) statistic images were thresholded using clusters determined by $Z > 2.3$ and a corrected cluster significance level of $p = 0.05$. Registration to high resolution T_1 structural images of each individual was carried out using FMRIB's Linear Registration Tool (FLIRT)[599] and both were co-registered to standard (Montreal Neurological Institute) space.

All higher-level analysis was carried out using FMRIB's Local Analysis of Mixed Effects (FLAME). [600] Z (Gaussianised T/F) statistic images were thresholded using clusters determined by $Z > 2.3$ and an adjusted corrected cluster significance threshold of $p = 0.05$, corrected for multiple comparisons.[601]

(2) The second-level voxel-wise GLM analysis was performed to determine whether each subject activated, on average, across two sessions for each condition. Second-level, voxel-wise, fixed-effects GLM analyses were conducted separately for each participant to determine the mean COPE across both sessions in each condition (smoking and abstinent).

(3) The third-level voxel-wise GLM analyses examined whether or not the entire group activated on average. In our model, two third-level analyses were performed with group

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[599] Jenkinson, M., Bannister, P., Brady, M., Smith, S. (2002). 'Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images', *Neuroimage*, **17**(2): pp. 825-41.
[600] Beckmann, C.F., Jenkinson, M., Smith, S. M. (2003); Woolrich, M.W., Behrens, T. E., Beckmann, C. F., Jenkinson, M., Smith, S. M. (2004).
[601] Worsley, K.J., Evans, A. C., Marrett, S., Neelin, P. (1992). 'A Three-Dimensional Statistical Analysis for CBF Activation Studies in Human Brain', *J Cereb Blood Flow Metab.* **12**(6): pp. 900-18.

random-effects GLM analyses conducted for the smoking condition and for the abstinent condition.

(4) The fourth-level voxel-wise GLM analysis examined whether or not there was a paired-difference in the mean COPE between smoking and abstinent conditions using FLAME. Two paired-analyses were performed examining (a) whether the mean COPE for the smoking condition was greater than the mean COPE for the abstinent condition and (b) whether the mean COPE for the abstinent condition was greater than the mean COPE for the smoking condition.

In addition to voxel-wise comparisons, region-of-interest-based analyses were conducted to test my hypotheses regarding the effects of smoking condition and genotype (independent variables) on mean VS/NAc COPE (the dependent variable). Region of interest (ROI) anatomical masks were defined using the averaged group T1 structural image normalised to standard space for the VS/NAc using FSL View software (www.fmrib.ox.ac.uk). The brain atlas by Duvernoy[602] was used as a guide for defining anatomical landmarks. I used the criteria for defining the mesolimbic VS published by Mawlawi and colleagues[603] and, as described in Chapter 2, (MNI coordinates X: ±4 to 10; Y: +6 to +18, 0 to -10) consistent with the landmarks of the "limbic-related" striatum as defined by Fudge and Haber.[604]

Specifically, the boundary between the VS/NAc inferiorly, dorsal caudate, and dorsal putamen superiorly was defined by a line joining the intersection between the outer edge of the putamen with a vertical line going through the most superior and lateral point of the

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[602] Duvernoy, H. (1999). *The Human Brain*, 2nd ed. (London: Springer-Verlab).
[603] Mawlawi, O., Martinez, D., Slifstein, M., Broft, A., Chatterjee, R., Hwang, D. R., Huang, Y., Simpson, N., Ngo, K., Van Heertum, R., Laruelle, M. (2001). 'Imaging Human Mesolimbic Dopamine Transmission with Positron Emission Tomography: I. Accuracy and Precision of D(2) Receptor Parameter Measurements in Ventral Striatum', *J Cereb Blood Flow Metab.* **21**(9): pp. 1034-57.
[604] Fudge, J.L., Haber, S. N. (2002). 'Defining the Caudal Ventral Striatum in Primates: Cellular and Histochemical Features', *J Neurosci.* **22**(23): pp. 10078-82.

internal capsule and centre of the portion of the anterior commissure transaxial plane overlying the striatum. This line was then extended to the internal edge of the caudate.[605]

4.3.1.6 Genotyping

All genotyping was performed prior to the fMRI study by the Cancer Research UK General Practice Research Group (GPRG). DNA extraction and genotyping for the 5-HTTLPR was performed using techniques previously described in Chapter 3 (Section 3.3.3) and in the literature.[606]

4.3.1.7 Statistical Methods for Hypothesis Testing

In order to test the hypotheses stated above, we conducted the following planned analyses. A multivariate analysis of variance (MANOVA) was performed with mean VS/NAc COPE within the voxels defined by the VS/NAc masked described above as the dependent variable, 5-HTTLPR genotype as a between-subjects independent variable and hemisphere, smoking condition, and session as within-subjects variables. The MANOVA permitted the examination of main effects of genotype and condition as well as interactions between genotype and condition on VS/NAc COPE.

4.4 Results

The final sample size consisted of 12 subjects who had been genotyped for the 5-HTTLPR polymorphism consisting of 10 females and 2 males. Eight subjects underwent all 4 fMRI scans (32 scans on separate days) and incomplete data are available on four subjects. Two

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[605] Ibid.

[606] Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., Lesch, K. P. (1996). 'Allelic Variation of Human Serotonin Transporter Gene Expression', *J Neurochem*, **66**(6): pp. 2621-4; Bunce, M., Fanning, G. C., Welsh, K. I. (1995). 'Comprehensive, Serologically Equivalent DNA Typing for HLA-B by PCR Using Sequence-Specific Primers (PCR-SSP)', *Tissue Antigens*. **45**(2): pp. 81-90.

subjects withdrew from the study, 1 subject cancelled his final scan, and 1 subject was excluded because of an unstable medical condition affecting his ability to complete scanning sessions safely (end-stage chronic obstructive pulmonary disease) and ventriculomegaly, thus markedly altering the anatomy of the VS. The four subjects who did not complete all sessions were excluded from the final analyses.

Of the total study population, 10 participants were female (83.3%) and 2 male (16.7%). Mean age was 54.1 years (SD = 6.3), mean height, 166.9 cm (SD = 9.9), mean weight, 64.5 kg (SD = 12.1), and mean age of onset of regular smoking was 16.8 years (SD = 3.9). Mean years smoked was 37.3 (SD = 6.3). Saliva cotinine was 113.3 (SD = 60.0) ng/ml, which corresponds to a mean of 19.5 (SD = 0.8) cigarettes per day.[607] Self-reported mean cigarettes/day was 17.9 (SD = 7.6), suggesting that smokers slightly underreported their daily cigarette consumption. The mean total Fagerström score was 6.8 (SD = 1.5).

Basic characteristics of the study participants who completed all trials and those who did not are presented in separate columns in Table 4.1 below. Of note, the sex distribution of 8 subjects who completed all 4 trials was 100% female. One of the reasons for the sex imbalance in recruitment was that I attempted to recruit as many homozygotes for the 5-HTTLPR (SS or LL genotypes) as possible and more female participants from this genetic subgroup agreed to participate in the study than male participants. Non-parametric comparisons of study completers and non-completers suggested that non-completers smoked more cigarettes per day than completers, however there were no significant differences between the groups with the exception of sex distribution (Table 4.1).

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[607] Estimation of mean cigarettes/day (cpd) based on linear regression equation [y (mean cpd) = 0.013 (saliva cotinine in ng/ml) + 18.0]. Garvey, S. (2005) 13 Aug, 2005: personal communication.

Table 4.1

Basic Characteristic of Study Participants

Characteristic	Completed all trials (N = 8)		Did not complete all trials (N = 4)		*t*-test of difference	
	Mean	SD	Mean	SD	*Z*	*P*
Age (years)	55.6	9.2	51	5.7	0.59	0.570
*Sex (female)	8	100%	2	50% (X^2)	4.40	0.036
Height (cm)	164.0	3.6	172.8	16.0	1.11	0.283
Weight (kg)	61.8	3.6	69.9	19.6	0.60	0.473
Age onset	17.6	4.3	15.3	2.5	0.57	0.461
Years smoked	38.0	7.1	35.8	5.0	0.43	0.683
Cigarettes/day	17.9	7.6	27.5	5.0	2.07	0.048
Fagerström	6.5	1.3	7.5	1.7	0.96	0.368
CO abstinent (p.p.m.)	4.6	2.2	8.8	4.8	0.79	0.533
CO smoking (p.p.m.)	14.6	4.1	23.3	15.3	1.44	0.178
**SERT 5-HTTLPR					*F*	*P*
LL	3	37.8%	2	50%	0.15	0.711
LS	1	12.5%	1	25%	0.26	0.624
SS	4	50%	1	25%	0.45	0.454

LEGEND: Data are presented separately for study completers and non-completers. *Data presented as frequency and percent. *Hypothesis testing: Chi-square with df=1 for sex; one-way analysis of variance for genotype. 'Fagerström' = mean total score on Fagerström Test of Nicotine Dependence[608]. 'CO abstinent' = mean exhaled carbon monoxide (CO) across both abstinent sessions. 'CO smoking' = mean exhaled CO across both smoking sessions. If subjects did not complete both sessions single session CO values are listed. 'SD' = standard deviation.

4.4.1 Imaging Data

Whole-brain mixed-effects group analysis with a corrected *z*-statistic of 2.3 ($p = 0.05$) for the smoking condition demonstrated two large bilateral clusters in the temporal-occipital region inclusive of right and left posterior fusiform gyri and inferior temoporal gyri

[608] Heatherton, T.F., Kozlowski, L. T., Frecker, R. C., Fagerstrom, K. O. (1991).

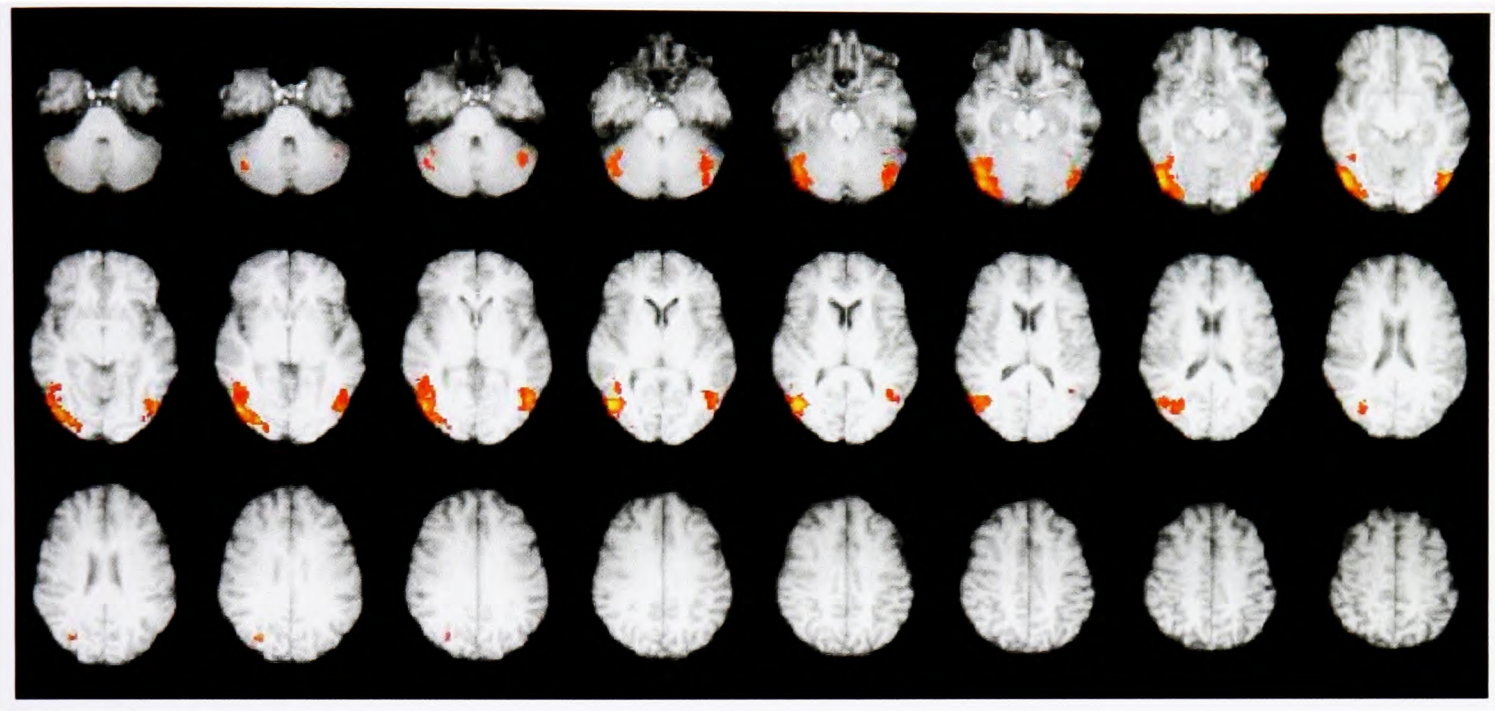
respectively, as seen in Figure 4.1. Centres of gravity (COG), coordinates in MNI space, and maximum *z*-statistic (max *z*-stat) and *p* values are presented in Table 4.2. In addition to the COGs of each cluster, local maxima were observed in the left posterior fusiform gyrus (PFG) (-44, -70, -8, max *z*-stat = 2.97), right inferior temporal gyrus (ITG) (52, -72, -12, max *z*-stat = 3.66) and left ITG (-50, -74, -12, max *z*-stat = 3.42). In a pattern similar to that observed in the smoking condition, mixed-effects group analysis of the same subjects in the abstinent condition revealed bilateral occipital-temporal clusters inclusive of right and left posterior fusiform gyri and inferior temporal gyri, respectively [Local maxima: right ITG (52, -64, -4, max *z*-stat = 3.45), left ITG (-48, -74, -12, max *z*-stat = 3.16), left PFG (-44, -68, -16, max *z*-stat = 3.16)].

Next, mixed-effects group comparisons of the mean COPE and its variance (VARCOPE) were conducted on a voxel-by-voxel basis within subjects between smoking sessions and abstinent sessions using FLAME. Differences in whole-brain activation were not observed at a corrected cluster *z*-threshold of 2.3 when we compared smoking to abstinent conditions (smoking minus abstinent) or abstinent to smoking conditions (abstinent minus smoking). However, there was bilateral VS activation within the NAc proper at an uncorrected *z*-threshold of 2.0 and $p < 0.05$. The maximum *z*-statistic in the right VS/NAc was 2.12 and in the left VS/NAc was 2.02 as shown in Figure 4.2.

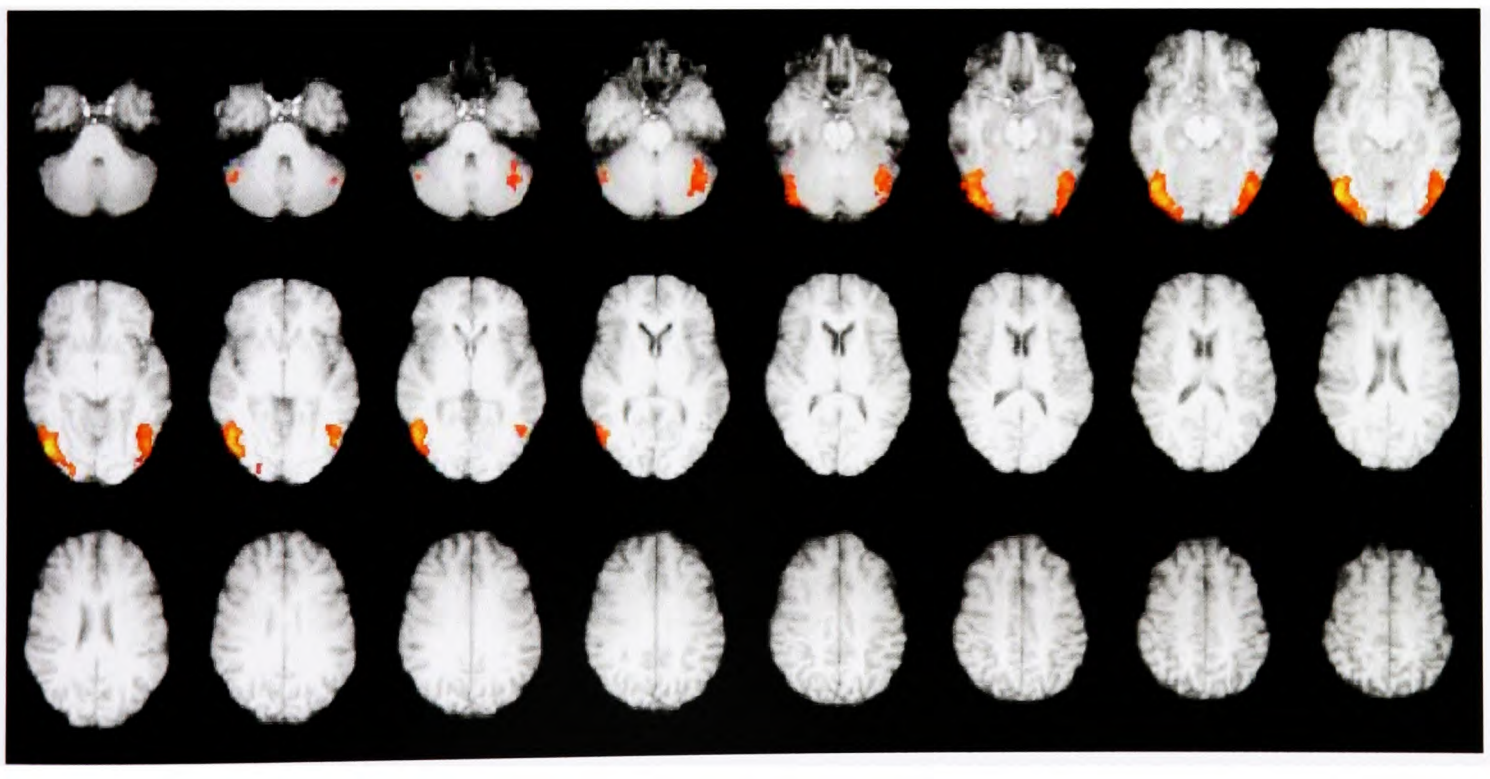
Figure 4.1

Mixed-Effects Group Analyses of Smokers in Smoking and Abstinent Conditions

A. Smoking condition



B. Abstinent condition



LEGEND: Random-effects group analyses of smokers in the smoking and neutral condition. Image depicts statistical maps of *z*-statistics at threshold of 2.3 using clusters determined by $Z > 2.3$ and a corrected cluster significance level of $p = 0.05$.

Table 4.2

Corrected Mixed Effects Cluster Analysis in Smokers by Condition

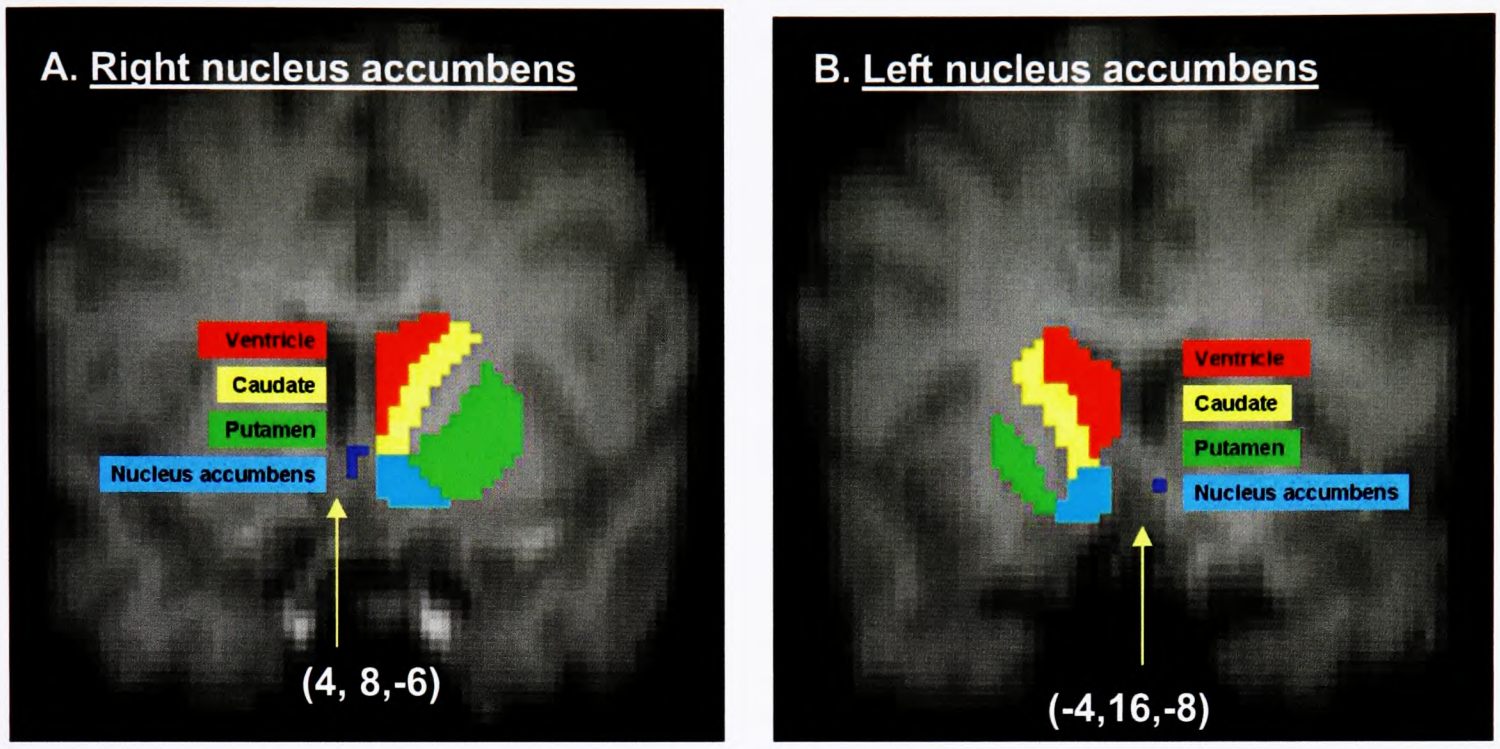
Cluster	COG *x* (mm)	COG *y* (mm)	COG *z* (mm)	Max *Z*-statistic	Mean COPE	*P*
Smoking condition						
Right posterior fusiform gyrus	44.6	-72.2	-6.61	3.66	34.2	1.59e-12
Left posterior fusiform gyrus	-44.5	-69.2	-10.40	3.42	30.4	5.66e-06
Abstinent condition						
Right inferior temporal gyrus	45.3	-79.3	-11.8	3.63	36.8	6.49e-10
Left posterior fusiform gyrus	-43.9	-69.5	-16.1	3.17	30.6	5.96e-08

LEGEND: Group mixed-effects analysis of smoking vs. neutral contrast demonstrated significant activation in three main clusters consisting of multiple local maxima. COG *x*, *y*, *z*, are the coordinates of the centres of gravity (COG) for each cluster. *P* represents the *p* value corresponding to the maximum *z*-statistic within each cluster.

Images of the VS/NAc masks and specific criteria for boundaries are demonstrated in Figure 4.3. Mean contrasts of the parameter estimates (COPE) for the smoking vs. neutral image contrast were calculated within the VS/NAc masks for both hemispheres. I then performed a multivariate analysis of variance (MANOVA) using these ROI COPEs as the dependent variable, with serotonin transporter (5-HTTLPR) genotype as a between-subjects variable and smoking condition, hemisphere, and session as within-subjects variables. The results indicated that there was greater mean VS/NAc COPE in the smoking condition than in the abstinent condition ($F = 6.45$; $df = 6,1$; $p = 0.044$) and a significant smoking condition by hemisphere interaction ($F = 8.71$; $df = 6,1$; $p = 0.026$). There was not a significant main effect of 5-HTTLPR genotype nor were there any significant interactions with 5-HTTLPR genotype. Figure 4.3 demonstrates that the active clusters observed were within the confines of the pre-defined VS/NAc mask.

Figure 4.2

Comparison of Nucleus Accumbens Activation between Smoking and Abstinent Conditions



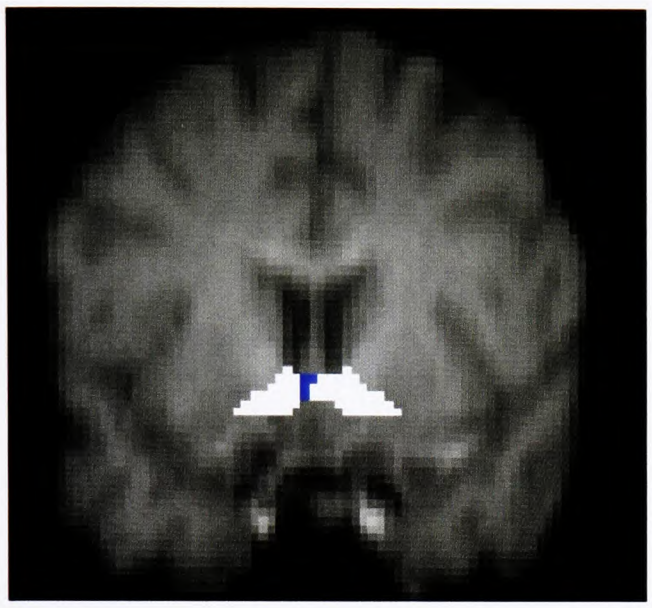
LEGEND: Statistical map of group comparison of smoking to abstinent conditions within subjects with (a) right NAc and (b) left NAc in coronal orientation. Activation clusters represent group within-subjects mixed-effects analyses comparing average activation to smoking vs. neutral cues between smoking and abstinent conditions. Thresholds were set at *z*-statistic of 2.0, $p = 0.05$ uncorrected. Anatomical regions are colour-coded to illustrate the locations of the NAc, putamen, caudate, and lateral ventricles contralateral to each activation cluster. The yellow arrows indicate active voxels and the location of maximum *z*-statistics for right and left NAc.

In order to determine the nature of the hemisphere by smoking condition interaction demonstrated in the MANOVA described above, I conducted a series of paired t-tests. Bi-hemispheric VS/NAc COPE was significantly greater in the smoking condition than in the non-smoking condition ($t = 2.39$, $df = 7$, $p = 0.048$). In addition, right hemisphere VS/NAc COPE was significantly greater in the smoking than in the abstinent conditions ($t = 3.38$, df = 7, $p = 0.012$). However, in the left hemisphere, the mean VS/NAc COPE was not significantly greater in the smoking condition than in the abstinent condition ($t = 1.30$, $df = 7$, $p = 0.235$), although the effect was in the same direction (i.e., smoking condition greater than abstinent condition).

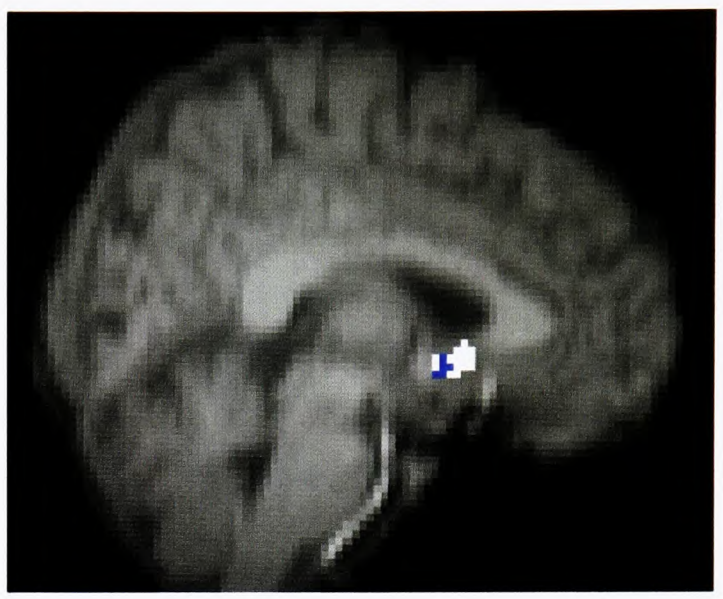
Figure 4.3

Activation within Ventral Striatum/Nucleus Accumbens Anatomical Mask

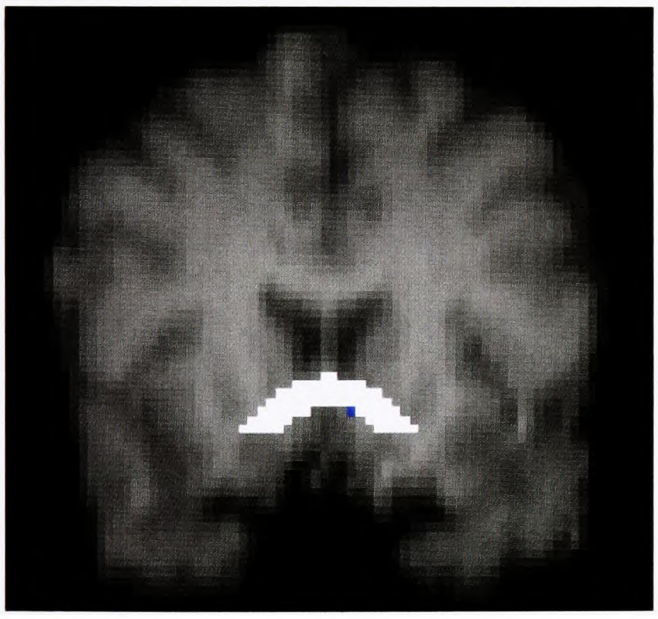
A. Right activation (coronal) within mask



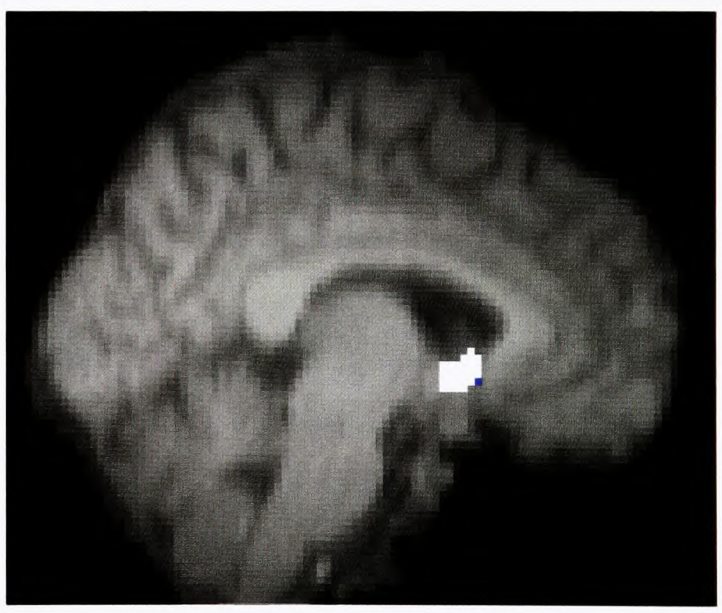
B. Right activation (sagittal) within mask



A. Left activation (coronal) within mask



B. Left activation (sagittal) within mask

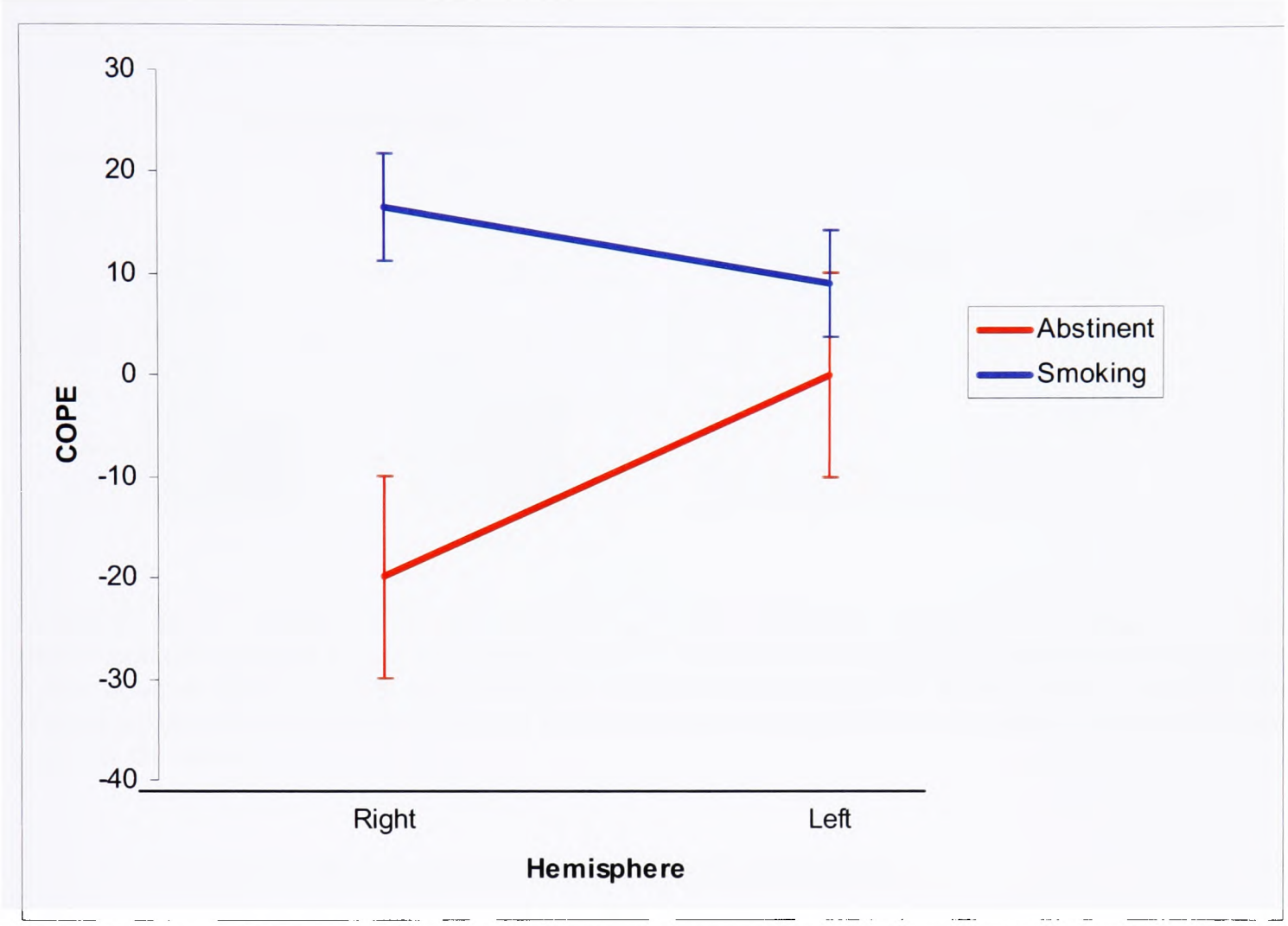


LEGEND: Statistical map of smoking condition compared with abstinent condition within pre-defined VS/NAc anatomical masks.

There were no significant differences in VS/NAc COPE between hemispheres for either condition. Figure 4.4 presents a histogram of the mean VS/NAc COPEs for each hemisphere in both conditions.

Figure 4.4

Ventral Striatum/Nucleus Accumbens Mean COPE by Hemisphere and Condition

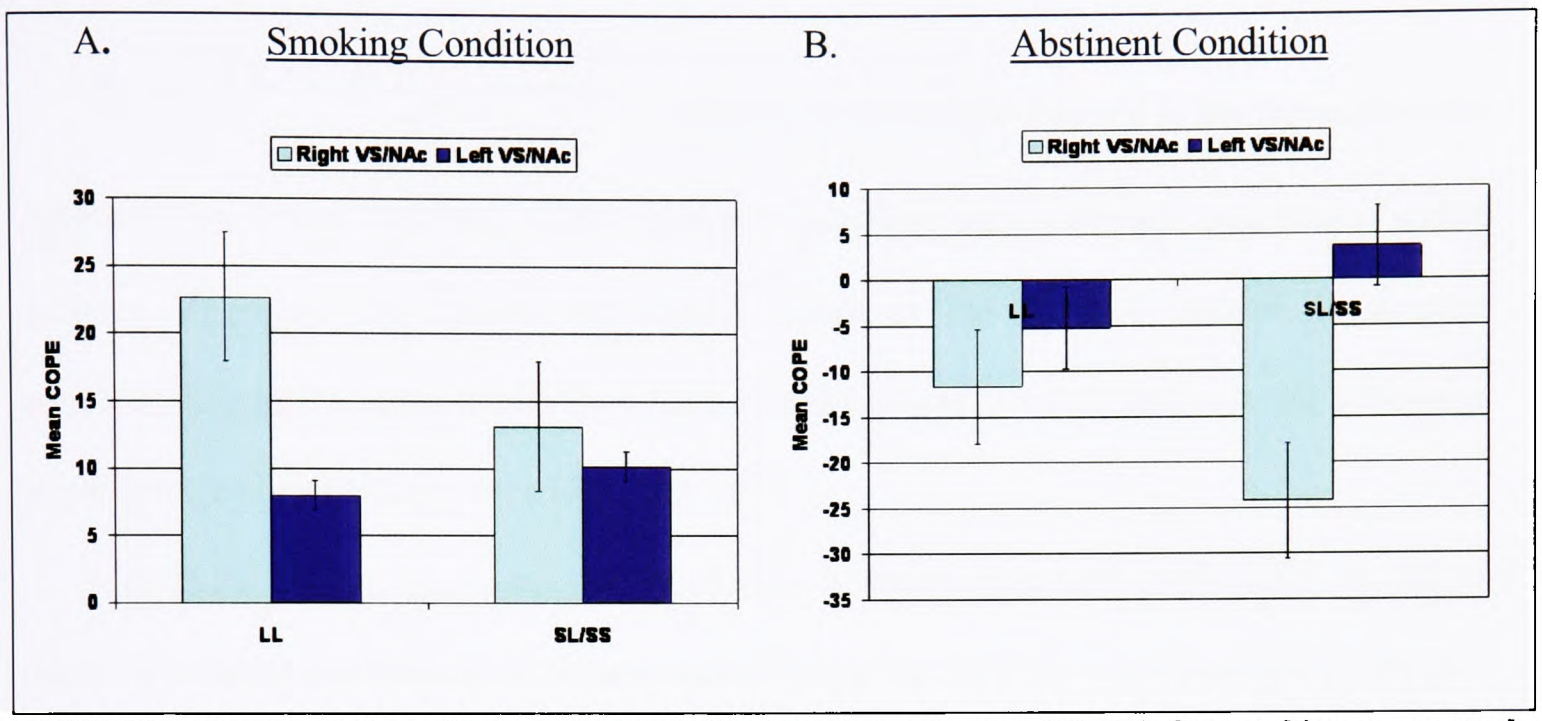


LEGEND: Mean COPE (smoking vs. neutral stimulus contrast) within the ventral striatum including the nucleus accumbens (VS/NAc) for right and left hemispheres and both conditions (smoking and abstinent). Error bars represent the standard error of the mean COPE for each hemisphere. COPE values were calculated within an anatomically-defined VS/NAc mask created with FSLview software as described in section 4.3.1.5.

As noted, there was not a significant effect of 5-HTTLPR genotype on VS/NAc COPE. Therefore, Figure 4.5 and Table 4.4, which present mean VS/NAc COPE within genetic strata and by smoking condition, are provided for descriptive purposes and as the basis for calculations of observed statistical power.

Figure 4.5

Graphical Representation of Mean COPE by Hemisphere and 5-HTTLPR Genotype



LEGEND: Representation of mean contrast of parameter estimates (COPE) for smoking vs. neutral picture stimuli averaged across two sessions for two conditions (smoking day and abstinent day). Bars represent mean VS/NAc COPE for participants with serotonin transporter 5-HTTLPR LL genotype and SL/SS genotype for right (light blue) and left (dark blue) hemispheres. Error bars represent standard errors of the mean.

4.4.1.1 Sample Size Estimates for Testing Genetic Hypotheses

In order to inform future research, sample size estimates for comparing across conditions within genotypic strata (within-subjects) and between between genotypic strata (between-subjects) were determined based on observed statistical power. Given the sample size and lack of male participants, these estimates should be considered with caution given these potential threats to external validity.

Within-Subjects:

I chose to calculate the minimum sample size required to detect a difference in percentage signal change of 0.10% beween smoking and abstinent conditions in the VS/NAc within each genetic stratum, which is consistent with the difference in BOLD contrast

between smoking and neutral conditions observed by McClernon and colleagues.[609] The first sample size estimate used the formula for paired data:

$$n = \frac{\sigma_d{}^2 (Z_{1-\alpha/2} + Z_{1-\beta})^2}{\Delta^2}$$, where n is the sample size, σ_d^2 is the variance of the difference in mean VS/NAc COPE between smoking and abstinent conditions within subjects. $Z_{1-\alpha/2}$ and $Z_{1-\beta}$ denote the critical values of the standard normal distribution corresponding to the error rate α (Type I error) and β (Type II error), and Δ is the difference in percentage signal change we wish to detect.[610]

5-HTTLPR S/S or S/L Genotype: A minimum sample size of 9 participants would be required to detect a difference in percent signal change of 0.10 at α =0.05 and power of 0.80.

5-HTTLPR L/L Genotype: A minimum sample size of 40 participants would be required to detect a difference in percent signal change of 0.10 at α =0.05 and power of 0.80.

Between-Subjects:

The next calculation was an estimate of the minimum sample size required to detect a desired difference in BOLD contrast associated with smoking-related cues between genetic strata for both conditions (smoking and abstinent). The following formula was applied for between-subjects hypothesis testing. A difference in percent signal change of 0.10 is based on the difference observed by Hariri and colleagues comparing participants with LL genotypes to L/S or S/S genotypes engaged in a face recognition task.[611]

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[609] McClernon, F.J., Hiott, F. B., Huettel, S. A., Rose, J. E. (2005). 'Abstinence-Induced Changes in Self-Report Craving Correlate with Event-Related fMRI Responses to Smoking Cues', *Neuropsychopharmacology.*

[610] Belle, G., Fisher, L.D., Heagerty, P.J., Lumley, T., (ed.). (2004) *Biostatistics: A Methodology for the Health Sciences* (Hoboken, New Jersey, USA: John Wiley & Sons).

[611] Hariri, A.R., Drabant, E. M., Munoz, K. E., Kolachana, B. S., Mattay, V. S., Egan, M. F., Weinberger, D. R. (2005). 'A Susceptibility Gene for Affective Disorders and the Response of the Human Amygdala', *Arch Gen Psychiatry*, **62**(2): pp. 146-52.

$$n = \frac{2\sigma^2 (Z_{1-\alpha/2} + Z_{1-\beta})^2}{(\mu_1 - \mu_2)^2}$$, where σ^2 is the estimated variance, μ1 and μ2 are the mean VS/NAc COPEs for participants with L/L or LS/LL genotypes, respectively, $Z_{1-\alpha/2}$ and $Z_{1-\beta}$ are the critical values of the standard normal distribution corresponding to the error rate α (Type I error) and β (Type II error), and Δ is the difference in percentage signal change we wish to detect[612].

Calculations are based on the following data for mean VS/NAc COPE, which have been converted to percent signal change (in order to compare with other studies) by estimating the height of the signal intensity for both explanatory variables (EVs) = 0.8, multiplying by the mean COPE, and dividing by the baseline signal (10,000): Mean global VS/NAc COPEs and standard deviations (SD) in the smoking condition for L/L genotype (15.35, SD = 10.87) and S allele carriers (11.62, SD = 19.66); and mean global VS/NAc COPEs and SDs in the abstinent condition for L/L genotype (1.85, SD = 9.07) and S allele carriers (0.54, SD = 16.29). These data are also presented in Table 4.4.

Abstinent Condition:

A minimum sample size of 31 participants per group would be required to detect a difference in percent signal change of 0.10 between participants with 5-HTTLPR LL vs. LS/SS genotypes at α =0.05 and power of 0.80.

Smoking Condition:

A minimum sample size of 29 participants per group would be required to detect a difference in percent signal change of 0.10 between participants with 5-HTTLPR LL vs. LS/SS genotypes at α = 0.05 and power of 0.80.

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[612] Belle, G., Fisher, L.D., Heagerty, P.J., Lumley, T. (2004).

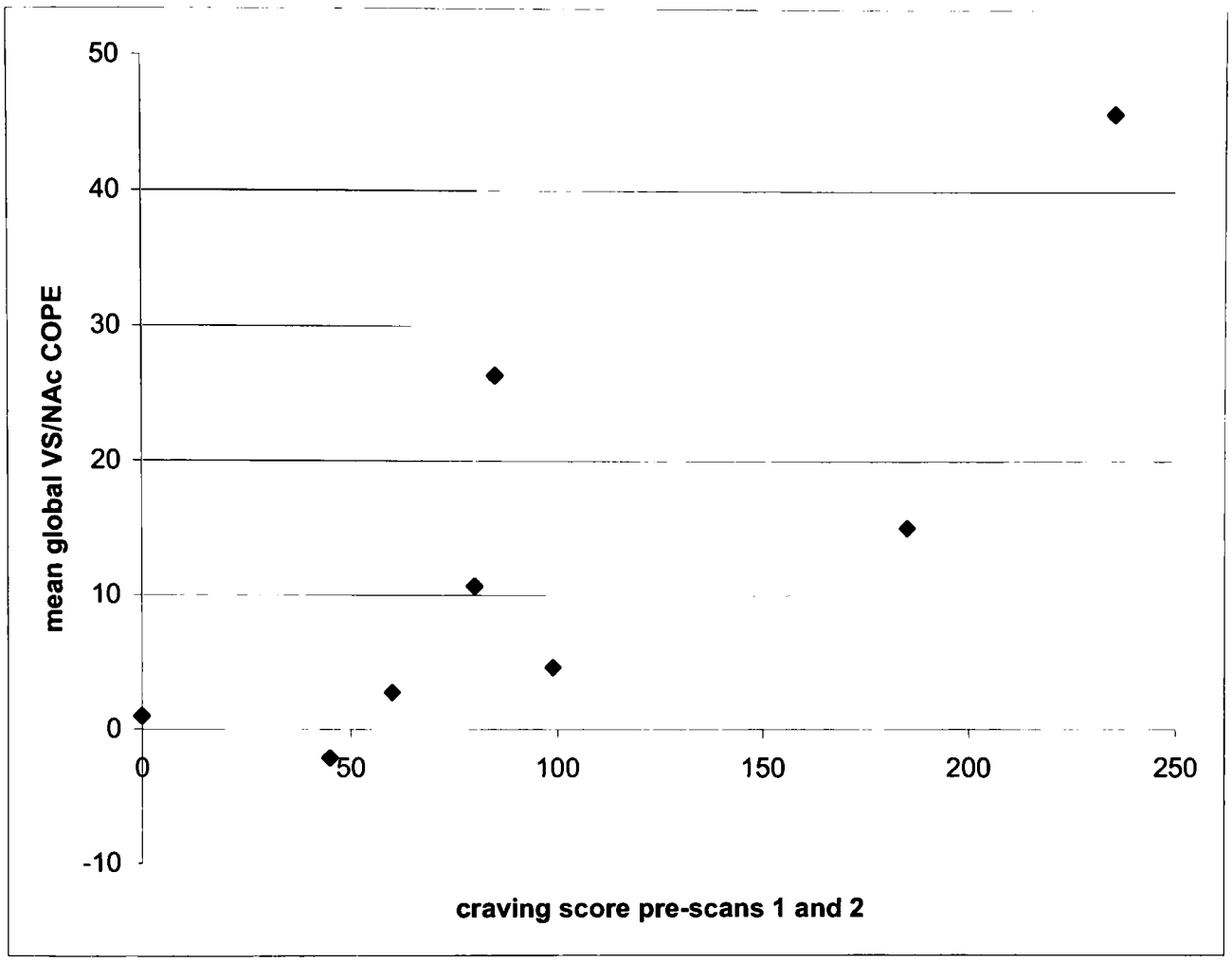
4.4.2 Behavioural Data

As expected, total nicotine withdrawal scores and craving scores were lower in the smoking condition than the abstinent condition before and after the scanning sessions (Table 4.3). There were no significant differences in subjective ratings of craving or withdrawal before and after the scans, respectively: [craving average pre scans: (smoking sessions, 98.63, SD = 76.24; abstinent sessions 339.18, SD = 156.74), $t = 4.31$, $df = 7$, $p = 0.004$; [craving average post scans: (smoking sessions 152.06, SD = 98.95; abstinent sessions 321.43, SD = 179.59), $t = 3.14$, $df = 7$, $p = 0.016$]; [withdrawal average pre scans: (smoking sessions 4.03, SD = 2.70; abstinent sessions 11.25, SD = 4.55), $t = 4.14$, $df = 7$, $p = 0.004$]; [withdrawal average post scans: (smoking sessions 6.19, SD = 4.51; abstinent sessions 10.3, SD = 5.93, $t = 2.49$, $df = 7$, $p = 0.042$].

Bivariate non-parametric correlational analysis was performed examining VS/NAc BOLD contrast with craving and withdrawal to better characterise the relationship between the fMRI activation and self-reported affect. There was a significant and positive correlation between the average pre-scan craving score and the global bi-hemispheric VS/NAc COPE (Spearman's $r = 0.86$, $p = 0.007$) as demonstrated in Figure 4.6. There were no correlations between VS/NAc COPE and craving in the abstinent condition. Nicotine withdrawal scores were not significantly correlated with VS/NAc COPE for either condition.

Figure 4.6

Correlation of Global VS/NAc COPE and Craving in Smoking Condition



LEGEND: Scatter plot of total score on the Shiffman-Jarvick Craving Scale prior to picture presentation averaged across both scanning sessions on the *x*-axis and mean bi-hemispheric COPE averaged across both smoking sessions for the VS including the nucleus accumbens (VS/NAc) on the *y*-axis.

In order to provide confirmation of the internal validity of our study and demonstrate external validity consistent with other published literature,[613] a MANOVA was performed with reaction time (RT) as the dependent variable, and cue type (smoking or neutral), condition (smoking or abstinent day), and session (1^{st} or 2^{nd}) as within-subjects independent variables. A significant

[613] Trimmel, M., Wittberger, S. (2004). 'Effects of Transdermally Administered Nicotine on Aspects of Attention, Task Load, and Mood in Women and Men', *Pharmacol Biochem Behav.* **78**(3): pp. 639-45; Havermans, R.C., Debaere, S., Smulders, F. T., Wiers, R. W., Jansen, A. T. (2003). 'Effect of Cue Exposure, Urge to Smoke, and Nicotine Deprivation on Cognitive Performance in Smokers', *Psychol Addict Behav.* **17**(4): pp. 336-9.

effect of session was observed indicating faster reaction times in the second sessions for both conditions ($F = 5.89$; $df = 7,1$; $p = 0.046$). Statistical trends were observed for a main effect for cue type, suggesting reaction times might be greater for smoking-related than neutral pictures ($F = 0.94$; $df = 7,1$; $p = 0.094$) and a cue type by condition interaction indicating a greater relative intereference of smoking-related cues compared to neutral cues in the abstinent condition compared to the satiated condition ($F = 3.81$; $df = 7,1$; $p = 0.092$). Given the potential confound of time within each of the smoking sessions on RT because of the short half-life of nicotine, we performed a MANOVA with RT as the dependent variable and condition, word type, and time (first half vs. second half of experiment) as independent variables. There was a significant main effect of time ($F = 5.63$; $df = 7,1$; $p = 0.049$). However, there were no interactions between time and condition ($F = 0.09$; df = 7,1; p = 0.775), time and word type ($F = 0.02$; $df = 7,1$; $p = 0.884$), or time, word type, and condition ($F = 0.76$; $df = 7,1$; $p = 0.411$).

Table 4.3 demonstrates results of a series of paired *t*-tests to further explore the nature of the statistical trends for cue type and cue type by condition. As seen below, the RT for smoking cues were significantly greater than the RTs for neutral cues in the abstinent condition. The RT was greater for smoking cues than neutral cues in the smoking condition but the difference was not statistically significant. Furthermore, comparisons of RTs for each cue type (smoking vs. neutral) were performed for each of the first- and second-session diads (i.e., smoking session 1 smoking cues vs. smoking session 2 smoking cues; smoking session 2 smoking cues vs. smoking session 2 neutral cues, etc.) and there were no significant differences across conditions (student t-test, ps>0.400).

Table 4.3

Reaction Times by Condition, Session and Cue Type

Session	Condition	Reaction times (ms)		Paired t-test
		Smoking cues	Neutral cues	*Df* = 7, *P*
1	Smoking	1079.23 (161.04)	1069.59 (287.47)	0.903
2		708.60 (443.41)	673.26 (426.49)	0.363
1	Abstinent	1164.82 (403.14)	1052.30 (346.56)	0.010
2		903.85 (407.21)	811.46 (362.29)	0.001
Average of sessions 1 & 2	Smoking	1050.67 (148.34)	1040.19 (289.30)	0.892
	Abstinent	1098.58 (283.91)	992.10 (241.06)	0.002

LEGEND: Mean and standard deviations (sd) for reaction times in milliseconds (ms) for smoking and neutral picture cues. Data are presented for each of two sessions for two conditions (smoking and abstinent days). Subjects are asked to indicate whether the picture displayed is male of female with a forced key press.

4.5 Discussion

This study was intended to explore the influence of smoking condition compared with abstinence and the 5-HTTLPR polymorphism on cue-elicited activation of the VS/NAc. The demonstration of greater NAc activation in the smoking condition than abstinent condition with whole brain mixed-effects group analysis, albeit at an uncorrected *z*-statistic threshold, suggests that the effect of smoking condition was remarkably consistent in this sample of eight female smokers.

I had predicted greater activation in the VS/NAc in the abstinent state given those higher ratings of tobacco craving in this and other studies are observed in the nicotine abstinent state.[614] However, since the conception of this study, the results of a recently published fMRI experiment by McClernon and colleagues suggest otherwise.[615] Although they do not present the following results in their publication, Dr. McClernon provided me with data demonstrating that the contrast between smoking and neutral cues in the VS/NAc was

[614] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002); McClernon, F.J., Hiott, F. B., Huettel, S. A., Rose, J. E. (2005). (manuscript in press).
[615] McClernon, F.J., Hiott, F. B., Huettel, S. A., Rose, J. E. (2002).

greater in the smoking condition than the abstinent condition.[616] Also, while they report correlations between other brain regions and craving in the abstinent condition, they found no correlation between craving in the abstinent state and VS/NAc activation. McClernon and colleagues do not present correlational analyses in the smoking condition and thus we do not know whether or not they observed a correlation between craving and haemodynamic response to smoking-related vs. neutral cues.

The correlation between craving and VS/NAc COPE in the smoking condition can be explained in the context of prevailing nicotine dependence models. First, smoking results in DA overflow to the core of the NAc, and when coupled with specific environmental contexts associated with obtaining the drug, is theorised to stimulate a sub-component of craving that Robinson and Berridge have termed "drug wanting". Chronic nicotine use results in neuroadaptions which alter the smoking experience from one of drug liking to aversive subjective experiences such as drug wanting and motivation to seek the drug at the expense of other activities. This may explain why active smokers, who are not in a state of nicotine withdrawal, seek out the drug despite negative social and physical consequences and even though the smoking experience provides less hedonic reward.

The MANOVA demonstrated a significant hemisphere by smoking interaction. However, given the sample size, it would be premature to assert that there was an effect of smoking condition on lateralisation. First of all, there were no significant differences in VS/NAc COPE between hemispheres in either condition. Qualitatively, it appears that the mean COPE is greater in the right hemisphere than the left hemisphere in the smoking condition and greater in the left hemisphere than the right hemisphere in the abstinent condition. I can

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[616] McClernon, F.J. Parameter estimates for smoking and neutral pictorial cues in the ventral striatum using fMRI. Personal communication (14 July 2005).

not make any generalisations or conclusions given the sample size in this study. That said, differential lateralisation according to smoking condition would not be inconsistent with other studies or biologically implausible. Animal studies have demonstrated right lateralisation in striatal DA concentration.[617] Stein and colleagues demonstrated right lateralisation in the NAc using fMRI when they administered nicotine to addicted smokers.[618] Although left lateralisation has not been described per se for cue-elicited fMRI in nicotine dependence, Heinz and colleagues demonstrated left lateralisation in the NAc when they administered alcohol-related picture cues using fMRI to alcoholics in alcohol withdrawal.[619] Furthermore, the core of the NAc is more closely linked to the sensorimotor function of the basal ganglia whilst the shell is considered a part of the extended Am. Thus, left lateralisation in the abstinent condition may reflect a pre-conscious motor process, which would be expected to lateralise to the contralateral hemisphere in these right-handed participants.[620]

The measurement of craving has been a contentious issue in the nicotine research community[621] as, even as craving is a sub-component of withdrawal, craving can exist in the absence of the nicotine withdrawal symptoms and when withdrawal symptoms have been

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[617] Bortolozzi, A., Duffard, R., de Duffard, A. M. (2003). 'Asymmetrical Development of the Monoamine Systems in 2,4-Dichlorophenoxyacetic Acid Treated Rats', *Neurotoxicology*, **24**(1): pp. 149-57; Schneider, L.H., Murphy, R. B., Coons, E. E. (1982). 'Lateralization of Striatal Dopamine (D2) Receptors in Normal Rats', *Neurosci Lett.* **33**(3): pp. 281-4.

[618] Stein, E.A., Pankiewicz, J., Harsch, H. H., Cho, J. K., Fuller, S. A., Hoffmann, R. G., Hawkins, M., Rao, S. M., Bandettini, P. A., Bloom, A. S. (1998).

[619] Heinz, A., Jones, D. W., Mazzanti, C., Goldman, D., Ragan, P., Hommer, D., Linnoila, M., Weinberger, D. R. (2000). 'A Relationship Between Serotonin Transporter Genotype and *In Vivo* Protein Expression and Alcohol Neurotoxicity', *Biol Psychiatry*, **47**(7): pp. 643-9.

[620] Zahm, D.S., Heimer, L., (1988). Ventral Striatopallidal Parts of the Basal Ganglia in the Rat:I. Neurochemical Compartmentation as Reflected by the Distributions of Neurotensin and Substance P Immunoreactivity', *J Comp Neurol.*, **272**(4):516-35.

[621] Sayette, M.A., Shiffman, S., Tiffany, S. T., Niaura, R. S., Martin, C. S., Shadel, W. G. (2000). 'The Measurement of Drug Craving', *Addiction*, **95 Suppl 2**: pp. S189-210.

treated with nicotine replacement therapy.[622] Thus, the measurement of craving during nicotine withdrawal and during nicotine satiation may, in fact, be capturing different neuropsychological processes. For this reason, it is interesting that there was a significantly positive correlation between tobacco craving and VS/NAc activation in the smoking condition but not in the abstinent condition. Although the craving scores were lower in the smoking condition than the abstinent condition, it could be argued that craving in this condition may be a more valid measure as the subjective experience is not confounded by the constellation of symptoms composing the nicotine withdrawal syndrome such as anxiety, irritability, impatience, depression, and sleep disturbances.[623]

That VS/NAc activation was greater in the smoking condition than the abstinent condition may seem surprising given the results presented in Chapter 2 of abstinent smokers who demonstrated VS/NAc activation. However, as the scatter plot in Figure 4.2 indicates, there was wide variability in effect sizes for the VS/NAc in both conditions. Thus, some smokers exhibited mean COPEs for the VS/NAc in the abstinent condition similar to those observed in the pilot study. Furthermore, the different sex composition, age, and years of smoking between smokers in the two studies may explain, in part, the difference in distribution of VS/NAc activation effect sizes. One finding that did provide some reassurance of replication was the observation of posterior fusiform gyrus activation in both study populations. As cue-elicited fusiform activation has been described in the only other published study of smoking-related cue reactivity using fMRI in abstinent smokers, this observation provides further confirmation of the notion that extrastriate visual pathways are

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[622] Waters, A.J., Shiffman, S., Sayette, M. A., Paty, J. A., Gwaltney, C. J., Balabanis, M. H. (2004). 'Cue-Provoked Craving and Nicotine Replacement Therapy in Smoking Cessation', *J Consult Clin Psychol.* **72**(6): pp. 1136-43.

[623] Sayette, M.A., Martin, C. S., Wertz, J. M., Shiffman, S., Perrott, M. A. (2001). 'A Multi-Dimensional Analysis of Cue-Elicited Craving in Heavy Smokers and Tobacco Chippers', *Addiction*, **96**(10): pp. 1419-32.

integrated with the mesolimbic system to process cue-elicited activation to smoking-related picture cues.[624]

The observation of statistical trends for cue type and cue type by condition interactions is reassuring, as *post-hoc* testing demonstrated that, as expected, reaction times to smoking-related cues were significantly greater than to neutral cues in the abstinent condition, which is consistent with results of other studies and suggestive of incentive sensitisation to smoking-related pictures.[625] Furthermore, the observation of faster reaction times in the second session is expected, as subjects have become accustomed to the task. Although not statistically significant, the difference in reaction times in the smoking condition was in the expected direction and does not obviate the possibility of a smaller effect of cue type not reflecting nicotine deprivation.

As the sample sizes were insufficient, I am not able to confidently accept or reject the null hypotheses regarding genetic differences in VS/NAc activation. Descriptively, it appears that, in the smoking condition, right lateralisation was more marked in smokers with LL genotypes and conversely, left lateralisation was more marked in smokers with LS or SS genotypes in the abstinent condition. With the current sample size, however, it would not be appropriate to speculate as to whether or not there was an influence of the 5-HTTLPR on cue-elicited activation in the VS/NAc or apparent interactions with smoking condition or hemisphere, particularly as the MANOVA found no significant main or interaction effects.

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[624] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002); David, S.P., Munafo', M., Johansen-Berg, H., Smith, S.M., Rogers, R.D., Matthews, P.M., Walton, R.T. (2005). 'Ventral Striatum/Nucleus Accumbens Activation to Smoking-Related Pictorial Cues in Smokers and Non-Smokers: An fMRI study', *Biol Psychiatry*, **58**(6):488-94.

[625] Trimmel, M., Wittberger, S. (2004); Havermans, R.C., Debaere, S., Smulders, F. T., Wiers, R. W., Jansen, A. T. (2003).

4.6 Future Directions

I found that recruitment of the sample was more difficult than expected. Many of the previously available subjects meeting genetic criteria had moved out of the region, changed telephone numbers, had quit smoking, or were no longer interested in participating in the research. Therefore, in future work I will invest substantial effort to assiduously confirming the availability of a sufficient number of study participants before initiating studies.

Another important observation that came from this study was that unexpected results can be every bit as compelling and important as results predicted by prior hypotheses. I was surprised to observe greater activation in the smoking condition than the abstinent condition. However, in light of the extant corpus of research and prevailing animal models the observation is, in fact, biologically plausible and consistent with clinical observations.

In conclusion, the primary findings from this study were that there was significantly greater activation to smoking-related vs. neutral cues in the VS/NAc in the smoking condition than in the abstinent condition. Next, there was a significant smoking by hemisphere interaction suggesting right lateralisation in the smoking condition and left lateralisation in the abstinent condition. Furthermore, there was a statistically significant positive correlation between tobacco craving and global mean VS/NAc COPE in the smoking condition. Finally, reaction times demonstrated statistical trends for main effects of cue type and cue type by condition interactions.

These trends were all in the predicted direction suggesting that subjects demonstrated incentive-sensitisation to smoking-related picture cues. Although the sample size was not large enough to detect significant differences in activation by 5-HTTLPR genotype,

descriptive data were informative in projecting necessary sample sizes for planned future work.

Chapter 5 will apply the conclusions from the sum of the work presented in this chapter, and from Chapters 2 and 3 toward the development of a theoretical model of nicotine dependence with implications for the development more efficacious smoking cessation therapies.

Chapter 5.

DISCUSSION AND TRANSLATIONAL APPLICATIONS

5.1 Conclusions

The work described in the preceding chapters addressed each of the specific aims presented in Chapter 1. These aims were to:

1. Identify novel nicotine dependence endophenotypes using functional magnetic resonance imaging (fMRI);

2. Examine the functional significance of polymorphisms in the serotonin 5-HT_{1A} receptor and serotonin transporter genes utilising positron emission tomography (PET);

3. Triangulate results from both arms of research in order to examine whether or not a trait variable (5-HTTLPR genotype) or a state variable (smoking condition) would significantly influence cue-elicited activation of the ventral striatum including the nucleus accumbens.

In this concluding chapter I describe how I met each of these aims, conclusions derived from the research, lessons learned, and applications of these data to future research with mentors and collaborators.

Clearly, nicotine dependence represents a large array of complex behaviours, each of which must be defined in specific behavioural and biological terms in order to advance our understanding of the precise mechanisms underlying this powerful addiction.

My first aim was to identify novel nicotine dependence endophenotypes utilising fMRI. I chose to examine cue-elicited activation in the ventral striatum including the nucleus accumbens VS/NAc and other reward-signalling regions because human research has demonstrated that cue reactivity to drug-associated stimuli reliably predicts relapse to cigarette smoking and other drug use.[626] As such, a more precise understanding of the

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[626] Niaura, R., Shadel, W. G., Abrams, D. B., Monti, P. M., Rohsenow, D. J., Sirota, A. (1998). 'Individual Differences in Cue Reactivity Among Smokers Trying to Quit: Effects of Gender and Cue Type', *Addict Behav.* **23**(2): pp. 209-24; Niaura, R., Abrams, D. B., Monti, P. M., Pedraza, M. (1989). 'Reactivity to High Risk Situations and Smoking Cessation Outcome', *J Subst Abuse*, **1**(4): pp. 393-405; Rohsenow, D.J., Niaura,

biological processes underlying cue-elicited activation of mesolimbic reward regions might therefore identify anatomical and molecular targets for future drug development and specific, well-defined endophenotypes for examination of genetic influences on nicotine dependence.

The research described in Chapter 2 suggested that, in this population of addicted smokers undergoing nicotine withdrawal, smoking-related pictorial stimuli, when compared to neutral stimuli, activated the anterior cingulate cortex, orbitofrontal cortex, superior frontal gyrus, and VS/NAc.[627] Furthermore, no activation was observed in non-smoking controls and the effect size for VS activation was significantly greater for smokers than non-smokers. These data were consistent with prevailing models of nicotine addiction derived from animal[628] and pre-clinical human studies of cocaine and alcohol addiction.[629]

Although these data are preliminary and require replication, they suggest that cue-elicited activation of the VS (including the nucleus accumbens) can be demonstrated in addicted smokers *in vivo* and that this activation is specific to smokers when compared with non-

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R. S., Childress, A. R., Abrams, D. B., Monti, P. M. (1990). 'Cue Reactivity in Addictive Behaviors: Theoretical and Treatment Implications', *Int J Addict*, **25**(7A-8A): pp. 957-93; Hutchison, K.E., Niaura, R., Swift, R. (1999). 'Smoking Cues Decrease Prepulse Inhibition of the Startle Response and Increase Subjective Craving in Humans', *Exp Clin Psychopharmacol.* **7**(3): pp. 250-6.

[627] David, S.P., Munafo', M., Johansen-Berg, H., Smith, S.M., Rogers, R.D., Matthews, P.M., Walton, R.T. (2005). 'Ventral Striatum/Nucleus Accumbens Activation to Smoking-Related Pictorial Cues in Smokers and Non-Smokers: An fMRI study', *Biol Psychiatry*, **58**(6):488-94.

[628] Robinson, T.E., Berridge, K. C. (1993). 'The Neural Basis of Drug Craving: An Incentive-Sensitization Theory of Addiction', *Brain Res Brain Res Rev.* **18**(3): pp. 247-91; Koob, G.F., Le Moal, M. (2001). 'Drug Addiction, Dysregulation of Reward, and Allostasis', *Neuropsychopharmacology*, **24**(2): pp. 97-129.

[629] Garavan, H., Pankiewicz, J., Bloom, A., Cho, J. K., Sperry, L., Ross, T. J., Salmeron, B. J., Risinger, R., Kelley, D., Stein, E. A. (2000). 'Cue-Induced Cocaine Craving: Neuroanatomical Specificity for Drug Users and Drug Stimuli', *Am J Psychiatry*, **157**(11): pp. 1789-98; Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004). 'Correlation between Dopamine D(2) Receptors in the Ventral Striatum and Central Processing of Alcohol Cues and Craving', *Am J Psychiatry*, **161**(10): pp. 1783-9; Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002). 'Activation in Mesolimbic and Visuospatial Neural Circuits Elicited by Smoking Cues: Evidence from Functional Magnetic Resonance Imaging', *Am J Psychiatry*, **159**(6): pp. 954-60; Volkow, N.D., Fowler, J. S., Wang, G. J. (2003). 'The Addicted Human Brain: Insights from Imaging Studies', *J Clin Invest.* **111**(10): pp. 1444-51.

smoking controls. Therefore, the event-related stimulus paradigm has potential applications for human studies of the pharmacodynamics of nicotine, and for the development or identification of novel compounds for smoking cessation. Furthermore, the integration of functional neuroimaging and pharmacogenetics will permit investigators to gather more precise information on how various genotypes affect a very specific smoking-related endophenotype. Therefore, by developing a reliable fMRI experiment for smoking-related cue reactivity, I believe I successfully achieved my first specific aim. In addition, it is worth noting that the process of developing, piloting, and refining the paradigm was extremely useful in developing a new skill set (i.e., study design and fMRI analysis), and I believe that the lessons learned helped in sharpening my research skills in general.

Having identified an informative endophenotype for nicotine dependence, and with the serotonin 5-HT_{1A} receptor being implicated in the behavioural sensitisation process to nicotine (as described in Chapter 1), my next aim was to examine the functional significance of polymorphisms in the serotonin $5HT_{1A}$ receptor and serotonin transporter genes utilising PET. I hypothesised that a functional polymorphisms in the $5HT_{1A}$ gene would affect binding potential (BP) both pre-synaptically and post-synaptically and that any genetic effect of the $5HT_{1A}$ –1018 C/G polymorphism would be modified by the serotonin transporter 5-HTTLPR polymorphism. We found, however, that instead of a functional candidate gene polymorphism in the gene ($5HT_{1A}$ -1018 C/G), a polymorphism in the 5-HTT gene (5-HTTLPR) significantly affected $5HT_{1A}$ receptor BP. Had it not been for the study by Li and colleagues[630] in knockout mice suggesting a genetic effect of the 5-HTT gene on $5HT_{1A}$

[630] Li, Q., Wichems, C., Heils, A., Lesch, K. P., Murphy, D. L. (2000). 'Reduction in the Density and Expression, but not G-Protein Coupling, of Serotonin Receptors (5-HT1A) in 5-HT Transporter Knock-Out Mice: Gender and Brain Region Differences', *J Neurosci.* **20**(21): pp. 7888-95.

receptor expression, I would not have included this genotype in the analysis. Thus, the application of rigorous pharmacological research from animal studies to hypothesis generation was critical to identify a biologically plausible mechanism in humans for the observed association of the 5-HTTLPR with nicotine dependence as well as mood disorders and personality traits that are highly co-morbid with nicotine dependence.

Therefore, as I methodically examined genetic influences in serotonergic genes on 5-HT_{1A} receptor binding and identified an association with the 5-HTTLPR polymorphism, I believe I was successful in achieving my second specific aim.

The next logical step in my research, then, was to examine, in preliminary fashion, whether the 5-HTTLPR influences cue-elicited activation to smoking-related stimuli in addicted smokers and, as the receptor is implicated in mediating the nicotine withdrawal syndrome and behavioural sensitisation to nicotine, whether cigarette smoking influenced fMRI activation in reward-signalling brain regions.

Thus, having identified a technique for examining a relevant endophenotype and a biologically plausible candidate gene, my next aim was to examine whether or not a trait variable (5-HTTLPR genotype) or a state variable (smoking condition) would significantly influence cue-elicited activation of the VS/NAc utilising fMRI.

Therefore, as described, addicted smokers who had been genotyped for the 5-HTTLPR underwent an event-related paradigm, which like the study described in Chapter 2, presented smoking-related and neutral pictorial cues from the International Smoking Image Series.[631] The primary conclusions from this triangulation study were that there was a significant main effect of smoking condition on VS/NAc activation to smoking-related vs. neutral pictorial

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[631] Gilbert, D.G., Rabinovich, N.E. (1999). *International Smoking Images Series (With Neutral Counterparts).* (Southern Illinois University: Integrative Neuroscience Laboratory, Department of Psychology) [on CD-ROM].

cues. Moreover, there was a significant smoking condition by hemisphere interaction such that following *ad libitum* cigarette smoking, right hemisphere lateralisation was suggested, whilst left lateralisation was suggested in the abstinent condition.

The doubling of the number of observations for each condition increased the reliability of inferences regarding mean VS/NAc COPE when compared to the pilot study described in Chapter 2. Thus, even as significant lateralisation was not observed in the abstinent condition in said study, this observation may be the result of insufficient statistical power rather than non-replication per se. Moreover, with the repeated measures design I was able to detect statistical trends for main effects of cue type and interaction effects for cue type by condition on reaction times such that, as expected, reaction times were significantly greater in response to smoking-related pictures than neutral pictures in the abstinent/nicotine withdrawal condition.[632] Furthermore, whilst qualitatively greater in the smoking condition, reaction times to smoking-related cues were not significantly greater than neutral cues in the smoking condition. Therefore, these results indicate that, in this group of addicted smokers, incentive sensitisation to smoking-related pictures was observed when compared to neutral pictures and that, as expected, the effect was exaggerated in the withdrawal condition as a likely effect of nicotine deprivation.[633]

Finally, whilst observed power analyses indicated that substantially larger sample sizes are needed for sufficient statistical power to detect genetic effects on cue-elicited fMRI BOLD response to smoking-related vs. neutral cues, preliminary observations may

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[632] Trimmel, M., Wittberger, S. (2004). 'Effects of Transdermally Administered Nicotine on Aspects of Attention, Task Load, and Mood in Women and Men', *Pharmacol Biochem Behav.* **78**(3): pp. 639-45; Havermans, R.C., Debaere, S., Smulders, F. T., Wiers, R. W., Jansen, A. T. (2003). 'Effect of Cue Exposure, Urge to Smoke, and Nicotine Deprivation on Cognitive Performance in Smokers', *Psychol Addict Behav.* **17**(4): pp. 336-9.
[633] Ibid.

reasonably generate tentative hypotheses about potential influences of the 5-HTTLPR on the effects of smoking condition on VS/NAc lateralisation.

As I did triangulate the results from the fMRI pilot study described in Chapter 1 and the PET study of 5-HT_{1A} receptor function described in Chapter 2 and examined the influences of a trait (5-HTTLPR polymorphism) variable and state (satiation vs. abstinence) variable on cue-elicited activation in the VS/NAc, I believe I have achieved the third and final specific aim of this course of research.

Therefore, the following preliminary conclusions can be drawn from the results of the three studies.

- First, in this population of addicted smokers, smoking-related pictorial cues activated a distributed reward network including the VS/NAc.
- Second, cue-elicited activation of brain reward regions was observed uniquely in addicted smokers and not in non-smokers.
- Third, that smoking-related VS/NAc activation was greater in smokers than non-smokers.
- Next, that a well-supported candidate gene polymorphism associated with nicotine dependence ubiquitously affects serotonin binding to 5-HT_{1A} receptors throughout the brain including cortical and subcortical regions implicated in reward signalling.
- Furthermore, that we did not detect a significant effect of 5-HTTLPR genotype on cue-elicited activation to smoking-related vs. neutral cues but that, given *post hoc* sample size estimates, that larger sample sizes would be required to have sufficient statistical power to reject the null hypothesis.

- Finally, that VS/NAc activation to smoking-related cues was significantly greater following cigarette smoking than during nicotine withdrawal/abstinence as verified by exhaled CO.

Whilst there are no published studies employing precisely the same methods, other fMRI studies of drug dependence including nicotine may provide useful benchmarks for determining whether the work reported in this thesis is consistent with that of other investigators.

McClernon and colleagues conducted an event-related fMRI study of smoking-related picture cues taken from the standardised set of images used in my studies. Subjects were evaluated on two occasions (after smoking *ad libitum* 'satiated' condition and after overnight abstinence 'abstinent' condition).[634] These investigators found that smoking cues elicited significantly greater activation than neutral cues in the VS/NAc, anterior cingulate, superior frontal gyrus, and other cortical areas in both conditions. Although they do not report the contrast of the smoking and neutral parameter estimates in their paper, the mean COPE for smoking vs. neutral cues in the VS was greater in the smoking condition than in the neutral condition.[635] This study was not published until the time of completion of the triangulation study described in Chapter 4 and therefore could not inform my hypothesis generation. However, the observation of greater activation to smoking vs. neutral cues in the satiated state is consistent with the findings in our study. Furthermore, Brody and colleagues, utilising [11C]-raclopride PET, demonstrated that cigarette smoking resulted in significantly

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[634] McClernon, F.J., Hiott, F. B., Huettel, S. A., Rose, J. E. (2005). 'Abstinence-Induced Changes in Self-Report Craving Correlate with Event-Related fMRI Responses to Smoking Cues', *Neuropsychopharmacology* (manuscript in press).

[635] McClernon, F.J. (2005). 'Effect Sizes for Smoking and Neutral Pictorial Cues in the Ventral Striatum Using fMRI', [from private correspondence].

increased VS release of DA when compared to abstinence.[636] Heinz and colleagues, using combined approaches of [11C]-raclopride PET and a paradigm of alcohol-related vs. neutral pictorial cues, demonstrated that cue-elicited BOLD response to such stimuli reflected DA dysfunction and observed a negative correlation between alcohol craving and fMRI BOLD contrast in the VS/NAc. In addition, Heinz and colleagues observed left lateralisation in the abstinent state.[637] Heinz and colleagues did not compare fMRI BOLD contrast between alcohol satiated and abstinent states, thus we do not know if a positive correlation would have been observed.

However, the results of these three studies may shed light on the observations presented in this thesis and suggest consistency in overall results. As such, the greater activation of VS/NAc in the smoking condition may reflect relative DA overflow following cigarette smoking and behavioural sensitisation to smoking-related pictorial cues when coupled with NAc DA overflow. That VS/NAc activation was positively and significantly correlated with cigarette craving following cigarette smoking would indicate a relationship between intensity of craving and VS/NAc activation during DA overflow in the NAc core without the potentially confounding anxiogenic effects of nicotine withdrawal contributing to the overall craving rating.

That statistical trends for reaction times indicated incentive sensitisation to smoking-related pictures reinforces the inference that the observed activation in VS/NAc is a function of incentive sensitisation. Furthermore, robust activation of the posterior fusiform and inferior temporal gyri, which are extrastriate regions implicated in recognition of faces and

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[636] Brody, A.L., Olmstead, R. E., London, E. D., Farahi, J., Meyer, J. H., Grossman, P., Lee, G. S., Huang, J., Hahn, E. L., Mandelkern, M. A. (2004). 'Smoking-Induced Ventral Striatum Dopamine Release', *Am J Psychiatry*, **161**(7): pp. 1211-8.

[637] Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004).

manmade objects[638] respectively, suggests greater salience of smoking-related picture cues than neutral cues in these smokers and is consistent with a similar study, previously discussed, by Due and colleagues.[639]

The results of the study described in Chapter 3 indicate, as previously discussed, that the 5-HTTLPR significantly influences 5-HT_{1A} BP presynaptically and postsynaptically. Also, as previously discussed, the lower 5-HT_{1A} BP may plausibly result from increased lifelong 5-HT tone. These observations have implications for the pharmacological management of smoking cessation and may explain why individuals with the S allele do not respond as well to selective serotonin reuptake inhibitors (SSRIs). As addressed in the previous chapter, the putative mechanism for SSRIs is desensitisation of inhibitory 5-HT_{1A} receptors.[640] With effectively reduced BP resulting from the 5-HTTLPR S allele, it stands to reason that the antidepressant effects of SSRIs may be less efficacious in individuals with 5-HTTLPR S alleles than those with LL genotypes[641] because of a relatively reduced effect of SSRIs on 5-HT_{1A} receptor desensitisation in S allele carriers.

Likewise, the observation in the PATCH II population that only smokers with the S allele had significant benefits from nicotine patch therapy in the first four weeks following a quit attempt[642] is consistent with these subjects having reduced 5-HT_{1A} BP and may explain in

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[638] Ishai, A., Ungerleider, L. G., Martin, A., Schouten, J. L., Haxby, J. V. (1999). 'Distributed Representation of Objects in the Human Ventral Visual Pathway', *Proc Natl Acad Sci U S A*, **96**(16): pp. 9379-84.

[639] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002).

[640] Blier, P., Ward, N. M. (2003). 'Is there a Role for 5-HT1A Agonists in the Treatment of Depression?' *Biol Psychiatry*, **53**(3): pp. 193-203; Pineyro, G., Blier, P. (1999). 'Autoregulation of Serotonin Neurons: Role in Antidepressant Drug Action', *Pharmacol Rev.* **51**(3): pp. 533-91; Rueter, L.E., Blier, P. (1999). 'Electrophysiological Examination of the Effects of Sustained Flibanserin Administration on Serotonin Receptors in Rat Brain', *Br J Pharmacol.* **126**(3): pp. 627-38.

[641] Smits, K.M., Smits, L. J., Schouten, J. S., Stelma, F. F., Nelemans, P., Prins, M. H. (2004). 'Influence of SERTPR and STin2 in the Serotonin Transporter Gene on the Effect of Selective Serotonin Reuptake Inhibitors in Depression: A Systematic Review', *Mol Psychiatry*, **9**(5): pp. 433-41.

[642] David, S.P., Murthy, N.V., Rabiner, E.A., Munafo', M., Jacob, R., Johnstone, E., Grasby, P.M. (2004). 'Serotonin Transporter Polymorphism Linked to Personality Traits Affects Serotonin (5-HT1A) Receptor

part observations from the fMRI triangulation study. As 5-HT_{1A} receptor stimulation attenuates the anxiolytic effects of nicotine, smokers with greater 5-HT_{1A} receptor availability in the raphe and in postsynaptic 5-HT terminal fields would experience more attenuation of the anxiolytic effects of nicotine than smokers with lower 5-HT_{1A} availability. Conversely, smokers with S alleles would not theoretically experience as much inhibition of the anxiolytic effects of nicotine and would therefore derive more benefit from nicotine replacement therapy. It is worth repeating, however, that we did not find significantly greater short-term effectiveness of the nicotine patch in the S allele group, but that significant efficacy at four weeks following a quit attempt was observed only in the S allele subgroup. Although not of sufficient sample size to draw broad, generalisable conclusions, the fMRI study described in Chapter 4 demonstrated results consistent with this observation. In the smoking condition, smokers with LL genotype appeared to demonstrate greater VS/NAc activation to smoking-related pictorial cues than those with S alleles. Whilst larger sample size is needed to determine whether this qualitative observation can be replicated and whether it would be statistically significant, the effect is in the expected direction.

Thus, as S allele carriers may have greater genetic liability for behavioural sensitisation, they may also experience greater relief of the anxiety component of nicotine withdrawal and therefore be less prone to relapse on the nicotine patch. This assertion is consistent with the observation that $5HT_{1A}$ receptor stimulation inhibits the anxiolytic effects of nicotine. Therefore, nicotine replacement would theoretically result in less inhibition of anxiety by nicotine as a result of reduced $5HT_{1A}$ stimulation. Conversely, smokers with LL genotypes may derive relatively less relief of nicotine withdrawal from nicotine replacement therapy. In

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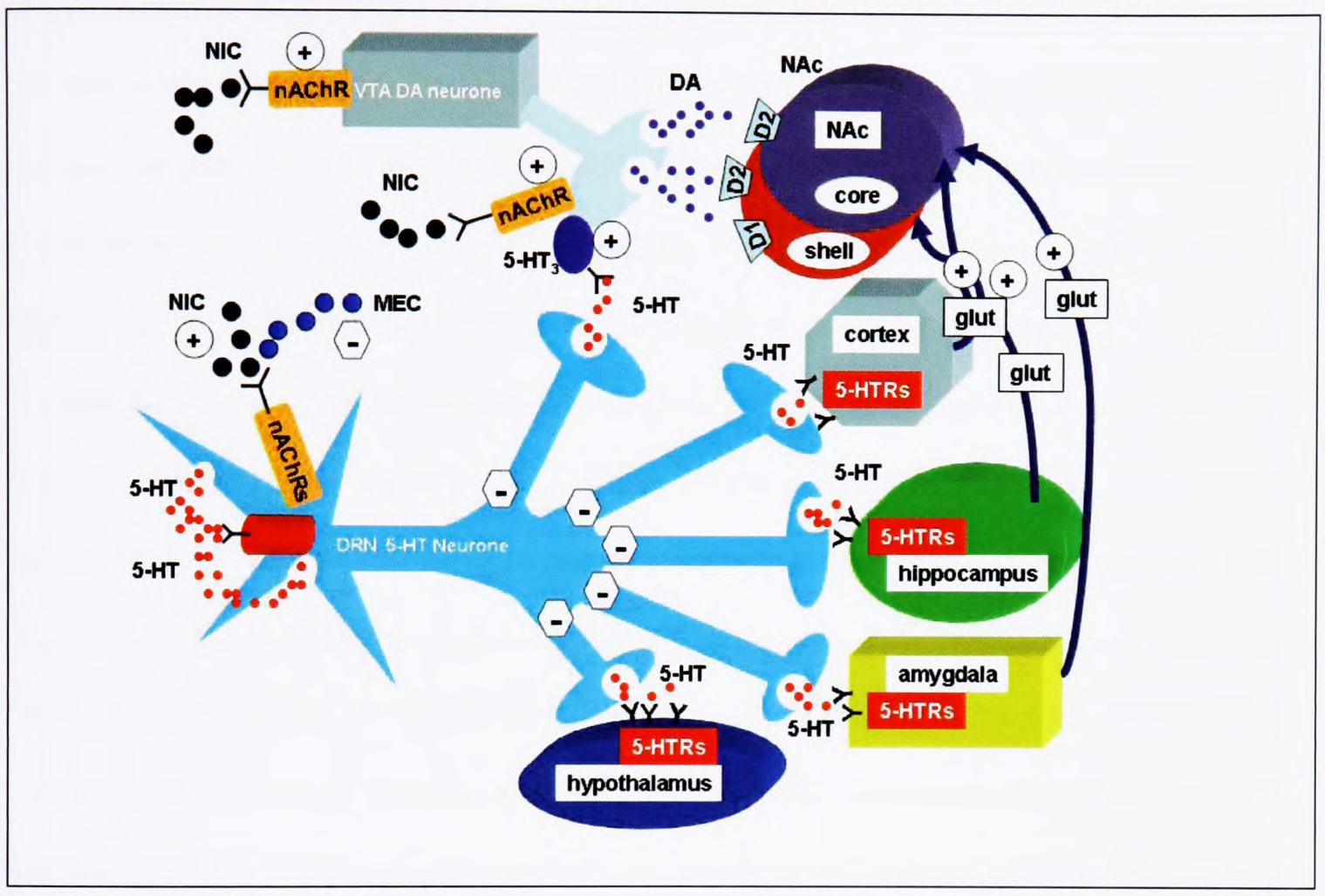
Function in Positron Emission Tomography Study', paper presented at the North American Primary Care Research Group's (NAPCRG) 32nd annual meeting in Orlando, FL, USA.

the same way, smokers with the 5-HTTLPR LS or SS genotype and greater genetic liability for behavioural sensitisation would be expected to demonstrate greater activation in the withdrawal condition than those with LL genotypes. This model, as shown in figure 5.1, clearly requires further examination in a larger study but is, I believe, a plausible explanation for my collective observations.

Thus, as proposed by Olausson and colleagues, nicotine dependence results, in part, from a disruption of a delicate balance between 5-HT and DA systems due to nicotine-induced neuroadaptations. The result of the 5-HT-DA imbalance is enhanced incentive sensitisation. Therefore, reduced 5-HT_{1A} receptor expression, as seen in individuals with the 5-HTTLPR, would theoretically increase the risk of developing nicotine dependence because of greater incentive sensitisation.

Figure 5.1

Proposed Mechanism for 5-HT-DA Interactions in the Development of Incentive Sensitisation and Implications for Genetic Liability to Nicotine Dependence



LEGEND: Proposed model for serotonin-dopamine interactions in nicotine dependence. Evidence suggests that nicotine (NIC) binds to nicotinic acetylcholine receptors (nAChRs) on serotonergic cell bodies in the dorsal raphe nucleus (DRN) and dopaminergic cell bodies in the ventral tegmental area (VTA). Nicotine infusion into the DRN stimulates release of serotonin (5-HT) in terminal fields but intra-DRN nicotine also leads to release of 5-HT, presumably due to somatodendritic exocytosis, which binds to inhibitory 5-HT_{1A} receptors on DRN cell bodies. The result is inhibition of 5-HT release in terminal fields, which may explain why chronic nicotine administration results in decreased 5-HT in the hippocampus and why 5-HT_{1A} stimulation in the DRN inhibits the anxiolytic effects of nicotine, effects reversed by the 5-HT_{1A} receptor antagonist WAY-100635 and by the α4β2 antagonist DhβE. In addition, 5-HT_3 receptors are located presynaptically on DA neurones in the NAc. Stimulation of 5-HT_3 neurones enhances the amount of nicotine-induced DA release in the NAc. Therefore, theoretically, 5-HT_{1A} stimulation in the DRN would attenuate 5-HT release in the NAc resulting in less augmentation of nicotine-induced NAc DA release. The NAc also receives glutamatergic (glut) innervation from the amygdala and hippocampus. A proposed mechanism explaining why 5-HT_{1A} receptor stimulation inhibits behavioural sensitisation would be that in smokers with normal 5-HT_{1A} receptor distribution (i.e., 5-HTTLPR LL genotype), stimulation results reduced 5-HT release in terminal fields including the NAc, hippocampus, amygdala, and hypothalamus. As a result, NAc DA receptor stimulation would theoretically be reduced in smokers with LL genotype compared to LS/SS genotypes because of reduced NAc core stimulation by DA and glutamine. Furthermore, less stimulation of the hypothalamus would reduce the coupling of NAc stimulation with HPA activation.

5.2 Limitations of Research and Lessons Learned

I have discussed some of the limitations of the research in the preceding experimental chapters. Each of these studies some aspects of the experimental designs were novel and had not previously been reported in published work. Thus, in each case, replication of the findings are needed. In the first study, which was presented in Chapter 2, 9 smokers and 11 non-smokers were included in the final analysis. The sample size was adequate to detect between-group differences in VS/NAc fMRI BOLD contrast for the smoking vs. neutral stimulus paradigm using a more sensitive region of interest (ROI) approach with voxel-wise analysis. However, it is possible that a larger sample size might permit the detection of differences in BOLD contrast between groups using whole-brain mixed-effects analysis. The limitations of time and resources did not permit a larger pilot study but, as seen in other cue reactivity fMRI studies, sample sizes of similar or slightly greater numbers of subjects have only reported significant between-group differences using voxel-wise ROI approaches.[643] Similarly with the fMRI triangulation study, as described in Chapter 4, a larger sample size would have permitted me to examine genetic hypotheses with greater statistical power and would have also made it possible to examine effects of gender on cue-elicited BOLD response to smoking-related cues.

In addition, further methodological modifications to the fMRI protocol might theoretically improve sensitivity. As mentioned in Chapter 2, the VS/NAc borders on regions prone to signal loss due to susceptibility artefact.[644] Figure 5.2 below demonstrates signal

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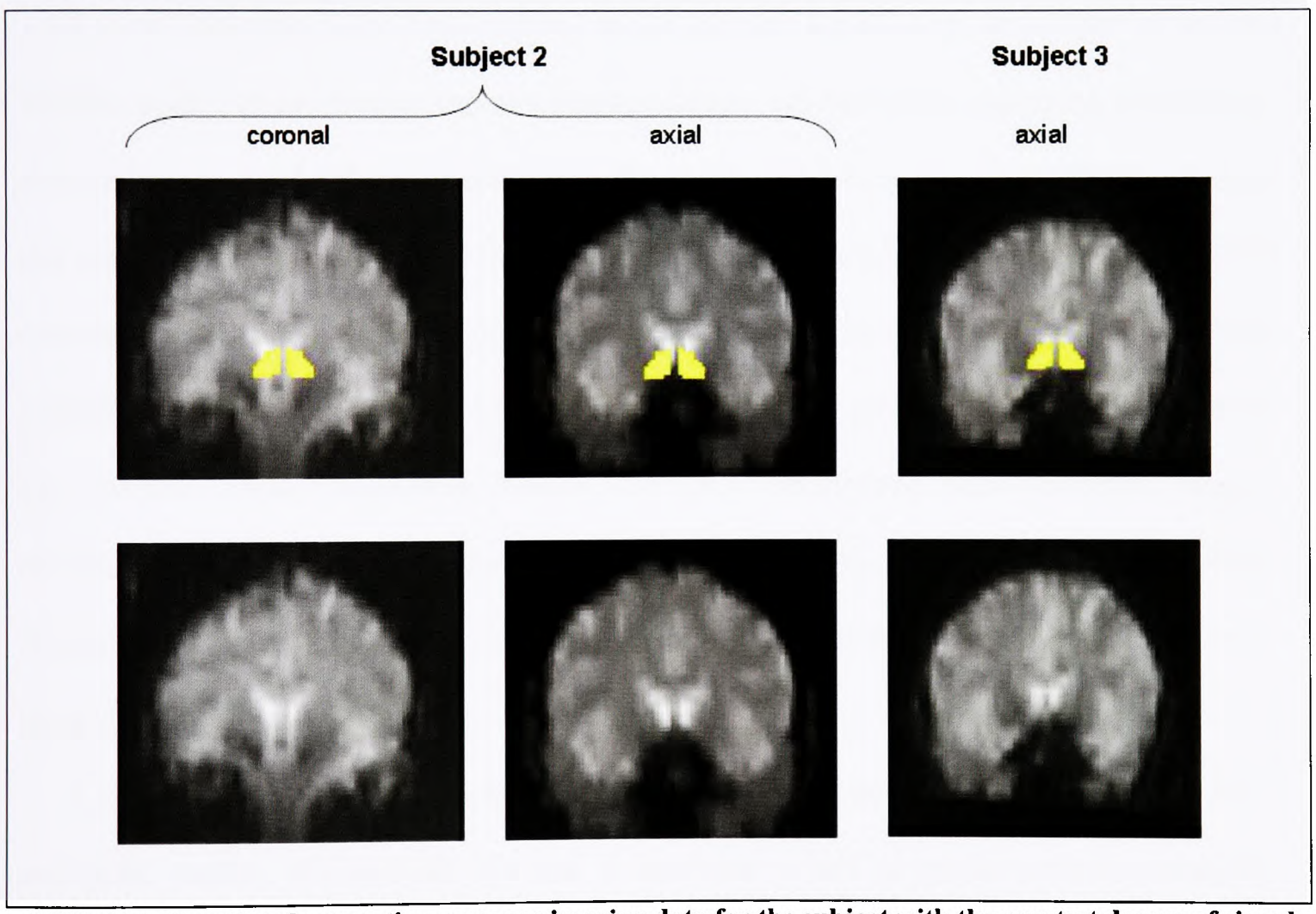
[643] Garavan, H., Pankiewicz, J., Bloom, A., Cho, J. K., Sperry, L., Ross, T. J., Salmeron, B. J., Risinger, R., Kelley, D., Stein, E. A. (2000); Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004).

[644] Rogers, R.D., Ramnani, N., Mackay, C., Wilson, J. L., Jezzard, P., Carter, C. S., Smith, S. M. (2004). 'Distinct Portions of Anterior Cingulate Cortex and Medial Prefrontal Cortex Are Activated by Reward Processing in Separable Phases of Decision-Making Cognition', *Biol Psychiatry*, **55**(6): pp. 594-602; Ojemann,

drop-out using axial and coronal acquisitions for the same individual (subject 2) with the same experimental paradigm (described in Chapter 2), and taken on the same day (left) from the fMRI study described in Chapter 4. It is clear in this case that there is substantial signal dropout of the functional data using axial acquisitions when compared to coronal scans. However, the degree of signal dropout varies markedly across subjects as demonstrated with subject 3 on the right.

Figure 5.2

Susceptibility Artifact with Axial and Coronal fMRI Acquisitions



LEGEND: Functional magnetic resonance imaging data for the subject with the greatest degree of signal dropout (subject 2) and the least (subject 3) using axial acquisitions. Comparisons are made between use of axially- and coronally-oriented slices to demonstrate potential reductions in signal dropout when utilizing coronal acquisitions. Top row images include ventral striatum/nucleus accumbens (VS/NAc) mask (described in Chapter 4); bottom row shows same functional data without the VS/NAc mask.

J.G., Akbudak, E., Snyder, A. Z., McKinstry, R. C., Raichle, M. E., Conturo, T. E. (1997). 'Anatomic Localization and Quantitative Analysis of Gradient Refocused Echo-Planar fMRI Susceptibility Artifacts', *Neuroimage*, **6**(3): pp. 156-67.

The relatively few published fMRI cue reactivity studies of nicotine dependence are not consistent with respect to whether or not the use of coronal slices are more sensitive than axial slices for detecting haemodynamic response in the VS/NAc. Rather, the data are mixed. For example, McClernon and colleagues used an event-related cue presentation with axial slices and demonstrated VS/NAc activation to smoking-related pictorial cues,[645] and, as described in Chapters 2 and 4, I also used axial scanning and observed VS/NAc activation.[646] However, using the identical protocol in the same laboratory, Due and colleagues employed coronal slices and actually found deactivation in the VS/NAc.[647]

As I will describe in the next section, one of the next logical steps would be to examine whether modifications such as use of a blocked design, coronal slices, shimming localised to anterior orbitofrontal regions, or different pulse sequences, will reduce susceptibility artefact and optimise signal to noise ratio in orbitofrontal brain regions.[648] A systematic approach to considering changes to the study protocol would necessarily involve determination of which combination of the following parameters would result in the greatest signal to noise ratio in the VS/NAc. These parameters include: the slice orientation, pulse sequence, phase-encoding, head positioning, signal strength (e.g., 1.5 T vs. 3 T), and use of specific shimming methods. Furthermore, sensitivity would be expected to improve with use of a block design.[649]

Another issue worth mentioning is the ability to examine gender effects in both the PET and fMRI studies. Whilst I did not find a significant effect of gender on the association

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[645] McClernon, F.J., Hiott, F. B., Huettel, S. A., Rose, J. E. (2005).
[646] David, S.P., Munafo', M., Johansen-Berg, H., Smith, S.M., Rogers, R.D., Matthews, P.M., Walton, R.T. (2005).
[647] Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002).
[648] Rogers, R.D., Ramnani, N., Mackay, C., Wilson, J. L., Jezzard, P., Carter, C. S., Smith, S. M. (2004).
[649] Matthews, P.M. (2001). 'Introduction', in *Functional MRI--An Introduction to Methods*, ed. by Jezzard, P., Matthews, P.M., Smith, S.M. (Oxford, UK: Oxford University Press), pp. 1-34.

between 5-HTTLPR and 5-HT_{1A} BP, it is possible that larger sample sizes for each gender might detect effects of gender.[650] In the final study only female smokers were available from the limited population of subjects who had been genotyped. There are mixed results regarding the question of whether or not there are gender effects of nicotine replacement therapy (NRT) on smoking cessation. A recent meta-analysis in which I participated did not find a significant gender effect on NRT efficacy.[651] However, even if nicotine replacement therapy efficacy is not moderated by gender, it is possible that there are gender effects on cue-elicited VS/NAc activation, which could in turn be influenced by smoking condition or genotype. Therefore, substantially larger sample sizes would be needed to examine effects of gender and genotype on VS/NAc activation to smoking-related picture cues.

I believe that the process of conceptualising, designing, executing, and analysing the research in this thesis was of immeasurable value. An important lesson has been how critically important it is to thoroughly pilot new experimental protocols prior to beginning a new experiment. I was fortunate to have the advice of extremely experienced advisors in neuroimaging and psychology. Even so, the process of protocol refinement was one of trial and error requiring several months and a steep learning curve. Fortunately, as a result of initial pilot work, the final fMRI protocol demonstrated reliability in detecting VS reactivity to smoking-related pictorial cues and the findings of the study are consistent with other published work. Future work will incorporate thorough pilot testing.

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[650] Lee, M., Bailer, U. F., Frank, G. K., Henry, S. E., Meltzer, C. C., Price, J. C., Mathis, C. A., Putnam, K. T., Ferrell, R. E., Hariri, A. R., Kaye, W. H. (2005). 'Relationship of a 5-HT Transporter Functional Polymorphism to 5-HT(1A) Receptor Binding in Healthy Women', *Mol Psychiatry*, **10**(8): pp. 715-6; Li, Q., Wichems, C., Heils, A., Van De Kar, L. D., Lesch, K. P., Murphy, D. L. (1999). 'Reduction of 5-Hydroxytryptamine (5-HT)(1A)-Mediated Temperature and Neuroendocrine Responses and 5-HT(1A) Binding Sites in 5-HT Transporter Knockout Mice', *J Pharmacol Exp Ther*. **291**(3): pp. 999-1007.

[651] Munafo', M., Bradburn, M., Bowes, L., David, S. (2004). 'Are There Sex Differences in Transdermal Nicotine Replacement Therapy Patch Efficacy? A meta-analysis', *Nicotine Tob Res*. **6**(5): pp. 769-76.

Furthermore, I was surprised to find significant main effects of the 5-HTTLPR on 5-HT_{1A} BP in the PET study and that VS activation to smoking-related cues was greater in the smoking condition in the fMRI triangulation study. However, in hindsight, these observations are in fact well explained in light of the since published literature. Thus, even when I feel prepared with well-supported *a priori* hypotheses, I must be prepared for the possibility of unexpected results, which, in this case, should inform future research and contribute to the advancement of research into pharmacogenetic influences on nicotine dependence.

5.3 Next Steps

The next logical steps in this line of research have been discussed and planned extensively with Professor Matthews. Postdoctoral pilot work with FMRIB will examine whether several modifications to the current fMRI protocol will enhance the sensitivity and reliability of examining cue-elicited VS activation. The specific modifications we will examine in future piloting include comparison of coronal and axial slices, shorter time to echo (TE), anterior shimming, and use of a scanner with lower field strength (i.e., 1.5 Tesla scanner at the Oxford Centre for Clinical Magnetic Resonance).

With the assistance of Dr. Kate Watkins, Dr. Clare Mackay, Professor Matthews, and others, I have begun the process of systematically examining optimal scanning parameters for examining NAc BOLD signal. Although preliminary, an examination was conducted of one healthy volunteer using a 1.5 Tesla scanner at OCMR. The following components of fMRI Echo Planar Imaging (EPI) methodology were examined for optimal SNR in the VS/NAc: Scan acquisition orientation, phase encoding, TE, voxel size, head positioning, and

use of a mouth shield. In summary, SNR maps were calculated for variations in each parameter mentioned above for the NAc and compared with the putamen. The techniques resulting in the highest SNR included coronal acquisition, TR of 3000 ms, TE of 40 ms, left to right phase encoding, shimming localised to cerebrum anterior to the anterior commissure, and an isotropic voxel size of 3 x 3 x 3 mm^3. Furthermore, I developed a cue reactivity presentation using a balanced blocked design of smoking-related and neutral pictures. Application of the blocked design in one abstinent smoker demonstrated no sign of signal dropout in the NAc and activation in the orbitofrontal cortex.

Whilst these results are preliminary, I believe they are the starting point for planned post-doctoral pilot work to develop a fully optimised, sensitive, and reliable cue reactivity protocol that would be readily applicable to pharmacodynamic evaluation of novel smoking cessation compounds.

In addition, collaborative work with Professor Matthews will continue for the next five years with a study of genetic influences on nicotine dependence funded by a centre of excellence grant at my home institution (Brown University, Providence, Rhode Island, U.S.A.) from the National Institutes of Health. In this study, a total of 120 smokers and non-smokers will be recruited (Smokers = 60, Non-Smokers = 60) to examine effects of genotype and nicotine administration on activation to N-Back, continuous performance, cue-reactivity, and emotional regulation tasks and stimuli. We hypothesise that different brain regions will show maximal activation during the attention (frontal) and emotional processing (mesial temporal and mesial frontal). We hypothesise that on the cue reactivity task, activation of both the attention and emotional systems will occur.

5.4 Translational Applications of Research

This work has immediate and future translational applications. Given the effect of the 5-HTTLPR on 5-HT_{1A} receptor BP, an immediate application will be the generation of novel hypotheses regarding the pathogenesis of nicotine dependence, mood disorders and some personality traits. Complex behaviours including nicotine dependence are clearly under the influence of multiple genes and environmental variables.[652] However, the observation of concurring associations between the 5-HTTLPR and the abovementioned behaviours and 5-HT_{1A} receptor BP beg the question of precisely how the 5-HT_{1A} receptor influences behaviour. Thus further pharmacogenetic research may ultimately lead to clues informing drug development.

A larger study such as that planned in the United States and mentioned in the previous section would be required in order to justify study of agents such as 5-HT_{1A} receptor agonists for smoking cessation in smokers with the 5-HTTLPR. However, if the next stage of research is conclusive in establishing greater activation to smoking-related pictorial cues in abstinent smokers with the 5-HTTLPR, pharmacogenetic studies examining 5-HT_{1A} receptor agonists would be a novel and well-supported translational application.

There is evidence that 5-HT_{1A} receptors are potentially worthy targets for examining the efficacy of pharmacological smoking cessation agents. However, the evidence to date suggests that 5-HT_{1A} receptor manipulation may only benefit subgroups based on severity of depression and anxiety disorders. For example, meta-analyses of SSRIs do not demonstrate significant efficacy benefits for smoking cessation. However, Niaura and colleagues

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[652] David, S.P., Munafo', M.R., Walton, R.T. (2005). 'Chapter 21. Pharmacogenomics Research in Nicotine Addiction and Smoking Cessation', in *Clinical Pharmacogenomics and Introduction to PharmacoProteomics*, ed. by Wong, S.H.Y., Linder, M., Valdes, R. (Washington, D.C., U.S.A: American Association for Clinical Chemistry (AACC) Press).

observed that the SSRI fluoxetine had a significant benefit for smoking cessation when compared to placebo but only in subjects who adhered to the prescribed regimen of fluoxetine. Those most likely to adhere to the treatment protocol were of male gender and men who did not experience weight gain.[653] The 5-HT_{1A} receptor partial agonist buspirone has demonstrated inconsistent results for smoking cessation and relief of withdrawal symptoms.[654] However, there is limited evidence that smoking cessation efficacy is affected by severity of anxiety, with the most anxious smokers having the highest efficacy at short-term (4-week follow-up from quit date) but no long-term benefit (12-month follow-up).[655]

Unfortunately, studies of selective 5-HT_{1A} receptor agonists such as ipsapirone and flesinoxan have not been reported in the published literature. Furthermore, even though the effects of nicotine on several behaviours in rats, including acoustic startle,[656] tail tremor,[657]

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[653] Niaura, R., Spring, B., Borrelli, B., Hedeker, D., Goldstein, M. G., Keuthen, N., DePue, J., Kristeller, J., Ockene, J., Prochazka, A., Chiles, J. A., Abrams, D. B. (2002) 'Multicenter Trial of Fluoxetine as an Adjunct to Behavioral Smoking Cessation Treatment', *J Consult Clin Psychol.* **70**(4): pp. 887-96; Hitsman, B., Spring, B., Borrelli, B., Niaura, R., Papandonatos, G. D. (2001). 'Influence of Antidepressant Pharmacotherapy on Behavioral Treatment Adherence and Smoking Cessation Outcome in a Combined Treatment Involving Fluoxetine', *Exp Clin Psychopharmacol.* **9**(4): pp. 355-62; Borrelli, B., Spring, B., Niaura, R., Hitsman, B., Papandonatos, G. (2001). 'Influences of Gender and Weight Gain on Short-Term Relapse to Smoking in a Cessation Trial', *J Consult Clin Psychol.* **69**(3): pp. 511-5.

[654] Hughes, J.R., Stead, L. F., Lancaster, T. (2004). 'Antidepressants for Smoking Cessation'. Cochrane Database Syst Rev. (4): p. CD000031.pub2; Robinson, M.D., Pettice, Y. L., Smith, W. A., Cederstrom, E. A., Sutherland, D. E., Davis, H. (1992). 'Buspirone Effect on Tobacco Withdrawal Symptoms: A Randomized Placebo-Controlled Trial', *J Am Board Fam Pract.* **5**(1): pp. 1-9; Hilleman, D.E., Mohiuddin, S. M., Delcore, M. G. (1994). 'Comparison of Fixed-Dose Transdermal Nicotine, Tapered-Dose Transdermal Nicotine, and Buspirone in Smoking Cessation', *J Clin Pharmacol.* **34**(3): pp. 222-4; Hilleman, D.E., Mohiuddin, S. M., Del Core, M. G., Sketch, M. H., Sr. (1992). 'Effect of Buspirone on Withdrawal Symptoms Associated with Smoking Cessation', *Arch Intern Med.* **152**(2): pp. 350-2; Cinciripini, P.M., Lapitsky, L., Seay, S., Wallfisch, A., Meyer, W. J., 3rd, van Vunakis, H. (1995). 'A Placebo-Controlled Evaluation of the Effects of Buspirone on Smoking Cessation: Differences between High- and Low-Anxiety Smokers', *J Clin Psychopharmacol.* **15**(3): pp. 182-91; West, R., Hajek, P., McNeill, A. (1991). 'Effect of Buspirone on Cigarette Withdrawal Symptoms and Short-Term Abstinence Rates in a Smokers Clinic', *Psychopharmacology* (Berl), **104**(1): pp. 91-6.

[655] Cinciripini, P.M., Lapitsky, L., Seay, S., Wallfisch, A., Meyer, W. J., 3rd, van Vunakis, H. (1995).

[656] Rasmussen, K., Kallman, M. J., Helton, D. R. (1997). 'Serotonin-1A Antagonists Attenuate the Effects of Nicotine Withdrawal on the Auditory Startle Response', *Synapse*, **27**(2): pp. 145-52.

[657] Suemaru, K., Araki, H., Gomita, Y. (2000). 'Involvement of 5-Hydroxytryptamine(1A) Receptors in Nicotine-Induced Tail Tremor in Rats', *Eur J Pharmacol.* **408**(1): pp. 19-23.

and anxiety,[658] appear to be mediated by 5-HT_{1A}, the role of the receptor in nicotine-seeking behaviour has not been studied extensively in animals and humans and may represent a promising future direction for drug development.

Furthermore, the observation of greater VS/NAc activation in the satiated state is the first reported study of this kind and, if replicated, may provide additional clues to enhancing the efficacy of smoking cessation therapy. Specifically, the relatively low efficacy of NRT for long-term smoking cessation may result, in part, from the possibility that NRT only really attenuates withdrawal symptoms whilst smokers on NRT may be more prone to cue-elicited activation of the VS/NAc. If this notion is upheld in future studies, it may be incumbent upon researchers to explore therapies to augment NRT efficacy by targeting the mesolimbic reward system to inhibit cue-elicited activation of the NAc.

An additional application of this research is pharmacogenomics.[659] Thus, with planned future research as mentioned above, it will be possible to combine functional imaging with genome-wide linkage studies to explore new molecular targets for smoking cessation therapy and to examine the pharmacodynamic properties of novel compounds designed to attenuate cue-elicited reward system activation, reduce craving, and prevent relapse to smoking cessation interventions.

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[658] File, S.E., Cheeta, S., Kenny, P. J. (2000). 'Neurobiological Mechanisms by which Nicotine Mediates Different Types of Anxiety', *Eur J Pharmacol.* **393**(1-3): pp. 231-6; File, S.E., Kenny, P. J., Cheeta, S. (2000). 'The Role of the Dorsal Hippocampal Serotonergic and Cholinergic Systems in the Modulation of Anxiety', *Pharmacol Biochem Behav.* **66**(1): pp. 65-72; Cheeta, S., Irvine, E. E., Kenny, P. J., File, S. E. (2001). 'The Dorsal Raphe Nucleus is a Crucial Structure Mediating Nicotine's Anxiolytic Effects and the Development of Tolerance and Withdrawal Responses', *Psychopharmacology* (Berl), **155**(1): pp. 78-85.

[659] David, S.P., Munafo', M.R., Walton, R.T. (2005); David, S.P. (2004). 'Pharmacogenetics', *Prim Care*, **31**(3): pp. 543-559.

GLOSSARY

Abstinence. To refrain from use of a drug or behaviour.
Acetyl choline. Parasympathetic neurotransmitter .
Activation. Haemodynamic response to stimulus inferring increased neural activity.
Ad libitum. (to smoke) as much as one desires or according to routine.
Allele. A form of a gene at a specific locus.
A priori. Hypothesis or presumption made prior to a study.
Autocorrelation. Significant temporal correlation in fMRI analysis resulting from low frequency physiological fluctuations in signal, such as those resulting from respiration, which can lead to elevated false positive (type I error) rates.
Axial. Transverse plane parallel to x-y axis.
Base-pair. A pair of complementary nucleotides or nucleosides held occurring in sequences in strands of DNA and RNA molecules, respectively.
Binding potential (BP). Quantification of availability of receptor binding sites as evaluated with positron emission tomography. The basic BP potential equation is $BP = f_2 B_{Avail} / K_D$ where f_2 = free fraction of the radioligand in the tissue, B_{Avail} = concentration of available binding sites and K_D = equilibrium dissociation rate constant of the radioligand.
Blood-oxygen-level-dependent (BOLD) signal. Magnetic resonance signal produced by change inferromagnetic properties of haemoglobin resulting from alterations in ratio of deoxyhaemoglobin to oxyhaemoglobin, which in turn is a function of local arterial autoregulation or vasodilation.
Bupropion. Antidepressant medication that inhibits dopamine and norepinephrine transporters
Confidence interval. Range of values in which there is a specified probability (typically 95%)that a parameter (e.g., mean, odds ratio, percent) of a population lies within it.
Cotinine. Major metabolite of nicotine.
Conditioned place preference. Behavioural test commonly used to study motivational properties of nicotine and other drugs of abuse in which animals are placed in unique environments, given the drug or a neutral vehicle, and observed for preference of places associated with either drug or neutral vehicle.
Coronal. Plane paralelle with x-z axis and coronal suture.
Cue reactivity. Physiological reaction to environmental cues of heightened emotional or cognitive salience-often referring to drug-related stimuli.
Depolorisation. Increase in voltage of a cell, which may trigger an action potential.
Dopamine. Monoamine neurotransmitter with central and peripheral pharmacodynamic properties.
Efficacy. Effectiveness of a drug in producing desired clinical effect.
Endophenotype. Subclassification of a more generalised phenotype
Gaussian kernal. Spatial filtering with each voxel intensity being replaced by a weighted average of neighbouring intensities.
Gaussianised. Mathematical conversion of *t* statistic to *z* statistic.
Genotype. Genetic constitution of an individual, often referring to the specific pair of alleles at a locus on a chromosome.

General linear model (GLM). Mulitple linear regression model used to examine correlation between explanatory variables of interest and haemodynamic response. The basic GLM equation is $Y = \mu 1 + \beta X + \varepsilon$ with Y = data, β = parameter estimate, X = explanatory variable, and ε = residuals.
Hardy-Weinberg Equalilibrium. Assuming the absence of migration, mutation, natural selection, or random drift, there is a stable frequency distribution of genotypes such that AA, Aa, and aa are in the proportions p2, 2pq, and q2, respectively. AA, Aa, and aa are the genotype frequencies of alleles A and a.
Hedonic dysregulation. Alteration of brain reward systems resulting in loss of control and compulsive use of a drug of abuse.
Heterozygote. An individual with different alleles at a specific locus.
Homozygote. An individual with identical alleles at a specific locus.
Hydroxybupropion. Major metabolite of bupropion.
Incentive sensitization. Model of addiction characterized by neuroadaptations in brain reward systems rendering these systems hypersensitive to drugs and drug-associated stimuli and mediating drug "wanting".
In vitro. (Experiments) conducted outside the living body.
In vivo. (Experiments) conducted on the living body.
Linkage. Region of chromosome that does not assort independently of a trait or phenotype suggesting an association between a gene within that chromosomal region and the phenotype of interest.
Mesocorticolimbic. Brain pathways consisting primarily, but not exclusively, of dopaminergic neurones originating in the ventral tegmental area of the midbrain and projecting to the amygdala, hippocampus, medial prefrontal cortex, and nucleus accumbens shell implicated in reward processing and motivation.
Meta-analysis. Analysis of data from a number of independent studies of the same subject in order to determine overall trends and significance.
Neuroadaptation. Cellular and molecular adaptations in the brain to persistent environmental challenges such as repetitive drug use.
Neurone. A nerve cell including its processes (axons and dendrites).
Nicotine. Alkaloid occurring naturally in tobacco with addictive properties.
Nicotine dependence. A syndrome characterised by a maladaptive pattern of nicotine use, leading to clinically significant impairment or distress, tolerance, withdrawal, and continued use despite negative consequences.
Nigrostriatal. A pathway of dopaminergic neurones emanating from the substantia nigra pars compacta and terminating in the striatum.
Nortriptyline. Tricyclic antidepressant with established efficacy for smoking cessation.
Odds ratio. An effect size which is calculated from the ratio of relative odds of a disease or behaviour in exposed compared to non-exposed individuals.
OXCHECK. Population-based multiple risk factor reduction study conducted by the Imperial Cancer Research Fund General Practice Research Group.
PATCH. Nicotine replacement patch clinical trial conducted by the Imperial Cancer Research Fund General Practice Research Group.
Phenotype. Observable characteristics of an individual regarded as a consequence of the interaction between genotype and environment.

Polymerase chain reaction. A technique for producing multiple copies of a chosen sequence of genomic DNA using repeated cycles of denaturation, annealing with primer, and replication with DNA polymerase.
Polymorphism. Variation in gene consisting of one or more base-pairs, insertions, deletions, or repetitive sequences .
Post hoc. After the fact.
Radioligand. Radioactively-labelled ligand for receptor used in positron emission tomography studies.
Receptor. Chemical structure present on cells that receive chemical stimuli such as neurotransmitters and generates postsynaptic cellular responses including activation of second messenger systems leading to cell depolarization .
Region of interest. (Brain) region selected for analysis of response to a stimulus or structure.
Sagittal. Plane parallel with y-z axis and sagittal suture (median antero-posterior suture between the two parietal bones).
Tachyphylaxis. Acute tolerance to a drug.
Tolerance. A need for markedly increased amounts of the substance to achieve intoxication or desired effect or a markedly diminished effect with continued use of the same amount of the substance.
Transcription. The process of copying genetic information as represented by the sequence of nucleotides from DNA to messenger RNA.
Translation. The process of transferring genetic information from messenger RNA to transfer RNA for subsequent expression of proteins.
Translational. Basic scientific research with direct clinical applications.
Transporter. A transmembrane protein involved in transport of neurotransmitters from the synaptic cleft to the synaptic terminal.
Voxel. Discrete volume in anatomical space used for MRI analysis.

BIBLIOGRAPHY

Afifi, A.K. (2003). 'The Basal Ganglia: A Neural Network with More than Motor Function', *Semin Pediatr Neurol.* **10**(1): pp. 3-10

Andrews, A.M., Murphy, D. L. (1993). 'Fluoxetine and Desipramine Selectively Attenuate 2'-NH2-MPTP-Induced Depletions in Serotonin and Norepinephrine', *Eur J Pharmacol.* **250**(2): pp. 215-21

Andrews, A.M., Murphy, D. L. (1993). 'Sustained Depletion of Cortical and Hippocampal Serotonin and Norepinephrine but not Striatal Dopamine by 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine (2'-NH2-MPTP): A Comparative Study with 2'-CH3-MPTP and MPTP', *J Neurochem.* **60**(3): pp. 1167-70

Anguelova, M., Benkelfat, C., Turecki, G. (2003). 'A Systematic Review of Association Studies Investigating Genes Coding for Serotonin Receptors and the Serotonin Transporter: II. Suicidal behavior', *Mol Psychiatry*, **8**(7): pp. 646-53

APA (2002). *Diagnostic and Statistical Manual of Mental Disorders [DSM-IV-TR (text revision 2002)]*, 4th edn (Washington, D.C: American Psychiatric Association, American Psychiatric Press)

Ascher, J.A., Cole, J.O, Collin, J.N., Feighner, J.P., Ferris, R.M., Fibiger, H.C. et al. (1995). 'Bupropion: A Review of its Mechanism of Antidepressant Activity', *J Clin Psychiatry*, 56(**9**): 395-401

ASH-UK (2000). 'Basic Facts', <http://www.ash.org.uk/> [accessed 20 May 2005]

Avila, M.T., Sherr, J. D., Hong, E., Myers, C. S., Thaker, G. K. (2003). 'Effects of Nicotine on Leading Saccades During Smooth Pursuit Eye Movements in Smokers and Nonsmokers with Schizophrenia', *Neuropsychopharmacology*, **28**(12): pp. 2184-91

Badiani, A., Oates, M. M., Day, H. E., Watson, S. J., Akil, H., Robinson, T. E. (1998). 'Amphetamine-Induced Behavior, Dopamine Release, and c-fos mRNA Expression: Modulation by Environmental Novelty', *J Neurosci.* **18**(24): pp. 10579-93

Bahk, J.Y., Li, S., Park, M. S., Kim, M. O. (2002). 'Dopamine D1 and D2 Receptor mRNA Up-Regulation in the Caudate-Putamen and Nucleus Accumbens of Rat Brains by Smoking', *Prog Neuropsychopharmacol Biol Psychiatry*, **26**(6): pp. 1095-104

Balfour, D.J. (2002). 'Neuroplasticity Within the Mesoaccumbens Dopamine System and Its Role in Tobacco Dependence', *Curr Drug Targets CNS Neurol Disord*, **1**(4): pp. 413-21

Balfour, D.J., Benwell, M. E., Birrell, C. E., Kelly, R. J., Al-Aloul, M. (1998). 'Sensitization of the Mesoaccumbens Dopamine Response to Nicotine', *Pharmacol Biochem Behav.* **59**(4): pp. 1021-30

Balfour, D.J., Graham, C. A., Vale, A. L. (1986). 'Studies on the Possible Role of Brain 5-HT Systems and Adrenocortical Activity in Behavioural Responses to Nicotine and Diazepam in an Elevated X-maze', *Psychopharmacology* (Berl), **90**(4): pp. 528-32

Balfour, D.J., Ridley, D. L. (2000). 'The Effects of Nicotine on Neural Pathways Implicated in Depression: A Factor in Nicotine Addiction?' *Pharmacol Biochem Behav.* **66**(1): pp. 79-85

Barnes, N.M., Costall, B., Kelly, M. E., Onaivi, E. S., Naylor, R. J. (1990). 'Ketotifen and Its Analogues Reduce Aversive Responding in the Rodent', *Pharmacol Biochem Behav*. **37**(4): pp. 785-93

Barnes, N.M., Sharp, T. (1999). 'A Review of Central 5-HT Receptors and their Function', *Neuropharmacology*, **38**(8): pp. 1083-152

Barrass, B.C., Blackburn, J. W., Brimblecombe, R. W., Rich, P. (1969). 'Modification of Nicotine Toxicity by Pretreatment with Different Drugs', *Biochem Pharmacol.* **18**(9): pp. 2145-52

Battersby, S., Ogilvie, A. D., Blackwood, D. H. Shen, S., Muqit, M. M., Muir, W. J., Teague, P., Goodwin, G. M., Harmar, A. J. (1999). 'Presence of Multiple Functional Polyadenylation Signals and a Single Nucleotide Polymorphism in the 3' Untranslated Region of the Human Serotonin Transporter Gene', *J Neurochem.* **72**(4): pp.1384-8

Beckmann, C.F., Jenkinson, M., Smith, S. M. (2003). 'General Multilevel Linear Modeling for Group Analysis in FMRI', *Neuroimage*, **20**(2): pp. 1052-63

Belle, G., Fisher, L.D., Heagerty, P.J., Lumley, T., (ed.). (2004) *Biostatistics: A Methodology for the Health Sciences* (Hoboken, New Jersey, USA: John Wiley & Sons)

Benloucif, S., Keegan, M. J., Galloway, M. P. (1993). 'Serotonin-Facilitated Dopamine Release *In Vivo*: Pharmacological Characterization', *J Pharmacol Exp Ther*. **265**(1): pp. 373-7

Benowitz, N.L. (1999). 'Nicotine addiction', *Prim Care*, **26**(3): pp. 611-31

Benowitz, N.L., Hall, S. M., Herning, R. I., Jacob, P., 3rd, Jones, R. T., Osman, A. L. (1983). 'Smokers of Low-Yield Cigarettes Do Not Consume Less Nicotine', *N Engl J Med.* **309**(3): pp. 139-42

Benowitz, N.L., Jacob, P. (1984), 3rd, 'Daily Intake of Nicotine During Cigarette Smoking', *Clin Pharmacol Ther*. **35**(4): pp. 499-504

Benowitz, N.L., Jacob, P., 3rd, (1994). 'Metabolism of Nicotine to Cotinine Studied by a Dual Stable Isotope Method', *Clin Pharmacol Ther*. **56**(5): pp. 483-93

Benowitz, N.L., Jacob, P., 3rd, Jones, R. T., Rosenberg, J. (1982). 'Interindividual Variability in the Metabolism and Cardiovascular Effects of Nicotine in Man', *J Pharmacol Exp Ther*. **221**(2): pp. 368-72

Benowitz, N.L., Kuyt, F., Jacob, P., 3rd, (1982). 'Circadian Blood Nicotine Concentrations During Cigarette Smoking', *Clin Pharmacol Ther*. **32**(6): pp. 758-64

Benowitz, N.L., Kuyt, F., Jacob, P., 3rd, Jones, R. T., Osman, A. L. (1983). 'Cotinine Disposition and Effects', *Clin Pharmacol Ther*. **34**(5): pp. 604-11

Benowitz, N.L., Perez-Stable, E. J., Fong, I., Modin, G., Herrera, B., Jacob, P., 3rd, (1999). 'Ethnic Differences in N-Glucuronidation of Nicotine and Cotinine', *J Pharmacol Exp Ther*. **291**(3): pp. 1196-203

Benowitz, N.L., Pomerleau, O. F., Pomerleau, C. S., Jacob, P., 3rd, (2003). 'Nicotine metabolite ratio as a predictor of cigarette consumption', *Nicotine Tob Res*. **5**(5): pp. 621-4

Benowitz, N.L., Porchet, H., Sheiner, L., Jacob, P., 3rd, (1988). 'Nicotine Absorption and Cardiovascular Effects with Smokeless Tobacco Use: Comparison with Cigarettes and Nicotine Gum', *Clin Pharmacol Ther*. **44**(1): pp. 23-8

Benwell, M.E., Balfour, D. J. (1992). 'The Effects of Acute and Repeated Nicotine Treatment on Nucleus Accumbens Dopamine and Locomotor Activity', *Br J Pharmacol.* **105**(4): pp. 849-56

Benwell, M.E., Balfour, D. J. (1979). 'Effects of Nicotine Administration and Its Withdrawal on Plasma Corticosterone and Brain 5-hHydroxyindoles', *Psychopharmacology* (Berl). **63**(1): pp. 7-11

Benwell, M.E., Balfour, D. J. (1982). 'The Effects of Nicotine Administration on 5-HT Uptake and Biosynthesis in Rat Brain', *Eur J Pharmacol.* **84**(1-2): pp. 71-7

Benwell, M.E., Balfour, D. J., Anderson, J. M. (1990). 'Smoking-Associated Changes in the Serotonergic Systems of Discrete Regions of Human Brain', *Psychopharmacology* (Berl). **102**(1): pp. 68-72

Benwell, M.E., Balfour, D. J., Birrell, C. E. (1995). 'Desensitization of the Nicotine-Induced Mesolimbic Dopamine Responses During Constant Infusion with Nicotine', *Br J Pharmacol.* **114**(2): pp. 454-60

Bergen, A.W., Korczak, J. F., Weissbecker, K. A., Goldstein, A. M. (1999). 'A Genome-Wide Search for Loci Contributing to Smoking and Alcoholism', *Genet Epidemiol.* **17 Suppl 1**: pp. S55-60

Bergqvist, P.B., Bouchard, C., Blier, P. (1999). 'Effect of Long-Term Administration of Antidepressant Treatments on Serotonin Release in Brain Regions Involved in Obsessive-Compulsive Disorder', *Biol Psychiatry*, **45**(2): pp. 164-74

Berkman, C.E., Park, S. B., Wrighton, S. A., Cashman, J. R. (1995). '*In Vitro-In Vivo* Correlations of Human (S)-Nicotine Metabolism', *Biochem Pharmacol.* **50**(4): pp. 565-70

Berridge, K.C., Robinson, T. E. (1998). 'What is the Role of Dopamine in Reward: Hedonic Impact, Reward Learning, or Incentive Salience?' *Brain Res Brain Res Rev.* **28**(3): pp. 309-69

Bhagwagar, Z., Rabiner, E. A., Sargent, P. A., Grasby, P. M., Cowen, P. J. (2004). 'Persistent Reduction in Brain Serotonin1A Receptor Binding in Recovered Depressed Men Measured by Positron Emission Tomography with [11C]WAY-100635', *Mol Psychiatry*, **9**(4): pp. 386-92

Bierut, L.J., Rice, J. P., Goate, A., Hinrichs, A. L., Saccone, N. L., Foroud, T., Edenberg, H. J., Cloninger, C. R., Begleiter, H., Conneally, P. M., Crowe, R. R., Hesselbrock, V., Li, T. K., Nurnberger, J. I., Jr., Porjesz, B., Schuckit, M. A., Reich, T. (2004). 'A Genomic Scan for Habitual Smoking in Families of Alcoholics: Common and Specific Genetic Factors in Substance Dependence', *Am J Med Genet A.* **124**(1): pp. 19-27

Blier, P., Chaput, Y., de Montigny, C. (1988). 'Long-Term 5-HT Reuptake Blockade, but not Monoamine Oxidase Inhibition, Decreases the Function of Terminal 5-HT Autoreceptors: An Electrophysiological Study in the Rat Brain', *Naunyn Schmiedebergs Arch Pharmacol.* **337**(3): pp. 246-54

Blier, P., De Montigny, C. (1983). 'Electrophysiological Investigations on the Effect of Repeated Zimelidine Administration on Serotonergic Neurotransmission in the Rat', *J Neurosci.* **3**(6): pp. 1270-8

Blier, P., Pineyro, G., el Mansari, M., Bergeron, R., de Montigny, C. (1998). 'Role of Somatodendritic 5-HT Autoreceptors in Modulating 5-HT Neurotransmission', *Ann N Y Acad Sci.* **861**: pp. 204-16

Blier, P., Ward, N. M. (2003). 'Is there a Role for 5-HT1A Agonists in the Treatment of Depression?' *Biol Psychiatry*, **53**(3): pp. 193-203

Blum, K., Braverman, E. R., Holder, J. M., Lubar, J. F., Monastra, V. J., Miller, D., Lubar, J. O., Chen, T. J., Comings, D. E. (2000). 'Reward Deficiency Syndrome: A Biogenetic Model for the Diagnosis and Treatment of Impulsive, Addictive, and Compulsive Behaviors', *J Psychoactive Drugs*, **32 Suppl**: pp. i-iv, 1-112

Borrelli, B., Spring, B., Niaura, R., Hitsman, B., Papandonatos, G. (2001). 'Influences of Gender and Weight Gain on Short-Term Relapse to Smoking in a Cessation Trial', *J Consult Clin Psychol.* **69**(3): pp. 511-5

Bortolozzi, A., Duffard, R., de Duffard, A. M. (2003). 'Asymmetrical Development of the Monoamine Systems in 2,4-Dichlorophenoxyacetic Acid Treated Rats', *Neurotoxicology*, **24**(1): pp. 149-57

Bradbury, J. (1998). 'UK "White Paper on Tobacco" Welcomed', *Lancet*, **352**(9145): p. 1991

Bradley, B., Field, M., Mogg, K., De Houwer, J. (2004). 'Attentional and Evaluative Biases for Smoking Cues in Nicotine Dependence: Component Processes of Biases in Visual Orienting', *Behav Pharmacol.* **15**(1): pp. 29-36

Brandange, S., Lindblom, L. (1979). 'The Enzyme "Aldehyde Oxidase" is an Iminium Oxidase. Reaction with Nicotine Delta 1'(5') Iminium Ion', *Biochem Biophys Res Commun.* **91**(3): pp. 991-6

Breiter, H.C., Rosen, B. R. (1999). 'Functional Magnetic Resonance Imaging of Brain Reward Circuitry in the Human', *Ann N Y Acad Sci.* **877**: pp. 523-47

Brody, A.L., Mandelkern, M. A., Lee, G., Smith, E., Sadeghi, M., Saxena, S., Jarvik, M. E., London, E. D. (2004). 'Attenuation of Cue-Induced Cigarette Craving and Anterior Cingulate Cortex Activation in Bupropion-Treated Smokers: A Preliminary Study', *Psychiatry Res.* **130**(3): pp. 269-81

Brody, A.L., Mandelkern, M. A., London, E. D., Childress, A. R., Lee, G. S., Bota, R. G., Ho, M. L., Saxena, S., Baxter, L. R., Jr., Madsen, D., Jarvik, M. E. (2002). 'Brain Metabolic Changes During Cigarette Craving', *Arch Gen Psychiatry*, **59**(12): pp. 1162-72

Brody, A.L., Olmstead, R. E., London, E. D., Farahi, J., Meyer, J. H., Grossman, P., Lee, G. S., Huang, J., Hahn, E. L., Mandelkern, M. A. (2004). 'Smoking-Induced Ventral Striatum Dopamine Release', *Am J Psychiatry*, **161**(7): pp. 1211-8

Brunnemann, K.D., Hoffmann, D. (1974). 'The pH of Tobacco Smoke', *Food Cosmet Toxicol.* **12**(1): pp. 115-24

Bunce, M., Fanning, G. C., Welsh, K. I. (1995). 'Comprehensive, Serologically Equivalent DNA Typing for HLA-B by PCR Using Sequence-Specific Primers (PCR-SSP)', *Tissue Antigens.* **45**(2): pp. 81-90

Burnet, P.W., Eastwood, S. L., Harrison, P. J. (1997). '[3H]WAY-100635 for 5-HT1A Receptor Autoradiography in Human Brain: A Comparison with [3H]8-OH-DPAT and Demonstration of Increased Binding in the Frontal Cortex in Schizophrenia', *Neurochem Int.* **30**(6): pp. 565-74

Burnet, P.W., Eastwood, S. L., Harrison, P. J. (1996). '5-HT1A and 5-HT2A Receptor mRNAs and Binding Site Densities Are Differentially Altered in Schizophrenia', *Neuropsychopharmacology*, **15**(5): pp. 442-55

Byrd, G.D., Chang, K. M., Greene, J. M., deBethizy, J. D. (1992). 'Evidence for Urinary Excretion of Glucuronide Conjugates of Nicotine, Cotinine, and Trans-3'-Hydroxycotinine in Smokers', *Drug Metab Dispos*. **20**(2): pp. 192-7

Calabresi, P., Lacey, M. G., North, R. A. (1989). 'Nicotinic Excitation of Rat Ventral Tegmental Neurones *In Vitro* Studied by Intracellular Recording', *Br J Pharmacol.* **98**(1): pp. 135-40

Carlezon, W.A., Jr., Haile, C. N., Coppersmith, R., Hayashi, Y., Malinow, R., Neve, R. L., Nestler, E. J. (2000). 'Distinct Sites of Opiate Reward and Aversion within the Midbrain Identified Using a Herpes Simplex Virus Vector Expressing GluR1', *J Neurosci.* **20**(5): pp. RC62

Carmelli, D., Swan, G. E., Robinette, D., Fabsitz, R. R. (1990). 'Heritability of Substance Use in the NAS-NRC Twin Registry', *Acta Genet Med Gemellol* (Roma), **39**(1): pp. 91-8

Cashman, J.R., Park, S. B., Yang, Z. C., Wrighton, S. A., Jacob, P., 3rd, Benowitz, N. L. (1992). 'Metabolism of Nicotine by Human Liver Microsomes: Stereoselective Formation of Trans-Nicotine N'-Oxide', *Chem Res Toxicol.* **5**(5): pp. 639-46

Castro, E., Romon, T., Castillo, J. (1997). 'Identification and Characterisation of a New Serotonergic Recognition with High Affinity for 5-Carboxamidotryptamine in Mammalian Brain', *J neurochem.* **69**: pp. 2123-2131

Chambers, R.A., Taylor, J. R., Potenza, M. N. (2003). 'Developmental Neurocircuitry of Motivation in Adolescence: A Critical Period of Addiction Vulnerability', *Am J Psychiatry*, **160**(6): pp. 1041-52

Champagne, F.A., Chretien, P., Stevenson, C. W., Zhang, T. Y., Gratton, A., Meaney, M. J. (2004). 'Variations in Nucleus Accumbens Dopamine Associated with Individual Differences in Maternal Behavior in the Rat', *J Neurosci.* **24**(17): pp. 4113-23

Champtiaux, N., Changeux, J. P. (2002). 'Knock-Out and Knock-In Mice to Investigate the Role of Nicotinic Receptors in the Central Nervous System', *Curr Drug Targets CNS Neurol Disord*, **1**(4): pp. 319-30

Champtiaux, N., Han, Z. Y., Bessis, A., Rossi, F. M., Zoli, M., Marubio, L., McIntosh, J. M., Changeux, J. P. (2002). 'Distribution and Pharmacology of Alpha 6-Containing Nicotinic Acetylcholine Receptors Analyzed with Mutant Mice', *J Neurosci.* **22**(4): pp. 1208-17

Chaput, Y., de Montigny, C., Blier, P. (1986). 'Effects of a Selective 5-HT Reuptake Blocker, Citalopram, on the Sensitivity of 5-HT Autoreceptors: Electrophysiological Studies in the Rat Brain', *Naunyn Schmiedebergs Arch Pharmacol.* **333**(4): pp. 342-8

Charara, A., Grace, A. A. (2003). 'Dopamine Receptor Subtypes Selectively Modulate Excitatory Afferents from the Hippocampus and Amygdala to Rat Nucleus Accumbens Neurons', *Neuropsychopharmacology*, **28**(8): pp. 1412-21

Charpantier, E., Barneoud, P., Moser, P., Besnard, F., Sgard, F. (1998). 'Nicotinic Acetylcholine Subunit mRNA Expression in Dopaminergic Neurons of the Rat Substantia Nigra and Ventral Tegmental Area', *Neuroreport*, **9**(13): pp. 3097-101

Cheeta, S., Irvine, E., File, S. E. (2001). 'Social Isolation Modifies Nicotine's Effects in Animal Tests of Anxiety', *Br J Pharmacol.* **132**(7): pp. 1389-95

Cheeta, S., Irvine, E. E., Kenny, P. J., File, S. E. (2001). 'The Dorsal Raphe Nucleus is a Crucial Structure Mediating Nicotine's Anxiolytic Effects and the Development of

Tolerance and Withdrawal Responses', *Psychopharmacology* (Berl), **155**(1): pp. 78-85

Cheeta, S., Kenny, P. J., File, S. E. (2000). 'The Role of 5-HT1A Receptors in Mediating the Anxiogenic Effects of Nicotine Following Lateral Septal Administration', *Eur J Neurosci.* **12**(10): pp. 3797-802

Cheeta, S., Tucci, S., File, S. E. (2001). 'Antagonism of the Anxiolytic Effect of Nicotine in the Dorsal Raphe Nucleus by Dihydro-Beta-Erythroidine', *Pharmacol Biochem Behav.* **70**(4): pp. 491-6

Cidis Meltzer, C., Drevets, W. C., Price, J. C., Mathis, C. A., Lopresti, B., Greer, P. J., Villemagne, V. L., Holt, D., Mason, N. S., Houck, P. R., Reynolds, C. F., 3rd, DeKosky, S. T. (2001). 'Gender-Specific Aging Effects on the Serotonin 1A Receptor', *Brain Res*, **895**(1-2): pp. 9-17

Cinciripini, P.M., Lapitsky, L., Seay, S., Wallfisch, A., Meyer, W. J., 3rd, van Vunakis, H. (1995). 'A Placebo-Controlled Evaluation of the Effects of Buspirone on Smoking Cessation: Differences between High- and Low-Anxiety Smokers', *J Clin Psychopharmacol.* **15**(3): pp. 182-91

Cohen, R., Sweet, L., David, S.P., Niaura, R. (2004). 'Nicotine Satiation and Abstinence Effects on fMRI Brain Activation During Verbal Working Memory', paper presented at 10th annual Society for Research on Nicotine and Tobacco meeting, Scotsdale, AZ

Colditz, G.A. (2000). 'Illnesses Caused by Smoking Cigarettes', *Cancer Causes Control*, **11**(1): pp. 93-7

Colquhoun, L.M., Patrick, J. W. (1997), 'Pharmacology of Neuronal Nicotinic Acetylcholine Receptor Subtypes', *Adv Pharmacol.* **39**: pp. 191-220

Comroe, J.H., Jr. (1960). 'The Pharmacological Actions of Nicotine', *Ann N Y Acad Sci.* **90**: pp. 48-51

Cordero-Erausquin, M., Marubio, L. M., Klink, R., Changeux, J. P. (2000). 'Nicotinic Receptor Function: New Perspectives from Knockout Mice', *Trends Pharmacol Sci.* **21**(6): pp. 211-7

Corrigall, W.A. (1999). 'Nicotine Self-Administration in Animals as a Dependence Model', *Nicotine Tob Res.* **1**(1): pp. 11-20

Corrigall, W.A., Coen, K. M., Adamson, K. L. (1994). 'Self-Administered Nicotine Activates the Mesolimbic Dopamine System through the Ventral Tegmental Area', *Brain Res.* **653**(1-2): pp. 278-84

Corrigall, W.A., Coen, K. M., Adamson, K. L., Chow, B. L., Zhang, J. (2000). 'Response of Nicotine Self-Administration in the Rat to Manipulations of Mu-Opioid and Gamma-Aminobutyric Acid Receptors in the Ventral Tegmental Area', *Psychopharmacology* (Berl), **149**(2): pp. 107-14

Corrigall, W.A., Franklin, K. B., Coen, K. M., Clarke, P. B. (1992). 'The Mesolimbic Dopaminergic System is Implicated in the Reinforcing Effects of Nicotine', *Psychopharmacology* (Berl), **107**(2-3): pp. 285-9

Costall, B., Jones, B. J., Kelly, M. E., Naylor, R. J., Onaivi, E. S., Tyers, M. B. (1990). 'Sites of Action of Ondansetron to Inhibit Withdrawal from Drugs of Abuse', *Pharmacol Biochem Behav.* **36**(1): pp. 97-104

Courtney, S.M., Ungerleider, L. G., Keil, K., Haxby, J. V. (1997). 'Transient and Sustained Activity in a Distributed Neural System for Human Working Memory', *Nature*, **386**(6625): pp. 608-11

Dani, J.A., Radcliffe, K. A., Pidoplichko, V. I. (2000). 'Variations in Desensitization of Nicotinic Acetylcholine Receptors from Hippocampus and Midbrain Dopamine Areas', *Eur J Pharmacol.* **393**(1-3): pp. 31-8

David, S.P. (2004). 'Pharmacogenetics', *Prim Care*, **31**(3): pp. 543-559

David, S.P. (2001a). 'Should We Recommend Nicotine Replacement Therapy?' *Am Fam Physician*, **63**(11): pp. 2245-7

David, S.P. (2001b). 'Smoking Cessation for the Primary Care Physician', *Primary Care Reports*, **7**(24): pp. 211-221

David, S.P., Brown, R.A., Papandonatos, G.D., Lloyd-Richardson, E.L., Munafo', M.R., Shields, P.G., Lerman, C., Strong, D.R., McCaffery, J.M., Niaura, R. (2005). 'Pharmacogenetic Clinical Trial of Sustained-Release Bupropion for Smoking Cessation', *Pharmacogenomics J.* (manuscript under review)

David, S.P., Munafo', M., Johansen-Berg, H., Smith, S.M., Rogers, R.D., Matthews, P.M., Walton, R.T. (2005). 'Ventral Striatum/Nucleus Accumbens Activation to Smoking-Related Pictorial Cues in Smokers and Non-Smokers: An fMRI study', *Biol Psychiatry*, **58**(6):488-94

David, S.P., Munafo', M.R., Walton, R.T. (2005). 'Chapter 21. Pharmacogenomics Research in Nicotine Addiction and Smoking Cessation', in *Clinical Pharmacogenomics and Introduction to PharmacoProteomics*, ed. by Wong, S.H.Y., Linder, M., Valdes, R. (Washington, D.C., U.S.A: American Association for Clinical Chemistry (AACC) Press)

David, S.P., Murthy, N.V., Rabiner, E.A., Munafo', M.R., Johnstone, E.C., Jacob, R., Walton, R.T., Grasby, P.M. (2005). 'A Functional Genetic Variation of the Serotonin (5-HT) Transporter Affects 5-HT1A Receptor Binding in Humans', *J Neurosci.* **25**(10): pp. 2586-90

David, S.P., Murthy, N.V., Rabiner, E.A., Munafo', M., Jacob, R., Johnstone, E., Grasby, P.M. (2004). 'Serotonin Transporter Polymorphism Linked to Personality Traits Affects Serotonin (5-HT1A) Receptor Function in Positron Emission Tomography Study', paper presented at the North American Primary Care Research Group's (NAPCRG) 32nd annual meeting in Orlando, FL, USA

David, S.P., Niaura, R., Papandonatos, G., Shadel, W., Burkholder, G., Britt, D., et al. (2003). 'Does the DRD2-Taq1 A Polymorphism Influence Treatment Response to Bupropion Hydrochloride for Reduction of the Nicotine Withdrawal Syndrome?' *Nicotine Tob Res.* **5**(6): 935-42

de Montigny, C., Chaput, Y., Blier, P. (1990). 'Modification of Serotonergic Neuron Properties by Long-Term Treatment with Serotonin Reuptake Blockers', *J Clin Psychiatry*, **51 Suppl B**: pp. S4-8

Depatie, L., O'Driscoll, G. A., Holahan, A. L., Atkinson, V., Thavundayil, J. X., Kin, N. N., Lal, S. (2002). 'Nicotine and Behavioral Markers of Risk for Schizophrenia: A Double-Blind, Placebo-Controlled, Cross-Over Study', *Neuropsychopharmacology*, **27**(6): pp. 1056-70

Diana, M., Brodie, M., Muntoni, A., Puddu, M. C., Pillolla, G., Steffensen, S., Spiga, S., Little, H. J. (2003). 'Enduring Effects of Chronic Ethanol in the CNS: Basis for Alcoholism', *Alcohol Clin Exp Res.* **27**(2): pp. 354-61

Di Chiara, G., Imperato, A. (1988). 'Drugs Abused by Humans Preferentially Increase Synaptic Dopamine Concentrations in the Mesolimbic System of Freely Moving Rats', *Proc Natl Acad Sci U S A*, **85**(14): pp. 5274-8

Doll, R. (2000). 'Fifty Years of Research on Tobacco', *J Epidemiol Biostat.* **5**(6): pp. 321-9

Doll, R., Hill, A. B. (1954). 'The Mortality of Doctors in Relation to their Smoking Habits; a Preliminary Report', *Br Med J.* **4877**: pp. 1451-5

Doll, R., Peto, R., Boreham, J., Sutherland, I. (2004). 'Mortality in Relation to Smoking: 50 Years' Observations on Male British Doctors', *BMJ.* **328**(7455): pp. 1519

Donaldson, D.I., Buckner, R.L. (2001). 'Effective Paradigm Design', in *Functional MRI – An Introduction to Methods*, ed. by Jezzard, P., Matthews, P.M., Smith, S.M. (Oxford, UK: Oxford University Press)

Doulton, T.W., He, F. J., MacGregor, G. A. (2005). 'Systematic Review of Combined Angiotensin-Converting Enzyme Inhibition and Angiotensin Receptor Blockade in Hypertension', *Hypertension*, **45**(5): pp. 880-6

Drevets, W.C., Frank, E., Price, J. C., Kupfer, D. J., Holt, D., Greer, P. J., Huang, Y., Gautier, C., Mathis, C. (1999). 'PET Imaging of Serotonin 1A Receptor Binding in Depression', *Biol Psychiatry*, **46**(10): pp. 1375-87

D'Souza, D.N., Zhang, Y., Garcia, F., Battaglia, G., Van De Kar, L. D. (2002). 'Destruction of Serotonergic Nerve Terminals Prevents Fluoxetine-Induced Desensitization of Hypothalamic 5-HT(1A) Receptors', *Psychopharmacology* (Berl). **164**(4): pp. 392-400

Due, D.L., Huettel, S. A., Hall, W. G., Rubin, D. C. (2002). 'Activation in Mesolimbic and Visuospatial Neural Circuits Elicited by Smoking Cues: Evidence from Functional Magnetic Resonance Imaging', *Am J Psychiatry*, **159**(6): pp. 954-60

Duggirala, R., Almasy, L., Blangero, J. (1999). 'Smoking Behavior is Under the Influence of a Major Quantitative Trait Locus on Human Chromosome 5q', *Genet Epidemiol.* **17 Suppl 1**: pp. S139-44

Duvernoy, H. (1999). *The Human Brain*, 2nd ed. (London: Springer-Verlab)

Edwards, K.L., Austin, M. A., Jarvik, G. P. (1995). 'Evidence for Genetic Influences on Smoking in Adult Women Twins', *Clin Genet.* **47**(5): pp. 236-44

Edwards, J.E., Moore, R. A. (2003). 'Statins in Hypercholesterolaemia: A Dose-Specific Meta-Analysis of Lipid Changes in Randomised, Double Blind Trials', *BMC Fam Pract.* **4**(1): p. 18

Engberg, G., Erhardt, S., Sharp, T., Hajos, M. (2000). 'Nicotine Inhibits Firing Activity of Dorsal Raphe 5-HT Neurones *In Vivo*', *Naunyn Schmiedebergs Arch Pharmacol.* **362**(1): pp. 41-5

Engel, J.A., Fahlke, C., Hard, E., Johannessen, K., Svensson, L., Soderpalm, B. (1992). 'Serotonergic and Dopaminergic Involvement in Ethanol Intake', *Clin Neuropharmacol.* **15 Suppl 1 Pt A**: pp. 64A-65A

Epping-Jordan, M.P., Watkins, S. S., Koob, G. F., Markou, A. (1998). 'Dramatic Decreases in Brain Reward Function During Nicotine Withdrawal', *Nature*, **393**(6680): pp. 76-9

Erdmann, J., Shimron-Abarbanell, D., Cichon, S., Albus, M., Maier, W., Lichtermann, D., Minges, J., Reuner, U., Franzek, E., Ertl, M. A. and et al. (1995). 'Systematic Screening for Mutations in the Promoter and the Coding Region of the 5-HT1A Gene', *Am J Med Genet.* **60**(5): pp. 393-9

Faucette, S.R., Hawke, R.L., Lecluyse, E.L., Shord, S.S., Yan, B., Laethem, R.M., et al. (2000). 'Validation of Bupropion Hydroxylation as a Selective Marker of Human Cytochrome P450 2B6 Catalytic Activity', *Drug Metab Dipos.* **28**(10): 1222-30

Fayolle, C., Fillion, M. P., Barone, P., Oudar, P., Rousselle, J. C., Fillion, G. (1988). '5-Hydroxytryptamine Stimulates Two Distinct Adenylate Cyclase Activities in Rat Brain: High-Affinity Activation is Related to a 5-HT1 Subtype Different from 5-HT1A, 5-HT1B, and 5-HT1C', *Fundam Clin Pharmacol.* **2**(3): pp. 195-214

Fichtenberg, C.M. and Glantz, S.A. (2000). 'Association of the California Tobacco Control Program with Declines in Cigarette Consumption and Mortality from Heart Disease', *N Engl J Med.* **343**(24): pp. 1772-7

File, S.E., Cheeta, S., Kenny, P. J. (2000). 'Neurobiological Mechanisms by which Nicotine Mediates Different Types of Anxiety', *Eur J Pharmacol.* **393**(1-3): pp. 231-6

File, S.E., Kenny, P. J., Cheeta, S. (2000). 'The Role of the Dorsal Hippocampal Serotonergic and Cholinergic Systems in the Modulation of Anxiety', *Pharmacol Biochem Behav.* **66**(1): pp. 65-72

File, S.E., Kenny, P. J., Ouagazzal, A. M. (1998). 'Bimodal Modulation by Nicotine of Anxiety in the Social Interaction Test: Role of the Dorsal Hippocampus', *Behav Neurosci.* **112**(6): pp. 1423-9

Fiore, M.C. (2000). 'US Public Health Service Clinical Practice Guideline: Treating Tobacco Use and Dependence', *Respir Care.* **45**(10): pp. 1200-62

Fiore, M.C., Bailey, W.C., Cohen, S.J., et al. (2000). *Treating Tobacco Use and Dependence: Clinical Practice Guideline* (Rockville, MD, USA: US Department of Health and Human Services, Public Health Service)

Fitzgerald, L.W., Kaplinsky, L., Kimelberg, H. K. (1990). 'Serotonin Metabolism by Monoamine Oxidase in Rat Primary Astrocyte Cultures', *J Neurochem.* **55**(6): pp. 2008-14

Flattem, N. L., Blakely, R. D. (2000). 'Modified Structure of the Human Serotonin Transporter Promoter', *Mol Psychiatry*, **5**(1): pp.110-5

Forman, S.D., Cohen, J.D., Fitzgerald, M., Eddy, W.F., Mintun, M.A., Noll, D.C. (1995). 'Improved Assessment of Significant Activation in Functional Magnetic Resonance Imaging (fMRI): Use of a Cluster-Size Threshold', *Magn Reson Med.*, **33**(5): pp. 636–47

Friston, K.J., Worsley, K.J., Frackowiak, R.S., Mazziotta, J.C., Evans, A.C. (1994). 'Assessing the Significance of Focal Activations Using their Spatial Extent', *Hum Brain Mapp.* **1**: pp. 214–20

Fryer, J.D., Lukas, R.J. (1999). 'Non-Competitive Functional Inhibition at Diverse, Human Nicotinic Acetylcholine Receptor Subtypes by Bupropion, Phencyclidine, and Ibogaine', *J Pharmacol Exp Ther.* 288(1): pp. 88-92

Fu, Y., Matta, S. G., Brower, V. G., Sharp, B. M. (2001). 'Norepinephrine Secretion in the Hypothalamic Paraventricular Nucleus of Rats during Unlimited Access to Self-Administered Nicotine: An *In Vivo* Microdialysis Study', *J Neurosci.* **21**(22): pp. 8979-89

Fudge, J.L., Haber, S. N. (2002). 'Defining the Caudal Ventral Striatum in Primates: Cellular and Histochemical Features', *J Neurosci.* **22**(23): pp. 10078-82

Gaiardi, M., Bartoletti, M., Bacchi, A., Gubellini, C., Costa, M., Babbini, M. (1991). 'Role of Repeated Exposure to Morphine in Determining its Affective Properties: Place and Taste Conditioning Studies in Rats', *Psychopharmacology* (Berl), **103**(2): pp. 183-6

Garavan, H., Pankiewicz, J., Bloom, A., Cho, J. K., Sperry, L., Ross, T. J., Salmeron, B. J., Risinger, R., Kelley, D., Stein, E. A. (2000). 'Cue-Induced Cocaine Craving: Neuroanatomical Specificity for Drug Users and Drug Stimuli', *Am J Psychiatry*, **157**(11): pp. 1789-98

Garreffa, G., Bianciardi, M., Hagberg, G. E., Macaluso, E., Marciani, M. G., Maraviglia, B., Abbafati, M., Carni, M., Bruni, I., Bianchi, L. (2004). 'Simultaneous EEG-fMRI Acquisition: How Far is it from Being a Standardized Technique?' *Magn Reson Imaging*, **22**(10): pp. 1445-55

Garzon, M., Vaughan, R. A., Uhl, G. R., Kuhar, M. J., Pickel, V. M. (1999). 'Cholinergic Axon Terminals in the Ventral Tegmental Area Target a Subpopulation of Neurons Expressing Low Levels of the Dopamine Transporter', *J Comp Neurol.* **410**(2): pp. 197-210

Geddes, J.R., Freemantle, N., Mason, J., Eccles, M. P., Boynton, J. (2000). 'SSRIs Versus Other Antidepressants for Depressive Disorder'. Cochrane Database Syst Rev. (2): p. CD001851

Gelernter, J., Kranzler, H., Cubells, J. F. (1997). 'Serotonin Transporter Protein (SLC6A4) Allele and Haplotype Frequencies and Linkage Disequilibria in African- and European-American and Japanese Populations and in Alcohol-Dependent Subjects', *Hum Genet.* **101**(2): pp. 243-6

Gelernter, J., Liu, X., Hesselbrock, V., Page, G. P., Goddard, A., Zhang, H. (2004). 'Results of a Genomewide Linkage Scan: Support for Chromosomes 9 and 11 Loci Increasing Risk for Cigarette Smoking', *Am J Med Genet B Neuropsychiatr Genet.* **128**(1): pp. 94-101

Ghosheh, O.A., Dwoskin, L. P., Miller, D. K., Crooks, P. A. (2001). 'Accumulation of Nicotine and its Metabolites in Rat Brain after Intermittent or Continuous Peripheral Administration of [2'-(14)C]Nicotine', *Drug Metab Dispos.* **29**(5): pp. 645-51

Gilbert, D.G., Rabinovich, N.E. (1999). *International Smoking Images Series (With Neutral Counterparts).* (Southern Illinois University: Integrative Neuroscience Laboratory, Department of Psychology) [on CD-ROM]

Girod, R., Barazangi, N., McGehee, D., Role, L. W. (2000). 'Facilitation of Glutamatergic Neurotransmission by Presynaptic Nicotinic Acetylcholine Receptors', *Neuropharmacology*, **39**(13): pp. 2715-25

Goldstein, R.Z., Volkow, N. D. (2002). 'Drug Addiction and its Underlying Neurobiological Basis: Neuroimaging Evidence for the Involvement of the Frontal Cortex', *Am J Psychiatry*, **159**(10): pp. 1642-52

Goode, E.L., Badzioch, M. D., Kim, H., Gagnon, F., Rozek, L. S., Edwards, K. L., Jarvik, G. P. (2003). 'Multiple Genome-Wide Analyses of Smoking Behavior in the Framingham Heart Study', *BMC Genet.* **4 Suppl 1**: p. S102

Gori, G.B., Benowitz, N. L., Lynch, C. J. (1986). 'Mouth Versus Deep Airways Absorption of Nicotine in Cigarette Smokers', *Pharmacol Biochem Behav.* **25**(6): pp. 1181-4

Gorrod, J.W., Hibberd, A. R. (1982). 'The Metabolism of Nicotine-Delta 1'(5')-Iminium Ion, *In Vivo* and *In Vitro*', *Eur J Drug Metab Pharmacokinet.* **7**(4): pp. 293-8

Gorrod, J.W., Jenner, P. (1975). 'The Metabolism of Tobacco Alkaloids', in *Essays in Toxicology* (New York: Academic), pp. 35-78

Gotti, C., Fornasari, D., Clementi, F. (1997). 'Human Neuronal Nicotinic Receptors', Prog *Neurobiol.* **53**(2): pp. 199-237

Gourlay, S.G., Stead, L. F., Benowitz, N. L. (2004). 'Clonidine for Smoking Cessation'. Cochrane Database Syst Rev. (3): p. CD000058

Grady, S.R., Meinerz, N. M., Cao, J., Reynolds, A. M., Picciotto, M. R., Changeux, J. P., McIntosh, J. M., Marks, M. J., Collins, A. C. (2001). 'Nicotinic Agonists Stimulate Acetylcholine Release from Mouse Interpeduncular Nucleus: A Function Mediated by a Different nAChR than Dopamine Release from Striatum', *J Neurochem.* **76**(1): pp. 258-68

Grenhoff, J., Aston-Jones, G., Svensson, T. H. (1986). 'Nicotinic Effects on the Firing Pattern of Midbrain Dopamine Neurons', *Acta Physiol Scand.* **128**(3): pp. 351-8

Gritz, E.R., Baer-Weiss, V., Benowitz, N. L., Van Vunakis, H., Jarvik, M. E. (1981). 'Plasma Nicotine and Cotinine Concentrations in Habitual Smokeless Tobacco Users', *Clin Pharmacol Ther.* **30**(2): pp. 201-9

Gurevich, E.V., Joyce, J. N. (1997), 'Alterations in the Cortical Serotonergic System in Schizophrenia: A Postmortem Study', *Biol Psychiatry*, **42**(7): pp. 529-45

Hajek, P., Stead, L. F., West, R., Jarvis, M. (2005). 'Relapse Prevention Interventions for Smoking Cessation'. Cochrane Database Syst Rev. (1): p. CD003999

Haley, N.J., Axelrad, C. M., Tilton, K. A. (1983). 'Validation of Self-Reported Smoking Behavior: Biochemical Analyses of Cotinine and Thiocyanate', *Am J Public Health*, **73**(10): pp. 1204-7

Halldin, C., Nagren, K., Swahn, C. G., Langstrom, B., Nyback, H. (1992). '(S)- and (R)-[11C]Nicotine and the Metabolite (R/S)-[11C]Cotinine. Preparation, Metabolite Studies and *In Vivo* Distribution in the Human Brain Using PET', *Int J Rad Appl Instrum B.* **19**(8): pp. 871-80

Harada, S., Okubo, T., Tsutsumi, M., Takase, S., Muramatsu, T. (1996). 'Investigation of Genetic Risk Factors Associated with Alcoholism', *Alcohol Clin Exp Res.* **20**(9 Suppl): pp. 293A-296A

Hariri, A.R., Drabant, E. M., Munoz, K. E., Kolachana, B. S., Mattay, V. S., Egan, M. F., Weinberger, D. R. (2005). 'A Susceptibility Gene for Affective Disorders and the Response of the Human Amygdala', *Arch Gen Psychiatry*, **62**(2): pp. 146-52

Hariri, A.R., Mattay, V. S., Tessitore, A., Kolachana, B., Fera, F., Goldman, D., Egan, M. F., Weinberger, D. R. (2002). 'Serotonin Transporter Genetic Variation and the Response of the Human Amygdala', *Science*, **297**(5580): pp. 400-3

Hariri, A.R., Weinberger, D. R. (2003). 'Functional Neuroimaging of Genetic Variation in Serotonergic Neurotransmission', *Genes Brain Behav.* **2**(6): pp. 341-9

Hashimoto, T., Nishino, N., Nakai, H., Tanaka, C. (1991). 'Increase in Serotonin 5-HT1A Receptors in Prefrontal and Temporal Cortices of Brains from Patients with Chronic Schizophrenia', *Life Sci.* **48**(4): pp. 355-63

Havermans, R.C., Debaere, S., Smulders, F. T., Wiers, R. W., Jansen, A. T. (2003). 'Effect of Cue Exposure, Urge to Smoke, and Nicotine Deprivation on Cognitive Performance in Smokers', *Psychol Addict Behav.* **17**(4): pp. 336-9

Heath, A.C., Cates, R., Martin, N. G., Meyer, J., Hewitt, J. K., Neale, M. C., Eaves, L. J. (1993). 'Genetic Contribution to Risk of Smoking Initiation: Comparisons Across Birth Cohorts and Across Cultures', *J Subst Abuse*, **5**(3): pp. 221-46

Heath, A.C., Kirk, K. M., Meyer, J. M., Martin, N. G. (1999). 'Genetic and Social Determinants of Initiation and Age at Onset of Smoking in Australian Twins', *Behav Genet.* **29**(6): pp. 395-407

Heath, A.C., Madden, P. A., Martin, N. G. (1998). 'Statistical Methods in Genetic Research on Smoking', *Stat Methods Med Res.* **7**(2): pp. 165-86

Heath, A.C., Madden, P.A.F. (1995). 'Genetic Influences on Smoking Behavior', in *Behavior Genetics Approaches in Behavioral Medicine*, ed. by Turner, J.R., Cardon, L.R., Hewitt, J.K. (New York, NY, USA: Plenum Press), pp. 45-66

Heath, A.C. and Martin, N.G. (1993). 'Genetic Models for the Natural History of Smoking: Evidence for a Genetic Influence on Smoking Persistence', *Addict Behav.* **18**(1): pp. 19-34

Heatherton, T.F., Kozlowski, L. T., Frecker, R. C., Fagerstrom, K. O. (1991). 'The Fagerstrom Test for Nicotine Dependence: A Revision of the Fagerstrom Tolerance Questionnaire', *Br J Addict*, **86**(9): pp. 1119-27

Heils, A., Teufel, A., Petri, S., Stober, G., Riederer, P., Bengel, D., Lesch, K. P. (1996). 'Allelic Variation of Human Serotonin Transporter Gene Expression', *J Neurochem*, **66**(6): pp. 2621-4

Heimer, L., Zahm, D.S., Churchill, L., Kalivas, P.W., Wohltmann, C. (1991). 'Specificity in the Projection Patterns of Accumbal Core and Shell in the Rat', *Neuroscience*, **41**(1): 89-125

Heinz, A., Jones, D. W., Mazzanti, C., Goldman, D., Ragan, P., Hommer, D., Linnoila, M., Weinberger, D. R. (2000). 'A Relationship Between Serotonin Transporter Genotype and *In Vivo* Protein Expression and Alcohol Neurotoxicity', *Biol Psychiatry*, **47**(7): pp. 643-9

Heinz, A., Siessmeier, T., Wrase, J., Hermann, D., Klein, S., Grusser-Sinopoli, S. M., Flor, H., Braus, D. F., Buchholz, H. G., Grunder, G., Schreckenberger, M., Smolka, M. N., Rosch, F., Mann, K., Bartenstein, P. (2004). 'Correlation between Dopamine D(2) Receptors in the Ventral Striatum and Central Processing of Alcohol Cues and Craving', *Am J Psychiatry*, **161**(10): pp. 1783-9

Heishman, S.J., Henningfield, J. E. (2000). 'Tolerance to repeated nicotine administration on performance, subjective, and.physiological responses in nonsmokers', *Psychopharmacology* (Berl), **152**(3): pp. 321-33

Hesse, L.M., Venkatakrishnan, K., Court, M. H., von Moltke, L. L., Duan, S. X., Shader, R. I., Greenblatt, D. J. (2000). 'CYP2B6 Mediates the *In Vitro* Hydroxylation of Bupropion: Potential Drug Interactions with Other Antidepressants', *Drug Metab Dispos.* **28**(10): pp. 1176-83

Higgins, G.A., Bradbury, A. J., Jones, B. J., Oakley, N. R. (1988). 'Behavioural and Biochemical Consequences Following Activation of 5HT1-Like and GABA Receptors in the Dorsal Raphe Nucleus of the Rat', *Neuropharmacology*, **27**(10): pp. 993-1001

Higgins, G.A., Wang, Y., Corrigall, W. A., Sellers, E. M. (1994). 'Influence of 5-HT3 Receptor Antagonists and the Indirect 5-HT Agonist, Dexfenfluramine, on Heroin Self-Administration in Rats', *Psychopharmacology* (Berl), **114**(4): pp. 611-9

Hildebrand, B.E., Nomikos, G. G., Bondjers, C., Nisell, M., Svensson, T. H. (1997). 'Behavioral Manifestations of the Nicotine Abstinence Syndrome in the Rat: Peripheral Versus Central Mechanisms', *Psychopharmacology* (Berl), **129**(4): pp. 348-56

Hildebrand, B.E., Nomikos, G. G., Hertel, P., Schilstrom, B., Svensson, T. H. (1998). 'Reduced Dopamine Output in the Nucleus Accumbens but not in the Medial Prefrontal Cortex in Rats Displaying a Mecamylamine-Precipitated Nicotine Withdrawal Syndrome', *Brain Res.* **779**(1-2): pp. 214-25

Hilleman, D.E., Mohiuddin, S. M., Delcore, M. G. (1994). 'Comparison of Fixed-Dose Transdermal Nicotine, Tapered-Dose Transdermal Nicotine, and Buspirone in Smoking Cessation', *J Clin Pharmacol.* **34**(3): pp. 222-4

Hilleman, D.E., Mohiuddin, S. M., Del Core, M. G., Sketch, M. H., Sr. (1992). 'Effect of Buspirone on Withdrawal Symptoms Associated with Smoking Cessation', *Arch Intern Med.* **152**(2): pp. 350-2

Hitsman, B., Spring, B., Borrelli, B., Niaura, R., Papandonatos, G. D. (2001). 'Influence of Antidepressant Pharmacotherapy on Behavioral Treatment Adherence and Smoking Cessation Outcome in a Combined Treatment Involving Fluoxetine', *Exp Clin Psychopharmacol.* **9**(4): pp. 355-62

Hogarth, L.C., Mogg, K., Bradley, B. P., Duka, T., Dickinson, A. (2003). 'Attentional Orienting towards Smoking-Related Stimuli', *Behav Pharmacol.* **14**(2): pp. 153-60

Hogg, S., Andrews, N., File, S. E. (1994). 'Contrasting Behavioural Effects of 8-OH DPAT in the Dorsal Raphe Nucleus and Ventral Hippocampus', *Neuropharmacology*, **33**(3-4): pp. 343-8

Horger, B.A., Giles, M. K., Schenk, S. (1992). 'Preexposure to Amphetamine and Nicotine Predisposes Rats to self-administer a low dose of cocaine', *Psychopharmacology* (Berl), **107**(2-3): pp. 271-6

Horger, B.A., Shelton, K., Schenk, S. (1990). 'Preexposure Sensitizes Rats to the Rewarding Effects of Cocaine', *Pharmacol Biochem Behav.* **37**(4): pp. 707-11

Horvitz, J.C. (2000). 'Mesolimbocortical and Nigrostriatal Dopamine Responses to Salient Non-Reward Events', *Neuroscience*, **96**(4): pp. 651-6

Houlihan, M., Pritchard, W., Robinson, J. (2001). 'EEG effects of smoking: Is there tachyphylaxis?' *Neuropsychobiology*, **44**(1): pp. 54-8

Houlihan, M.E., Pritchard, W. S., Robinson, J. H. (2001). Effects of Smoking/Nicotine on Performance and Event-Related Potentials During a Short-Term Memory Scanning Task', *Psychopharmacology* (Berl), **156**(4): pp. 388-96

Howland, J.G., MacKenzie, E. M., Yim, T. T., Taepavarapruk, P., Phillips, A. G. (2004). 'Electrical Stimulation of the Hippocampus Disrupts Prepulse Inhibition in Rats: Frequency- and Site-Dependent Effects', *Behav Brain Res.* **152**(2): pp. 187-97

Huang, Y.Y., Battistuzzi, C., Oquendo, M. A., Harkavy-Friedman, J., Greenhill, L., Zalsman, G., Brodsky, B., Arango, V., Brent, D. A., Mann, J. J. (2004). 'Human 5-HT1A Receptor C(-1019)G Polymorphism and Psychopathology', *Int J Neuropsychopharmacol*, **7**(4): pp. 441-51

Huettal, S.A., McCarthy, G. (2004). 'What is odd in the oddball task? Prefrontal cortex is activated by dynamic changes in response strategy.' *Neuropsychologia* **42**(3):379-386

Hughes, J.R., Hatsukami, D. (1986). 'Signs and Symptoms of Tobacco Withdrawal', *Arch Gen Psychiatry*, **43**(3): pp. 289-94

Hughes, J.R., Stead, L. F., Lancaster, T. (2000). 'Anxiolytics for Smoking Cessation'. Cochrane Database Syst Rev. (4): p. CD002849

Hughes, J.R., Stead, L. F., Lancaster, T. (2004). 'Antidepressants for Smoking Cessation'. Cochrane Database Syst Rev. (4): p. CD000031.pub2

Hukkanen, J., Jacob, P., 3rd, Benowitz, N. L. (2005). 'Metabolism and Disposition Kinetics of Nicotine', Pharmacol Rev. **57**(1): pp. 79-115

Hurt, R. D., Sachs, D. P., Glover, E.D., Offord, K.P., Johnstone, J.A., Dale, L.C. et al. (1997). 'A Comparison of Sustained-Release Bupropion and Placebo for Smoking Cessation', *N Engl J Med.* **337**(17): pp. 1195-1202

Hutchison, K.E., McGeary, J., Wooden, A., Blumenthal, T., Ito, T. (2003). 'Startle Magnitude and Prepulse Inhibition: Effects of Alcohol and Attention', *Psychopharmacology* (Berl), **167**(3): pp. 235-41

Hutchison, K.E., Niaura, R., Swift, R. (2000). 'The Effects of Smoking High Nicotine Cigarettes on Prepulse Inhibition, Startle Latency, and Subjective Responses', *Psychopharmacology* (Berl), **150**(3): pp. 244-52

Hutchison, K.E., Niaura, R., Swift, R. (1999). 'Smoking Cues Decrease Prepulse Inhibition of the Startle Response and Increase Subjective Craving in Humans', *Exp Clin Psychopharmacol.* **7**(3): pp. 250-6

ICRF (1995). 'Effectiveness of Health Checks Conducted by Nurses in Primary Care: Final Results of the OXCHECK Study. Imperial Cancer Research Fund OXCHECK Study Group', *BMJ.* **310**(6987): pp. 1099-104

ICRF (1994). 'Effectiveness of Health Checks Conducted by Nurses in Primary Care: Results of the OXCHECK Study after One Year. Imperial Cancer Research Fund OXCHECK Study Group'. *BMJ.* **308**(6924): pp. 308-12

Imperato, A., Mulas, A., Di Chiara, G. (1986). 'Nicotine Preferentially Stimulates Dopamine Release in the Limbic System of Freely Moving Rats', *Eur J Pharmacol.* **132**(2-3): pp. 337-8

Irvine, E.E., Cheeta, S., File, S. E. (2001). 'Development of Tolerance to Nicotine's Anxiogenic Effect in the Social Interaction Test', *Brain Res.* **894**(1): pp. 95-100

Irvine, E.E., Cheeta, S., File, S. E. (1999). 'Time-Course of Changes in the Social Interaction Test of Anxiety Following Acute and Chronic Administration of Nicotine', *Behav Pharmacol.* **10**(6-7): pp. 691-7

Ishai, A., Ungerleider, L. G., Martin, A., Schouten, J. L., Haxby, J. V. (1999). 'Distributed Representation of Objects in the Human Ventral Visual Pathway', *Proc Natl Acad Sci U S A*, **96**(16): pp. 9379-84

Ito, R., Dalley, J.W., Howes, S.R., Robbins, T.W., Everitt, B.J. (2000). 'Dissociation in Conditioned Dopamine Release in the Nucleus Accumbens Core and Shell in Response to Cocaine Cues and During Cocaine-Seeking Behavior in Rats', *J Neurosci.* **20**(19):7489-95

Iyaniwura, T.T., Wright, A.E., Balfour, D.J. (2001). 'Evidence that Mesoaccumbens Dopamine and Locomotor Responses to Nicotine in the Rat are Influenced by Pretreatment Dose and Strain', *Psychopharmacology (Berl)*, **158**(1):73-9

Jacobsen, L.K., Gore, J. C., Skudlarski, P., Lacadie, C. M., Jatlow, P., Krystal, J. H. (2002). 'Impact of Intravenous Nicotine on BOLD Signal Response to Photic Stimulation', *Magn Reson Imaging*. **20**(2): pp. 141-5

James I, king of England (1604). *A Counterblaste to Tobacco by King James I* <http://www.tobacco.org/resources/history/Tobacco_Historynotes.html#aa4> [accessed 27 December 2004]

Janhunen, S., Ahtee, L. (2004). 'Comparison of the Effects of Nicotine and Epibatidine on the Striatal Extracellular Dopamine', *Eur J Pharmacol*. **494**(2-3): pp. 167-77

Jarvis, M., Tunstall-Pedoe, H., Feyerabend, C., Vesey, C., Salloojee, Y. (1984). 'Biochemical Markers of Smoke Absorption and Self Reported Exposure to Passive Smoking', *J Epidemiol Community Health*, **38**(4): pp. 335-9

Javors, M.A., Hatch, J. P., Lamb, R. J. (2005). 'Cut-off Levels for Breath Carbon Monoxide as a Marker for Cigarette Smoking', *Addiction*, **100**(2): pp. 159-67

Jenkinson, M., Bannister, P., Brady, M., Smith, S. (2002). 'Improved Optimization for the Robust and Accurate Linear Registration and Motion Correction of Brain Images', *Neuroimage*, **17**(2): pp. 825-41

Jenkinson, M., Smith, S. (2001). 'A Global Optimisation Method for Robust Affine Registration of Brain Images', *Med Image Anal*, **5**(2): pp. 143-56

Jiang, L.H., Ashby, C. R., Jr., Kasser, R. J., Wang, R. Y. (1990). 'The Effect of Intraventricular Administration of the 5-HT3 Receptor Agonist 2-Methylserotonin on the Release of Dopamine in the Nucleus Accumbens: An *In Vivo* Chronocoulometric Study', *Brain Res*. **513**(1): pp. 156-60

Johnson, L.R., Aylward, R. L., Hussain, Z., Totterdell, S. (1994). 'Input from the Amygdala to the Rat Nucleus Accumbens: Its Relationship with Tyrosine Hydroxylase Immunoreactivity and Identified Neurons', *Neuroscience*, **61**(4): pp. 851-65

Jones, R.A. (1986). 'Individual Differences in Nicotine Sensitivity', *Addict Behav*. **11**(4): pp. 435-8

Jones, S., Sudweeks, S., Yakel, J. L. (1999). 'Nicotinic Receptors in the Brain: Correlating Physiology with Function', *Trends Neurosci*. **22**(12): pp. 555-61

Jorenby, D.E., Leischow, S. J., Nides, M. A., Rennard, S. I., Johnston, J. A., Hughes, A. R., Smith, S. S., Muramoto, M. L., Daughton, D. M., Doan, K., Fiore, M. C., Baker, T. B. (1999). 'A Controlled Trial of Sustained-Release Bupropion, a Nicotine Patch, or Both for Smoking Cessation', *N Engl J Med*. **340**(9): pp. 685-91

Kadakia, S.C., De La Baume, H. R., Shaffer, R. T. (1996). 'Effects of Transdermal Nicotine on Lower Esophageal Sphincter and Esophageal Motility', *Dig Dis Sci*. **41**(11): pp. 2130-4

Kadakia, S.C., Kikendall, J. W., Maydonovitch, C., Johnson, L. F. (1995). 'Effect of Cigarette Smoking on Gastroesophageal Reflux Measured by 24-h Ambulatory Esophageal pH Monitoring', *Am J Gastroenterol*. **90**(10): pp. 1785-90

Kalivas, P.W. (1993). 'Neurotransmitter Regulation of Dopamine Neurons in the Ventral Tegmental Area', *Brain Res Brain Res Rev*. **18**(1): pp. 75-113

Kalivas, P.W. and Stewart, J. (1991). 'Dopamine Transmission in the Initiation and Expression of Drug- and Stress-Induced Sensitization of Motor Activity', *Brain Res Brain Res Rev*. **16**(3): pp. 223-44

Kalman, D. (2002). 'The Subjective Effects of Nicotine: Methodological Issues, a Review of Experimental Studies, and Recommendations for Future Research', *Nicotine Tob Res*. **4**(1): pp. 25-70

Katz, B., Thesleff, S. (1957). 'A Study of the Desensitization Produced by Acetylcholine at the Motor End-Plate', *J Physiol*. **138**(1): pp. 63-80

Kawanishi, Y., Harada, S., Tachikawa, H., Okubo, T., Shiraishi, H. (1998). 'Novel Mutations in the Promoter and Coding Region of the Human 5-HT1A Receptor Gene and Association Analysis in Schizophrenia', *Am J Med Genet*. **81**(5): pp. 434-9

Kendler, K.S., Neale, M. C., Sullivan, P., Corey, L. A., Gardner, C. O., Prescott, C. A. (1999). 'A Population-Based Twin Study in Women of Smoking Initiation and Nicotine Dependence', *Psychol Med*. **29**(2): pp. 299-308

Kendler, K.S., Thornton, L. M., Pedersen, N. L. (2000). 'Tobacco Consumption in Swedish Twins Reared Apart and Reared Together', *Arch Gen Psychiatry*, **57**(9): pp. 886-92

Kenny, P.J., File, S. E., Neal, M. J. (2000). 'Evidence for a Complex Influence of Nicotinic Acetylcholine Receptors on Hippocampal Serotonin Release', *J Neurochem*. **75**(6): pp. 2409-14

Kenny, P.J., File, S. E., Rattray, M. (2001). 'Nicotine Regulates 5-HT(1A) Receptor Gene Expression in the Cerebral Cortex and Dorsal Hippocampus', *Eur J Neurosci*. **13**(6): pp. 1267-71

Khan, Z.U., Gutierrez, A., Martin, R., Penafiel, A., Rivera, A., de la Calle, A. (2000). 'Dopamine D5 Receptors of Rat and Human Brain', *Neuroscience*, **100**(4): pp. 689-99

Kiba, H., Jayaraman, A. (1994). 'Nicotine Induced c-fos Expression in the Striatum is Mediated Mostly by Dopamine D1 Receptor and Is Dependent on NMDA Stimulation', *Brain Res Mol Brain Res*. **23**(1-2): pp. 1-13

Kirino, E., Belger, A., Goldman-Rakic, P.l., McCarthy, G. (2000). 'Prefrontal Activation Evoked by Infrequent Target and Novel Stimuli in a Visual Target Detection Task: An Event-Related Functional Magnetic Resonance Imaging Study', *J Neurosci*. **20**(17): pp. 6612-8

Klink, R., de Kerchove d'Exaerde, A., Zoli, M., Changeux, J. P. (2001). 'Molecular and Physiological Diversity of Nicotinic Acetylcholine Receptors in the Midbrain Dopaminergic Nuclei', *J Neurosci*. **21**(5): pp. 1452-63

Kobilka, B.K., Frielle, T., Collins, S., Yang-Feng, T., Kobilka, T. S., Francke, U., Lefkowitz, R. J., Caron, M. G. (1987). 'An Intronless Gene Encoding a Potential Member of the Family of Receptors Coupled to Guanine Nucleotide Regulatory Proteins', *Nature*, **329**(6134): pp. 75-9

Koechlin, E., Ody, C., and Kouneiher, F. (2003). 'The Architecture of Cognitive Control in the Human Prefrontal Cortex', *Science*, **302**(5648): pp. 1181-5

Koob, G.F. (1999). 'The Role of the Striatopallidal and Extended Amygdala Systems in Drug Addiction', *Ann N Y Acad Sci*. **877**: pp. 445-60

Koob, G.F., Le Moal, M. (1997). 'Drug Abuse: Hedonic Homeostatic Dysregulation', *Science*, **278**(5335): pp. 52-8

Koob, G.F., Le Moal, M. (2001). 'Drug Addiction, Dysregulation of Reward, and Allostasis', *Neuropsychopharmacology*, **24**(2): pp. 97-129

Koop, C.E. (2003). 'Tobacco Addiction: Accomplishments and Challenges in Science, Health, and Policy', *Nicotine Tob Res*. **5**(5): pp. 613-9

Koop, C.E., Luoto, J. (1982). "'The Health Consequences of Smoking: Cancer', Overview of a Report of the Surgeon General", *Public Health Rep*. **97**(4): pp. 318-24

Kozlowski, L.T. (1981). 'The Determinants of Tobacco Use: Cigarette Smoking in the Context of Other Forms of Tobacco Use', *Can J Public Health*, **72**(6): pp. 396-401

Kumari, V., Gray, J. A., ffytche, D. H., Mitterschiffthaler, M. T., Das, M., Zachariah, E., Vythelingum, G. N., Williams, S. C., Simmons, A., Sharma, T. (2003). 'Cognitive Effects of Nicotine in Humans: An fMRI Study', *Neuroimage*, **19**(3): pp. 1002-13

Lam, S., Shen, Y., Nguyen, T., Messier, T. L., Brann, M., Comings, D., George, S. R., O'Dowd, B. F. (1996). 'A Serotonin Receptor Gene (5HT1A) Variant Found in a Tourette's Syndrome Patient', *Biochem Biophys Res Commun*. **219**(3): pp. 853-8

Lancaster, T., Stead, L. (2005). 'Individual Behavioural Counselling for Smoking Cessation'. Cochrane Database Syst Rev. (2): p. CD001292

Lancaster, T., Stead, L. (2004). 'Physician Advice for Smoking Cessation'. Cochrane Database Syst Rev. (4): p. CD000165

Lanfumey, L., Hamon, M., (2000). 'Central 5-HT(1A) Receptors: Regional Distribution and Functional Characteristics', Nucl Med Biol. **27**(5): pp. 429-35

Larson, P.S., Haag, H.B. (1943). 'Studies on the Fate of Nicotine in the Body III. On the Pharmacology of Some Methylated and Demethylated Derivatives of Nicotine', *J Pharmacol Exp Ther*. **77**: pp. 343-349

Lautenbach, B.F. (1876). 'On a New Function of the Liver', *Philad Med Times*, **7**(7): pp. 387-39

Laviolette, S.R., van der Kooy, D. (2003). 'Blockade of Mesolimbic Dopamine Transmission Dramatically Increases Sensitivity to the Rewarding Effects of Nicotine in the Ventral Tegmental Area', *Mol Psychiatry*, **8**(1): pp. 50-9

Laviolette, S.R., van der Kooy, D. (2004). 'The Neurobiology of Nicotine Addiction: Bridging the Gap from Molecules to Behaviour', *Nat Rev Neurosci*. **5**(1): pp. 55-65

Lawrence, N.S., T.J. Ross, and E.A. Stein (2002). 'Cognitive Mechanisms of Nicotine on Visual Attention', *Neuron*. **36**(3): pp. 539-48

Lee, M., Bailer, U. F., Frank, G. K., Henry, S. E., Meltzer, C. C., Price, J. C., Mathis, C. A., Putnam, K. T., Ferrell, R. E., Hariri, A. R., Kaye, W. H. (2005). 'Relationship of a 5-HT Transporter Functional Polymorphism to 5-HT(1A) Receptor Binding in Healthy Women', *Mol Psychiatry*, **10**(8): pp. 715-6

Le Foll, B., Diaz, J., Sokoloff, P. (2003). 'Increased Dopamine D3 Receptor Expression Accompanying Behavioral Sensitization to Nicotine in Rats', *Synapse*, **47**(3): pp. 176-83

Legros, J.J., Conte-Devolx, B., Rougon-Rapuzzi, G., Millet, Y., Franchimont, P. (1977). '[Simultaneous Liberation of Vasopressin (ADH) and of Neurophysins During Nicotine Perfusion in Man]', *C R Seances Soc Biol Fil*. **171**(2): pp. 478-83

LeMarquand, D., Pihl, R. O., Benkelfat, C. (1994). 'Serotonin and Alcohol Intake, Abuse, and Dependence: Findings of Animal Studies', *Biol Psychiatry*, **36**(6): pp. 395-421

Lemonde, S., Du, L., Bakish, D., Hrdina, P., Albert, P. R. (2004). 'Association of the C(-1019)G 5-HT1A Functional Promoter Polymorphism with Antidepressant Response', *Int J Neuropsychopharmacol*, **7**(4): pp. 501-6

Lemonde, S., Turecki, G., Bakish, D., Du, L., Hrdina, P. D., Bown, C. D., Sequeira, A., Kushwaha, N., Morris, S. J., Basak, A., Ou, X. M., Albert, P. R. (2003). 'Impaired Repression at a 5-Hydroxytryptamine 1A Receptor Gene Polymorphism Associated with Major Depression and Suicide', *J Neurosci.* **23**(25): pp. 8788-99

Le Poul, E., Laaris, N., Doucet, E., Laporte, A. M., Hamon, M., Lanfumey, L. (1995). 'Early Desensitization of Somato-Dendritic 5-HT1A Autoreceptors in Rats Treated with Fluoxetine or Paroxetine', *Naunyn Schmiedebergs Arch Pharmacol*, **352**(2): pp. 141-8

Lerman, C., Berrettini, W. (2003). 'Elucidating the Role of Genetic Factors in Smoking Behavior and Nicotine Dependence', *Am J Med Genet B Neuropsychiatr Genet.* **118**(1): pp. 48-54

Lerman, C., Berrettini, W. (2003). 'Elucidating the Role of Genetic Factors in Smoking Behavior and Nicotine Dependence', *Am J Med Genet.* **118B**(1): pp. 48-54

Lerman, C., Caporaso, N. E., Audrain, J., Main, D., Boyd, N. R., Shields, P. G. (2000). 'Interacting Effects of the Serotonin Transporter Gene and Neuroticism in Smoking Practices and Nicotine Dependence', *Mol Psychiatry*, **5**(2): pp. 189-92

Lerman, C., Roth, D., Kaufman, V., Audrian, J., Hawk, L., Liu, A., et al. (2002). 'Mediating Mechanisms for the Impact of Bupropion in Smoking Cessation Treatment', *Drug Alcohol Depend.* **67**(2): pp. 219-23

Lerman, C., Shields, P. G., Audrain, J., Main, D., Cobb, B., Boyd, N. R., Caporaso, N. (1998). 'The Role of the Serotonin Transporter Gene in Cigarette Smoking', *Cancer Epidemiol Biomarkers Prev.* **7**(3): pp. 253-5

Lerman, C., Shields, P. G., Wileyto, E. P., Audrain, J., Pinto, A., Hawk, L., Krishnan, S., Niaura, R., Epstein, L. (2002). 'Pharmacogenetic Investigation of Smoking Cessation Treatment', *Pharmacogenetics*, **12**(8): pp. 627-634

Lerman, C., Niaura, R., Collins, B.N., Wileyto, P., Audrain-McGovern, J., Pinto, A., Hawk, L., Epstein, L.H. (2003) 'Effects of Dopamine Transporter and Receptor Polymorphisms on Smoking Cessation in a Bupropion Clinical Trial', *Health Psychol.* **22**(5):541-8

Lesch, K.P., Balling, U., Gross, J., Strauss, K., Wolozin, B. L., Murphy, D. L., Riederer, P. (1994). 'Organization of the Human Serotonin Transporter Gene', *J Neural Transm Gen Sect.* **95**(2): pp. 157-62

Lesch, K.P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., Murphy, D. L. (1996). 'Association of Anxiety-Related Traits with a Polymorphism in the Serotonin Transporter Gene Regulatory Region', *Science*, **274**(5292): pp. 1527-31

Lesch, K.P., Gutknecht, L. (2004). 'Focus on the 5-HT1A Receptor: Emerging Role of a Gene Regulatory Variant in Psychopathology and Pharmacogenetics', *Int J Neuropsychopharmacol*, **7**(4): pp. 381-5

Lester, R.A., Dani, J. A. (1995). 'Acetylcholine Receptor Desensitization Induced by Nicotine in Rat Medial Habenula Neurons', *J Neurophysiol.* **74**(1): pp. 195-206

Lett, B.T. (1989). 'Repeated Exposures Intensify Rather than Diminish the Rewarding Effects of Amphetamine, Morphine, and Cocaine', *Psychopharmacology* (Berl), **98**(3): pp. 357-62

Levitt, P., Pintar, J. E., Breakefield, X. O. (1982). 'Immunocytochemical Demonstration of Monoamine Oxidase B in Brain Astrocytes and Serotonergic Neurons', *Proc Natl Acad Sci U S A*, **79**(20): pp. 6385-9

Levy, D.L., Holzman, P. S., Matthysse, S., Mendell, N. R. (1993). 'Eye Tracking Dysfunction and Schizophrenia: A Critical Perspective', *Schizophr Bull.* **19**(3): pp. 461-536

Lewis, S.J., et al. (2003). 'Cognitive Iimpairments in Early Parkinson's Disease are Accompanied by Reductions in Activity in Frontostriatal Neural Circuitry', *J Neurosci.* **23**(15): pp. 6351-6

Li, M.D., Cheng, R., Ma, J. Z., Swan, G. E. (2003). 'A Meta-Analysis of Estimated Genetic and Environmental Effects on Smoking Behavior in Male and Female Adult Twins', *Addiction*, **98**(1): pp. 23-31

Li, M.D., Ma, J. Z., Cheng, R., Dupont, R. T., Williams, N. J., Crews, K. M., Payne, T. J., Elston, R. C. (2003). 'A Genome-Wide Scan to Identify Loci for Smoking Rate in the Framingham Heart Study Population', *BMC Genet.* **4 Suppl 1**: p. S103

Li, Q., Muma, N. A., Battaglia, G., Van de Kar, L. D. (1997). 'A Desensitization of Hypothalamic 5-HT1A Receptors by Repeated Injections of Paroxetine: Reduction in the Levels of G(i) and G(o) Proteins and Neuroendocrine Responses, but not in the Density of 5-HT1A Receptors', *J Pharmacol Exp Ther.* **282**(3): pp. 1581-90

Li, Q., Muma, N. A., van de Kar, L. D. (1996). 'Chronic Fluoxetine Induces a Gradual Desensitization of 5-HT1A Receptors: Reductions in Hypothalamic and Midbrain Gi and G(o) Proteins and in Neuroendocrine Responses to a 5-HT1A Agonist', *J Pharmacol Exp Ther.* **279**(2): pp. 1035-42

Li, Q., Wichems, C., Heils, A., Lesch, K. P., Murphy, D. L. (2000). 'Reduction in the Density and Expression, but not G-Protein Coupling, of Serotonin Receptors (5-HT1A) in 5-HT Transporter Knock-Out Mice: Gender and Brain Region Differences', *J Neurosci.* **20**(21): pp. 7888-95

Li, Q., Wichems, C., Heils, A., Van De Kar, L. D., Lesch, K. P., Murphy, D. L. (1999). 'Reduction of 5-Hydroxytryptamine (5-HT)(1A)-Mediated Temperature and Neuroendocrine Responses and 5-HT(1A) Binding Sites in 5-HT Transporter Knockout Mice', *J Pharmacol Exp Ther.* **291**(3): pp. 999-1007

Li, Y., Acerbo, M.J., Robinson, T.E. (2004). 'The Induction of Behavioural Sensitisatiopn is associated with Cocaine-Induced Structural Plasticity in the Core (but not Shell) of the Nucleus Accumbens' *Eur J Neurosci.* **20**(6):1647-54

Lim, H.K., Pae, C. U., Joo, R. H., Yoo, S. S., Choi, B. G., Kim, D. J., Lee, C., Lee, C. U. (2005). 'fMRI Investigation on Cue-Induced Smoking Craving', *J Psychiatr Res.* **39**(3): pp. 333-5

Little, K.Y., McLaughlin, D. P., Zhang, L., Livermore, C. S., Dalack, G. W., McFinton, P. R., DelProposto, Z. S., Hill, E., Cassin, B. J., Watson, S. J., Cook, E. H. (1998). 'Cocaine, Ethanol, and Genotype Effects on Human Midbrain Serotonin Transporter Binding Sites and mRNA Levels', *Am J Psychiatry*, **155**(2): pp. 207-13

Luellen, B.A., Miller, D. B., Chisnell, A. C., Murphy, D. L., O'Callaghan, J. P., Andrews, A. M. (2003). 'Neuronal and Astroglial Responses to the Serotonin and Norepinephrine Neurotoxin: 1-methyl-4-(2'-aminophenyl)-1,2,3,6-tetrahydropyridine', *J Pharmacol Exp Ther.* **307**(3): pp. 923-31

Maldonado, R., Saiardi, A., Valverde, O., Samad, T. A., Roques, B. P., Borrelli, E. (1997). 'Absence of Opiate Rewarding Effects in Mice Lacking Dopamine D2 Receptors', *Nature*, **388**(6642): pp. 586-9

Malin, D.H., Lake, J. R., Newlin-Maultsby, P., Roberts, L. K., Lanier, J. G., Carter, V. A., Cunningham, J. S., Wilson, O. B. (1992). 'Rodent Model of Nicotine Abstinence Syndrome', *Pharmacol Biochem Behav*. **43**(3): pp. 779-84

Mansvelder, H.D., De Rover, M., McGehee, D. S., Brussaard, A. B. (2003). 'Cholinergic Modulation of Dopaminergic Reward Areas: Upstream and Downstream Targets of Nicotine Addiction', *Eur J Pharmacol*. **480**(1-3): pp. 117-23

Mansvelder, H.D., Keath, J. R., McGehee, D. S. (2002). 'Synaptic Mechanisms Underlie Nicotine-Induced Excitability of Brain Reward Areas', *Neuron*. **33**(6): pp 905-19

Markstein, R., Hoyer, D., Engel, G. (1986). '5-HT1A-Receptors Mediate Stimulation of Adenylate Cyclase in Rat Hippocampus', *Naunyn Schmiedebergs Arch Pharmacol*. **333**(4): pp. 335-41

Martin-Soelch, C., Missimer, J., Leenders, K. L., Schultz, W. (2003). 'Neural Activity Related to the Processing of Increasing Monetary Reward in Smokers and Nonsmokers', *Eur J Neurosci*. **18**(3): pp. 680-8

Mathieu-Kia, A.M., Pages, C., Besson, M. J. (1998) 'Inducibility of c-Fos Protein in Visuo-Motor System and Limbic Structures after Acute and Repeated Administration of Nicotine in the Rat', *Synapse*, **29**(4): pp. 343-54

Matsuda, L.A., Lolait, S. J., Brownstein, M. J., Young, A. C., Bonner, T. I. (1990). 'Structure of a Cannabinoid Receptor and Functional Expression of the Cloned cDNA', *Nature*, **346**(6284): pp. 561-4

Matta, S.G., Foster, C. A., Sharp, B. M. (1993a). 'Nicotine Stimulates the Expression of cFos Protein in the Parvocellular Paraventricular Nucleus and Brainstem Catecholaminergic Regions', *Endocrinology*, **132**(5): pp. 2149-56

Matta, S.G., Foster, C. A., Sharp, B. M. (1993b). 'Selective Administration of Nicotine into Catecholaminergic Regions of Rat Brainstem Stimulates Adrenocorticotropin Secretion', *Endocrinology*, **133**(6): pp. 2935-42

Matta, S.G., Fu, Y., Valentine, J. D., Sharp, B. M. (1998). 'Response of the Hypothalamo-Pituitary-Adrenal Axis to Nicotine', *Psychoneuroendocrinology*, **23**(2): pp. 103-13

Matta, S.G., McAllen, K. M., Sharp, B. M. (1990). 'Role of the Fourth Cerebroventricle in Mediating Rat Plasma ACTH Responses to Intravenous Nicotine', *J Pharmacol Exp Ther*. **252**(2): pp. 623-30

Matta, S.G., Valentine, J. D., Sharp, B. M. (1997). 'Nicotinic Activation of CRH Neurons in Extrahypothalamic Regions of the Rat Brain', *Endocrine*. **7**(2): pp. 245-53

Matthews, P.M. (2001). 'Introduction', in *Functional MRI--An Introduction to Methods*, ed. by Jezzard, P., Matthews, P.M., Smith, S.M. (Oxford, UK: Oxford University Press), pp. 1-34

Mawlawi, O., Martinez, D., Slifstein, M., Broft, A., Chatterjee, R., Hwang, D. R., Huang, Y., Simpson, N., Ngo, K., Van Heertum, R., Laruelle, M. (2001). 'Imaging Human Mesolimbic Dopamine Transmission with Positron Emission Tomography: I. Accuracy and Precision of D(2) Receptor Parameter Measurements in Ventral Striatum', *J Cereb Blood Flow Metab*. **21**(9): pp. 1034-57

Maziere, M., Comar, D., Marazano, C., Berger, G. (1976). 'Nicotine-11C: Synthesis and Distribution Kinetics in Animals', *Eur J Nucl Med*. **1**(4): pp. 255-8

Mazzanti, C.M., Lappalainen, J., Long, J. C., Bengel, D., Naukkarinen, H., Eggert, M., Virkkunen, M., Linnoila, M., Goldman, D. (1998). 'Role of the Serotonin Transporter Promoter Polymorphism in Anxiety-Related Traits', *Arch Gen Psychiatry*, **55**(10): pp. 936-40

McCarthy, G., Luby, M., Gore, J., Goldman-Rakic, P. (1997). 'Infrequent Events Transiently Activate Human Prefrontal and Parietal Cortex as Measured by Functional MRI', *J Neurophysiol.* **77**(3): pp. 1630-4

McClernon, F.J. (2005). 'Effect Sizes for Smoking and Neutral Pictorial Cues in the Ventral Striatum Using fMRI', [from private correspondence]

McClernon, F.J., Gilbert, D. G. (2004). 'Human Functional Neuroimaging in Nicotine and Tobacco Research: Basics, Background, and Beyond', *Nicotine Tob Res.* **6**(6): pp 941-59

McClernon, F.J., Hiott, F. B., Huettel, S. A., Rose, J. E. (2005). 'Abstinence-Induced Changes in Self-Report Craving Correlate with Event-Related fMRI Responses to Smoking Cues', *Neuropsychopharmacology* (manuscript in press)

McGehee, D.S., Heath, M. J., Gelber, S., Devay, P., Role, L. W. (1995). 'Nicotine Enhancement of Fast Excitatory Synaptic Transmission in CNS by Presynaptic Receptors', *Science*, **269**(5231): pp. 1692-6

McGehee, D.S., Role, L. W. (1995). 'Physiological Diversity of Nicotinic Acetylcholine Receptors Expressed by Vertebrate Neurons', *Annu Rev Physiol.* **57**: pp. 521-46

McNair, D.M., Loor, J., Droppelman, L.F. (1971). *Profile of Mood States* (San Diego, CA: Educational Testing Service)

Mendelson, J.H., Sholar, M. B., Goletiani, N., Siegel, A. J., Mello, N. K. (2005). 'Effects of Low- and High-Nicotine Cigarette Smoking on Mood States and the HPA Axis in Men', *Neuropsychopharmacology* (manuscript in press)

Mifsud, J.C., Hernandez, L., Hoebel, B. G. (1989). 'Nicotine Infused into the Nucleus Accumbens Increases Synaptic Dopamine as Measured by *In Vivo* Microdialysis', *Brain Res.* **478**(2): pp. 365-7

Mihailescu, S., Guzman-Marin, R., Drucker-Colin, R. (2001). 'Nicotine Stimulation of Dorsal Raphe Neurons: Effects on Laterodorsal and Pedunculopontine Neurons', *Eur Neuropsychopharmacol*, **11**(5): pp. 359-66

Mortensen, O. V., Thomassen, M., Larsen, M. B., Whittemore, S. R., Wiborg, O. (1999). 'Functional Analysis of a Novel Human Serotonin Transporter Gene Promoter in Immortalized Raphe Cells', *Brain Res Mol Brain Res.* **68**(1-2): pp.141-8

Mossner, R., Henneberg, A., Schmitt, A., Syagailo, Y. V., Grassle, M., Hennig, T., Simantov, R., Gerlach, M., Riederer, P., Lesch, K. P. (2001). 'Allelic Variation of Serotonin Transporter Expression is Associated with Depression in Parkinson's Disease', *Mol Psychiatry*, **6**(3): pp. 350-2.

Munafo', M. (2004). 'Replication Validity of Genetic Association Studies of Smoking Behavior: What Can Meta-Analytic Techniques Offer?' *Nicotine Tob Res.* **6**(2): pp. 381-2

Munafo', M., Bradburn, M., Bowes, L., David, S. (2004). 'Are There Sex Differences in Transdermal Nicotine Replacement Therapy Patch Efficacy? A meta-analysis', *Nicotine Tob Res.* **6**(5): pp. 769-76

Munafo', M., Clark, T., Johnstone, E., Murphy, M., Walton, R. (2004). 'The Genetic Basis for Smoking Behavior: A Systematic Review and Meta-Analysis', *Nicotine Tob Res*. **6**(4): pp. 583-97

Munafo', M.R., Clark, T. G., Moore, L. R., Payne, E., Walton, R., Flint, J. (2003). 'Genetic Polymorphisms and Personality in Healthy Adults: A Systematic Review and Meta-Analysis', *Mol Psychiatry*, **8**(5): pp. 471-84

Munafo', M.R., Clark, T.G., Murphy, M., Walton, R.T. (2004). 'The Genetic Basis for Smoking Behavior: A Systematic Review and Meta-Analysis', *Nicotine Tob Res*. **6**(4): pp. 583-598

Murphy, P.J. (1973). 'Enzymatic Oxidation of Nicotine to Nicotine 1'(5') Iminium Ion. A Newly Discovered Intermediate in the Metabolism of Nicotine', *J Biol Chem*. **248**(8): pp. 2796-800

Murphy, D.L., Lerner, A., Rudnick, G., Lesch, K. P. (2004). 'Serotonin Transporter: Gene, Genetic Disorders, and Pharmacogenetics', *Mol Interv*. **4**(2): pp. 109-23

Nader, K., van der Kooy, D. (1997). 'Deprivation State Switches the Neurobiological Substrates Mediating Opiate Reward in the Ventral Tegmental Area', *J Neurosci*. **17**(1): pp. 383-90

Nakajima, M., Yamamoto, T., Nunoya, K., Yokoi, T., Nagashima, K., Inoue, K., Funae, Y., Shimada, N., Kamataki, T., Kuroiwa, Y. (1996). 'Characterization of CYP2A6 Involved in 3'-Hydroxylation of Cotinine in Human Liver Microsomes', *J Pharmacol Exp Ther*. **277**(2): pp. 1010-5

Nakajima, M., Yamamoto, T., Nunoya, K., Yokoi, T., Nagashima, K., Inoue, K., Funae, Y., Shimada, N., Kamataki, T., Kuroiwa, Y. (1996). 'Role of Human Cytochrome P4502A6 in C-Oxidation of Nicotine', *Drug Metab Dispos*. **24**(11): pp. 1212-7

Nakhai, B., Nielsen, D. A., Linnoila, M., Goldman, D. (1995). 'Two Naturally Occurring Amino Acid Substitutions in the Human 5-HT1A Receptor: Glycine 22 to Serine 22 and Isoleucine 28 to Valine 28', *Biochem Biophys Res Commun*. **210**(2): pp. 530-6

Nayak, S.V., Ronde, P., Spier, A. D., Lummis, S. C., Nichols, R. A. (2000). 'Nicotinic Receptors Co-Localize with 5-HT(3) Serotonin Receptors on Striatal Nerve Terminals', *Neuropharmacology*, **39**(13): pp. 2681-90

Nesbitt, P.D. (1973). 'Smoking, Physiological Arousal, and Emotional Response', *J Pers Soc Psychol*. **25**(1): pp. 137-44

Neumeister, A., Bain, E., Nugent, A. C., Carson, R. E., Bonne, O., Luckenbaugh, D. A., Eckelman, W., Herscovitch, P., Charney, D. S., Drevets, W. C. (2004). 'Reduced Serotonin Type 1A Receptor Binding in Panic Disorder', *J Neurosci*. **24**(3): pp. 589-91

Niaura, R., Abrams, D. B., Monti, P. M., Pedraza, M. (1989). 'Reactivity to High Risk Situations and Smoking Cessation Outcome', *J Subst Abuse*, **1**(4): pp. 393-405

Niaura, R., Shadel, W. G., Abrams, D. B., Monti, P. M., Rohsenow, D. J., Sirota, A. (1998). 'Individual Differences in Cue Reactivity Among Smokers Trying to Quit: Effects of Gender and Cue Type', *Addict Behav*. **23**(2): pp. 209-24

Niaura, R., Shadel, W. G., Goldstein, M. G., Hutchinson, K. E., Abrams, D. B. (2001). 'Individual Differences in Responses to the First Cigarette Following Overnight Abstinence in Regular Smokers', *Nicotine Tob Res*. **3**(1): pp. 37-44

Niaura, R., Spring, B., Borrelli, B., Hedeker, D., Goldstein, M. G., Keuthen, N., DePue, J., Kristeller, J., Ockene, J., Prochazka, A., Chiles, J. A., Abrams, D. B. (2002)

'Multicenter Trial of Fluoxetine as an Adjunct to Behavioral Smoking Cessation Treatment', *J Consult Clin Psychol.* **70**(4): pp. 887-96

Niaura, R.S., Rohsenow, D. J., Binkoff, J. A., Monti, P. M., Pedraza, M., Abrams, D. B. (1988). 'Relevance of Cue Reactivity to Understanding Alcohol and Smoking Relapse', *J Abnorm Psychol.* **97**(2): pp. 133-52

Nirenberg, M.J., Chan, J., Pohorille, A., Vaughan, R.A., Uhl, G.R., Kuhar, M.J., Pickel, V.M. (1997). 'The Dopamine Transporter: Comparitive Ultrastructure of Dopaminergic Axons in Limbic and Motor Compartments of the Nucleus Accumbens', *J Neurosci.* **17**(18):6899-907

Nisell, M., Nomikos, G. G., Svensson, T. H. (1994). 'Systemic Nicotine-Induced Dopamine Release in the Rat Nucleus Accumbens is Regulated by Nicotinic Receptors in the Ventral Tegmental Area', *Synapse.* **16**(1): pp. 36-44

Nishiguchi, N., Shirakawa, O., Ono, H., Nishimura, A., Nushida, H., Ueno, Y., Maeda, K. (2002). 'Lack of an Association Between 5-HT1A Receptor Gene Structural Polymorphisms and Suicide Victims', *Am J Med Genet.* **114**(4): pp. 423-5

Noble, E.P., St Jeor, S. T., Ritchie, T., Syndulko, K., St Jeor, S. C., Fitch, R. J., Brunner, R. L., Sparkes, R. S. (1994). 'D2 Dopamine Receptor Gene and Cigarette Smoking: A Reward Gene?' *Med Hypotheses.* **42**(4): pp. 257-60

North, R.A., Uchimura, N. (1989). '5-Hydroxytryptamine Acts at 5-HT2 Receptors to Decrease Potassium Conductance in Rat Nucleus Accumbens Neurones', *J Physiol.* **417**: pp. 1-12

Nwosu, C.G., Crooks, P. A. (1988). 'Species Variation and Stereoselectivity in the Metabolism of Nicotine Enantiomers', *Xenobiotica*, **18**(12): pp. 1361-72

Ochoa, E.L., Chattopadhyay, A., McNamee, M. G. (1989). 'Desensitization of the Nicotinic Acetylcholine Receptor: Molecular Mechanisms and Effect of Modulators', *Cell Mol Neurobiol.* **9**(2): pp. 141-78

O'Doherty, J., Kringlebach, M.L., Rolls, E.T., Hornak, J., Andrews, C. (2001). 'Abstract Reward and Punishment Representations in the Human Orbitofrontal Cortex', *Nat Neurosci.* **4**(1): pp. 95-102

O'Doherty, J., Rolls, E.T., Francis, S., Bowtell, R., McGlone, F., Kobal, G., Renner, B., Ahne, G. (2000). 'Sensory-Specific Satiety-Related Olfactory Activation of the Human Orbitofrontal Cortex', *Neuroreport*, **11**(4): pp. 893-7

Ojemann, J.G., Akbudak, E., Snyder, A. Z., McKinstry, R. C., Raichle, M. E., Conturo, T. E. (1997). 'Anatomic Localization and Quantitative Analysis of Gradient Refocused Echo-Planar fMRI Susceptibility Artifacts', *Neuroimage*, **6**(3): pp. 156-67

Olausson, P., Akesson, P., Engel, J. A., Soderpalm, B. (2001). 'Effects of 5-HT1A and 5-HT2 Receptor Agonists on the Behavioral and Neurochemical Consequences of Repeated Nicotine Treatment', *Eur J Pharmacol.* **420**(1): pp. 45-54

Olausson, P., Akesson, P., Petersson, A., Engel, J. A., Soderpalm, B. (2001). 'Behavioral and Neurochemical Consequences of Repeated Nicotine Treatment in the Serotonin-Depleted Rat', *Psychopharmacology* (Berl), **155**(4): pp. 348-61

Olausson, P., Engel, J. A., Soderpalm, B. (1999). 'Behavioral Sensitization to Nicotine is Associated with Behavioral Disinhibition; Counteraction by Citalopram', *Psychopharmacology* (Berl), **142**(2): pp. 111-9

Olausson, P., Engel, J. A., Soderpalm, B. (2002). 'Involvement of Serotonin in Nicotine Dependence: Processes Relevant to Positive and Negative Regulation of Drug Intake', *Pharmacol Biochem Behav.* **71**(4): pp. 757-71

Olincy, A., Johnson, L. L., Ross, R. G. (2003). 'Differential Effects of Cigarette Smoking on Performance of a Smooth Pursuit and a Saccadic Eye Movement Task in Schizophrenia', *Psychiatry Res.* **117**(3): pp. 223-36

Olincy, A., Ross, R. G., Young, D. A., Roath, M., Freedman, R. (1998). 'Improvement in Smooth Pursuit Eye Movements after Cigarette Smoking in Schizophrenic Patients', *Neuropsychopharmacology*, **18**(3): pp. 175-85

Opitz, K., Weischer, M. L. (1988). 'Volitional Oral Intake of Nicotine in Tupaias: Drug-Induced Alterations', *Drug Alcohol Depend*, **21**(2): pp. 99-104

Oscarson, M., McLellan, R. A., Asp, V., Ledesma, M. and M.L. Ruiz, Sinues, B., Rautio, A., Ingelman-Sundberg, M. (2002). 'Characterization of a Novel CYP2A7/CYP2A6 Hybrid Allele (CYP2A6*12) that Causes Reduced CYP2A6 Activity', *Hum Mutat.* **20**(4): pp. 275-83

Ouagazzal, A.M., Kenny, P. J., File, S. E. (1999). 'Modulation of Behaviour on Trials 1 and 2 in the Elevated Plus-Maze Test of Anxiety after Systemic and Hippocampal Administration of Nicotine', *Psychopharmacology* (Berl), **144**(1): pp. 54-60

Parks, C.L., Shenk, T. (1996). 'The Serotonin 1a Receptor Gene Contains a TATA-less Promoter that Responds to MAZ and Sp1', *J Biol Chem.* **271**(8): pp. 4417-30

Parsey, R.V., Oquendo, M. A., Simpson, N. R., Ogden, R. T., Van Heertum, R., Arango, V., Mann, J. J. (2002). 'Effects of Sex, Age, and Aggressive Traits in Man on Brain Serotonin 5-HT1A Receptor Binding Potential Measured by PET Using [C-11]WAY-100635', *Brain Res.* **954**(2): pp. 173-82

Perkins, K.A., Fonte, C., Meeker, J., White, W., Wilson, A. (2001). 'The Discriminative Stimulus and Reinforcing Effects of Nicotine in Humans Following Nicotine Pretreatment', *Behav Pharmacol.* **12**(1): pp. 35-44

Perkins, K.A., Gerlach, D., Broge, M., Grobe, J. E., Wilson, A. (2000). 'Greater Sensitivity to Subjective Effects of Nicotine in Nonsmokers High in Sensation Seeking', *Exp Clin Psychopharmacol.* **8**(4): pp. 462-71

Perkins, K.A., Jetton, C., Stolinski, A., Fonte, C., Conklin, C. A. (2003). 'The Consistency of Acute Responses to Nicotine in Humans', *Nicotine Tob Res.* **5**(6): pp. 877-84

Peto, R., Darby, S., Deo, H., Silcocks, P., Whitley, E. and Doll, R. (2000). 'Smoking, Smoking Cessation, and Lung Cancer in the UK since 1950: Combination of National Statistics with Two Case-Control Studies', *BMJ.* **321**(7257): pp. 323-9

Peto, R., Lopez, A. D., Boreham, J., Thun, M., Heath, C., Jr. (1992). 'Mortality from Tobacco in Developed Countries: Indirect Estimation from National Vital Statistics', *Lancet*, **339**(8804): pp. 1268-78

Peto, R., Lopez, A. D., Boreham, J., Thun, M., Heath, C., Jr., Doll, R. (1996). 'Mortality from Smoking Worldwide', *Br Med Bull.* **52**(1): pp. 12-21

Pezawas, L., Meyer-Lindenberg, A., Drabant, E. M., Verchinski, B. A., Munoz, K. E., Kolachana, B. S., Egan, M. F., Mattay, V. S., Hariri, A. R., Weinberger, D. R. (2005). '5-HTTLPR Polymorphism Impacts Human Cingulate-Amygdala Interactions: A Genetic Susceptibility Mechanism for Depression', *Nat Neurosci.* **8**(6): pp. 828-34

Phillips, P.E., Stuber, G. D., Heien, M. L., Wightman, R. M., Carelli, R. M. (2003). 'Subsecond Dopamine Release Promotes Cocaine Seeking', *Nature*, **422**(6932): pp. 614-8

Pianezza, M.L, Sellers, E. M, Tyndale, R.F. (1998). 'A Common Genetic Defect in Nicotine Metabolism Defect Reduces Smoking', *Nature* **393**(6687): p. 750

Piazza, P.V., Deminiere, J. M., Le Moal, M., Simon, H. (1989). 'Factors that Predict Individual Vulnerability to Amphetamine Self-Administration', *Science*, **245**(4925): pp. 1511-3

Piazza, P.V., Deminiere, J. M., Le Moal, M., Simon, H. (1990). 'Stress- and Pharmacologically-Induced Behavioral Sensitization Increases Vulnerability to Acquisition of Amphetamine Self-Administration', *Brain Res.* **514**(1): pp. 22-6

Picazo, O., Lopez-Rubalcava, C., Fernandez-Guasti, A. (1995). 'Anxiolytic Effect of the 5-HT1A Compounds 8-Hydroxy-2-(di-n-propylamino) Tetralin and Ipsapirone in the Social Interaction Paradigm: Evidence of a Presynaptic Action', *Brain Res Bull.* **37**(2): pp. 169-75

Picciotto, M.R., Zoli, M., Rimondini, R., Lena, C., Marubio, L. M., Pich, E. M., Fuxe, K., Changeux, J. P. (1998). 'Acetylcholine Receptors Containing the Beta2 Subunit Are Involved in the Reinforcing Properties of Nicotine', *Nature*, **391**(6663): pp. 173-7

Pidoplichko, V.I., DeBiasi, M., Williams, J. T., Dani, J. A. (1997). 'Nicotine Activates and Desensitizes Midbrain Dopamine Neurons', *Nature*, **390**(6658): pp. 401-4

Pierre, P.J., Vezina, P. (1998). 'D1 Dopamine Receptor Blockade Prevents the Facilitation of Amphetamine Self-Administration Induced by Prior Exposure to the Drug', *Psychopharmacology* (Berl), **138**(2): pp. 159-66

Pierre, P.J., Vezina, P. (1997). 'Predisposition to Self-Administer Amphetamine: The Contribution of Response to Novelty and Prior Exposure to the Drug', *Psychopharmacology* (Berl), **129**(3): pp. 277-84

Pike, V.W., McCarron, J. A., Lammerstma, A. A., Hume, S. P., Poole, K., Grasby, P. M., Malizia, A., Cliffe, I. A., Fletcher, A., Benclı, C. J. (1995). 'First Delineation of 5-HT1A Receptors in Human Brain with PET and [11C]WAY-100635', *Eur J Pharmacol*, **283**(1-3): pp. R1-3

Pineyro, G., Blier, P. (1999). 'Autoregulation of Serotonin Neurons: Role in Antidepressant Drug Action', *Pharmacol Rev.* **51**(3): pp. 533-91

Pomerleau, O.F. (1995). 'Individual Differences in Sensitivity to Nicotine: Implications for Genetic Research on Nicotine Dependence', *Behav Genet.* **25**(2): pp 161-77

Pool, W.F., Godin, C.S., Crooks, P.A. (1985). 'Nicotine Racemization During Cigarette Smoking (Abstract)', *Toxicologist*, **5**: p. 232

Porrino, L.J., Ritz, M. C., Goodman, N. L., Sharpe, L. G., Kuhar, M. J., Goldberg, S. R. (1989). 'Differential Effects of the Pharmacological Manipulation of Serotonin Systems on Cocaine and Amphetamine Self-Administration in Rats', *Life Sci.* **45**(17): pp. 1529-35

Prochaska, J.O., DiClemente, C. C. (1983). 'Stages and Processes of Self-Change of Smoking: Toward an Integrative Model of Change', *J Consult Clin Psychol.* **51**(3): pp. 390-5

Prokhorov, A.V., et al. (1996). 'Measuring Nicotine Dependence among High-Risk Adolescent Smokers', *Addict Behav.* **21**(1): pp. 117-27

Quick, M.W., Lester, R. A. (2002). 'Desensitization of Neuronal Nicotinic Receptors', *J Neurobiol.* **53**(4): pp. 457-78

Raap, D.K., Evans, S., Garcia, F., Li, Q., Muma, N. A., Wolf, W. A., Battaglia, G., Van De Kar, L. D. (1999). 'Daily Injections of Fluoxetine Iinduce Dose-Dependent Desensitization of Hypothalamic 5-HT1A Receptors: Reductions in Neuroendocrine Responses to 8-OH-DPAT and in Levels of Gz and Gi Proteins', *J Pharmacol Exp Ther.* **288**(1): pp. 98-106

Rabiner, E.A., Messa, C., Sargent, P. A., Husted-Kjaer, K., Montgomery, A., Lawrence, A. D., Bench, C. J., Gunn, R. N., Cowen, P., Grasby, P. M. (2002). 'A Database of [(11)C]WAY-100635 Binding to 5-HT(1A) Receptors in Normal Male Volunteers: Normative Data and Relationship to Methodological, Demographic, Physiological, and Behavioral Variables', Neuroimage, **15**(3): pp. 620-32

Rabiner, E.A., Wilkins, M. R., Turkheimer, F., Gunn, R. N., de Haes, J. U., de Vries, M., Grasby, P. M. (2002). '5-Hydroxytryptamine1A Receptor Occupancy by Novel Full Antagonist 2-[4-[4-(7-chloro-2,3-dihydro-1,4-benzdioxyn-5-yl)-1-piperazinyl]butyl]-1, 2-benzisothiazol-3-(2H)-one-1,1-dioxide: a[11C][O-methyl-3H]-N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2-py ridinyl)cyclohexanecarboxamide trihydrochloride (WAY-100635) Positron Emission Tomography Study in Humans', *J Pharmacol Exp Ther.* **301**(3): pp. 1144-50

Rada, P.V., Mark, G. P., Hoebel, B. G. (1993). '*In Vivo* Modulation of Acetylcholine in the Nucleus Accumbens of Freely Moving Rats: I. Inhibition by Rerotonin', *Brain Res.* **619**(1-2): pp. 98-104

Rang, H.P., Dale, M.M., Ritter, J.M., Moore, P.K. (ed.) (2003). *Pharmacology*, 5th edn. (London: Churchill Livingstone)

Rasmussen, K., Calligaro, D. O., Czachura, J. F., Dreshfield-Ahmad, L. J., Evans, D. C., Hemrick-Luecke, S. K., Kallman, M. J., Kendrick, W. T., Leander, J. D., Nelson, D. L., Overshiner, C. D., Wainscott, D. B., Wolff, M. C., Wong, D. T., Branchek, T. A., Zgombick, J. M., Xu, Y. C. (2000). 'The Novel 5-Hydroxytryptamine(1A) Antagonist LY426965: Effects on Nicotine Withdrawal and Interactions with Fluoxetine', *J Pharmacol Exp Ther.* **294**(2): pp. 688-700

Rasmussen, K., Kallman, M. J., Helton, D. R. (1997). 'Serotonin-1A Antagonists Attenuate the Effects of Nicotine Withdrawal on the Auditory Startle Response', *Synapse*, **27**(2): pp. 145-52

Reavill, C., Stolerman, I. P. (1990). 'Locomotor Activity in Rats after Administration of Nicotinic Agonists Intracerebrally', *Br J Pharmacol.* **99**(2): pp. 273-8

Reuben, M., Clarke, P. B. (2000). 'Nicotine-Evoked [3H]5-Hydroxytryptamine Release from Rat Striatal Synaptosomes', *Neuropharmacology*, **39**(2): pp. 290-9

Rice, V.H., Stead, L. F. (2001). 'Nursing Interventions for Smoking Cessation'. Cochrane Database Syst Rev. (3): p. CD001188

Riederer, P., Konradi, C., Schay, V., Kienzl, E., Birkmayer, G., Danielczyk, W., Sofic, E., Youdim, M. B. (1987). 'Localization of MAO-A and MAO-B in Human Brain: A Step in Understanding the Therapeutic Action of L-Deprenyl', *Adv Neurol.* **45**: pp. 111-8

Rivera, A., Cuellar, B., Giron, F. J.,Grandy, D. K., de la Calle, A., Moratalla, R. (2002). 'Dopamine D4 Receptors are Heterogeneously Distributed in the Striosomes/Matrix Compartments of the Striatum', *J Neurochem.* **80**(2): pp. 219-29

Roberts, D.C., Loh, E. A., Baker, G. B., Vickers, G. (1994). 'Lesions of Central Serotonin Systems Affect Responding on a Progressive Ratio Schedule Reinforced Either by Intravenous Cocaine or by Food', *Pharmacol Biochem Behav.* **49**(1): pp. 177-82

Robinson, M.D., Pettice, Y. L., Smith, W. A., Cederstrom, E. A., Sutherland, D. E., Davis, H. (1992). 'Buspirone Effect on Tobacco Withdrawal Symptoms: A Randomized Placebo-Controlled Trial', *J Am Board Fam Pract.* **5**(1): pp. 1-9

Robinson, T.E., Berridge, K. C. (2001). 'Incentive-Sensitization and Addiction', *Addiction*, **96**(1): pp. 103-14

Robinson, T.E., Berridge, K. C. (1993). 'The Neural Basis of Drug Craving: An Incentive-Sensitization Theory of Addiction', *Brain Res Brain Res Rev.* **18**(3): pp. 247-91

Rogers, R.D., Ramnani, N., Mackay, C., Wilson, J. L., Jezzard, P., Carter, C. S., Smith, S. M. (2004). 'Distinct Portions of Anterior Cingulate Cortex and Medial Prefrontal Cortex Are Activated by Reward Processing in Separable Phases of Decision-Making Cognition', *Biol Psychiatry*, **55**(6): pp. 594-602

Rohsenow, D.J., Niaura, R. S., Childress, A. R., Abrams, D. B., Monti, P. M. (1990). 'Cue Reactivity in Addictive Behaviors: Theoretical and Treatment Implications', *Int J Addict*, **25**(7A-8A): pp. 957-93

Role, L.W., Berg, D. K. (1996) 'Nicotinic Receptors in the Development and Modulation of CNS Synapses', *Neuron.* **16**(6): pp. 1077-85

Rolls, E.T. (2004). 'Convergence of Sensory Systems in the Orbitofrontal Cortex in Primates and Brain Design for Emotion', *Anat Rec A Discov Mol Cell Evol Biol.* **281**(1): pp. 1212-25

Rolls, E.T. (2000). 'The Orbitofrontal Cortex and Reward', *Cereb Cortex*, **10**(3): pp. 284-94

Rothe, C., Gutknecht, L., Freitag, C., Tauber, R., Mossner, R., Franke, P., Fritze, J., Wagner, G., Peikert, G., Wenda, B., Sand, P., Jacob, C., Rietschel, M., Nothen, M. M., Garritsen, H., Fimmers, R., Deckert, J., Lesch, K. P. (2004). 'Association of a Functional 1019C>G 5-HT1A Receptor Gene Polymorphism with Panic Disorder with Agoraphobia', *Int J Neuropsychopharmacol*, **7**(2): pp. 189-92

Rueter, L.E., Blier, P. (1999). 'Electrophysiological Examination of the Effects of Sustained Flibanserin Administration on Serotonin Receptors in Rat Brain', *Br J Pharmacol.* **126**(3): pp. 627-38

Ryan, R.E., Loiacono, R. E. (2001). 'Nicotine Regulates Alpha7 Nicotinic Receptor Subunit mRNA: Implications for Nicotine Dependence', *Neuroreport*, **12**(3): pp. 569-72

Saccone, N.L., Neuman, R. J., Saccone, S. F., Rice, J. P. (2003). 'Genetic Analysis of Maximum Cigarette-Use Phenotypes', *BMC Genet.* **4 Suppl 1**: p. S105

Saito, D.N., Okada, T., Morita, Y., Yonekura, Y., Sadato, N. (2003). 'Tactile-Visual Cross-Modal Shape Matching: A Functional MRI Study', *Brain Res Cogn Brain Res.* **17**(1): pp. 14-25

Salamone, F.N., Zhou, M., Auerbach, A. (1999). 'A Re-Examination of Adult Mouse Nicotinic Acetylcholine Receptor Channel Activation Kinetics', *J Physiol.* **516 (Pt 2)**: pp. 315-30

Sammut, S., Dec, A., Mitchell, D., Linardakis, J., Ortiguela, M., West, A. R. (2005). 'Phasic Dopaminergic Transmission Increases NO Efflux in the Rat Dorsal Striatum via a Neuronal NOS and a Dopamine D(1/5) Receptor-Dependent Mechanism', *Neuropsychopharmacology*. (electronic publication in press)

Sargent, P.A., Kjaer, K. H., Bench, C. J., Rabiner, E. A., Messa, C., Meyer, J., Gunn, R. N., Grasby, P. M., Cowen, P. J. (2000). 'Brain Serotonin1A Receptor Binding Measured by Positron Emission Tomography with [11C]WAY-100635: Effects of Depression and Antidepressant Treatment', *Arch Gen Psychiatry*, **57**(2): pp. 174-80

Sato, S., Nishimura, K., Koyama, H.Tsukino, M., Oga, T., Hajiro, T., Mishima, M. (2003). 'Optimal Cutoff Level of Breath Carbon Monoxide for Assessing Smoking Status in Patients with Asthma and COPD', *Chest*, **124**(5): pp. 1749-54

Sayette, M.A., Martin, C. S., Wertz, J. M., Shiffman, S., Perrott, M. A. (2001). 'A Multi-Dimensional Analysis of Cue-Elicited Craving in Heavy Smokers and Tobacco Chippers', *Addiction*, **96**(10): pp. 1419-32

Sayette, M.A., Shiffman, S., Tiffany, S. T., Niaura, R. S., Martin, C. S., Shadel, W. G. (2000). 'The Measurement of Drug Craving', *Addiction*, **95 Suppl 2**: pp. S189-210

Schilstrom, B., Svensson, H. M., Svensson, T. H., Nomikos, G. G. (1998). 'Nicotine and Food Induced Dopamine Release in the Nucleus Accumbens of the Rat: Putative Role of Alpha7 Nicotinic Receptors in the Ventral Tegmental Area', *Neuroscience*, **85**(4): pp. 1005-9

Schneider, L.H., Murphy, R. B., Coons, E. E. (1982). 'Lateralization of Striatal Dopamine (D2) Receptors in Normal Rats', *Neurosci Lett*. **33**(3): pp. 281-4

Schoedel, K.A., Sellers, E. M., Palmour, R., Tyndale, R. F. (2003). 'Down-Regulation of Hepatic Nicotine Metabolism and a CYP2A6-Like Enzyme in African Green Monkeys after Long-Term Nicotine Administration', *Mol Pharmacol*. **63**(1): pp. 96-104

Schuldiner, S., Steiner-Mordoch, S., Yelin, R., Wall, S. C., Rudnick, G. (1993). 'Amphetamine Derivatives Interact with Both Plasma Membrane and Secretory Vesicle Biogenic Amine Transporters', *Mol Pharmacol*. **44**(6): pp. 1227-31

Seaton, M.J., Vesell, E. S., Luo, H., Hawes, E. M. (1993). 'Identification of Radiolabeled Metabolites of Nicotine in Rat Bile. Synthesis of S-(-)-Nicotine N-Glucuronide and Direct Separation of Nicotine-Derived Conjugates Using High-Performance Liquid Chromatography', *J Chromatogr*. **621**(1): pp. 49-53

Secker-Walker, R.H., Gnich, W., Platt, S., Lancaster, T. (2002). 'Community Interventions for Reducing Smoking among Adults'. Cochrane Database Syst Rev. (3): p. CD001745

Sen, S., Burmeister, M., Ghosh, D. (2004). 'Meta-Analysis of the Association Between a Serotonin Transporter Promoter Polymorphism (5-HTTLPR) and Anxiety-Related Personality Traits', *Am J Med Genet B Neuropsychiatr Genet*. **127**(1): pp. 85-9

Serova, L., Sabban, E. L. (2002). 'Involvement of Alpha 7 Nicotinic Acetylcholine Receptors in Gene Expression of Dopamine Biosynthetic Enzymes in Rat Brain', *J Pharmacol Exp Ther*. **303**(3): pp. 896-903

Serretti, A., Artioli, P., Lorenzi, C., Pirovano, A., Tubazio, V., Zanardi, R. (2004). 'The C(-1019)G Polymorphism of the 5-HT1A Gene Promoter and Antidepressant Response in Mood Disorders: Preliminary Findings', *Int J Neuropsychopharmacol*, 7(4): pp. 453-60

Sesack, S.R., Pickel, V. M. (1992). 'Prefrontal Cortical Efferents in the Rat Synapse on Unlabeled Neuronal Targets of Catecholamine Terminals in the Nucleus Accumbens Septi and on Dopamine Neurons in the Ventral Tegmental Area', *J Comp Neurol.* **320**(2): pp. 145-60

Seth, P., Cheeta, S., Tucci, S., File, S. E. (2002). 'Nicotinic--Serotonergic Interactions in Brain and Behaviour', *Pharmacol Biochem Behav.* **71**(4): pp. 795-805

Sharp, B.M., Beyer, H. S. (1986). 'Rapid Desensitization of the Acute Stimulatory Effects of Nicotine on Rat Plasma Adrenocorticotropin and Prolactin', *J Pharmacol Exp Ther.* **238**(2): pp. 486-91

Shenker, A., Maayani, S., Weinstein, H., Green, J. P. (1983). 'Enhanced Serotonin-Stimulated Adenylate Cyclase Activity in Membranes from Adult Guinea Pig Hippocampus', *Life Sci.* **32**(20): pp. 2335-42

Sherr, J.D., Myers, C., Avila, M. T., Elliott, A., Blaxton, T. A., Thaker, G. K. (2002). 'The Effects of Nicotine on Specific Eye Tracking Measures in Schizophrenia', *Biol Psychiatry*, **52**(7): pp. 721-8

Shiffman, S.M., Jarvik, M. E. (1976). 'Smoking Withdrawal Symptoms in Two Weeks of Abstinence', *Psychopharmacology* (Berl), **50**(1): pp. 35-9

Shiffman, S., West, R., Gilbert, D. (2004). 'Recommendation for the Assessment of Tobacco Craving and Withdrawal in Smoking Cessation Trials', *Nicotine Tob Res.* **6**(4): pp. 599-614

Shim, I., Javaid, J. I., Wirtshafter, D., Jang, S. Y., Shin, K. H., Lee, H. J., Chung, Y. C., Chun, B. G. (2001). 'Nicotine-Induced Behavioral Sensitization is Associated with Extracellular Dopamine Release and Expression of c-Fos in the Striatum and Nucleus Accumbens of the Rat', *Behav Brain Res.* **121**(1-2): pp. 137-47

Shippenberg, T.S., Heidbreder, C. (1995). 'Sensitization to the Conditioned Rewarding Effects of Cocaine: Pharmacological and Temporal Characteristics', *J Pharmacol Exp Ther.* **273**(2): pp. 808-15

Shippenberg, T.S., Heidbreder, C., Lefevour, A. (1996). 'Sensitization to the Conditioned Rewarding Effects of Morphine: Pharmacology and Temporal Characteristics', *Eur J Pharmacol.* **299**(1-3): pp. 33-9

Shippenberg, T.S., LeFevour, A., Heidbreder, C. (1996). 'Kappa-Opioid Receptor Agonists Prevent Sensitization to the Conditioned Rewarding Effects of Cocaine', *J Pharmacol Exp Ther.* **276**(2): pp. 545-54

Shoaib, M., Baumann, M. H., Rothman, R. B., Goldberg, S. R., Schindler, C. W. (1997). 'Behavioural and Neurochemical Characteristics of Phentermine and Fenfluramine Administered Separately and as a Mixture in Rats', *Psychopharmacology* (Berl), **131**(3): pp. 296-306

Silagy, C., Lancaster, T., Stead, L., Mant, D., Fowler, G. (2004). 'Nicotine Replacement Therapy for Smoking Cessation'. Cochrane Database Syst Rev. (3): p. CD000146

Simon, J.A., Duncan, C., Carmody, T.P., Hudes, E.S. (2002). 'Bupropion for Smoking Cessation: A randomized Trial', paper presented at the National Conference on Tobacco or Health in San Francisco, CA, U.S.A.

Simpson, M.D., Lubman, D. I., Slater, P., Deakin, J. F. (1996). 'Autoradiography with [3H]8-OH-DPAT Reveals Increases in 5-HT(1A) Receptors in Ventral Prefrontal Cortex in Schizophrenia', *Biol Psychiatry*, **39**(11): pp. 919-28

Smith, G.S., Dewey, S. L., Brodie, J. D., Logan, J., Vitkun, S. A., Simkowitz, P., Schloesser, R., Alexoff, D. A., Hurley, A., Cooper, T., Volkow, N. D. (1997). 'Serotonergic Modulation of Dopamine Measured with [11C]raclopride and PET in Normal Human Subjects', *Am J Psychiatry*, **154**(4): pp. 490-6

Smith, S.M. (2002). 'Fast Robust Automated Brain Extraction', *Hum Brain Mapp*. **17**(3): pp. 143-55

Smits, K.M., Smits, L. J., Schouten, J. S., Stelma, F. F., Nelemans, P., Prins, M. H. (2004). 'Influence of SERTPR and STin2 in the Serotonin Transporter Gene on the Effect of Selective Serotonin Reuptake Inhibitors in Depression: A Systematic Review', *Mol Psychiatry*, **9**(5): pp. 433-41

Sprouse, J.S., Aghajanian, G. K. (1987). 'Electrophysiological Responses of Serotoninergic Dorsal Raphe Neurons to 5-HT1A and 5-HT1B Agonists', *Synapse*, **1**(1): pp. 3-9

Stalhandske, T. (1970). 'Effects of Increased Liver Metabolism of Nicotine on its Uptake, Elimination and Toxicity in Mice', *Acta Physiol Scand.* **80**(2): pp. 222-34

Stalke, J., Hader, O., Bahr, V., Hensen, J., Scherer, G., Oelkers, W. (1992). 'The Role of Vasopressin in the Nicotine-Induced Stimulation of ACTH and Cortisol in Men', *Clin Investig*. **70**(3-4): pp. 218-23

Stapleton, J.A., Sutherland, G. (2005). 'Effective NHS Smoking Cessation Services' <http://bmj.bmjjournals.com/cgi/eletters/330/7494/760#100868> [accessed 20 May 2005]

Stead, L., Lancaster, T. (2005). 'Group Behaviour Therapy Programmes for Smoking Cessation'. Cochrane Database Syst Rev. (2): p. CD001007

Stein, E.A., Pankiewicz, J., Harsch, H. H., Cho, J. K., Fuller, S. A., Hoffmann, R. G., Hawkins, M., Rao, S. M., Bandettini, P. A., Bloom, A. S. (1998). 'Nicotine-Induced Limbic Cortical Activation in the Human Brain: A Functional MRI Study', *Am J Psychiatry*, **155**(8): pp. 1009-15

Stockmeier, C.A. (2003). 'Involvement of Serotonin in Depression: Evidence from Postmortem and Imaging Studies of Serotonin Receptors and the Serotonin Transporter', *J Psychiatr Res*. **37**(5): pp. 357-73

Stolerman, I.P., Bunker, P., Jarvik, M. E. (1974). 'Nicotine Tolerance in Rats; Role of Dose and Dose Interval', *Psychopharmacologia*, **34**(4): pp. 317-24

Stolerman, I.P., Garcha, H. S., Mirza, N. R. (1995). 'Dissociations Between the Locomotor Stimulant and Depressant Effects of Nicotinic Agonists in Rats', *Psychopharmacology* (Berl), **117**(4): pp. 430-7

Straub, R.E., Sullivan, P. F., Ma, Y., Myakishev, M. V., Harris-Kerr, C., Wormley, B., Kadambi, B., Sadek, H., Silverman, M. A., Webb, B. T., Neale, M. C., Bulik, C. M., Joyce, P. R., Kendler, K. S. (1999). 'Susceptibility Genes for Nicotine Dependence: A Genome Scan and Followup in an Independent Sample Suggest that Regions on Chromosomes 2, 4, 10, 16, 17 and 18 Merit Further Study', *Mol Psychiatry*, **4**(2): pp. 129-44

Stuss, D.T. and Alexander, M.P. (2000). 'Executive Functions and the Frontal Lobes: A Conceptual View', *Psychol Res*. **63**(3-4): pp. 289-98

Su, C. (1982). 'Actions of Nicotine and Smoking on Circulation', *Pharmacol Ther*. **17**(1): pp. 129-41

Suemaru, K., Araki, H., Gomita, Y. (2000). 'Involvement of 5-Hydroxytryptamine(1A) Receptors in Nicotine-Induced Tail Tremor in Rats', *Eur J Pharmacol.* **408**(1): pp. 19-23

Sullivan, P.F., Neale, B. M., van den Oord, E., Miles, M. F., Neale, M. C., Bulik, C. M., Joyce, P. R., Straub, R. E., Kendler, K. S. (2004). 'Candidate Genes for Nicotine Dependence Via Linkage, Epistasis, and Bioinformatics', *Am J Med Genet B Neuropsychiatr Genet.* **126**(1): pp. 23-36

Swan, G.E., Benowitz, N. L., Lessov, C. N., Jacob, P., 3rd, Tyndale, R. F., Wilhelmsen, K. (2005). 'Nicotine Metabolism: The Impact of CYP2A6 on Estimates of Additive Genetic Influence', *Pharmacogenet Genomics*, **15**(2): pp. 115-25

Swan, G.E., Carmelli, D., Cardon, L. R. (1996). 'The Consumption of Tobacco, Alcohol, and Coffee in Caucasian Male Twins: A Multivariate Genetic Analysis', *J Subst Abuse*, **8**(1): pp. 19-31

Swan, G.E., Carmelli, D., Cardon, L. R. (1997). 'Heavy Consumption of Cigarettes, Alcohol and Coffee in Male Twins', *J Stud Alcohol.* **58**(2): pp. 182-90

Swan, G.E., Carmelli, D., Rosenman, R. H., Fabsitz, R. R., Christian, J. C. (1990). 'Smoking and Alcohol Consumption in Adult Male Twins: Genetic Heritability and Shared Environmental Influences', *J Subst Abuse*, **2**(1): pp. 39-50

Talbot, P.S., Laruelle, M. (2002). 'The Role of *In Vivo* Molecular Imaging with PET and SPECT in the Elucidation of Psychiatric Drug Action and New Drug Development', *Eur Neuropsychopharmacol*, **12**(6): pp. 503-11

Tauscher, J., Bagby, R. M., Javanmard, M., Christensen, B. K., Kasper, S., Kapur, S. (2001). 'Inverse Relationship Between Serotonin 5-HT(1A) Receptor Binding and Anxiety: A [(11)C]WAY-100635 PET Investigation in Healthy Volunteers', *Am J Psychiatry*, **158**(8): pp. 1326-8

Tauscher, J., Kapur, S., Verhoeff, N. P., Hussey, D. F., Daskalakis, Z. J., Tauscher-Wisniewski, S., Wilson, A. A., Houle, S., Kasper, S., Zipursky, R. B. (2002). 'Brain Serotonin 5-HT(1A) Receptor Binding in Schizophrenia Measured by Positron Emission Tomography and [11C]WAY-100635', *Arch Gen Psychiatry*, **59**(6): pp. 514-20

Tauscher, J., Verhoeff, N. P., Christensen, B. K., Hussey, D., Meyer, J. H., Kecojevic, A., Javanmard, M., Kasper, S., Kapur, S. (2001). 'Serotonin 5-HT1A Receptor Binding Potential Declines with Age as Measured by [11C]WAY-100635 and PET', *Neuropsychopharmacology*, **24**(5): pp. 522-30

Thiel, C.M., Zilles, K., Fink, G. R. (2005). 'Nicotine Modulates Reorienting of Visuospatial Attention and Neural Activity in Human Parietal Cortex', *Neuropsychopharmacology*, **30**(4): pp. 810-20

Totterdell, S., Smith, A. D. (1989). 'Convergence of Hippocampal and Dopaminergic Input onto Identified Neurons in the Nucleus Accumbens of the Rat', *J Chem Neuroanat.* **2**(5): pp. 285-98

Tregellas, J.R., Tanabe, J. L., Martin, L. F., Freedman, R. (2005). 'FMRI of Response to Nicotine During a Smooth Pursuit Eye Movement Task in Schizophrenia', *Am J Psychiatry*, **162**(2): pp. 391-3

Trimmel, M., Wittberger, S. (2004). 'Effects of Transdermally Administered Nicotine on Aspects of Attention, Task Load, and Mood in Women and Men', *Pharmacol Biochem Behav.* **78**(3): pp. 639-45

True, W.R., Heath, A. C., Scherrer, J. F., Waterman, B., Goldberg, J., Lin, N., Eisen, S. A., Lyons, M. J., Tsuang, M. T. (1997). 'Genetic and Environmental Contributions to Smoking', *Addiction*, **92**(10): pp. 1277-87

True, W.R., Xian, H., Scherrer, J. F., Madden, P. A., Bucholz, K. K., Heath, A. C., Eisen, S. A., Lyons, M. J., Goldberg, J., Tsuang, M. (1999). 'Common Genetic Vulnerability for Nicotine and Alcohol Dependence in Men', *Arch Gen Psychiatry*, **56**(7): pp. 655-61

Tsukada, H., Miyasato, K., Kakiuchi, T., Nishiyama, S., Harada, N., Domino, E. F. (2002). 'Comparative Effects of Methamphetamine and Nicotine on the Striatal [(11)C]Raclopride Binding in Unanesthetized Monkeys', *Synapse*, **45**(4): pp. 207-12

Tucci, S., Genn, R. F., Marco, E., File, S. E. (2003). 'Do Different Mechanisms Underlie Two Anxiogenic Effects of Systemic Nicotine?' *Behav Pharmacol.* **14**(4): pp. 323-9

Tugay, M., Yildiz, F., Utkan, Z., Utkan, T., Sarioglu, Y. (2003). 'Impaired Gastric Motility in the Gastroesophageal Reflux Rat Model: An *In Vitro* Study', *J Surg Res.* **115**(2): pp. 272-8

Twigg, L., Moon, G., Walker, S. (2004). *The Smoking Epidemic in England* (National Health Service: The Health Development Agency)

Ugedo, L., Grenhoff, J., Svensson, T. H. (1989). 'Ritanserin, a 5-HT2 Receptor Antagonist, Activates Midbrain Dopamine Neurons by Blocking Serotonergic Inhibition', *Psychopharmacology* (Berl), **98**(1): pp. 45-50

Unwin, N. (1995). 'Acetylcholine Receptor Channel Imaged in the Open State', *Nature*, **373**(6509): pp. 37-43

Unwin, N. (2005). 'Refined Structure of the Nicotinic Acetylcholine Receptor at 4A Resolution', *J Mol Biol.* **346**(4): pp. 967-89

USPHS (2000). Chapter 4, 'Management of Nicotine Addiction', in *Reducing Tobacco Use: A Report of the Surgeon General* (Bethesda, MD, U.S.A United States Department of Health and Human Services, Public Health Service)

USPHS (1998). *The Health Consequences of Smoking: Nicotine Addiction: A Report of the Surgeon General* (Rockville, MD, USA: United States Department of Health and Human Services: Public Health Service)

Valadez, A., Schenk, S. (1994). 'Persistence of the Ability of Amphetamine Preexposure to Facilitate Acquisition of Cocaine Self-Administration', *Pharmacol Biochem Behav.* **47**(1): pp. 203-5

Vezina, P., Blanc, G., Glowinski, J., Tassin, J. P. (1992). 'Nicotine and Morphine Differentially Activate Brain Dopamine in Prefrontocortical and Subcortical Terminal Fields: Effects of Acute and Repeated Injections', *J Pharmacol Exp Ther.* **261**(2): pp. 484-90

Vink, J.M., Beem, A. L., Posthuma, D., Neale, M. C., Willemsen, G., Kendler, K. S., Slagboom, P. E., Boomsma, D. I. (2004) 'Linkage Analysis of Smoking Initiation and Quantity in Dutch Sibling Pairs', *Pharmacogenomics J.* **4**(4): pp. 274-82

Volkow, N.D., Fowler, J. S., Ding, Y. S., Wang, G. J., Gatley, S. J. (1999). 'Imaging the Neurochemistry of Nicotine Actions: Studies with Positron Emission Tomography', *Nicotine Tob Res.* **1 Suppl 2**: pp. S127-32; discussion S139-40

Volkow, N.D., Fowler, J. S., Wang, G. J. (2003). 'The Addicted Human Brain: Insights from Imaging Studies', *J Clin Invest.* **111**(10): pp. 1444-51

Volkow, N.D., Fowler, J. S., Wang, G. J., Goldstein, R. Z. (2002). 'Role of Dopamine, the Frontal Cortex and Memory Circuits in Drug Addiction: Insight from Imaging Studies', *Neurobiol Learn Mem.* **78**(3): pp. 610-24

Volkow, N.D., Rosen, B., Farde, L. (1997). 'Imaging the Living Human Brain: Magnetic Resonance Imaging and Positron Emission Tomography', *Proc Natl Acad Sci U S A*, **94**(7): pp. 2787-8

Wacholder, S., Chanock, S., Garcia-Closas, M., El Ghormli, L., Rothman, N. (2004). 'Assessing the Probability that a Positive Report is False: An Approach for Molecular Epidemiology Studies', *J Natl Cancer Inst.* **96**(6): pp. 434-32

Wang, D., Ma, J. Z., Li, M. D. (2005). 'Mapping and Verification of Susceptibility Loci for Smoking Quantity Using Permutation Linkage Analysis', *Pharmacogenomics J.* **5**(3): pp. 215-6

Waters, A.J., Shiffman, S., Sayette, M. A., Paty, J. A., Gwaltney, C. J., Balabanis, M. H. (2004). 'Cue-Provoked Craving and Nicotine Replacement Therapy in Smoking Cessation', *J Consult Clin Psychol.* **72**(6): pp. 1136-43

Watkins, S.S., Stinus, L., Koob, G. F., Markou, A. (2000). 'Reward and Somatic Changes During Precipitated Nicotine Withdrawal in Rats: Centrally and Peripherally Mediated Effects', *J Pharmacol Exp Ther.* **292**(3): pp. 1053-64

Wenzel, D.G., Azmeh, N., Clark, I. J. (1971). 'Studies on the Acute and Chronic Depressor Actions of Nicotine in the Rat', *Arch Int Pharmacodyn Ther.* **193**(1): pp. 23-36

Werle, E., Uschold, E. (1948). 'Ueber Fermentative Nicotinentgiftung durch Tierisches Gewebe', *Biochem Z.* **318**: pp. 531-537

West, R., Hajek, P., McNeill, A. (1991). 'Effect of Buspirone on Cigarette Withdrawal Symptoms and Short-Term Abstinence Rates in a Smokers Clinic', *Psychopharmacology* (Berl), **104**(1): pp. 91-6

West, R., Shiffman, S. (2001). 'Effect of Oral Nicotine Dosing Forms on Cigarette Withdrawal Symptoms and Craving: A Systematic Review', *Psychopharmacology* (Berl), **155**(2): pp. 115-22

Whiteaker, P., Peterson, C. G., Xu, W., McIntosh, J. M., Paylor, R., Beaudet, A. L., Collins, A. C., Marks, M. J. (2002). 'Involvement of the Alpha3 Subunit in Central Nicotinic Binding Populations', *J Neurosci.* **22**(7): pp. 2522-9

WHO. (2005). 'Why is Tobacco a Public Health Priority?' <http://www.who.int/tobacco/health_priority/en/> [accessed 20 May 2005]

Willeit, M., Praschak-Rieder, N., Neumeister, A., Zill, P., Leisch, F., Stastny, J., Hilger, E., Thierry, N., Konstantinidis, A., Winkler, D., Fuchs, K., Sieghart, W., Aschauer, H., Ackenheil, M., Bondy, B., Kasper, S. (2003). 'A Polymorphism (5-HTTLPR) in the Serotonin Transporter Promoter Gene is Associated with DSM-IV Depression Subtypes in Seasonal Affective Disorder', *Mol Psychiatry*, **8**(11): pp. 942-6

Woolrich, M.W., Behrens, T. E., Beckmann, C. F., Jenkinson, M., Smith, S. M. (2004). 'Multilevel Linear Modelling for FMRI Group Analysis Using Bayesian Inference', Neuroimage, **21**(4): pp. 1732-47

Woolrich, M.W., Ripley, B. D., Brady, M., Smith, S. M. (2001). 'Temporal Autocorrelation in Univariate Linear Modeling of FMRI Data', *Neuroimage*, **14**(6): pp. 1370-86

World Bank, The (1999). *Curbing the Epidemic: Governments and the Economics of Tobacco Control* (Washington, D.C., USA: The World Bank)

Worsley, K.J., Evans, A. C., Marrett, S., Neelin, P. (1992). 'A Three-Dimensional Statistical Analysis for CBF Activation Studies in Human Brain', *J Cereb Blood Flow Metab*. **12**(6): pp. 900-18

Woolverton, W.L., Goldberg, L. I., Ginos, J. Z. (1984). 'Intravenous Self-Administration of Dopamine Receptor Agonists by Rhesus Monkeys', *J Pharmacol Exp Ther*p. **230**(3): p. 678-83

Wu, S., Comings, D. E. (1999). 'A Common C-1018G Polymorphism in the Human 5-HT1A Receptor Gene', *Psychiatr Genet*. **9**(2): pp. 105-6

Yoshiura, T., Zhong, J., Shibata, D. K., Kwok, W. E., Shrier, D. A., Numaguchi, Y. (1999). 'Functional MRI Study of Auditory and Visual Oddball Tasks', *Neuroreport*, **10**(8): pp. 1683-8

Yudkin, P., Hey, K., Roberts, S., Welch, S., Murphy, M., Walton, R. (2003). 'Abstinence from Smoking Eight Years after Participation in Randomised Controlled Trial of Nicotine Patch', *BMJ*. **327**(7405): pp. 28-9

Yudkin, P., Walton, R.T., Hey, K., Roberts, S.J., Welch, S.J., Johnstone, E.C., Griffiths, S.E., Jones L.J., Murphy, M.F. (2001). 'Is There an Inherited Basis for Smoking Behaviour?' paper preented at the Society for Research on Nicotine and Tobacco's 7th annual conference in Seattle, Washington

Zhang, W.N., Bast, T., Feldon, J. (2002). 'Prepulse Inhibition in Rats with Temporary Inhibition/Inactivation of Ventral or Dorsal Hippocampus', *Pharmacol Biochem Behav*. **73**(4): pp. 929-40

Zahm, D.S., (1989). 'The Ventral Striatopallidal Parts of the Basal Ganglia in the Rat: II. Compartmentation of Ventral Pallidal Efferents', *Neuroscience*, **30**(1):33-50

Zahm, D.S., Heimer, L., (1988). Ventral Striatopallidal Parts of the Basal Ganglia in the Rat:I. Neurochemical Compartmentation as Reflected by the Distributions of Neurotensin and Substance P Immunoreactivity', *J Comp Neurol*., **272**(4):516-35

Zahm, D.S., Heimer, L., (1990). 'Two Transpallidal Pathways Originating in the Rat Nucleus Accumbens', *J Comp Neurol*, **302**(3):437-46

Zucker, D., Hopkins, R. S., Sly, D. F., Urich, J., Kershaw, J. M., Solari, S. (2000). 'Florida's "Truth" Campaign: A Counter-Marketing, Anti-Tobacco Media Campaign', *J Public Health Manag Pract*. **6**(3): pp. 1-6