The Dopaminergic response to drugs of abuse in healthy humans: insights from [^{11}C]raclopride studies

By

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PREFACE

The present dissertation examines the effects of drugs of abuse in healthy volunteers using positron emission tomography (PET) and the neuroreceptor ligand [^{11}C]raclopride. I have chosen the option provided in the "Guidelines for the Thesis Preparation" to present a manuscript based-dissertation. In compliance with the regulations provided by McGill Faculty of Graduate Studies and Research, the following text is required to be sited in full below:

"As an alternative to the traditional thesis format, the dissertation can consist of a collection of papers of which the student is an author or co-author. These papers must have a cohesive, unitary character making them a report of a single program of research. The structure for the manuscript-based thesis must conform to the following:

Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly-duplicated text (not the reprints) of one or more published papers. These texts must conform to the "Guidelines for Thesis Preparation" with respect to font size, line spacing and margin sizes and must be bound together as an integral part of the thesis. (Reprints of

published papers can be included in the appendices at the end of the thesis.)

The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensure that the thesis has continuity, connecting texts that provide logical bridges preceding and following each manuscript are mandatory.

The thesis must conform to all other requirements of the "Guidelines for Thesis Preparation" in addition to the manuscripts.

As manuscripts for publication are frequently very concise documents, where appropriate, additional material must be provided (e.g., in appendices) in sufficient detail to allow a clear and precise judgment to be made of the importance and originality of the research reported in the thesis.

In general, when co-authored papers are included in a thesis the candidate must have made a substantial contribution to all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to

what extent. This statement should appear in a single section entitled "Contributions of Authors" as a preface to the thesis. The supervisor must attest to the accuracy of this statement at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to clearly specify the responsibilities of all the authors of the co-authored papers.

When previously published copyright material is presented in a thesis, the candidate must include signed waivers from the publishers and submit these to the Graduate and Postdoctoral Studies Office with the final deposition, if not submitted previously. The candidate must also include signed waivers from any co-authors of unpublished manuscripts."

This thesis will consist of **VII chapters**

1. **Chapter I**

INTRODUCTION I: *Dopamine & Drugs of Abuse: Amphetamine & Alcohol.* This chapter will provide an overview of the dopamine neurotransmission system in mammalian brain, specifically covering its anatomical organization. The mechanism of drug action (pharmacology) of addictive drugs (amphetamine and alcohol), including their similarities and differences will be presented and the

existing literature regarding the involvement of the dopamine system in drug and natural reward processes will be briefly reviewed.

2. Chapter II

INTRODUCTION II: *Sensitization to psychostimulants.* This chapter reviews the experimental data concerning the phenomenon of sensitization. Specifically this section provides a brief description of the defining features of sensitization to amphetamine, which served in building our model of sensitization in humans.

2. **Chapter III**

METHODOLOGICAL OVERVIEW: *Positron emission tomography & [^{11}C]raclopride.* Provides a general, introductory, and technically brief description of the methodological procedures involved using positron emission tomography research. The focus of this chapter is on the methods developed to investigate the dopamine system with [C^{11}]raclopride.

3. **Chapter IV**

RESULTS I: *Amphetamine-induced increases in extracellular dopamine, drug wanting, and novelty seeking: a PET/[^{11}C]raclopride study in healthy men.* This chapter presents results presented in **manuscript 1**, which

particularly pertain to the effects of acute amphetamine administration on dopamine release in the human brain *in vivo.*

4. **Chapter V**

RESULTS II: *Alcohol promotes dopamine release in the human nucleus accumbens.* This chapter presents results presented in **manuscript 2,** which particularly pertain to the effects of acute alcohol administration on dopamine release in the human brain *in vivo.*

5. **Chapter VI**

RESULTS III: *Modeling Sensitization to Stimulants in Humans: A [^{11}C]raclopride / PET Study in Healthy Volunteers.* This chapter presents results presented in **manuscript 3,** which particularly pertain to the effects of repeated amphetamine administration on dopamine release in the human brain *in vivo.*

6. **Chapter VII**

DISCUSSION: This chapter will briefly discuss the general findings of the thesis with relation to previous studies and future direction of research is proposed.

STATEMENT OF ORIGINALITY

The intellectual content of the present thesis, and the research to which it refers are the product of my own work supervised and assisted by others (see next section on *contribution of authors*). Any ideas or quotations from the work of other people published or otherwise, are fully acknowledged in accordance with standard referencing practices. Three **(3)** distinct papers are included in the present thesis, which have been published/submitted for publication in refereed scientific journals. The **3** publications included are composed of an abstract, a summary presentation of the problem, an outline of the goals, a description of the experimental method as well as result and discussion sections.

1. Leyton M, **Boileau I,** Benkelfat C, Diksic M, Baker G, Dagher A. Amphetamine-induced increases in extracellular dopamine, drug wanting, and novelty seeking: a PET/[^{11}C]raclopride study in healthy men. *Neuropsychopharmacology* 2002; 27(6):1027-35.
2. **Boileau I,** Assaad JM, Pihl RO, Benkelfat C, Leyton M, Diksic M, et al. Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse* 2003; 49(4): 226-31.
3. **Boileau I,** Dagher A, Leyton M, Gunn R, Baker G, Diksic M, Benkelfat C. Modeling Sensitization to Stimulants in Humans: A [^{11}C]raclopride / PET Study in Healthy Volunteers. *Archives of General Psychiatry* 2006; in press.

CONTRIBUTION OF AUTHORS

McGill University requires that in the case where papers in a thesis have been published an explicit statement be made regarding the contribution of each author

PUBLICATION 1 describes the effects of amphetamine on the human dopamine system *in vivo* using PET and [^{11}C]raclopride. The first author (M Leyton) contributed equally with the co-author (A Dagher, C Benkelfat) to the overall concept and design of the study. The first author (M Leyton) made the initial experiments, which were continued by the author of the thesis (I Boileau) under the supervision of A Dagher and C Benkelfat. The analysis of the data, the methodology related to the computation of the statistical maps, and visualization of the results was mainly the responsibility of the author of the thesis (I Boileau) under the supervision of A Dagher and C Benkelfat. For publication, the author of the thesis (I Boileau) refined the method. The first author has written the publication the input of the author of the thesis (I Boileau) consisted of segments of text.

Leyton M, **Boileau I,** Benkelfat C, Diksic M, Baker G, Dagher A. Amphetamine-induced increases in extracellular dopamine, drug wanting, and novelty seeking: a PET/[^{11}C]raclopride study in healthy men. *Neuropsychopharmacology* 2002; 27(6): 1027-35.

PUBLICATION 2 describes the effects of alcohol on the human dopamine system *in vivo* using PET and [^{11}C]raclopride. The work for this publication was a joint effort between the departments of Psychology and Neurology, Neurosurgery and Psychiatry. During a first testing time, the department of Psychology (JM Assaad, RO Pihl, R Tremblay) tested a group of subjects drawn from a large cohort of low socio-economic male francophone (R Tremblay) twice in an alcohol protocol where physiological responses to alcohol consumption were measured. This preliminary study enabled to recruit subjects for the PET / [^{11}C]raclopride experiments based on heart rate reactivity to alcohol. All co-authors participated equally in the conception and design of the overall study as well as in drafting and providing critical revision of the manuscript. The acquisition, analysis and interpretation of the data were mainly the responsibility of the author of the thesis (I Boileau) under the supervision of A Dagher and C Benkelfat. The first author has written the publication.

Boileau I, Assaad JM, Pihl RO, Benkelfat C, Leyton M, Diksic M, Tremblay R, Dagher A. Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse* 2003; 49(4): 226-31

PUBLICATION 3 describes the effects of repeated amphetamine on the human dopamine system *in vivo* using PET and [^{11}C]raclopride. All co-authors participated equally with the author of the thesis, in the conception and design of the overall study. The acquisition, analysis and

interpretation of the data were mainly the responsibility of the author of the thesis (I Boileau) under the supervision of A Dagher and C Benkelfat. The first author has written the publication although all co-authors participated in drafting and providing critical revision of the manuscript.

Boileau I, Dagher A, Leyton M, Gunn R, Baker G, Diksic M, Benkelfat C. Modeling Sensitization to Stimulants in Humans: A [^{11}C]raclopride / PET Study in Healthy Volunteers. *Archives of General Psychiatry* 2006; in press.

ABBREVIATIONS

^{11}C	Carbon isotope
3D	Three dimensional
ADHD	Attention deficit hyperactivity disorder
AMPT	α-methyl-para-tyrosine
BDNF	Brain derived neurotrophic factor
BGO	Bismuth germanium oxide
B_{max}	Maximum binding sites (mol ml-1 or mg-1)
BP	Binding Potential
C_1	Blood compartment
C_2	Non-specifically bound compartment
C_3	Specifically bound compartment
CAM-KII	Calcium-calmodulin dependent kinase
cAMP	Cyclic adenosine monophosphate
Cdk5	Cyclin-dependent kinase-5
CREB	cAMP response element binding protein
CS	Conditioned stimuli
DA	Dopamine
DAT	Dopamine transporter
D_1	Dopamine type 1 receptor
$D_{2/3}$	Dopamine type 2 and 3 receptors.
DOPA	3,4-dihydroxyphenyl-L-alanine
DOPAC	3,4-dihydroxyphenylacetic acid
DC	Dorsal caudate nucleus
DP	Dorsal putamen
FOV	Field of View
FWHM	Full-width-at-half-maximum
GABA	Gamma-aminobutyric acid
GC	Glucocorticoids
GPe	External globus pallidus
GPi	Internal globus pallidus
GPCRs	G-protein coupled receptors
G-protein	Guanine-nucleotide binding protein
GTM	Geometric transfer method
HPA	Hypothalamus-pituitary-adrenal stress axis
IBZM	(S)-(-)-3-iodo-2-hydroxy-6-methoxy-N-(1-ethyl-2-pyrrolidinyl) methylbenzamide
IEG	Immediate early genes
IPSP	Inhibitory post-synaptic potential
K_1	Unidirectional blood-brain clearance (mL g-1 min-1)
k_2	Brain-blood diffusion rate constant; min-1

k_3	Association rate constant; min-1
k_4	Dissociation rate constant; min-1
K_d	Dissociation constant
KO	Knock-out
K_{off}	Rate of dissociation
K_{on}	Rate of association
L-dopa	L-dihydroxyphenylalanine
LTP	Long-term potentiation
MAP	Mitogen-activated protein kinase
MD	Mediodorsal thalamus
MRI	Magnetic resonance imaging
NAS	Nucleus accumbens septi
NMDA	N-methyl-D-aspartate
NMSP	N-methylspiperone
NPA	N-propyl-norapomorphine
NS	Novelty-seeking
OFC	Orbitofrontal cortex
PDP	Posterior dorsal putamen
PHNO	propyl-hexahydronaphtho-oxazin
PET	Positron emission tomography
PFC	Prefrontal cortex
PPN	Pedoculopontine nucleus
PVC	Partial volume correction
PVE	Partial volume effect
SNc	Substantia nigra pars compacta
SNr	Substantia nigra pars reticulata
SRTM	Simplified reference tissue model
STN	Subthalamic nucleus
TPQ	Tridimensional personality questionnaire
US	Unconditioned stimuli
VL	Ventro-lateral thalamus
VS	Ventral striatum
VTA	Ventral tegmental area

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amphetamine (dose 4) (n = 10), and **(B)** at one-year follow-up (n=7). VS: ventral limbic striatum; DC: associative pre-commissural dorsal caudate; PDP: post-commissural dorsal putamen. (*) Significantly different from dose 1 ($p<0.05$), (†) significantly different from dose 4 ($p<0.05$).

Figure 5 Relationship between novelty seeking personality and sensitization. **A)** Scatter plots and **B)** voxel-wise regression maps illustrating the relationship between Novelty Seeking personality trait and sensitization-induced changes in [11C]raclopride BP (% decreases from dose 1) measured during amphetamine doses 4 (x=13, y=15, z=7) and 5 (x=11, y=15, z=15)

CHAPTER VII

Figure 1 Schematic of the internalization of receptors. Loss of surface receptor number through endocytosis which occurs secondary to agonist treatment is a mechanism believed to explain changes in [^{11}C]raclopride binding.

Figure 2 Two-state model of $D_{2/3}$ receptors affinity illustrating that $D_{2/3}$ receptors are divided into two states for agonists (high affinity and low affinity sites), with each site contributing to 50% of the population. Under baseline conditions (prior to the challenge), 10% of the sites are occupied by DOPAMINE, and not available to [^{11}C]raclopride. Adapted from (Laruelle, 2000).

Figure 3 Schematic of the nonreciprocal striatonigrostriatal subcircuits which provides a hypothetical model for the preferential effect of amphetamine in the VS and PDP. VTA: ventral tegmental; SNc: substantia nigra compacta, SNr: substantia nigra reticulata; Pre DCA: anterior dorsal caudate; Pre DPU: anterior dorsal putamen; Post CA: posterior dorsal caudate; Post PU: posterior dorsal putamen. The limbic loop is in *red*, the associative loop is in *green* and the sensorimotor loop is in *blue*. Ascending arrows represent dopamine projections from the midbrain descending arrows represent GABA projections from the striatum. Adapted from (Martinez, 2003; Haber, 2000).

Figure 4 **a)** Scatter plots and **b)** voxel-wise regression maps illustrating the relationship between self-rated positive effects of amphetamine (euphoria) during initial dose and changes in [^{11}C]raclopride BP at subsequent exposure (dose 4 and dose 5).

ABSTRACT

Radioligand to receptor binding assessed with Positron Emission Tomography (PET) can be used to investigate dopamine (DA) concentration *in vivo*. The present dissertation focuses on the use of the PET DA $D_{2/3}$ receptor ligand [^{11}C]raclopride to study DA release following the administration of drugs of abuse: amphetamine and alcohol.

A number of experimental animal paradigms have indicated that DA transmission is generally potently engaged by the acute administration of addictive drugs (Di Chiara and Imperato 1988; Weiss, Markou et al. 1992), deficient during drug withdrawal (Markou and Koob 1991; Schulteis, Markou et al. 1995; Epping-Jordan, Watkins et al. 1998) and facilitated or *sensitized* when drug use is reinstated after repetitive exposure (Kalivas 1993; Pierce and Kalivas 1997). Despite the abundance of data establishing the role of mesolimbic DA in the acute reinforcing effects of drugs the exact nature of DA's contribution to reward or addiction is still a subject of debate.

Studies I and II present direct evidence in humans that amphetamine and alcohol promote DA release (decreased [^{11}C]raclopride binding) preferentially in the limbic striatum when given acutely by

mouth. Changes in binding correlated with self-reported *drug-wanting* and heart rate but not with *drug liking* or *euphoria* and were positively related to the personality trait of novelty seeking a known vulnerability factor in alcohol and drug dependence (Howard, Kivlahan et al. 1997).

Study III is primarily concerned with alterations in psychological function and in brain response (DA release) resulting from repeated drug exposure. In healthy human subjects, re-exposure to amphetamine 14 and 365 days after 3 repeated doses had greater psychomotor effects and a concomitant reduction of [^{11}C]raclopride binding compared to an initial dose. This result is interpreted as sensitization. We found that trait novelty seeking was a predictor of subsequent drug-induced DA release and of neurochemical sensitization.

Together our studies indicated that drugs abused by humans increase DA release in the striatum, and when given repeatedly their administration may lead to sensitization, expressed in the form of persistent changes in brain DA neurochemistry. Acute and sensitized DA responses were related to trait novelty seeking and to the psychomotor effects of drugs but not to its pleasurable effects; however enjoyment of the drug-experience seems to alter DA response at subsequent drug-use. The model provides a framework for analyzing the neurochemical

alterations associated with repeated drug-use and also provides useful information regarding the development and triggering of chronic relapsing disorders such as drug addiction and psychosis.

Di Chiara, G. and A. Imperato (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): 5274-8.

Epping-Jordan, M. P., S. S. Watkins, et al. (1998). "Dramatic decreases in brain reward function during nicotine withdrawal." Nature **393**(6680): 76-9.

Howard, M. O., D. Kivlahan, et al. (1997). "Cloninger's tridimensional theory of personality and psychopathology: applications to substance use disorders." J Stud Alcohol **58**(1): 48-66.

Kalivas, P. W. (1993). "Neurotransmitter regulation of dopamine neurons in the ventral tegmental area." Brain Res Brain Res Rev **18**(1): 75-113.

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Pierce, R. C. and P. W. Kalivas (1997). "A circuitry model of the expression of behavioral sensitization to amphetamine-like psychostimulants." Brain Res Brain Res Rev **25**(2): 192-216.

Schulteis, G., A. Markou, et al. (1995). "Decreased Brain Reward Produced by Ethanol Withdrawal." PNAS **92**(13): 5880-5884.

Weiss, F., A. Markou, et al. (1992). "Basal extracellular dopamine levels in the nucleus accumbens are decreased during cocaine withdrawal after unlimited-access self-administration." Brain Res **593**(2): 314-8.

RÉSUMÉ

La tomography par émission de positons (TEP) et le radioligand [^{11}C]raclopride permettent de mesurer les propriétés des récepteurs centraux de la dopamine (DA). De cette façon, l'union compétitive entre ce radioligand et la DA endogène sert à évaluer *in vivo*, dans l'espace synaptique, les variations de concentration de la DA secondaire à une intervention pharmacologique ou suite à une tâche mentale (cognitive). La présente dissertation est principalement centrée sur l'investigation de la libration de DA suite à l'administration aigue et à la prise répétée de drogues d'abus.

Un ensemble de données de la neurobiologie ont permis de montrer que la transmission de la DA est amplifiée par les drogues qui déclenchent une dépendance chez l'homme (Di Chiara and Imperato 1988; Weiss, Markou et al. 1992) et que celle-ci est déficiente, après l'arrêt de la consommation, pendant la période de sevrage (Markou and Koob 1991; Schulteis, Markou et Al. 1995; Epping-Jordan, Watkins et Al. 1998). De plus, la re-administration de ces substances résulte en une augmentation progressive de la réponse dopaminergique communément appelé sensibilisation (Kalivas 1993; Pierce and Kalivas 1997). En dépit du grand nombre de données corroborant le rôle crucial de la DA mesolimbique

dans le renforcement positif et l'addiction aux drogues, la nature exacte de ce rôle n'a pas encore été entièrement élucidée.

Les **études 1** et **2** de cette dissertation présentent des évidences directes chez l'humain démontrant que l'administration aigue d 'amphétamine et d'alcool entraîne une augmentation transitoire de DA dans le striatum limbique. Cette augmentation (déplacement du radioligand) est corrélée au désir pour la drogue (*drug-wanting)* et à l'augmentation du rythme cardiaque, et non à la réponse aux propriétés euphorisantes de la drogue (*drug liking / euphoria).* Aussi l'augmentation de DA est corrélée à un trait de personnalité marqué par la recherche de nouveauté (*novelty seeking*). Ce trait a été associé au développement des addictions (Howard, Kivlahan et al. 1997).

Etude 3 de cette dissertation est principalement centrée sur la réponse à un régime répétée d'amphétamines. Chez le sujet sain, la re-administration d'amphétamine 14 et 365 jours après 3 doses d'amphétamine, augmente l'activité psychomotrice et le taux de déplacement du [^{11}C]raclopride dans le striatum. Ce résultat suggère une sensibilisation aux effets des amphétamines. Aussi le trait de personnalité *novelty-seeking* prédit l'apparition de la sensibilisation.

Nos données suggèrent que l'administration de drogues d'abus augmente la DA dans le striatum. Avec une administration répétée, l'effet des amphétamines se traduit en sensibilisation psychomotrice et dopaminergique. La réponse aux amphétamines, en aigue et après sensibilisation est corrélée au trait de personnalité *novelty seeking* et aux effets psychomoteurs de la drogue et non au plaisir associé à la consommation aigue. Par contre, ce dernier modifie la réponse dopaminergique au cours d'administrations subséquentes. Ce model représente un paradigme par lequel nous pouvons étudier le développement des addictions.

Di Chiara, G. and A. Imperato (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): 5274-8.

Epping-Jordan, M. P., S. S. Watkins, et al. (1998). "Dramatic decreases in brain reward function during nicotine withdrawal." Nature **393**(6680): 76-9.

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CHAPTER I: Introduction I

Dopamine & Drugs of Abuse: Amphetamine & Alcohol

1.0. OVERVIEW

Since it has been postulated as a chemical neurotransmitter (Von Euler and Lishajko 1957; Carlsson, Lindqvist et al. 1958), dopamine (DA)'s role in brain and peripheral functions has been extensively studied. Perturbation of DA neurotransmission can result in profound neurological, psychiatric, or physiological signs and symptoms. Therapeutic modulation of DA receptor function provides the most efficacious therapy for many disorders, including schizophrenia and Parkinson's disease. DA research spans nearly a half-century, spurred by the discovery of chlorpromazine in the early 1950's (Delay, Deniker et al. 1952; Carlsson and Lindqvist 1963). DA continues to be of great interest in present day research, and remains a critical component in the therapy of many of the most serious psychiatric and neurological disorders. In this regard, positron emission tomography (PET; see chapter 3) has lead to a renewal of interest on the nature and involvement of DA neurotransmission in number of functions, in particular, in reward processing, in drug-reinforcement and in disorders such as addiction and psychosis.

The DA system is a major target of drugs of abuse and it is considered to convey the affect-related effects of pharmacologically distinct drug classes. Although drugs produce their in vivo effects on DA nerve terminals primarily via different mechanisms, the resulting increase in synaptic levels of DA may

ultimately result in the same regulatory changes, which might make way to the complex signs of addiction (craving, sensitization). After describing anatomical aspects of the dopaminergic system, this chapter will briefly review the involvement of this neurotransmitter system in natural and drug reward processing and focus on the mechanism of action of addictive drugs investigated in this thesis (amphetamine and alcohol). A brief description of theories of addiction will be provided.

1.1. OVERVIEW OF THE DOPAMINE SYSTEM

1.1.1 THE NEUROTRANSMITTER DOPAMINE

DA is classified chemically as a catecholamine. DA synthesis occurs in both the cytosol as well as in the presynaptic terminals of DA neurons though a two-step biosynthetic pathway. The first step is hydroxylation of the essential amino acid tyrosine, catalyzed by the enzyme tyrosine hydroxylase. Tyrosine is converted to L-dihydroxyphenylalanine (L-dopa) by the enzyme tyrosine hydroxylase, a reaction that also requires the tyrosine hydroxylase cofactor 6-tetrahydrobiopterin. This slower step is generally considered to be rate limiting for DA synthesis. The second step is the conversion of L-dopa to DA via the enzymatic action of aromatic amino acid decarboxylase. DA is sequestered into storage vesicles by vesicular monoamine transporter II (Liu, Peter et al. 1992).

Pulse-evoked DA release in the synaptic cleft diffuses in the extracellular space; the clearance rate, and thus the extracellular lifetime of DA is regulated by transporters located in the plasma membrane via a Na^+/Cl^- dependent process (Kuhar, Vaughan et al. 1998; Cragg and Rice 2004). Alternately DA in the extracellular space can be catabolized by monoamine oxidase (to 3,4,-dihydroxyphenylacetic acid) or catechol-*O*-methyltransferase (to 3-methoxytyramine). These enzymes are major mechanisms for inactivation of catecholamines. Action by both enzymes results in the formation of homovanillic acid (3-methoxy-4-hydroxy-phenylacetic acid).

1.1.2 DOPAMINE RECEPTORS

The diverse physiological actions of the neurotransmitter DA are mediated by the products of five genes, which are seven transmembrane G protein-coupled DA receptors (Missale, Nash et al. 1998). The receptors are categorized into two families, D_1-like and D_2-like, according to their ability to increase or inhibit the activity of the enzyme adenylcyclase, which generates the intracellular second messenger cyclic AMP (cAMP) (Kebabian and Greengard 1971). The D_2-like family receptors (D_2, D_3, and D_4) originate from three genes. They are prototypic G protein-coupled receptors, primarily coupled to Gi / Go proteins, they inhibit adenylate cyclase, activate K^+ channels and they have both

hetero and auto receptor functions. Alternative splicing gives rise to variants of D_2 and D_3 receptors in certain tissues. D_2 receptors can be subject to post-transcriptional processing, such that the final product can be expressed in a complete form (D_2-long), or after cleavage, in a short form (D_2-short) (Guiramand, Montmayeur et al. 1995). The D_1-like family (D_1 or D_{1A} and D_5 or D_{5B}) comes from intron-less genes (albeit D_5 pseudogenes exist), and the receptors generally couple to GOLF and Gs protein to activate adenylate cyclase. 1998). G-protein-coupled DA receptors, can occur in different conformations states that express different affinities for DA and other agonists. The high-affinity sites (D_2 high sites) are G-protein-coupled, whereas the low-affinity sites (D_2 low sites) are those uncoupled with G-proteins. DA receptors can convert between states with high or a low affinity for agonists, while retaining the same affinity for antagonists (Sibley, Leff et al. 1982). It has been proposed that receptor affinity might explain why PET competition studies with radiolabeled antagonists such as [^{11}C]raclopride have been unable to show a reduction in radiotracer binding greater than 40% i.e.: a ceiling effect (Laruelle 2000). Specifically, whereas the antagonist [^{11}C]raclopride can bind to receptors in both high and low affinity states, DA bind selectively to the high affinity receptors; therefore due to the fact that some proportion of the binding sites are entirely insensitive to DA, complete displacement of antagonist radioligands by DA cannot be detected *in vivo* (Hwang, Narendran et al. 2004; Narendran, Hwang et al. 2004).

Receptor localization in the central nervous system also differentiates one receptor subtype from another (Figure 1). The expression territories of the D_1 receptor subtype spread over telencephalic area, localized to many, but not all, brain regions receiving dopaminergic innervation. The highest amount of D_1 receptor mRNA has been found in the caudate-putamen, the olfactory tubercle, the piriform and entorhinal cortices and in limbic related regions including, nucleus accumbens, amygdala, medial prefrontal, infralimbic, prelimbic, anterior cingulate and in the insula. D_1 receptors in substantia nigra pars compacta or pars reticulata are present but sparsely distributed on non-DA containing cells (interneurons) and on afferent nerve terminals from other brain regions (Mansour, Meador-Woodruff et al. 1991). D_2 receptor mRNAs in the rat brain, as determined by *in situ* hybridization, has been found in DA containing neurons (as auto receptors) and in all associated projection areas: subsantia nigra, VTA, caudate, putamen, globus pallidus, nucleus accumbens, prefrontal cortex, cingulate cortex, entorhinal cortex, piriform cortex, septum, amygdala, zona incerta, hypothalalamus, olfactory bulb (Meador-Woodruff, Mansour et al. 1991). Within the D_2 -like receptor class, the D_2 receptor is the most highly expressed. The D_4 type overlaps with the D_2 type however with a more moderate level of expression in the nucleus accumbens and caudate-putamen (Rivera, Cuellar et al. 2002), whereas the D_3 type is found in a more restricted manner, in areas of lower D_2 concentration such as the island of Calleja, the nucleus accumbens and the

bed nucleus of stria terminalis (Bouthenet, Souil et al. 1991; Sokoloff, Giros et al. 1992).

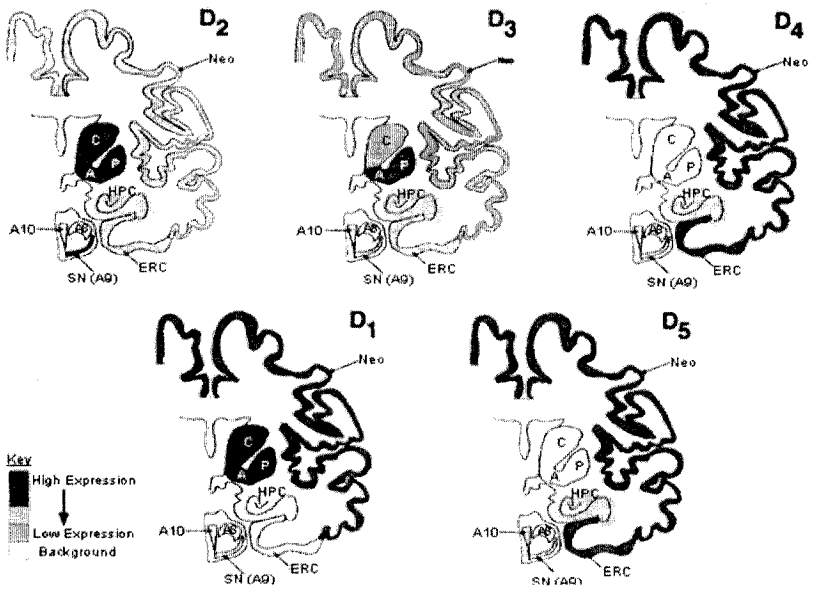


Figure 1 Receptor subtype distribution in the human brain.

1.1.3. ANATOMICAL ORGANIZATION

1.1.3.1. THE MESENCEPHALON DOPAMINE CELLS

Central nervous system DA neurons are for the most part gathered as a single continuous group of cells in the midbrain. In both rodents and primates (Figure 2) these mesencephalic DA cells have been compartmentally organized into three distinct cytoarchitectonic subdivisions: the A8 cell group roughly

corresponds to the reticulata retrorubral area; the A9 cells (the nigrostriatal dopaminergic system) are primarily gathered within the SNc with some cells found in the substantia nigra pars reticulata (SNr) and the A10 cell group (the mesolimbic and mesocortical dopaminergic systems) lies medial to the SNc in the VTA (Dahlstrom and Fuxe 1964). Retrograde tracing techniques have enabled neuroanatomists to identify the output topography of these cells, thus leading to their delineation into a dorsal and a ventral tier. The dorsal (and mediodorsal) part of the VTA and SNc(A 10) represents the *dorsal tier* (calcium-binding protein or calbindin-positive cells) and topographically innervates the ventrally located structures of the limbic striatum (matrix compartment) including the nucleus accumbens (NAS) (shell), septum, olfactory tubercle, piriform cortex and amygdala as well as the medial prefrontal, cingulate, perirhinal, and entorhinal cortices, whereas the ventral part of the VTA and SNc (A 9) composes the *ventral tier* (calbindin-negative cells) which primarily directs axons to the more dorsal neostriatum (lateral SNc to motor striatum and medial SNC and VTA to associative striatum; patch compartment) (Gerfen, Baimbridge et al. 1987). However, some neurons occupying the medial densocellular zone of the *ventral tier* have been recognized to have projections to the ventral striatum. The A8 cell group or retrorubral field innervates all striatal sub-compartments (for review see Fallon and Laughlin 1995). 85% of the VTA NAS projections contain DA (Swanson 1982). The non-dopaminergic part of the projection from the VTA to

the NAS and striatum is GABAergic and appears to terminate on cholinergic interneurons which in turn input to medium-size spiny neurons.

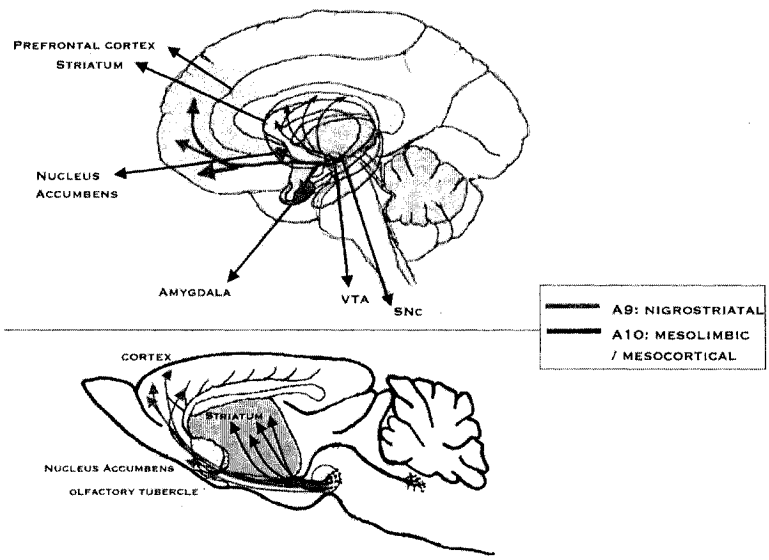


Figure 2 Schematic of the human (top) and rat brain (bottom) illustrating main dopaminergic projections from VTA cell group A10 (mesolimbic and mesocortical projections) and from SNc cell group A9 (nigrostriatal projections).

1.1.3.2. THE CORPUS STRIATUM

The striatum is a nuclear complex within the basal ganglia and a major recipient of DA input. Understanding the organization of the connections between the DA mesencephalic cells and the striatum is essential for unraveling

the role of DA in normal and pathological states such as schizophrenia, drug abuse and Parkinson's disease. The striatum is divided into three components. It includes the caudate nucleus, the putamen and the globus pallidus. The caudate and putamen together comprise the neostriatum which has a stripy appearance due to bands of white matter and is sometimes simply abbreviated *dorsal striatum* (Figure 3). The ventral (limbic) striatum (rostroventral caudate / putamen and nucleus accumbens) together with the ventral part of the pallidum (globus pallidus ventral to the anterior commissure) are recognized as forming part of a functional circuit distinct from the dorsal striatum. This classification is based on inputs received from the amygdala and cortical areas mediating emotion and motivation (cingulate, orbital, and superior temporal cortices) (Haber and McFarland 1999).

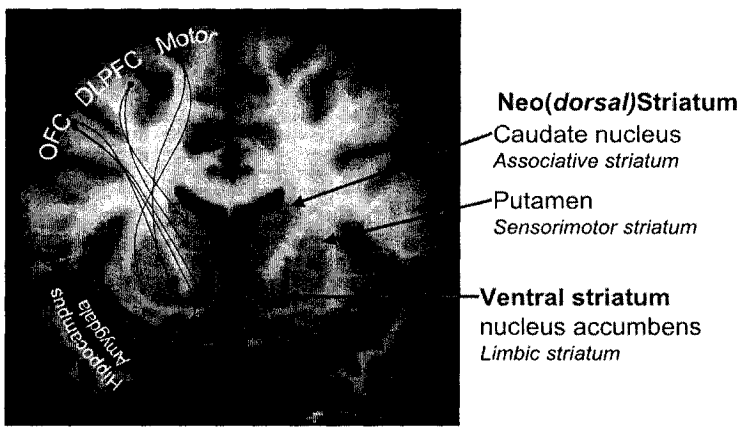


Figure 3 The tripartite anatomofunctional subdivisions of the striatum (sensorimotor, associative and limbic striata) based on the pattern of projections received from motor, limbic, and association cortices.

Most (90%) of the neurons composing the striatum have spiny dendrites and have a receptive/outflow (projection) function. They contain the inhibitory neurotransmitter gamma-aminobutyric acid (GABA). Other neurons have dendrites that lack spines and serve an associative or interneuronal function. They use the transmitter acetylcholine (Ach) and are excitatory. All neurons also contain various neuropeptides (enkephalin, substance P, neuropeptide Y) that are co-released with the neurotransmitters.

1.1.3.3. STRIATAL CONNECTIONS

Afferent connections to the striatum form the basis for its function. The major source of input to the striatum emerges from the cortex, particularly the frontal lobes projections (minor projections also arise from the intralaminar nuclei of the thalamus and from the pedoculopontine nucleus). Projections from the cortex to the striatum are excitatory, mediated by the neurotransmitter glutamate. The primary motor, premotor, supplementary motor and somatosensory areas of cortex project mainly to the putamen whereas the association cortex of the prefrontal and parietal lobes project to the caudate preferentially. These pathways contribute to control of movement speed and posture as well as to regulation and the organization of higher motor behavior and cognitive processes. The ventral striatum including the nucleus accumbens

receives input from areas that do not project to the dorsal striatum (motor and sensory areas of the frontal and parietal lobes), notably temporal cortex including the hippocampal formation, limbic, and orbitofrontal cortical areas as well as the basolateral amygdala (Figure 3). Its location at the interface of descending limbic projections from the amygdala, the hippocampus and the prefrontal cortex and of ascending DA fibers arising from the midbrain confers an important role for this region in integrating emotional (limbic) and motor information ("limbic-motor interface"). The striatum receives important dopaminergic (Figure 2) afferent projections from the SNC. The nigrostriatal tract are involved in the fine-tuning of movement. The breakdown of neurons in this pathway is associated with the tremors, rigidity, and slowness of movement characteristic of Parkinson's disease.

The major outflow from the striatum originates from the globus pallidus internal segment (GPi). Efferents from the GPi use GABA as a transmitter and are inhibitory and project to specific thalamic nuclei, which in turn outputs on and probably excite the primary motor, premotor and supplementary motor areas of the frontal lobe cortex. Conversely, the ventral striatum conveys information to the cingulate, orbitofrontal and prefrontal cortex and to the ventral tegmental area of the brainstem via projections through the ventral pallidum to dorsomedial and ventral anterior nuclei of the thalamus.

1.2. WHAT IS THE ROLE OF DOPAMINE?

1.2.1. DOPAMINE AND REWARD MOTIVATED BEHAVIOR

Olds and Milner incidental finding that in rats, electrical stimulation of DA fibers (medial forebrain bundle extending from the lateral hypothalamus to the VTA) reinforce arbitrary selected operant responses despite the absence of biological needs (Olds and Milner 1954). This finding has lead to the suggestion that meso-corticolimbic DA is a center for appetitive motivation and reward processes. Elucidating the role of DA in reward can provide insight into a number of psychopathologies, including depression, drug addiction, obsessive-compulsive and eating disorders. Presently, several lines of evidence from electrophysiological, microdialysis, or voltammetric studies in animals and recently PET studies in humans support the involvement of DA in a wide variety of adaptive (appetitive) behavioral actions including goal-directed locomotor activity, feeding (Mark, Smith et al. 1994; Kiyatkin 1995; Richardson and Gratton 1996; Small, Jones-Gotman et al. 2003), drinking (Young, Joseph et al. 1992), sexual (Pfaus, Damsma et al. 1995) and maternal behaviors (Stern and Lonstein 2001; Silva, Bernardi et al. 2003) as well as non-adaptive behaviors such as drug reinforcement (Di Chiara and Imperato 1988) (see 1.2), and brain-stimulation reward (Yokel and Wise 1976). However, precisely what role DA plays in sensing and responding to reward, both natural and drug-related, is still a matter of investigation (Berridge and Robinson 1998).

Wise initially introduced the *anhedonia hypothesis* to explain the reduction of reward-directed actions after pharmacological blockade of DA receptors (Wise and Bozarth 1982). Today, the idea that DA carries the pleasure or hedonic signal associated with reward has been replaced by views suggesting an anticipatory, attentional, learning, appetitive role for DA (i.e.: approach behavior as opposed to the consummatory behavior). In this regard, a series of recording studies in higher non-human primates (Schultz 1998) revealed that DA neurons within both the ventral tegmental area (VTA) and substantia nigra pars compacta (SNc) respond with burst firing to unexpected primary rewards (drops of juice) and to stimuli predictive of reward but not to primary rewards whose delivery is expected, due to being signaled by prior cues (Mirenowicz and Schultz 1994). These findings form the basis of the *prediction error theory*. This theory suggests that activity changes in DA neurons encode an error in the prediction of the time and amount of immediate and future rewards - thus contributing to learning (Schultz 1998). Increased dopaminergic activity is hypothesized to indicate that immediate or future reward is expected, while decreased dopaminergic activity signals the converse (Montague, Dayan et al. 1996). This formulation has been refined in a number of influential computational models (such as the temporal difference learning algorithm) believed to correspond to DA neurotransmission (Sutton and Barto 1981; Egelman, Person et al. 1998; Schultz 1998; McClure, Daw et al. 2003; Montague, Hyman et al. 2004).

In their formulation of the *incentive salience hypothesis* of dopaminergic function, Robinson and Berridge have proposed that DA release assigns incentive, or motivational, value (salience) to otherwise neutral objects or behavioral acts. In other words their hypothesis suggests that DA mediates the motivational rather than affective component of reward, i.e.: the *wanting* but not the *liking* (Robinson and Berridge 1993). In line with this, blockade of DA receptor function has been shown to inhibit the ability to use actions aimed at acquiring rewards, i.e.: instrumental responding to drug-associated cues, without influencing the behavioral measures of affective reactions to rewards (Robinson and Berridge 1993; Salamone, Cousins et al. 1997; Ikemoto and Panksepp 1999; Wyvell and Berridge 2001; Salamone, Correa et al. 2003). The latter has been proposed to involve opioid neurotransmission onto GABAergic spiny neurons within the shell region of the nucleus accumbens, since microinjections of opioid agonists into this region increase affective or hedonic facial responses to rewarding food (highly palatable sugars and fats) (Kelley, Bakshi et al. 2002).

1.2.2. SELF-ADMINISTERED DRUGS

Experimental disruption of DA transmission by discrete lesions and local intracerebral infusion of DA antagonists (especially in the shell region of the

nucleus accumbens) reduces approach towards biologically meaningful - and rewarding - stimuli or events. The general hypothesis that DA is involved in some aspect of reward and reinforcement has also built on the fact that a number of addictive drugs (e.g. amphetamine, alcohol, cocaine, morphine) seem to exert their primary action on the DA system through some combination of promoting release, preventing the transmitter's reuptake or mimicking its action in the brain (Di Chiara and Imperato 1988; Koob 1992). There is an overwhelming amount of data supporting the involvement of DA in psychostimulant reinforcement (Koob, Sanna et al. 1998). DA receptor blockers given during pairing of the drug with a specific environment impair conditioned place-preference, elicit an extinction-like increase of responding in trained animals and prevent acquisition of instrumental responding in untrained animals (Di Chiara 1995). The knock-out (KO) mice technology, although limited by a number of caveats (i.e.: phenotypic alterations due to adaptive changes) as also reinforced existing hypothesis regarding the role of DA in drug-induced reward and reward-related behaviors. Consistent with the effects of DA antagonist treatment on response to reward (Wise and Rompre 1989), deletion of DA receptors (D_1, $D_{2/3}$) either eliminates or markedly attenuates the voluntary consumption of drugs and conditioned place preference and operant responding for drugs (for review see (Holmes, Lachowicz et al. 2004). Neuroimaging techniques have provided clinical data, which favors the role of DA in the reinforcing effects of psychostimulants. For example, amphetamine-induced *euphoria* measurements in healthy humans

correlate with the reduced-binding (in the ventral striatum) of the DA receptor PET ligand [C^{11}]raclopride (Drevets, Gautier et al. 2001) (see chapter 3). In another PET study, DA transporter occupancy or blockade provoked by a single dose administration of cocaine in cocaine users was significantly associated with the magnitude of self-reported high (Volkow, Wang et al. 1997). Functional magnetic resonance imaging (fMRI) studies have also shown that the administration of cocaine to cocaine-addicted subjects increased drug-related *rush* together with regional cerebral activity in the VTA (BOLD signal).

1.3. PHARMACOLGY OF DRUGS OF ABUSE

The different molecular targets for almost every major drug of abuse have been identified (see table 1). Although drugs have a widely different repertoire of effects, which undoubtedly have different mechanisms and call into play different neurotransmitter systems, all drug targets identified are proteins that are involved in synaptic transmission and have a direct or indirect action on DA. This thesis is partly concerned with visualizing DA release following the acute administration of two of the most widely used and abused psychoactive substances: amphetamine and alcohol. The next sections will review the neuropharmacolgy of those drugs with regards to their acute mechanism of action on the DA system.

DRUG	INITIAL TARGET
Alcohol	Facilitates $GABA_A$ and inhibits NMDA glutamate receptor function
Amphetamine and derivatives	Dopamine transporters (DAT, VMAT)
Cocaine	Dopamine transporters
Opiates	Agonist at μ, δ, κ opioid peptide receptors
Nicotine	Agonist at nicotinic acetylcholine receptors
Cannabinoids	Agonist at cannabinoid receptors CB_1 and CB_2
Phencyclidine	Antagonist at NMDA glutamate receptors
Hallucinogens	Partial agonist at 5-HT_{2A} receptors
Inhalants	Unknown

Table 1 Pharmacology of drugs of abuse (websource, NIDA)

1.3.1. ALCOHOL

A variety of animal taxa consume alcohol on a regular basis through their fruit-based diet. In some cases drunkenness in wild elephants, primates, warthogs, birds and butterflies has been reported since ethanol content in sweet rotting fruits has been shown to be as high as 12% (although this represents an environmental extreme) (Dudley 2000; Dudley 2002). In this perspective, one can hypothesize that alcohol use or misuse in modern humans probably dates back to the nutritional behaviors of ancestral primates. Alcohol is considered to be a most reinforcing drug and hold an important dependence liability. Psychiatric epidemiological surveys reveal that alcohol abuse and dependency represent one of the most, if not the most, prevalent of psychiatric problems over one's lifetime.

Approximately 30% of young people get drunk regularly (consume more than 5 drinks of alcohol in a row at least every month) and 5% fit DSM IV criteria for alcohol abuse (http://www.niaaa.nih.gov/databases/).

Although the net effect of alcohol in humans is to produce sedation, anxiolytic and intoxicating effects similar to the effects seen with barbiturates and benzodiazepines, acute consumption of alcohol (depending on the dose) stimulates locomotor activity and produces a sense of well-being and mild euphoria (Charness, Simon et al. 1989). The acute effects of alcohol are subject to large inter-individual variability (heart rate, mood), the neurobiochemical basis of which has been suggested to reside in the functioning of DA mediated appetitive motivational system (Pihl and Peterson 1995).

1.3.1.1. ALCOHOL-INDUCED DA RELEASE MECHANISM

Several lines of evidence suggest a prominent role of the mesolimbic DA pathway in the acute reinforcing action of alcohol. For example rats self-administer ethanol in the VTA; a response shown to be modulated by the presence of DA agonist and antagonist in a direction that confirms the role of DA in ethanol reinforcement (Gatto, McBride et al. 1994). In vivo electrochemistry and microdialysis studies in un-anesthetized rats have indicated that acute

ethanol administration increases in a dose-dependent manner the dialysate level of striatal-DA. Furthermore, consistent with the topographic specificity of DA release observed during psychostimulants-induced reward, alcohol-induced DA release is preferential in the nucleus accumbens and the bed nucleus of the stria terminalis (Di Chiara and Imperato 1988; Carboni, Silvagni et al. 2000).

Many mechanisms are known to interact in alcohol-induced excitation of the mesolimbic reward system. On the one hand ethanol influences DA cell firing rate directly through its action on ion channels on membranes, particularly calcium and chloride (Brodie, Pesold et al. 1999). In particular, it inhibits calcium entry through voltage gated-channels thus inhibiting transmitter release. (Tabakoff and Hoffman 1996). On the other hand ethanol acts on DA neurotransmission through the indirect action of multiple neurotransmitter systems: opiatergic (κ & μ receptors), serotonergic (5-HT_3) and glutamatergic, acethylcholinergic (Nevo and Hamon 1995). In general, alcohol inhibits receptors for excitatory neurotransmitters and augments activity at receptors for inhibitory neurotransmitters. For example, similar to the action of benzodiazepine, alcohol is believed to affect brain function primarily by enhancing the function of GABA ($GABA_A$ receptors) (Koob 1992). Furthermore alcohol acts on NMDA receptors, inhibiting their functions and thereby diminishing glutamate-mediated neurotransmission (Rossetti, Hmaidan et al. 1992). A large body of studies has demonstrated that alcohol potentiates GABA inhibitory interneurons at synapses

leading to downstream release from tonic inhibition of DA containing neurons. Specifically, it reduces the firing rate of neurons in the substantia nigra pars reticulata which in turn is believed to causes a net increase in DA cell firing, and increased DA release in the striatum and nucleus accumbens (Mereu and Gessa 1985; Di Chiara and Imperato 1988). This action stimulates glutamatergic neurons that further excite DA cells burst firing (Gessa, Muntoni et al. 1985; Grace 2000).

1.3.2. AMPHETAMINE

Stimulant drugs such as the amphetamines are among the most widely used and abused of the many psychoactive compounds available. This class of drug is also referred to as *sympathomimetic* to indicate their potency to innervate the sympathetic nervous system. Their use to decrease fatigue and to heighten physical and mental abilities dates back to ancient China where people use to drink tea made with plants containing ephedrine (*Ephedra sinica*) called Ma huang which translates into "looking for trouble" (Sulzer, Sonders et al. 2005). Its medical use in Canada is restricted to the management of attention deficit hyperactivity disorder (ADHD), sleep-disorder (narcolepsy) and weight control. Nevertheless, illicit amphetamine is still widely available and extensively used for its stimulant and euphorigenic effects. Worldwide statistics indicate that 24

million people use amphetamine-type-stimulants for recreational use and the expansion of the amphetamine market is continuing (http://www.unodc.org/unodc/global_illicit_drug_trends.html).

Experimental models in animals have typically investigated the neural substrates that mediate the behavioral expression of stimulant action through measurements of locomotor activity. In humans, the main effects of amphetamine have been measured in locomotor and stereotyped activity, self-reported euphoria and excitements, appetite and mental function. Briefly, in animals and humans amphetamine initial produces hyperactivity, which at higher doses expresses itself as intense stereotyped behavior. Most often this period (of *binge*) is followed by a period of inactivity or *crash*. Continuous amphetamine treatment (at least 3 days in rats) at higher doses (48 mg/kg in rats) has been shown to be neurotoxic (Ricaurte, Seiden et al. 1984) and accompanied by hallucinatory-like behavior. This amphetamine neurotoxicity syndrome is believed to be different than amphetamine-induced psychosis (Ellison and Eison 1983). In this regard, amphetamine is known to be a potent psychotomimetic. This is based on the observation that their use in some patients with schizophrenia can intensify or precipitate psychotic symptoms (see chapter 2 and discussion).

1.3.2.1. AMPHETAMINE-INDUCED DA RELEASE MECHANISM

Amphetamine is a highly addictive psychostimulant, which promotes the release of DA, norepinephrine and serotonin. This drug crosses plasma membranes via lipophilic diffusion (Mack and Bonisch 1979). Once inside the cells, it can displace DA from secretory vesicles into the neuronal cytoplasm (Sulzer and Rayport 1990) and induce DA overflow into the synaptic cleft by facilitating outward exchange diffusion and triggering channel-like DA release (10,000 molecules per few milliseconds) through the pore of the Na^+ / Cl^- - dependent plasma membrane protein DAT (Staal, Mosharov et al. 2004). Although both synaptic vesicle and cytosolic DA pools contribute to the amount of DA released following amphetamine, the DA previously residing in the synaptic vesicles (vs DA in the cytosolic pool) represents the larger portion of amphetamine-mediated release (Jones, Gainetdinov et al. 1998). Like cocaine, amphetamine is also a substrate for the DA transporter (DAT) where it acts to inhibit DA up-take (Liang and Rutledge 1982) (Figure 4). This reversal (and blocking) of the uptake mechanism increases extracellular DA concentration thereby promoting $D_{2/3}$ autoreceptors activation which, through feedback mechanisms decreases DA cell firing (Bunney, Walters et al. 1973; Shi, Pun et al. 2000). For example decreased excitability of DA neurons has been recorded following the systemic intravenous administration of amphetamine at doses ranging from 0.25 to 5.0 mg/kg, (Groves, Fenster et al. 1981). In presence of $D_{2/3}$ autoreceptors blockers the amphetamine-induced inhibition of DA cell firing is

replaced by an excitation mediated in part through adrenergic α1 receptors (Shi, Pun et al. 2000). Amphetamine mediated DA neuron excitation is also believed to occur through the inhibition of metabotropic glutamate receptor-mediated IPSPs (Paladini, Fiorillo et al. 2001). However, under normal conditions DA release after amphetamine is impulse-independent and relies only to a small extend on calcium (Hurd and Ungerstedt 1989). DA-mediated feedback inhibition has been shown to be altered after chronic treatment and might play an important role in the development of behaviors associated with the abuse of these drugs (Wolf, White et al. 1993). Amphetamine derivatives are competitive inhibitors of MAO. Their inhibitory action on this enzyme increases DA available for release (Sulzer, Sonders et al. 2005). Amphetamine has also been noted to have an effect on tyrosine hydroxylase activity enhancing DA synthesis (Costa, Groppetti et al. 1972).

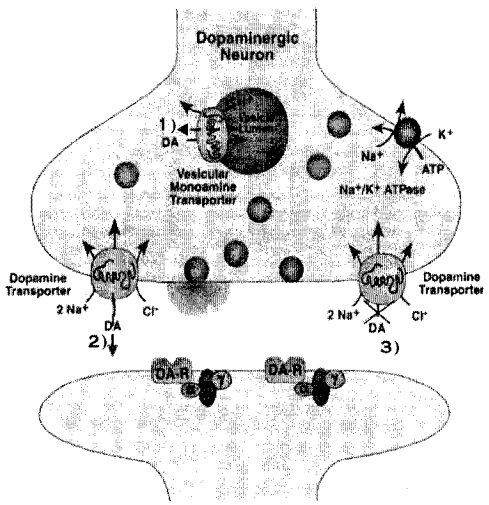


Figure 4 Schematic of the DA synapse illustrating the sites of action of amphetamine. **1**) Amphetamine acts directly on the vesicular monoamine carriers by a weak base mechanism to exacerbate vesicle depletion. **2**) Amphetamine facilitated outward exchange diffusion and triggers channel-like DA release (10,000 molecules per few ms) through the pore of the Na^+ / Cl^- -dependent plasma membrane protein DAT. **3**) Amphetamine is a substrate for the DAT, where it acts to prevent re-uptake.

1.4. DOPAMINE HYPOTHESES OF ADDICTION

Although drugs of abuse typically activate DA neural networks and neuroadaptations (including tolerance, withdrawal and sensitization: see chapter 2) induced by chronic administration, have been shown to occur downstream of DA receptors, it is unclear what neurophysiologic processes are critical for the transition from casual to addictive drug use. Addiction can be defined as overwhelming involvement with the use of a drug (compulsive use) and the loss of control over drug taking, despite adverse medical, relational or social consequences (APA 1994). A number of hypotheses have been formulated to describe how drugs of abuse, through their action on DA, might lead to addiction; most account for only some specific aspects of the whole process of addiction. A large amount of experimental models have been studied with the purpose of clarifying the link between DA and addiction, but a detailed account of the specific literature is beyond the scope of this thesis chapter. The reader is referred to the following reviews (Di Chiara 1999; Robbins and Everitt 1999; Hyman and Malenka 2001). The next paragraph briefly reviews some of the most influential theories.

Abstinence from chronic exposure to addictive drugs reduces *in vivo* DA transmission and induces a state of anhedonia that is expressed by a disinterest in normally rewarding stimuli (decreased sucrose consumption, ability to

associate rewards with a distinctive environment, and sensitivity to rewarding electrical brain stimulation) (Koob and Le Moal 1997). In line with this, early theories have suggested that withdrawal-induced anhedonia (physical / psychological dependence) fuels drug self-administration and addiction by a negative reinforcing mechanism i.e.: people use drugs to avoid the unpleasant withdrawal symptoms. This theory has been refined into the idea that the development of addiction involves counteradaptive mechanisms which change the hedonic set-point (allostasis) for reward (Solomon and Corbit 1973; Koob and Le Moal 2001). Others have argued that the excessive drive to take drugs results from an aberrant learning process (Tiffany 1990; Di Chiara 1998; Berke and Hyman 2000; Hyman and Malenka 2001). This is based on the observation that both normal learning and addiction involve some of the same mechanisms (long term potentiation; (Overton, Richards et al. 1999), molecular events (transcription factors such as ΔFosB and CREB; (Nestler 2001) and neurotransmitter systems (dopamine projections from the VTA and SNc to the nucleus accumbens and striatum, as well as glutamate inputs from the prefrontal cortex, amygdala and hippocampus). The power of drug-related cues (environment, priming dose of drug or stress) to induce a return to drug (relapse) is an example of the excessive strength of association between stimulus and response (and stimulus-reward) expressed in addiction (addiction memory)(Di Chiara 1998; Boening 2001). Furthermore, as contextual cues become associated and strengthened through repeated drug use, the neurocircuitry involved in emotional memory (obsessive

thoughts) and executive function (decision making and behavioral inhibition) become deficient. Indeed chronic exposure to drugs has been shown to impair functions of the prefrontal cortex and decrease frontocortical blood flow, glucose utilization and volume (Liu, Matochik et al. 1998)(for review see (Jentsch and Taylor 1999; Davidson, Putnam et al. 2000; Volkow 2004). In their *incentive sensitization* view of addiction Robinson and Berridge propose that the progression from initial drug use to addiction may evolve as repeated activation of the mesocorticolimbic pathway associated with repeated drug use promote an abnormal incentive state (abnormal wanting) which remain engaged even in the absence of ongoing drug use (Robinson and Berridge 2001). In a model that integrates findings from brain imaging and molecular studies, Volkow and her colleagues have suggested that in addiction while mesolimbic DA transmission is deficient, reducing responding for natural rewards, it is hyper responsive to drug-associated stimuli (increased D_1 signaling, glutamate on the PFC). This effect which would lead to an unmanageable motivation to seek drugs is though to result from cellular adaptations in the glutamatergic projection from the PFC to the NAS (Kalivas, Volkow et al. 2005). Clearly the entire scope of the addiction experience cannot be explained in one theory

CHAPTER II: Introduction II

Sensitization to psychostimulants

2.0. SENSITIZATION OVERVIEW

Altered dopamine (DA) neurotransmission is thought to play a critical role in the pathophysiology of psychotic states and addiction. However, the mechanism responsible for a dysregulated DA neurotransmission remain poorly understood. *Sensitization* is one such purported mechanism; by which repeated exposure to stimulants or stress would progressively result in heightened behavioural responsiveness, following re-exposure to stress or drug. This increased behavioural response is possibly related to increased dopaminergic neurotransmission. The study of *sensitization* in humans offers a novel perspective on the understanding of the neurobiology of the triggering and relapse of psychotic states, as well as the phenomenon of drug craving / drug seeking behaviour, consistent with several clinical observations: (1) ability of psychostimulants to induce psychosis in normal individuals and stimulant abusers, (2) ability of psychostimulants or environmental stressors to elicit disease exacerbation or relapse in stable patients with schizophrenia or bipolar affective disorder, (Lieberman, Sheitman et al. 1997; Laruelle 2000) (3) ability of environmental stressors to elicit relapse in abstinent drug users, (4) ability of drug-related cues to similarly elicit relapse. In the presented thesis we have tested the specific hypothesis that

sensitization, in healthy volunteers, as a result of repeated exposure to amphetamine, is associated with an increased DA release in the mesolimbic dopaminergic system. The present chapter provides a description of the phenomenon of sensitization along with core features and presents the theories that suggest that sensitization might play a role in chronic-relapsing disorders such as addiction and psychosis.

2.1. SENSITIZATION DEFINITION

Repeated exposure to drug (of abuse) often leads to a decreased reaction to their effects such that a larger dose is eventually required to achieve the same effect. This response known as *tolerance* is believed to results from the organism's effort to oppose the effect of the drug and maintain a homeostatic state. In some situations, *reverse-tolerance* or an increased sensitivity, i.e.: *sensitization* to the effects of drugs (or noxious stimuli) can occur. Sensitization has been defined as an experience-dependent type-plasticity, whereby repeated exposure to dependence-producing pharmacological challenge (or to a noxious stimulus) primarily functions to increase behavioural output; a simple example of this has been derived from the investigation of the gill and siphon withdrawal reflex of the Aplysia following repeated electrical stimulation (Kandel and

Schwartz 1982). An overwhelming amount of experiments have been conducted in animals to investigate the conditions that lead to behavioural sensitization. Landmark rodent models of stimulant sensitization have used intermittent moderate doses of cocaine or amphetamine pre-treatment followed by testing with a single dose in a (paired or unpaired) testing environment (Ungerstedt, Ljungberg et al. 1975; Post, Kopanda et al. 1976; Stripling and Ellinwood 1977; Post, Weiss et al. 1988; Vezina and Stewart 1989; Stewart and Badiani 1993; Vezina 1993). These early experiments have shown that behavioral sensitization to the locomotor activating and stereotypic effects of repeated stimulant administration is a replicable finding.

2.2. SENSITIZATION CORE FEATURES

The core features of sensitization are as follows (Robinson and Berridge 2000). **(1)** Sensitization results from the repeated administration of DA elevating drugs, though it has also been observed after a single dose administration (Grignaschi, Burbassi et al. 2004) and it cannot be explained by a change in the disposition of the drug (metabolism, accumulation in adipose tissue). Drugs able to induce sensitization primarily include psychostimulants, amphetamine and cocaine (Downs

and Eddy 1932; Robinson and Becker 1986; Kalivas and Stewart 1991), D_2 dopamine receptor agonists (Hoffman and Wise 1992; Mattingly, Rowlett et al. 1993), μ-opioids (Kalivas and Duffy 1987; Vezina, Kalivas et al. 1987; Kalivas and Stewart 1991), NMDA receptor antagonists (Wolf and Khansa 1991) but also nicotine (Johnson, Blomqvist et al. 1995; Cadoni and Di Chiara 2000; Miller, Wilkins et al. 2001; Shim, Javaid et al. 2001), cannabis, (Cadoni, Pisanu et al. 2001; Lamarque, Taghzouti et al. 2001) alcohol (Phillips, Roberts et al. 1997; Lessov and Phillips 1998; Correa, Arizzi et al. 2003) and caffeine (Cauli, Pinna et al. 2003).

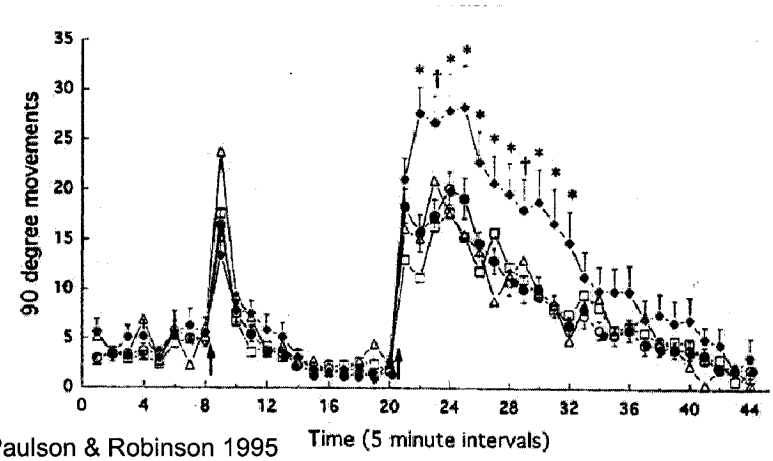


Figure 1 Locomotor-activating effects of a challenge dose of amphetamine (0.5 mg/kg), measured in hemiparkinsonian rats, 3 (empty squares), 7 (empty triangles), or 28 days after discontinuation from either saline (blue) or escalating-doses of amphetamine (red). Animals tested after 28 days of withdrawal were hypersensitive (sensitized) to the challenge dose of amphetamine (Paulson and Robinson 1995)

(2) Secondly, sensitization is characterized by an increased magnitude of behavioural response to subsequent exposure as well as a lower threshold for response triggering (ex: the progressive enhancement in rotational behaviour in rats). Explicitly the dose-response curve for behavioural effects is shifted to the left (Figure 1).

(3) Thirdly, sensitization is believed to be an enduring phenomenon; this defining feature of sensitization is mainly derived from the observation that chronic methamphetamine users remain hypersensitive to the psychotomimetic effects of amphetamine after years of abstinence (Sato, Chen et al. 1983). Behavioural sensitization to amphetamine persists undiminished for at least 12 months in rodents (Paulson, Camp et al. 1991), 28 months in non-human primates (Castner and Goldman-Rakic 1999). Behavioral changes have been associated with enduring (3.5 months), though perhaps reversible, structural reorganization of dendritic spines on medium spiny neurons of the nucleus accumbens and on pyramidal cells of the medial prefrontal cortex (Robinson and Kolb 1997; Li, Kolb et al. 2003; Li, Acerbo et al. 2004).

(4) Fourthly, sensitization is influenced by environmental context and conditioning; whereby, the exclusive repeated pairing of drug

administration with a *novel* environment facilitates the expression of sensitization (see section 2.6).

(5) Fifth, sensitization is dependent on dose and temporal pattern of drug treatment. In particular, robust sensitization is achieved by repeated intermittent low doses of pharmaceutical agents, rather than escalating or continuous doses (Vanderschuren, Tjon et al. 1997).

(6) Additionally, the phenomenon of sensitization is subject to cross-sensitization with stress. It is well established that repeated stress (maternal separation, social defeat stress, foot shock, tail-pinch, restraint) can induce heightened sensitivity to low doses of drugs (locomotor activation and drug self administration as well as neuroendocrine arousal and DA release) and inversely repeated drug exposure modify the stress response (Pani, Porcella et al. 2000; Stewart 2000; Barr, Hofmann et al. 2002; Nikulina, Covington et al. 2004; Kikusui, Faccidomo et al. 2005). The neurobiological substrate mediating cross-sensitization between stress and psychostimulants is thought to reside in the interaction between hypothalamus-pituitary-adrenal stress axis (HPA) and mesencephalic DA projections (Marinelli and Piazza 2002).

(7) Another characteristic of this phenomenon is the substantial inter-individual variability in the vulnerability to sensitize (see section 2.6). Typically, the magnitude of behavioural sensitization is greater in rats identified based on their locomotor responsiveness to a novel environment.

(8) Lastly, withdrawal plays an essential role in its development. Specifically, in the rodent the expression of neurochemical sensitization does not reach asymptote until at least two-weeks post-treatment (Paulson and Robinson 1995) (See section 2.4.2).

2.3. BEHAVIORAL CHARACTERISTICS

2.3.1. SENSITIZATION IN ANIMALS

In experimental animals, the most commonly studied drug responses showing sensitization are different forms of motor activation such as stereotyped and rotational behaviour, intra-cranial self-stimulation, and acoustic startle reflex at subsequent exposures to the same dose (Post and Kopanda 1976; Wolf and Khansa 1991; Pierce and Kalivas 1997; Tzschentke 2001).

Using alternate paradigms, a number of investigations have attempted to demonstrate sensitization to the reinforcing effects of drugs (and natural rewards) with repeated exposures. Specifically sensitization through repeated exposure to stress or psychostimulants, potentiates reward seeking behaviour: it increases instrumental responding for food, ethanol and sex (Cailhol and Mormede 2000; Wyvell and Berridge 2001), it enhances the acquisition of cue-driven (Pavlovian conditioned) amphetamine and cocaine self-administration (Woolverton, Cervo et al. 1984; Piazza, Deminiere et al. 1989; Horger, Shelton et al. 1990; Piazza, Deminiere et al. 1990; Horger, Giles et al. 1992; Robinson and Berridge 1993; Piazza and Le Moal 1996), it facilitates learning preference for places associated with drug administration (Lett 1989) and increases the breakpoint (amount of work an animal is willing to do to obtain reward) achieved on a progressive ratio schedule (Mendrek, Blaha et al. 1998; Lorrain, Arnold et al. 2000; Vezina, Lorrain et al. 2002). This and other evidence form the basis for the *incentive-sensitization theory of addiction*, suggesting that sensitization induced by drugs results in excessive "drug wanting" in face of reward cues, hence triggering *"compulsive drug pursuit"* transmission salient and wanted (Robinson and Berridge 1993; Robinson and Berridge 2001).

Behavioural sensitization has also been noted in nonhuman primate models, which might provide insight into the neural substrates of both drug-induced and idiopathic psychosis. In monkeys, a sensitizing regimen of amphetamine has been shown to enhance many amphetamine-induced behaviours and progressively induce hyper-vigilance, abnormal eye-tracking, grasping, and checking the environment for *stimuli that are not observable* to the experimenter (paranoid-like). Together these behaviours have been referred to has psychotomimetic or hallucinatory-like (Ellinwood, Sudilovsky et al. 1973; Snyder 1973; Ellison, Nielsen et al. 1981; Castner and Goldman-Rakic 1999; Castner, al-Tikriti et al. 2000; Castner, Goldman-Rakic et al. 2003). Thus far, these models have supported the role of sensitization in generating psychostimulant-induced psychosis.

2.3.2. SENSITIZATION IN HUMANS

Experimental evidence supporting the occurrence of *sensitization* in humans is limited (Szechtman, Cleghorn et al. 1988; Strakowski and Sax 1998). The most compelling is derived from longitudinal observations of methamphetamine abusers; following long periods of abstinence or remission from psychostimulants-induced psychosis, re-exposure to

methamphetamine (or stress) induces an increased behavioural response including re-occurrence of psychosis, even in lower doses than initially used (Sato, Chen et al. 1983; Yui, Goto et al. 1999).

There have been very few controlled experimental studies investigating the behavioural consequences of repeated psychostimulant exposure. Strakowski and colleagues have documented that in healthy volunteers, small oral doses of amphetamine (0.25 mg/Kg) repeated two or three times elicit a progressive increase in magnitude and duration of psychostimulants-induced eye-blink rate, motor activity and mood effects (Strakowski, Sax et al. 1996; Strakowski and Sax 1998). The observation of *sensitization* of the mood response to amphetamine was independently replicated by the same group in female healthy volunteers (Strakowski, Sax et al. 2001). As evidence of sensitization in humans, it was also proposed that PET / SPECT derived findings of enhanced DA release response to a single-dose of amphetamine reported in patients with schizophrenia could, in part, reflect *endogenous sensitization* (Breier, Su et al. 1997; Laruelle, Abi-Dargham et al. 1999).

2.4. NEURAL AND FUNCTIONAL CORRELATES

Although the process of sensitization is believed to include changes occurring over a wide network of brain areas, neuroadaptations of the mesoaccumbens DA system most likely underlie the emergence of locomotor sensitization following the repeated intermittent systemic administration of amphetamine. Sensitization involves an *induction* (see section 2.4.1) phase and an *expression* (see section 2.4.3) phase separated by a period of re-setting or *withdrawal* (see section 2.4.2) (Kalivas and Duffy 1993).

2.4.1. INDUCTION

Induction requires the stimulation of DA cell bodies in the ventral tegmental area (VTA) (A10 and/or A9 DA cell groups). In this regard, microinjection studies have shown that direct injections of psychostimulants (or opioids) in the VTA induce sensitization upon subsequent drug challenges, whereas injections at the level of DA cell terminal fields, the striatum or frontal cortex, do not (Kalivas and Stewart 1991; Hooks, Colvin et al. 1992; Kalivas and Duffy 1993; Kalivas, Sorg et al. 1993). DA receptor blockers, most consistently D_1 antagonists (Vezina and

Stewart 1989; Stewart and Druhan 1993) but also D_2 antagonists (Meng, Feldpaush et al. 1998), NMDA blockade (Karler, Calder et al. 1991; Karler, Turkanis et al. 1991; Karler, Finnegan et al. 1993; Stewart and Druhan 1993; Ranaldi, Munn et al. 2000) basic fibroblast growth factor (bFGF) antibody in the ventral tegmental area (VTA) (Flores, Samaha et al. 2000; Flores and Stewart 2000), removal of circulating corticosterone (Deroche, Marinelli et al. 1995) prevent the induction of sensitization.

Induction of sensitization is characterized by a post-receptor signalling cascade of molecular events, which includes protein phosphorylation and turnover, RNA translation and gene transcription. This stage, which starts at the site of action of the drug, is believed to ultimately result in the phenotype of structural changes associated with sensitization. For example, repeated treatment with psychostimulants, such as cocaine and amphetamine, are known to change the morphology of medium spiny neurons in the NAS, increasing the density and the number of branched spines on these neurons (and synaptic efficacy) (Robinson and Kolb 1997; Li, Kolb et al. 2003). The activation of several intracellular cascades is believed to be an instrumental mechanism of sensitization. Specifically, the downstream signalling of the cAMP-PKA pathway through G-protein coupled DA receptor stimulation, the Ca2+ calmodulin dependent kinase (CaM-K) cascade initiated by activation of

NMDA receptors and the Ras/mitogen-activated protein kinase (MAP) signal transduction cascade triggered by the transmitter brain derived neurotrophic factor (BDNF). In turn, these cascades activate programs of gene expression by inducing immediate early genes (IEG), c-Fos for example, and by up-regulating several transcription factors including cAMP response element binding protein (CREB) and ΔFosB (Nestler 2001). The induction of ΔFosB is particularly significant because it mediates stimulation of cyclin-dependent kinase-5 (Cdk5), which appears to be involved in the increased density of dendritic spines on NAS neurons after chronic cocaine exposure (Bibb, Chen et al. 2001).

2.4.2. WITHDRAWAL

The expression of sensitization is a delayed phenomenon, only becoming apparent during re-exposure to the stimulus, after a period of discontinuation (Paulson and Robinson 1995). Indeed, the most appropriate period for studying sensitization is long after acute withdrawal signs have dissipated. In fact, relative to short-term withdrawal, prolonged withdrawal periods are associated with an intensified drug reaction (Robinson and Becker 1986; Kalivas and Stewart 1991; Paulson and Robinson 1995).

Withdrawal may be considered as a sequence of phases beginning with a period of acute *crash* upon drug discontinuation and ending with long-term withdrawal; together the evolving stages of withdrawal encompass residual, transient as well as enduring behavioural and neurochemical signs. After psychostimulant binge depressive symptoms combined with irritability, anxiety and anhedonia have been noted (Gawin and Kleber 1986).

The neurochemical basis of withdrawal involves at least in part, mesocorticolimbic DA. A considerable body of evidence indicates that acute withdrawal from DA-elevating drugs disturbs compensatory processes geared at restoring steady baseline-state of DA (DA tone) (Grace 2000). Thought the spontaneous activity of VTA DA neurons does not seem to be altered during amphetamine withdrawal (Lee and Ellinwood 1989), a reduction of extracellular DA levels in the NAS as been estimated by microdialysis (Rossetti et al., 1992). In this respect, DA autoreceptor sensitivity is known to undergo time dependent changes during withdrawal; specifically, after short withdrawal somatodendritic D_2 autoreceptor regulation of impulse generation is believed to be desensitized (Kalivas and Stewart 1991). Withdrawal from repeated psychostimulant treatment has also been found to induce changes in DAT

mRNA and protein levels (Kuhar and Pilotte 1996). Again, these reported changes have been inconsistent. Activations of the HPA axis during withdrawal has also been shown to a be a key player in the resetting of biological parameters, priming the organism to sensitization (Deroche, Marinelli et al. 1995; Deroche, Marinelli et al. 1997).

2.4.3. EXPRESSION

The anatomical substrates for the expression of sensitization consists of changes at multiple sites, occurring over an entire circuit of interconnected cortical and sub cortical structures, deemed critical for the translation of biologically salient stimuli, into adapted motor and non-motor outputs (Pierce and Kalivas 1997). DA is a critical player of this system, and its projections to NAS and the prefrontal cortex (PFC) have been recognized as key neural substrates of behavioural sensitization (Le Moal and Simon 1991; Koob 1992; Pierce and Kalivas 1997). One general hypothesis is that sensitization is related to a progressively developing change in the balance of neurotransmitters within these systems such that the magnitude and threshold of a behavioural response is shifted (Pierce and Kalivas 1997). Sensitization-related changes in pre and postsynaptic neurotransmission include increased excitatory glutamatergic input from PFC and reduced GABAergic inhibition from NAS and ventral pallidum

to the cells of the VTA, both of which could contribute to increased DA release at the level of the NAS and striatum.

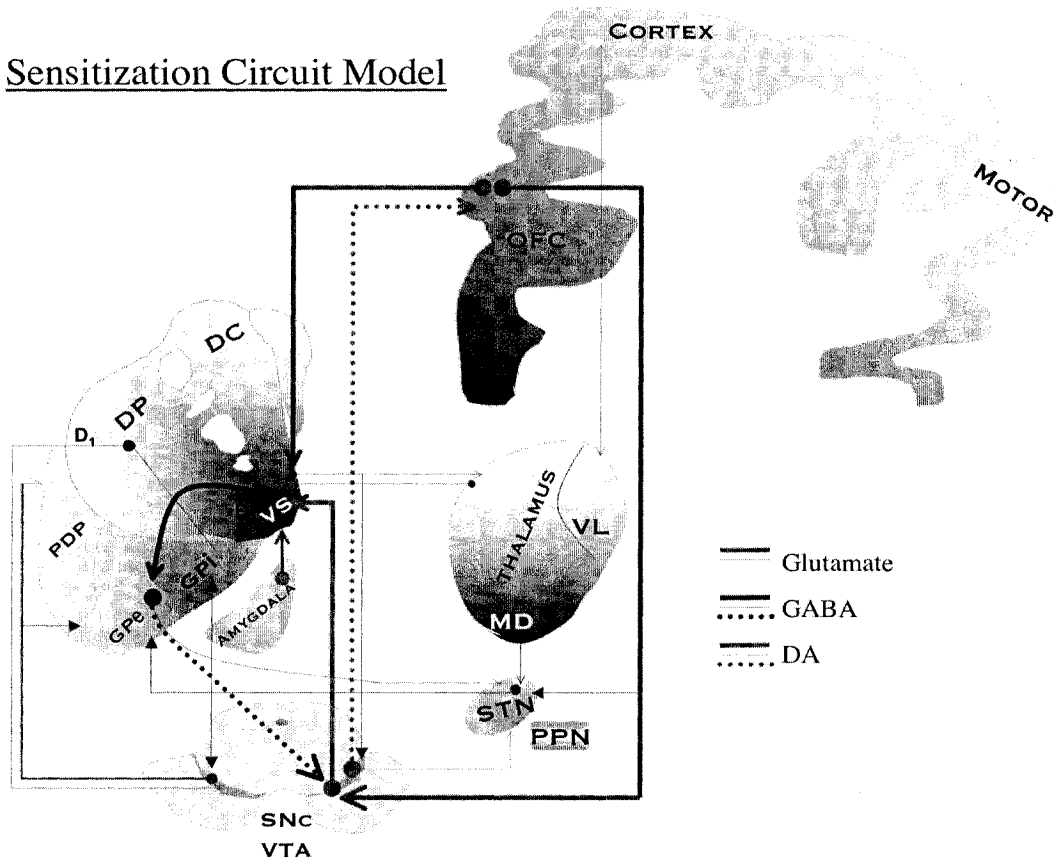


Figure 2 Box-and-arrow diagram, representing connections between sub-nuclei of the basal ganglia, limbic system, cerebral cortex and the thalamus. The primary neurotransmitter content and the major projections are represented by the colored arrows: blue, GABA; red, glutamate; green, DA. The changes in neurotransmission associated with the expression of behavioral sensitization are represented by the bold lines: continuous lines indicating increases, while dotted lines represent decreases in neurotransmission. GPe, external globus pallidus; GPi, internal globus pallidus; SNc, substantia nigra pars compacta; SNr substantia nigra pars reticulata; STN, subthalamic nucleus; VTA, ventral tegmental area; OFC, orbitofrontal cortex; PPN, pedoculopontine nucleus; VL, ventro-lateral, MD, mediodorsal thalamus; NAS, Nucleus accumbens.

2.4.4. DOPAMINERGIC MECHANISMS

One of the most common neurochemical correlates of sensitization is enhanced DA transmission at the level of the NAS upon re-exposure to a depolarizing or pharmacological challenge (for review see: (Pierce and Kalivas 1997) (Robinson and Becker 1986; Wolf 1998; Vanderschuren and Kalivas 2000) (Post, Weiss et al. 1988; Vezina 2004). Studies reveal that DA neurotransmission in the NAS has a time course characterized by reduced activity upon drug discontinuation, but hyper responsiveness following re-exposure to psychostimulants, after a week of withdrawal (Kolta, Shreve et al. 1985).

Different pre and post-synaptic events are thought to mediate the augmented DA transmission during the expression of sensitization. The sensitization-induced increase of DA release has, in part, been attributed to adaptive changes in DA transporter **1)** kinetics and **2)** expression (Izenwasser and Cox 1990; Benmansour, Tejani-Butt et al. 1992; Giros, Jaber et al. 1996) Findings show DAT upregulation upon withdrawal of psychostimulants, followed by a selective reduction following a longer drug-free period in the shell of the NAS (Kuhar and Pilotte 1996; Pilotte, Sharpe et al. 1996). Changes occurring in **3)** DA autoreceptor sensitivity and **4)** DA synthesis have also been reported, though less consistently

(Kalivas and Duffy 1988; Gifford and Johnson 1992). Changes in **5)** calcium conductance involving activation of calcium-calmodulin kinase (CAM-KII) have been reported. Findings suggest that repeated amphetamine administration leads to the emergence of a novel calcium-dependent mechanism for DA release because sensitized, but not acute DA responses to amphetamine are abolished when CAM-KII is blocked (Pierce and Kalivas 1997). CAM-KII-related mechanisms induced by amphetamine-sensitization include phosphorylation of synapsin I (Iwata, Ito et al. 1999). This mechanism is thought to have a role in augmenting the pool of DA-containing vesicles available for exocytosis. **6)** Long-term potentiation-like mechanisms thought to facilitate DA transmission (Overton, Richards et al. 1999), via increases in glutamate efflux on midbrain DA neurons, have been invoked.

Finally, although stimulation of post-synaptic receptors in the NAS is necessary for the expression of sensitization, the bulk of evidence suggests that neither the development nor expression of sensitization entails changes in D_1, D_2, DA receptor density (Kleven, Perry et al. 1990; Henry and White 1991; Farfel, Kleven et al. 1992; King, Ellinwood et al. 1994; Bonhomme, Cador et al. 1995; Zhang, Walsh et al. 2000) however D_3 over-expression has been reported (Guillin, Diaz et al. 2001). In addition,

affinity changes in sensitization have been reported by some (Seeman, Tallerico et al. 2002) but not others (Pierce and Kalivas 1997).

2.5. CONTEXT AND CONDITIONING

Conditioned stimuli, or secondary reinforcers (CS; discret cues or envrironmental / contextual cues) are stimuli with formerly motivationally neutral properties that gain incentive properties by their predictive association with primary reinforcers. Both human and non-human primates studies have shown the implication of the DA system in conditioning and reward prediction (Schultz, Dayan et al. 1997) (de la Fuente-Fernandez, Phillips et al. 2002). When drug administration (unconditioned stimuli, US) is paired with a distinct environmental context (CS) or a discrete cue the later acquire the ability to elicit conditional responses including drug-like psychomotor effects (Pavlov 1923; Pavlov 1927). By the same bias, repeated psychomotor stimulant treatment, in addition to producing sensitization to the psychomotor-activating effects of drugs, increases response (neurochemical and behavioral / motivational) to their associated cues (Robinson, Browman et al. 1998). Whether sensitization simply reflects the addition of the conditioned response to a stable unconditioned pharmacological response has been a subject of debate. There is considerable evidence showing that

the context in which drugs are administered largely impact on their ability to induce psychomotor sensitization (Badiani, Camp et al. 1997; Crombag, Badiani et al. 2000; Crombag, Badiani et al. 2001). Indeed several studies have shown that the expression of sensitization can be entirely context-specific i.e.: absent in an environment previously not paired with drug administration, even after treatment that induces robust sensitization (Stewart and Vezina 1991; Anagnostaras and Robinson 1996; Battisti, Uretsky et al. 2000). However, different paradigms geared at dissociating associative vs non-associative (non context-specific) sensitization have shown that the CS alone does not promote locomotor sensitization but rather other environmental properties would act to facilitate sensitization through mechanisms independent of associative learning. In this regard studies have shown that a distinct and relatively *novel* environment (not the home cage) potentiates sensitization even in specific conditions that degrade the ability of contextual stimuli to acquire CS properties i.e.: after habituation. The mechanism proposed for this facilitation might reside in that exposure to a novel environment activates the HPA axis, increasing the release of corticosterone (Badiani, Browman et al. 1995).

There is some evidence that the expression of sensitization depends on context, however there is also evidence for non-associative sensitization i.e.: an increased in the unconditioned response to the drug.

This is based on the findings that sensitization measured as increased DA release is observed in vitro from slices preparation (Robinson and Becker 1982), in rats anesthetized with chloral hydrate during pretreatment and in animals continuously housed in the test chambers (Segal and Kuczenski 1997).

2.6. INDIVIDUAL DIFFERENCES

From a large number of individuals who use psychostimulants only a few will ever develop addiction or drug-induced psychosis, hence the need to identify reliable predictors of individual response to drug and vulnerability to sensitization. There is a great deal of evidence to indicate that personality factors play an important part in understanding patterns of addiction. A number research has lead to evidence of a relationship between personality factors, and alcohol and drug addiction (de Wit and Richards 2004). Personality factors that appear to be central to this relationship are novelty seeking and impulsivity.

In animals, response to a novel environment is the most used experimental paradigm to distinguish high responders to drugs: highly reactive rats, as rated by their locomotor behavior when placed in a novel

environment, consistently show a greater propensity for drug self-administration, are more sensitive to reward by food, have a higher level of DA activity in the NAS under basal condition and demonstrate an increased and prolonged secretion of glucocorticoids (GC) in response to stress; unlike low reactive rats, high reactive rats show pronounced sensitization to locomotor properties of stimulants (Piazza et al., 1990, 1991; Dellu et al., 1996; Rouge-Pont et al., 1993, Hooks et al., 1991). Some of these biobehavioural markers in rats have been compared to Novelty Seeking (NS) personality traits in humans, characterized by a "tendency toward the activation of behavior" such as exploratory behavior in response to novelty and/or "impulsive decision making, extravagance in the approach to cues of reward, and quick loss of temper" (Dellu et al., 1996).

In humans, the Cloninger Tridimensional Personality Questionnaire (**TPQ**; Cloninger, 1987, 1988; Cloninger et al., 1991), the Eysenck Extroversion and Psychotism scale (**EEP**; Eysenck, 1953, 1990), and the Zuckerman's Sensation-Seeking Inventory (**SSI**; Zuckerman, 1994) are some of the most common psychometric instruments used to measure individual differences in response to novelty. Despite some subtle terminological and conceptual differences [the TPQ uses four novelty seeking subscales: [1] Exploratory-Excitability vs. Stoic-Reserve, [2]

Impulsiveness vs. Reflection, [3] Extravagance vs. Reserve, and [4] Disorderliness vs. Regimentation; the SSI, four axes: [1] Thrill and adventure-seeking, [2] Experience-seeking and [3] Disinhibition; the EEP three axes: [1] Extraversion, [2] Psychoticism, [2] Neuroticism], all three instruments are believed to refer to a common temperament, related to investigatory behaviour and sensitivity to novelty (Jaffee and Archer, 1987). Healthy volunteers scoring high on Novelty Seeking are prone to sensitization of the mood response to amphetamine (Sax and Strakowski 1998); likewise, detoxified alcoholics who score high on novelty seeking are more susceptible to relapse (Meszaros et al., 1999). Conversally, novelty seeking, in the absence of other risk factors, does not predict response to the reinforcing-effects of amphetamine (Corr and Kumari, 2000). It has been suggested that novelty seeking [Impulsivity / Exploratory-extraversion] might be related, in some significant part, to DA neurotransmission (Gerra et al. 2000): differences in novelty seeking would reflect interindividual variability in DA neurotransmission, hence a distinct disposition to engage in behaviors motivated by positive incentive and/or reward (Depue and Collins, 1999; Koob et al., 1993; Panksepp, 1986; Stewart et al., 1984, Bardo et al., 1996).

In vivo brain imaging studies have recently started to investigate the association between biological factors and personality traits. These

studies have mostly been interested in looking at the association between levels of D_2 dopamine receptor (and dopamine transporter) and personality. The main findings of these studies is that low levels of in vivo D_2 receptor binding in the striatum is associated with high *detachment* scores (high aloufness, social irritability) (Farde, Gustavsson et al. 1997; Laakso, Vilkman et al. 2000) as rated by the Karolinska Scales of Personality (Schalling, Asberg et al. 1987).

In the present studies (study 1, 2, 3), we have used the NS / TPQ subscore as a possible predictor of acute and sensitized DA responses to amphetamine. Using exploratory analyses, we examined the relationship between specific drug induced D_2 binding and personality traits from the Tridimensional Personality Questionnaire.

2.7. RELEVANCE TO CHRONIC-RELAPSING DISORDERS

It has been postulated that pathological forms of behavioral and neurochemical plasticity, i.e.: sensitization, might be a factor contributing to chronic relapsing disorders such as addiction, psychosis, post-traumatic stress-disorder – to name a few. Particularly, sensitization is believed to be

a vulnerability factor in the development of addictions (Robinson and Berridge 2001). In humans, some aspects of sensitization may increase craving for drugs even if, as a result of tolerance, the actual enjoyment of drugs is diminished. Another form of sensitization observed both in humans and in non-human primates is a syndrome of amphetamine-induced paranoia. The involvement of the DA neurotransmitter system in both the reinforcing effects of drugs of abuse (see chapter 1) and in psychosis and schizophrenia (see 2.7.1) has been demonstrated. Dopaminergic transmission sensitization presumably resulting from repeated drug (or stress) -induced DA stimulation may therefore underlie the development of both these disorders. We have presented the *incentive sensitization* view of addiction in chapter 1. The next section will serve to introduce the concept of *endogenous sensitization* as a model of psychosis development by first presenting the DA hypothesis of psychosis.

2.7.1. THE DA HYPOTHESIS OF PSYCHOSIS

For many decades the predominant biological theory has been that DA hyperactivity in the brain is the underlying cause of schizophrenia (Carlsson and Lindqvist 1963). This idea has been supported by indirect pharmacological evidence: the root of the hypothesis residing in the

discovery that the DA antagonist chlorpromazine reverses the symptoms of schizophrenia (Delay, Deniker et al. 1952). Since then several similar antipsychotic compounds have been produced all sharing the ability to antagonize D_2 receptors; in fact the potency of antipsychotic runs parallel to activity on D_2 receptors (Seeman and Lee 1975). The ability of drugs to induce psychosis by increasing dopaminergic tone constitutes a second line of evidence supporting the DA hypothesis of schizophrenia. Amphetamine, which release DA in the brain, can induce some psychosis-like symptoms in otherwise healthy individuals and trigger positive symptoms and relapse in remitting patients with schizophrenia (Angrist, Corwin et al. 1987); as well Parkinson's patients treated chronically with the precursor of DA L-dopa have been observed to exhibit periods of psychotic symptoms (Cummings 1992). Imaging studies have also contributed evidence in support of the DA hypothesis of psychosis. For one, studies investigating presynaptic DA metabolism with PET have shown that schizophrenia (drug-free) is associated with abnormal patterns of L-[^{11}C]dopa utilization in corticostriatal systems (Elkashef, Doudet et al. 2000; Gefvert, Lindstrom et al. 2003); specifically results indicate that the synthesis of DA is elevated within the striatum and parts of medial prefrontal cortex in schizophrenia (Lindstrom, Gefvert et al. 1999). PET studies using [^{11}C]raclopride have also shown an exaggerated amphetamine-provoked DA release along with positive symptom

exacerbation in schizophrenia (Laruelle, Abi-Dargham et al. 1996; Breier, Su et al. 1997; Abi-Dargham, Gil et al. 1998; Laruelle, Abi-Dargham et al. 1999). An extension to the DA hypothesis of schizophrenia has emerged to explain the negative symptoms of schizophrenia: hypofrontality lack of goal directed behaviors, aloofness, impaired cognitive function (working memory). This theory is based on the premise that schizophrenia is associated with a deficit in dorsolateral prefrontal cortex (DLPFC) DA function, presumably of neurodevelopmental origin (Weinberger 1987; Davis, Kahn et al. 1991; Friedman, Temporini et al. 1999). Indirect evidence has accumulated to support the involvement of altered prefrontal DA function in the pathophysiology of cognitive impairments associated with schizophrenia, including: decreased perfusion in frontal regions (Weinberger, Berman et al. 1992; Malaspina, Harkavy-Friedman et al. 2004), reduced tyrosine hydroxylase and length of DA transporter positive fibers (Akil, Pierri et al. 1999), upregulation of D_1 receptors correlating with poor working memory (Abi-Dargham, Mawlawi et al. 2002). In addition to possible contributions to negative symptoms and cognitive impairment, a deficit in prefrontal DA innervation in schizophrenia has been suggested to contribute to the disinhibition of subcortical DA (Laruelle, Abi-Dargham et al. 1996; Breier, Su et al. 1997; Abi-Dargham, Gil et al. 1998) through a negative feedback on VTA

activity (Pycock, Kerwin et al. 1980; Deutch 1990; Bubser and Koch 1994; Thompson and Moss 1995).

2.7.2. ENDOGENOUS SENSITIZATION

It has been postulated that one of the etiopathogenic processes involved in the emergence of positive symptoms of schizophrenia is a pathological form of neural plasticity. This pathophysiological model of schizophrenia, defined as *endogenous sensitization* has been put forth in a comprehensive review (Lieberman, Sheitman et al. 1997). This model builds upon the neurodevelopmental (genetic and epigenetic) theory of schizophrenia, which postulates that the disease is the consequence of a pathological process occurring *in utero* or early in development (perinatal) and proposes *neurochemical sensitization* has a mechanism explaining the late onset (early adulthood), the longitudinal course as well as the relapsing-remitting expression of the illness. Specifically, the illness would unfold in a series of stages. **(1)** Neuromaturational abnormalities (*fetal development*) in synaptic connectivity in the cortex are believed to be at the origin of the process of endogenous sensitization. These abnormalities would translate into cortico-subcortical connectivity deficiency and in a failure of the cortex (prefrontal glutamate hyofunction) to regulate DA

firing. In turn, this disconnectivity would lead to an excess of subcortical DA, which would result in sustained hyper stimulation of D_2 receptors making the system vulnerable to the development of sensitization. **(2)** Through the repeated failure of normal homeostatic and buffering mechanism, a deficient cellular adaptation to physiological events (stress) would occur during the prodromal phase. In other words, during this phase in adolescence the consequences of the disrupted neuronal system and of its repeated failure to inhibit DA release would progress from silent vulnerability to overt symptomatology (ie: the emergence of psychosis). **(3)** During the end phase the sensitization process has become self-perpetuating leading to the development of a neurotoxic residual illness state.

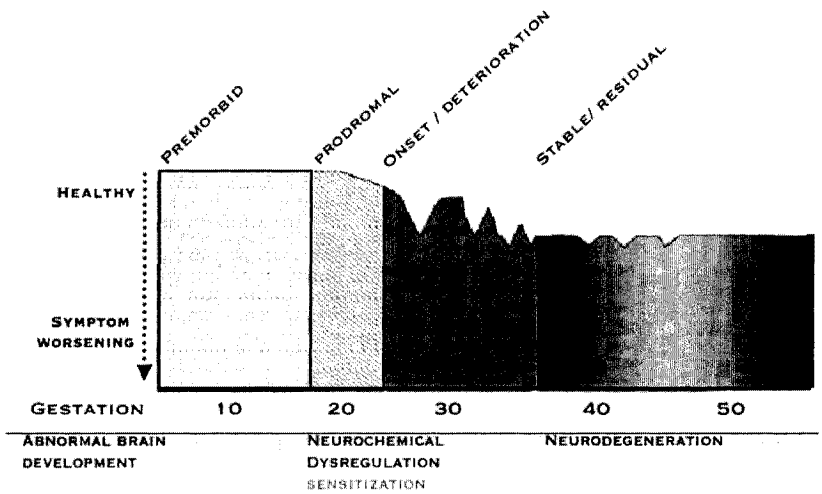


Figure 3 Course of Schizophrenia. Symptom worsening is represented as decreasing on the y axis. Adapted from Lieberman.

CHAPTER III: Methodological overview

Positron emission tomography & [^{11}C]raclopride.

3.0. POSITRION EMISSION TOMOGRAPHY & [^{11}C]RACLOPRIDE

Positron Emission Tomography (PET) produces three-dimensional (3D) images of a biochemical process *in vivo* by combining computed tomography with the tracer kinetic assay method (Phelps 1991). Essentially, PET allows visualizing the distribution and time-course of positron-emitting radioisotopes (tissue activity time course); among the most commonly used positron-emitting nuclides in PET are ^{11}Carbon, ^{13}Nitrogen, ^{15}Oxygen and ^{18}Fluorine. This technology makes use of biologically active compounds (eg.: receptor ligands) bearing short-lived positron-emitting isotopes, known as radiotracers and of appropriate mathematical models that describe the radiotracer's kinetics. The use of PET radiotracers has provided tremendous promise when probing processes such as membrane transport, metabolism, neurotransmitter synthesis and ligand-receptor interactions. PET has proven to be a powerful research, drug development and clinical tool, that can be used to elucidate the neurochemical underpinning of pathophysiological states, to provide diagnostic or prognostic information about disorders ranging from dementia and movement disorders to schizophrenia and drug abuse and to determine the principle of action

(drug kinetic, affinity and occupancy) and the efficacy of new pharmacotherapies.

This chapter will introduce the basic theoretical concepts of 3D PET imaging with a primary focus on the use of PET for imaging the dopamine (DA) neurotransmitter systems (which has been described in chapter 1) to probe the mysteries of how drugs such as stimulants affect the brain.

3.1. RADIOTRACER PRODUCTION

The first step in a PET experiment is the production of a radioactive isotope (radioisotope) by means of a particle accelerator known as a cyclotron. This machine creates radioactivity by disturbing the natural balance between neutrons and protons in the atomic nucleus. For example, the overall effect of bombarding carbon atoms with deuterons (ions of the stable hydrogen isotope, ^{2}H) in the cyclotron is to transform a stable isotope to a neutron-deficient element, which undergoes nuclear decay by spontaneously emitting a positron (a particle of equal mass and opposite charge to that of the electron: or anti-electron). Essentially the energy emitted from the nuclear decay is the signal that is recorded by the PET camera. The radioisotope is synthetically introduced into a

biologically relevant precursor molecule by means of a biosynthesizer; this operation produces what is called a radiopharmaceuticals. Radiopharmaceuticals with high selectivity for a specific class of neuroreceptors are conventionally called radioligands.

3.2. PROPERTIES OF AN *IN VIVO* RADIOLIGAND

A certain number of considerations need to be addressed before initiating research with a new radioligand. The standard in defining the suitability of a new radioligand resides not only in its selectivity for a receptor protein (pharmacological specificity) but also in a number of other requirements which, when appropriately combined insures that the specific physiological or pharmacological process of interest is adequately and safely measured. First amongst desirable properties is the tracer's brain penetrance. A radioligand must have a low molecular weigh (less then 400-600 Dalton) and a high degree of lipophilicity in order to pass the blood-brain-barrier. However excessive lipopilicity increases binding to plasma protein therefore reducing both brain up-take and the intra-cerebral ratio of tracer specifically bound to the receptor pool versus that which is bound to non-specific proteins. Thirdly, the affinity of the ligand should combine tight specific binding with fast clearance from the

receptor compartment. In other words, optimal affinity should allow equilibrium between the rate of association (K_{on}) and the rate of dissociation (K_{off}) from receptors during the physical half-life of the radioisotope (Innis 2002). Fourthly, a PET ligand must have good specific activity: high radioactive yield and small mass (usually < 0.01 μg/kg). Typically, but without clear consensus, the mass dose of a radioligand should occupancy no less then 5%, (ideally around 1%) of the receptor pool to be considered an appropriate tracer, meaning one which does not have pharmacological effects. Lastly a good PET ligand should have a high chemical purity. Table 1 lists examples of DA-related radioligands that have presently reached the clinical state; this list is not an exhaustive list.

Table 1 List of clinically available ligands for DA receptors

Isotope	Ligand	Receptor	Pharmacological effect
^{11}C	N-propyl-norapomorphine	$D_{2/3}$	Agonist
^{11}C	Raclopride	$D_{2/3}$	Antagonist
^{11}C / ^{18}F	Fallypride	$D_{2/3}$	Antagonist
^{11}C/^{18}F	Spiperon Methyl-spiperone	$D_{2/3}$	Antagonist
^{11}C	Pimozide	$D_{2/3}$	Antagonist
^{11}C	SKF 82957	D_1	Agonist
^{11}C	SCH 23390	D_1	Antagonist
^{11}C	NNC 112 9756	D_1	Antagonist

3.3. RECORDING POSITRON EMISSION

In clinical applications, PET radioligand studies require that a very small amount, or a tracer dose of a radiopharmaceutical be introduced into the subject, usually by intravenous injection. As mentioned, radioisotopes are unstable and decay by a process involving positron emission. Briefly, positrons emitted from a decaying nucleus travels a short distance in the surrounding tissue before encountering a free electron, The two particles combine in a matter-antimatter annihilation from which gamma rays (511 keV each) of opposite directions emerge. The tomograph's highly sensitive scintillation detectors made of dense crystalline materials (bismuth germanium oxide, BGO) captures the source of annihilation based on the external detection of coincidence rays between two detectors on opposite sides of the scanner. The angular and linear positions of the coincidence events are measured and stored into a sinogram (or matrix). Sophisticated algorithms are then used to reconstruct the sinogram data to an image that depicts the localization and concentration of the positron-emitting radioisotope. Only a fraction of gamma rays actually reach the external detectors; some are absorbed as they interact with head tissues of different density. Therefore, a transmission scan is performed to accurately compensate for attenuated photons. This static scan yields a map of the density that is then used to

correct the emission recording for attenuation. Figure 1 is a schematic of a PET experiment.

All experiments that compose this thesis were performed on a CTI/Siemens ECAT HR + PET (Knoxville, TN) camera with lead septa removed to allow for 3D acquisition. This high-resolution tomography with intrinsic resolution 4.8 X 4.8 X 5.6 mm FWHM provides 47 contiguous trans-axial slices. For all studies data acquisition is started at tracer injection after a 10-minute transmission scan. Images are reconstructed using a 128 X 128 matrix by applying a Hanning filter with cut off frequency of 0.4 cycles per pixel.

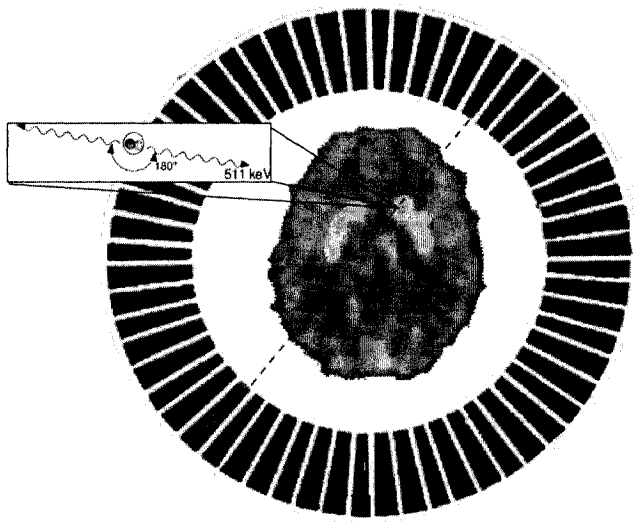


Figure 1 Schematic representation of a Positron Emission Tomography scan showing the annihilation event from which gamma rays (511 keV each) emerge, and the detection of the signal by scintillation detectors located 180^0 from each other.

3.4. KINETIC MODELS

Ultimately a full determination of the quantitative information available from the PET image requires a bio-mathematical kinetic model, which describes the different components in which the radiopharmaceutical participates and that contribute to the externally detected total signal. The analysis of a ligand's kinetics is understood under the theoretical framework of tissue compartments. A tissue compartment is a physiological space in which the tracer concentration is assumed to be homogeneous at all times. For instance, a three-compartment configuration has been described for brain-tissue: a blood compartment *(C1)*, an intracerebral compartment in which the tracer is free or nonspecifically bound *(C2)*, and a specifically bound compartment *(C3)*. The tracer's transport between compartments is described as a series of rate constants (K_1-K_4) (Figure 2).

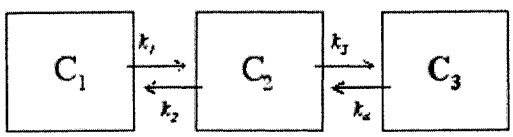


Figure 2 Compartmental descriptions of a radioactive tracer's concentration, uptake, transport and clearance in brain tissue. C_a blood compartment; C_2 displaceable compartment composed of free and non-specifically bound ligand; C_3 specifically-bound compartment. K_1 rate constant from blood to tissue; K_2 rate constants; K_3 bimolecular association rate constants (K_{on}); K_4 dissociation rate constants (K_{off}).

Analysis of reversible radioligand binding is based on a simple model, called the law of mass action. The later assumes a theoretical relationship between occupancy and ligand concentration where the rate of binding is proportional to the product of the concentration of receptors and ligand. The change in tracer concentration in any given compartment can be expressed in mathematical terms as the amount of tracer entering and leaving per unit time. For reversible ligands i.e.: that do not form a covalent bounds with the receptor (like the antagonist raclopride), the relationship between the specifically bound and the free and non-specifically bound tracer satisfies the Michaelis-Menten equation. Transfer between the bound and free compartments are expressed by the rate constants of association (k_{on}) and dissociation (k_{off}). The plot of the Michaelis-Menten equation illustrates that at receptor saturation the bound ligand is equal to the maximum number of available receptor (B_{max}) and the receptor dissociation constant (K_d) is equal to the free ligand concentration at 50% of maximal binding. It is therefore assumed that tissue tracer concentration is related to the number of receptors to which the radiotracer ligand binds selectively. However, methods are required to distill from images of radioactivity the parameter which reflects purely the process under study ie.: the binding potential of the ligand to the receptor. The term "binding potential" (defined as the ratio between B_{max} and K_d) was introduced as a PET outcome measure of the capacity of a given

tissue for ligand-binding site interaction, since B_{max} and K_d are inseparable in a single PET experiment (Mintun, Raichle et al. 1984).

3.4.1. SIMPLIFIED REFERENCE TISSUE MODEL

For experiments performed at tracer doses (high specific activity), the binding potential (BP) or equilibrium volume of distribution in the bound compartment (C_3) equals the product of receptor density and affinity (B_{max}/K_D). This can be expressed as:

$$B_{\max} f_2 / K_D^t \left(1 + N_f / K_D^d\right)$$

Where $B_{\max}$ is the total concentration of D_2 binding sites, f_2 the free fraction of radioligand in tissue, K_D^t the equilibrium dissociation constant of the radioligand, N_f the concentration of free dopamine in tissue, and K_D^d the equilibrium dissociation constant of dopamine at the D_2 receptor (Lammertsma and Hume 1996; Gunn, Lammertsma et al. 1997). As mentioned above (see section 3.4) quantification of receptor kinetics have to be derived from the total tissue equilibrium volume of distribution ($C_t = C_2 + C_3$; free + bound) and requires information from an input function. The input from arterial blood (arterial function) can be used, however one

robust approach, which circumvents arterial cannulation, relies on the use of a reference region with a negligible presence of binding sites. This reference-tissue model makes the assumption that the volume distribution in the free compartment (C_2) is constant across the brain therefore allowing the reduction of parameter (rate constants). Given this, the outcome measures (C_3) can be computed indirectly by subtracting C_t of a reference region from C_t in regions of interest. The simplified reference tissue method (SRTM), frequently referred to as the Lammertsma method, is well documented as a robust and stable reference tissue method for the estimation of [^{11}C]raclopride's binding to $D_{2/3}$ receptors after a bolus injection (Lammertsma and Hume 1996). In contrast to the original reference tissue model Lammertsma's one-tissue compartment method allows additional parameter reduction (3 parameters rather than 4).

3.5. PARAMETRIC MAPPING

One way to retain spatial information in the data is to apply the pharmacokinetic model (such as the SRTM described above) at the finest spatial scale, determined by the image digitization matrix - the pixel. By estimating a parameter at each pixel a map of the parameter spatial distribution, or parametric map can be made (Gunn, Lammertsma et al.

1997). The parametric image then contains the most complete yet succinct summary of the data. Parametric images can be investigated with statistical techniques to test the spatial difference in parameter distribution between conditions. A voxel-wise statistical mapping method using the SRTM model has been developed for dual scan subtraction studies (activation vs baseline). This method applies nonlinear least-squares theory on the scan's dynamic information to estimate the parameters of the kinetic model (SRTM) and utilizes the residuals to calculate their associated variance; thereby increasing the number of degrees of freedom and providing a better estimate of the (voxel-wise) standard deviation (Aston, Gunn et al. 2000) (figure 3).

3.6. ANATOMICAL DEFINITION

Anatomical areas cannot readily be identified on PET images. In order to make PET-derived data (parametric BP map) anatomically relevant, a structural (MRI) scan from individual subjects is used and aligned with each subject functional PET study using computer-assisted image co-registration techniques such as the one developed by Collins et al., (1994) (Collins, Neelin et al. 1994). Each subject participating in the studies that compose this thesis underwent a whole brain high-resolution

T_1-weighted MRI using a gradient echo pulse sequence (TR 9.7 ms, TE 4 ms, flip angle 12°, FOV 250, matrix 256 X 256). MR images were corrected for image intensity non-uniformity this adjustment eliminates anatomy-dependent field inhomogeneity or what is called the shading artifact (Sled, Zijdenbos et al. 1998). Images where then linearly and non-linearly resampled to an average MRI template (MNI average 305) in stereotactic space a process called spatial normalization. Images are automatically classified into labels of tissue type (gray matter, white matter, CSF, or background) and segmented into labels representing structural and functional anatomy (Collins DL 1995; Collins, Zijdenbos et al. 1999). These binary representations of anatomical and functional structures can then be matched to PET data.

3.7. PARTIAL VOLUME EFFECT

The degree of spatial diffusion of imaged radioactivity is such that each point source appears spread-out, distributed over a Gaussian curve. In the center of the Field of View (FOV), the spatial resolution of a multi-ring (16 rings of bismuth germanate oxide detectors) commercial PET scanner such as the HR+ camera (Siemens/CTI, Knoxville, TN) is 4mm

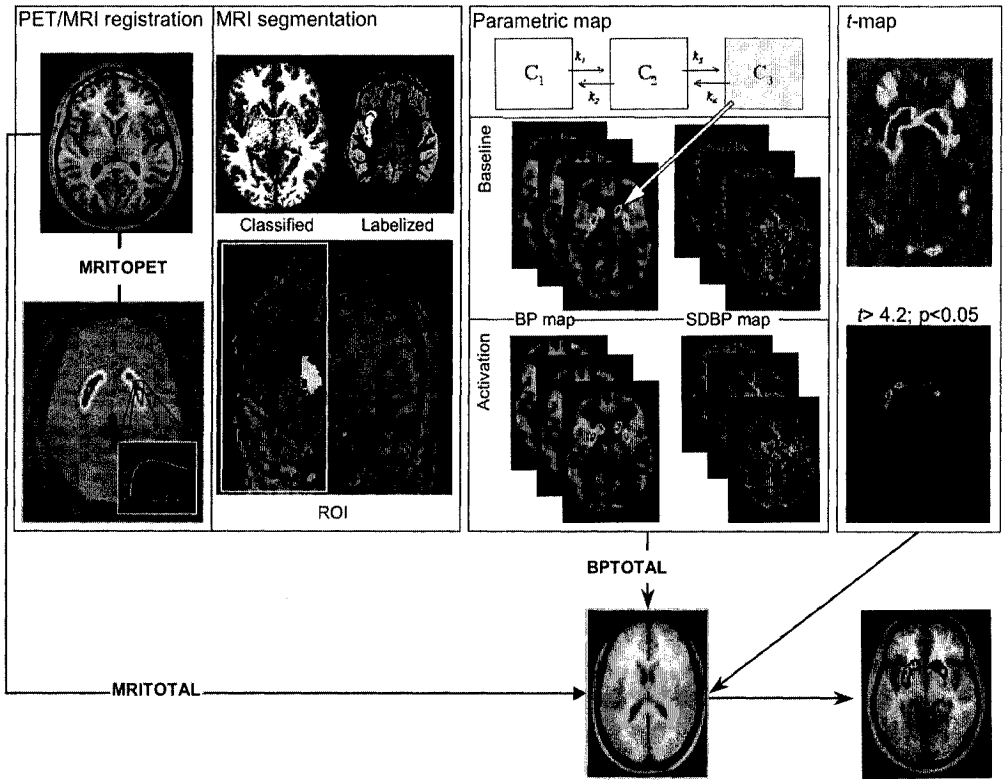


Figure 3 From left to right, ***PET / MRI registration***) T_1-weighted MRI linearly fitted to stereotaxic space (Talairach) using a standard brain template (MRITOTAL; MNI 305 average brain) and aligned with a single [^{11}C]raclopride PET image of which the 26-frame data (60 minutes line graph) has been collapsed into a single frame (MRITOPET using AIR algorithm; Collins et al., 1994). ***MRI segmentation)*** T_1-weighted MRI in Talairach space is corrected for intensity non-uniformity and classified into white matter, grey matter and cerebrospinal fluid. After the classification, the image is segmented into anatomical labels (ANIMAL). Next, the labelized image is manually reworked into specific ROI using predefined criteria. ***Parametric map)*** Voxel-by-voxel application of the SRTM yielding BP map with SDBP maps which can be transformed to a common space (Talairach) in view of statistical analysis. ***t-map)*** Voxel-wise *t*-statistic illustrating differences in radioligand binding between baseline and activation scans can be thresholded to statistically significant level and overlaid an average MRI.

full-width-at-half-maximum (FWHM) when operating in 3D acquisition mode; where FWHM indicates the distance at which two discrete point sources begin to be distinguishable (at half peak activity). Due to the limited resolution of the tomograph the measured voxel intensity in the PET image is the sum of the true activity at that voxel weighted by its regional spread function i.e.: by the loss of signal from the structure and contamination from adjacent brain regions (spill-over and tissue fraction effect). This point-spread artifact, which results in an over or under estimation of the true tracer activity, is called partial volume effect (PVE). Correction for the effect of partial volume is a crucial step to a better estimation of true local tissue concentration of radioactivity. The geometric transfer method (GTM) is an efficient and well-validated MRI-based algorithm, which assumes homogeneous true activity across anatomical domains and uses binary representation of anatomical structures to correct for the regional spread effect (Rousset, Ma et al. 1998; Aston, Cunningham et al. 2002). We used a partial volume correction (PVC) model, which can be directly applied to the parametric image (Aston, Cunningham et al. 2002). This method uses a more accurate noise model that includes correlated and uncorrelated components, as well as error estimates (obtained from background) used for weighting in the subsequent kinetic fitting.

3.8. DIRECT MEASUREMENT OF DOPAMINE IN HUMANS

The progression from *ex vivo* receptor binding methods (e.g. autoradiography) as means of exploring dopaminergic neurotransmission (REF), to the *in vivo* PET approach occurred two decades ago when the radiolabeled neuroleptic 3-N-[^{11}C]methylspiperone ([^{11}C]NMSP) was used for the first time to visualize the distribution of dopamine and serotonin receptors in brain (Wagner, Burns et al. 1983). The imaging of neuroleptic drug binding *in vivo* therefore presented the interesting possibility of measuring competitive binding with endogenous neurotransmitter present as ligands (Friedman, DeJesus et al. 1984). Since then this non-invasive imaging modality has allowed investigating dopaminergic transmission at the level of plasma membrane receptors with **1)** D_1 receptor antagonists including SCH-23390 and NNC (112 & 756) (Billard, Ruperto et al. 1984; Andersen, Gronvald et al. 1992); with **2)** $D_{2/3}$ receptor antagonists such as the butyrophenone [^{3}H]spiperone and its methylated analog [^{11}C]NMSP (Leysen, Gommeren et al. 1978; Wagner, Burns et al. 1983; Lyon, Titeler et al. 1986), the benzamide [^{11}C]raclopride (Ehrin, Farde et al. 1985; Kohler, Hall et al. 1985), the high-affinity antagonist ligand [^{18}F]fallypride (Mukherjee, Yang et al. 1997; Mukherjee, Yang et al. 1999); with **3)** the D_1 receptor agonist SKF-82957 (Zhou, Katki et al. 1991)

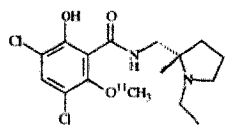
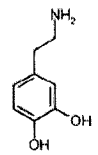
and with **4)** D_2 receptor agonists, N-[^{11}C]propyl-norapomorphine (NPA) and apomorphine (Seeman, Watanabe et al. 1985; Zijlstra, van der Worp et al. 1993). Several tracers also exist to map non-receptor proteins such as dopamine up-take sites ([^{11}C]Cocaine, b-[^{123}I]CIT, FE-[^{123}I]CIT, [^{123}I]/[^{18}F]/[^{11}C]FP-CIT, [^{18}F]/[^{11}C]CFT, [^{123}I]/[^{11}C]Altropane, [^{123}I]/[^{11}C]PE2I, and [^{11}C]methylphenidate; (Aquilonius, Bergstrom et al. 1987; Kilbourn, DaSilva et al. 1993; Stoessl and Ruth 1998; Bergstrom, Tupala et al. 2001) and enzymes, [^{18}F]DOPA (Gjedde, Reith et al. 1991; Cumming and Gjedde 1998). The development of these DA system ligands have made it possible to directly study disorders such as schizophrenia (Wong, Wagner et al. 1986; Laruelle, Abi-Dargham et al. 1996; Abi-Dargham, Gil et al. 1998; Abi-Dargham, Rodenhiser et al. 2000), Parkinson's disease (Brooks 1997), Tourette's syndrome (Wong, Ricaurte et al. 1998), drug addiction (Lindsey, Gatley et al. 2003), ADHD (Rosa Neto, Lou et al. 2002; Krause, Dresel et al. 2003; Jucaite, Fernell et al. 2005). It has allowed to investigate the response to pain (Pertovaara, Martikainen et al. 2004; Martikainen, Hagelberg et al. 2005), the neurochemical basis of personality (Breier, Kestler et al. 1998; Laakso, Vilkman et al. 2000) and the role of DA in behaviors such as feeding (Small, Jones-Gotman et al. 2003; Volkow, Wang et al. 2003) gambling (Zald, Boileau et al. 2004) and playing a video game (Koepp, Gunn et al. 1998). Table 3 provides a list of findings that have emerged from using radiotracers of the DA system in human.

Of particular interest to the focus of this thesis is the suitability of the substituted benzamide neuroleptic [^{11}C]raclopride to measure pharmacological and behavioral modulation of dopamine release in the intrasynaptic cleft (for review see (Laruelle 2000).

This agent is a $D_{2/3}$-like DA receptor antagonist, which has high affinity and selectivity for those receptors, in low and high receptor affinity states. In high receptor density regions (the striatum) [^{11}C]raclopride has been shown to provide an accurate and reliable estimate of the $D_{2/3}$ receptor density. Due to its low nanomolar receptor affinity and fast dissociation rate, [^{11}C]raclopride is highly sensitive to competition from endogenous DA within the synapse (K_D = 1.2 nM) (Kohler, Hall et al. 1985; Seeman, Guan et al. 1989) Table 2. This antagonist ligand competes with endogenous DA for overlapping sites on $D_{2/3}$ receptors; specifically the agonist (DA) binds in a pocket composed of the receptors transmembrane helices 3, 4, 5, and 6, whereas the antagonist binds in the same pocket but with minimal contact with transmembrane helix 5 (Javitch, Shi et al. 2000). Because DA shows selectivity for the activated receptor state and [^{11}C]raclopride does not, the competition between the ligand and the endogenous neurotransmitter is measured at the level of receptors in high-affinity state (Laruelle 2000). In principle, the

competition model assumes that changes in endogenous DA synaptic levels are translated into changes in the availability of binding sites or binding potential (BP) for the exogenous radioligand [^{11}C]raclopride.

Table 2 Characteristics of Raclopride and Dopamine as ligands for DA receptors

Raclopride	Specificity	MW	Affinity (Nm)	Lipophilicity (Log P)	Reference
	$D_{2/3}$ Antagonist	497	1.2	1.3	(Kohler, Hall et al. 1985)
Dopamine 	D_1 and $D_{2/3}$ Agonist	133	4	Hydrophilic	(Lidow, Goldman-Rakic et al. 1989)

This model has been supported by a variety of protocols using [^{11}C]raclopride. For example, when a tracer dose of [^{11}C]raclopride is administered conjointly or following an experimental procedure that elevates DA, be it a pharmacological challenge with amphetamine (Dewey, Smith et al. 1993), cocaine (Schlaepfer, Pearlson et al. 1997), methylphenidate (Volkow, Wang et al. 1994), nicotine(Brody, Olmstead et al. 2004), ketamine (Vollenweider, Vontobel et al. 2000; Kegeles, Martinez et al. 2002), psilocybin (Vollenweider, Vontobel et al. 1999), an experimental stress challenge (Pruessner, Champagne et al. 2004)(Trier

mathematical challenge test, (Kirschbaum, Pirke et al. 1993) a cognitive task (video game) (Koepp, Gunn et al. 1998) or expectation of reward (de la Fuente-Fernandez, Ruth et al. 2001; de la Fuente-Fernandez, Phillips et al. 2002), measurements of tracer receptor occupancy reveal a reduction in receptor BP, relative to baseline. Conversely, depleting endogenous DA (α-methyl-para-tyrosine (AMPT), β-hydroxybutyrate, reserpine, acute dietary phenylalanine-tyrosine depletion) leaves more receptors available for tracer binding (Ginovart, Farde et al. 1997; Leyton, Dagher et al. 2003; Montgomery, McTavish et al. 2003; Verhoeff, Christensen et al. 2003). Stability and reproducibility of [^{11}C]raclopride have been addressed through test retest studies. Both long-term stability of baseline [^{11}C]raclopride BP (11 months) (Hietala, Nagren et al. 1999) and within-subject reproducibility of amphetamine-induced decrease in ligand binding (Kegeles, Zea-Ponce et al. 1999) have been reliably found. Table 3 summarizes finding obtained from competition studies using [^{11}C]raclopride in humans.

Table 3 Changes in [^{11}C]raclopride binding potential induced by challenges interfering with dopamine transmission in humans

Challenge	Subjects *(n)*	ROI	Effect on SRTM-derived [^{11}C]raclopride BP	Reference
Amphetamine i.v. 0.3 mg /kg	14 Healthy controls	Ventral Striatum	-17.8 ± 13.8%	(Martinez, Slifstein et al. 2003)
Amphetamine i.v. 0.3 mg /kg	16 Healthy controls	L Ventral Striatum R Ventral Striatum	-10.6 ± 5.1 % -9.27 ± 5.6 %	(Oswald, Wong et al. 2005)
Amphetamine i.v. 0.3 mg /kg	7 Healthy controls	Ventral Striatum	-14.8 ± 11.5 %	(Drevets, Gautier et al. 2001)
Amphetamine p.o. 0.3 mg /kg	12 Healthy controls	Ventral Striatum	-13% ± 5 %	(Cardenas, Houle et al. 2004)
6 hours post-Amphetamine p.o. 0.3 mg /kg	12 Healthy controls	Ventral Striatum	-18% ± 6 %	(Cardenas, Houle et al. 2004)
Methylphenidate i.v. 0.5 mg /kg	7 Healthy controls	Ventral Striatum	-18.4 ± 8.7 %	(Wang, Volkow et al. 1999)
Cocaine i.v 48 mg.	11 Cocaine users	Ventral Striatum	N.R.	(Schlaepfer, Pearlson et al. 1997)
Nicotine 1 cigarette/ 1.1 mg	20 Smokers	Ventral Striatum	–25.9% to –36.6%	(Brody, Olmstead et al. 2004)
Nicotine 3-6 cigarettes	10 Smokers	Caudate	–21.2 to 17%	(Barrett, Boileau et al. 2004)
Psilocybin p.o. 0.25 mg/kg	7 Healthy controls	L Caudate R Caudate	-19.9 ± 5.5% -18.6 ± 5.4%	(Vollenweider, Vontobel et al. 1999)
Ketamine i.v. bolus 0.12 mg/kg + 0.65 mg/kg 1h constant infusion	9 Healthy controls	Ventral Striatum	-11.2 ± 8.9%	(Breier, Adler et al. 1998)
Phenylalanine Tyrosine depletion + Amphetamine 0.3 mg kg p.o.	8 Healthy controls	Ventral Striatum	11.8 ± 11.9%	(Leyton, Dagher et al. 2003)
Alpha-methyl-para-tyrosine p.o. 5250 mg (29h)	6 Healthy controls	Ventral Striatum	13.3 ± 5.9%	(Verhoeff, Christensen et al. 2003)
Experimental Cognitive Stress (30 min.)	10 Healthy controls / High and low parental bonding	Ventral Striatum	-0.02 ± 4.4 -16.7 ± 8.6	(Pruessner, Champagne et al. 2004)
Metabolic Stress	6	Striatum	-5.49 %	(Adler, Elman et

2-deoxy-glucose 40 mg/kg	Healthy controls			al. 2000)
Gambling monetary reward	10 Healthy controls	Lateral Putamen	−8.7 ± 11.1	(Zald, Boileau et al. 2004)
Video Game	Healthy controls	Striatum	N.R.	(Koepp, Gunn et al. 1998)
Non-hedonic food + 20 mg Methylphenidate	10 food-deprived healthy controls	Dorsal Caudate	-10 ± 7 %	(Wang, Volkow et al. 2002)
Reward Expectancy Placebo	6 Parkinson's patient	Ventral Striatum	≈ -23%	(de la Fuente-Fernandez, Phillips et al. 2002)
Yoga Nidra meditation	8 Meditation teachers	Ventral Striatum	-7.9 ± 7 %	(Kjaer, Bertelsen et al. 2002)

Direct measurements of amphetamine-induced DA release in primates using *in vivo* microdialysis have established a quantitative relationship between changes in DA release and changes in D_2 BP, such that, for every 1% change in BP, there appears to be a corresponding 44% change in synaptic DA concentration (Endres, Kolachana et al. 1997; Laruelle, Iyer et al. 1997). In humans, endogenous competition studies using amphetamine result in observations consistent with the occupancy model: an i.v. or oral dose of d-amphetamine (0.3 mg/kg) results in a 15%and 10% decrease in BP (Drevets, Price et al. 1999; Leyton, Boileau et al. 2002).

Yet, the mechanisms underlying the changes in BP in response to changes in synaptic DA concentrations are not fully elucidated, nor are all the data entirely consistent with the occupancy model. It is believed that

G-protein-coupled receptor internalization may be invoked in the interaction between the radioligand and $D_{2/3}$ receptors. The activation of these receptors by endogenous or artificial agonist ligands promotes multistep molecular and cellular events of which inhibition of the second messenger system, and desensitization and recycling of the receptors via phosphorylation-dephosphorylation processes (Dumartin, Caille et al. 1998). Rapid internalization of $D_{2/3}$ receptors in the endosomal compartment in the face of amphetamine-induced released dopamine, leads to fewer externalized receptors available for [^{11}C]raclopride binding (Sun, Ginovart et al. 2003). Hence, the distribution of receptors in the external and internal pool depends on the relative presence of the agonist. In vitro studies have demonstrated the occurrence of agonist mediated receptor internalization in several transfected cell lines for beta-adrenergic, muscarinic cholinergic, serontonergic, D_1 and $D_{2/3}$ dopamine receptors, delta-opiate and 5HT-1_A (Barton, Black et al. 1991) Vickery and Von Zastrow, 1999)(Riad, Zimmer et al. 2004; Zimmer, Riad et al. 2004).

PREFACE CHAPTER IV

The following section introduces the first manuscript (**study 1**) included in the present thesis. This manuscript entitled "*Amphetamine-induced increases in extracellular dopamine, drug wanting, and novelty seeking: a PET/[^{11}C]raclopride study in healthy men*" has been published in Neuropsychopharmacology 2002; 27(6):1027-35.

Changes in DA transmission induced by pathology or by pharmacological or cognitive challenges can be measured *in vivo* by recording positron emission following a [^{11}C]raclopride injection (see chapter 3). **Study 1** of the present thesis was carried out in an effort to test the feasibility as well as validate the use of PET and [^{11}C]raclopride as a new tool (in our laboratory) to investigate the DA system in humans *in vivo*. This study was a necessary starting point, which served to launch subsequent studies investigating the nature of DA in the subjective effects of drugs.

Over the years, the PET / [^{11}C]raclopride technique has lead to a renewal of interest with regards to the nature of DA release in drug-reinforcement and addiction. Where basic research studying prototypic

addictive drugs in animal models fall short, this technical advance has opened the possibility to investigate the role of DA in triggering and / or maintenance of drug-use with relation to many human factors such as personality characteristics, psychological stress, subjective pleasurable effects, social factors, self-perceived loss of control. Imaging studies in humans have for the most part corroborated the importance of DA release in the reinforcing effects of stimulant drugs after acute pharmacological challenges (see chapter 1). Specifically, the reinforcing, "euphorigenic" effects of cocaine (Volkow, Fowler et al. 1999) and amphetamine during acute administration (Drevets, Gautier et al. 2001) have respectively been associated with the magnitude of DA transporter blockade and that of synaptic DA release (decreased [^{11}C]raclopride BP).

In **study 1** of this thesis we report that amphetamine-induced DA release is related to "drug-wanting" and not to "drug-liking" *per se*. In contrast to existing neuroimaging findings, which for the most have supported the view that DA is related to the positive / pleasurable effects of drugs, results to **study 1** parts from this original view, in that it suggests that DA might have a role in driving the motivation to use drugs (motivational role). **Study 1** of the present thesis also asks the following questions: Can DAergic responses to drugs explain individual differences in the vulnerability to addiction? Do personality factors interact with brain

mechanisms to influence the development of an addiction? Results presented in the following manuscript suggest that indeed, differences in personality can substantially modulate the neural (DA) response as well as the desire for stimulant drugs ("drug-wanting").

Although the interaction between endogenous DA levels and [^{11}C]raclopride for binding on $D_{2/3}$ receptors is complex, involving both competitive and non-competitive mechanisms, it is believed that the observation of decreased [^{11}C]raclopride binding in the limbic striatum after amphetamine reported in **study 1** can be interpreted has increased synaptic DA. Thus **study 1** validates the suitability of this tool (in our laboratory) to investigate the effects of acute drugs on human behavior.

Amphetamine-Induced Increases in Extracellular Dopamine, Drug Wanting, and Novelty Seeking A PET/[^{11}C]Raclopride Study in Healthy Men

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Running Head: Dopamine, Wanting and Novelty Seeking

ABSTRACT

Animal studies suggest that psychostimulant drugs preferentially increase synaptic dopamine (DA) levels in the ventral striatum. Individual variability is substantial, and might be a trait related to both novelty seeking and drug seeking. Here, eight healthy men had two PET [^{11}C]raclopride studies, once with placebo, once with *d*-amphetamine (0.30 mg/kg, p.o.). PET data were analyzed using voxel-by-voxel brain parametric maps to identify statistically significant clusters and manually drawn regions of interest (ROI). Compared to placebo, *d*-amphetamine decreased [^{11}C]raclopride binding potential (BP) with preferential effects in bilateral ventral striatum. Change in BP in the statistically generated cluster correlated with self-reported drug-induced drug wanting ($r=0.83$, $p=0.01$) and the personality trait of Novelty Seeking-Exploratory Excitability ($r=0.79$, $p=0.02$). The same associations were seen in the manually drawn ROI in ventral striatum but not in dorsal putamen or caudate. Changes in extracellular DA did not correlate with mood. Mesolimbic DA might mediate interest in obtaining reward rather than reward, *per se*.

Key words: **addiction, reward, reinforcement, nucleus accumbens**

INTRODUCTION

Preclinical studies indicate that psychostimulant drugs increase extracellular dopamine (DA) levels with preferential effects in ventral striatum (Di Chiara and Imperato 1988). The behavioral significance of increased DA, though, remains a subject of debate. One influential theory suggests that mesolimbic DA mediates rewarding, possibly pleasurable effects of addictive drugs (Wise and Bozarth 1982). Other work suggests that DA signals interest in rewards (Stewart 1984), the expectation that a reward is forthcoming (Schultz 1998)or wanting reward as opposed to liking it (Robinson and Berridge 1993).

Significant individual variability in stimulant drug-induced DA release is apparent. Some evidence suggests that this variability reflects a pre-existing trait. Predictors of the variability include elevated exploratory behavior in novel environments (Hooks, Jones et al. 1991)high levels of sugar feeding (Sills and Crawley 1996) and a predisposition to rapidly acquire drug self-administration (Zocchi, Orsini et al. 1998).

In humans, psychostimulant drugs of abuse elicit a range of behavioral effects, including mood elevation and increased drug wanting (Uhlenhuth, Johanson et al. 1981; Jaffe, Cascella et al. 1989; Cousins,

Stamat et al. 2001). Functional neuroimaging studies with labeled $D_{2/3}$ benzamide ligands suggest that relatively high *d*-amphetamine doses (0.2 - 0.3 mg/kg, given i.v.) increase extracellular DA levels in human striatum (Laruelle, Abi-Dargham et al. 1995; Breier, Su et al. 1997; Drevets, Gautier et al. 2001). The magnitude of the drug-induced DA release correlates with the self-reported euphoria (Laruelle, Abi-Dargham et al. 1995; Drevets, Gautier et al. 2001); however, it remains unclear whether the mood-elevation is mediated by DA or instead reflects other effects of the drug. High amphetamine doses increase both catecholamine and serotonin release whereas lower doses primarily increase catecholamines (Kuczenski and Segal 1989). Mood-elevating effects of cocaine are reported to be reduced by lowering serotonin transmission (Aronson et al 1995). In comparison, stimulant drug-induced mood elevation is not decreased consistently by DA antagonists (Brauer and De Wit 1997).

The present study addressed three questions. First, could a relatively low oral dose of *d*-amphetamine elicit detectable changes in [^{11}C]raclopride binding? Second, would the effect on extracellular DA be larger in ventral than dorsal striatum? Third, would amphetamine-induced increases in extracellular DA be related to drug-induced drug wanting, drug-induced mood-elevation, or the personality trait of novelty seeking?

METHODS

SUBJECTS

Eight non-smoking men (Table 1) were recruited using newspaper advertisements. All were healthy, as determined by a physical exam, standard laboratory tests, and an interview with the SCID-NP. None had a personal or first-degree relative history of axis I psychiatric disorders. On the day of each PET scan, all tested negative on a urine drug screen sensitive to cocaine, opiates, PCP, LSD, THC and amphetamines (Triage Panel for Drugs of Abuse). The study was carried out in accordance with the Declaration of Helsinki and was approved by the Research Ethics Committee of the Montreal Neurological Institute. All subjects gave informed written consent.

PET AND MRI

All subjects were scanned with an ECAT HR+ tomograph (CTI/Siemens). 3D dynamic images were blurred to 4.2 mm FWHM in the transaxial direction using a Hanning filter. Prior to tracer injection,

transmission scans were performed using ^{68}Ga for attenuation correction. Sixty minutes before tracer injection ([^{11}C]raclopride, 10mCi, 2-min bolus), an intravenous catheter was inserted into the subject's arm, and an oral dose of *d*-amphetamine was administered (0.0 or 0.3 mg/kg). PET studies were conducted between 14:00 and 17:00.

Each subject underwent a magnetic resonance imaging (MRI) scan (160 slices, 1 mm thick) obtained with a Philips Gyroscan (1.5 Tesla). Each MRI was linearly re-sampled, transferred into standard stereotaxic space, co-registered to each subject's PET images (Collins, Neelin et al. 1994), and then transferred into Talairach space (Talairach and P 1988).

Voxelwise [^{11}C]raclopride binding potential ($BP=Bmax_{free}/Kd$) was calculated to generate parametric images (Gunn, Lammertsma et al. 1997). *t*-Maps were then constructed, representing voxel-by-voxel *t*-tests of change in [^{11}C]raclopride BP between the amphetamine *vs.* placebo scans (Aston, Gunn et al. 2000). Clusters of voxels with a $t > 4.2$ were considered statistically significant. BP values for each subject were extracted from the region identified by the *t*-map as well as from *a priori* identified regions of interest (ROI). ROI were drawn manually on aligned transverse slices from each subject's MRI in stereotaxic space on caudate nucleus, dorsal putamen, and ventral striatum bilaterally. The boundaries for each ROI

were labeled well within the gray matter of the structure to reduce artifact due to misalignment or partial volume effects (Figure 1). The rostrocaudal extent of the ROI relative to the anterior commissure was approximately the same for all subjects, z = 2 to 15 mm for dorsal caudate, z = 2 to 10 mm for dorsal putamen, and z = -4 to -8 mm for ventral striatum, corresponding approximately to nucleus accumbens (Talairach and P 1988). Left and right ROI values were combined. ROI drawn on five consecutive 1 mm slices in cerebellum served as the reference region.

SUBJECTIVE EFFECTS OF AMPHETAMINE

Subjective effects induced by *d*-amphetamine were measured with the Addiction Research Center Inventory (ARCI) Benzedrine scale (Haertzen, Hill et al. 1963), and 10 visual analog scales (VAS) labeled Want Drug, Like Drug, Euphoria, Mind Racing, Alert, Energetic, Excited, Rush, Anxiety, and High (Bond and Lader 1974). The VAS were administered at five time points, immediately before drug (or placebo), and at 30, 60, 90 and 120 minutes after drug. Changes in VAS measures were analyzed as the peak change from pre-drug administration to the end of the test session. The ARCI was administered only once each session, 120 minutes post-drug.

TRIDIMENSIONAL PERSONALITY QUESTIONAIRE

All subjects completed the Tridimensional Personality Questionnaire (TPQ; (Cloninger 1987; Cloninger, Przybeck et al. 1991). The TPQ measures four dimensions, novelty seeking, harm avoidance, reward dependence, and persistence. Cloninger has proposed that TPQ traits of novelty seeking, harm avoidance, and reward dependence are mediated in significant part by activity in dopamine, serotonin and norepinephrine pathways, respectively.

PLASMA AMPHETAMINE

Blood samples were drawn immediately before *d*-amphetamine administration and 90 minutes later, which corresponded to the mid-point of the 60-min PET scan. Plasma concentrations of amphetamine were analyzed with gas chromatography (Paetsch, Baker et al. 1992).

STATISTICS

Plasma *d*-amphetamine concentrations, self-report questionnaire data, and [^{11}C]raclopride region of interest (ROI) BP values were analyzed by within groups ANOVAs and, when appropriate, Newman-Keuls *post hoc* tests. Correlations were assessed with Pearson's correlation coefficient.

RESULTS

On the day that subjects received active drug, plasma levels of d-amphetamine increased from 0.0±0.0 ng/ml before drug administration to 10.5±8.2ng/ml 90 minutes later corresponding to the mid-point of the PET scan ($p = 0.001$). *d*-Amphetamine was not detected on the placebo test day.

ARCI scores were significantly higher on the *d*-amphetamine test day compared to placebo ($p = 0.02$). *d*-Amphetamine, compared to placebo, also lead to significantly greater peak changes on VAS labeled Alert ($p = 0.002$), Mind Racing ($p = 0.003$), Energetic ($p = 0.01$), and Excited ($p = 0.03$) (Table 2).

[^{11}C]Raclopride BP values varied with both region of interest (ROI) and drug administration. A drug x ROI ANOVA yielded significant main effects of drug ($F(1,7) = 55.6$, $p < 0.001$) and ROI ($F(2,14) = 31.2$, $p < 0.0001$), and a significant drug x ROI interaction ($F(2,14)=8.96$, $p = 0.003$). As seen

in post-mortem and previous PET studies (Hall, Sedvall et al. 1994; Mawlawi, Martinez et al. 2001), [^{11}C]raclopride BP values were significantly lower in ventral striatum, compared to dorsal caudate and putamen ($p < 0.001$) and this was evident on both the placebo and amphetamine test days ($p < 0.001$) (Table 3).

d-Amphetamine significantly decreased [^{11}C]raclopride BP in ventral striatum ($p = 0.05$) but not in dorsal caudate ($p = 0.68$) or putamen ($p = 0.96$) (Table 3). The same regional specificity was seen when change in BP was calculated as percent change from the placebo test day ($F(2,14) = 6.01$, $p = 0.01$). The change in BP was significantly greater in ventral striatum (-10.7±9.5%) than in dorsal caudate (-1.5±9.3%, $p = 0.02$) or putamen (0.4±9.0, $p = 0.02$). Parametric mapping analyses yielded the same results. Compared to placebo, a significant effect of *d*-amphetamine on [^{11}C]raclopride BP was restricted to bilateral ventral striatum (Peak Effect: x, y, z = 32.1, -3.2, -6.5, $t = 7.34$) (Figure 2). In this *t*-map identified cluster there was a mean -8.4±19.6% decrease in [^{11}C]raclopride BP.

Inter-regional correlations suggested that the effect of *d*-amphetamine on extracellular DA was qualitatively as well as quantitatively different in ventral *vs.* dorsal striatum. The change in [^{11}C]raclopride BP in dorsal caudate and putamen correlated with each

other ($r^2 = 0.88$, $p < 0.001$) but not with changes in ventral striatum or the *t*-map ($p > 0.70$). In comparison, there was a significant correlation between change in [^{11}C]raclopride BP in the *t*-map identified cluster and the manually drawn ventral striatum ($r^2 = 0.67$, $p = 0.01$) supporting the proposition that both methods of analysis identified the same changes in tracer binding. Finally, there was a non-significant trend for older age to be associated with reduced *d*-amphetamine-induced changes in [^{11}C]raclopride BP in the ventral striatum ($r^2 = -0.46$, $p = 0.06$) yet not in other regions ($p > 0.20$). *d*-Amphetamine-induced changes in [^{11}C]raclopride BP in the ventral striatum correlated significantly with two TPQ items, overall Novelty Seeking ($r^2 = 0.56$, $p = 0.03$) and the Novelty Seeking subscale, Exploratory Excitability ($r^2 = 0.55$, $p = 0.05$). Associations with changes in BP in the *t*-map identified region were similar (

Table 4 and Table 3). Change in BP in ventral striatum did not correlate significantly with any other TPQ scale or subscale ($p > 0.10$). Change in [^{11}C]raclopride BP in dorsal caudate or putamen did not correlate with any TPQ scale or subscale ($p > 0.10$). *d*-Amphetamine-induced increases in extracellular DA in the ventral striatum correlated significantly with only one reported effect of the drug, VAS Want Drug on the *d*-amphetamine test day (*t*-Map: $r^2 = 0.69$, $p = 0.01$; ROI: $r^2 = 0.38$, $p = 0.10$) (

Table 4; Figure 4) and Novelty seeking and drug-induced drug wanting were significantly correlated with each other. TPQ Exploratory Excitability predicted drug wanting on the drug administration test (r^2 = 0.74, p = 0.006), an association not seen on the placebo test (r^2 = 0.004, p = 0.89).

Drug-induced change in extracellular DA in dorsal caudate and putamen correlated negatively with a one reported effect of the drug, VAS mind racing (dorsal caudate: r^2 = -0.70, p = 0.009; dorsal putamen: r^2 = -0.55, p = 0.03); however, these negative correlations – not expected *a priori* - were driven by a single outlier, and the associations were no longer significant when the point was removed (dorsal caudate: p > 0.15; dorsal putamen: p > 0.65). Change in BP did not correlate significantly with any other self-report variable.

DISCUSSION

The present study suggests that (i) a moderately low oral dose of *d*-amphetamine (0.3 mg/kg) significantly increases extracellular DA in human striatum, (ii) this effect of *d*-amphetamine occurs preferentially in ventral striatum, (iii) *d*-amphetamine-induced increases in extracellular DA are more closely related to drug-induced drug wanting than mood-

elevation, and (iv) the personality trait of novelty seeking predicts greater amphetamine-induced DA release and amphetamine-induced drug wanting. A non-significant trend ($p = 0.06$) suggested that the ability of *d*-amphetamine to induce DA release in ventral striatum might decrease from age 20 to 30.

The effect of 0.3 mg/kg, given p.o. on [^{11}C]raclopride BP was smaller and more circumscribed than that reported to occur when the same dose is given intravenously. *d*-Amphetamine, 0.3 mg/kg, p.o. decreased [^{11}C]raclopride BP in the ventral striatum by an average of 10%; significant changes were not seen in dorsal caudate or putamen. In comparison, the same dose of *d*-amphetamine given i.v. decreases [^{11}C]raclopride BP by 10-20% in striatum as a whole (Breier, Su et al. 1997); (Drevets, Gautier et al. 2001). A recent report suggests that 0.3 mg/kg *d*-amphetamine given i.v. also has larger effects on [^{11}C]raclopride BP in the ventral striatum than other subcompartments (anteroventral striatum, -15.4±10.6%; dorsal putamen, -10.2±10.6%; dorsal caudate, -4.5±8.2%) (Drevets, Gautier et al. 2001). The present study with a low oral dose more clearly identifies preferential effects in ventral striatum, and this regional specificity is supported by both manually drawn ROI and statistically generated clusters.

There was substantial individual variability in the ability of *d*-amphetamine to decrease [^{11}C]raclopride BP. This variability in extracellular DA in ventral striatum was associated with variability in self-reported drug-induced drug wanting. To our knowledge, previous neuroimaging researchers have not assessed the association between DA release and drug wanting. In comparison, PET and fMRI studies suggest that both cue and drug-induced activation of the ventral striatum and interconnected limbic regions correlates with drug craving in cocaine dependent subjects (Grant, London et al. 1996; Breiter, Gollub et al. 1997; Childress, Mozley et al. 1999; Kilts, Schweitzer et al. 2001). Moreover, accumulating findings in the animal literature also suggest a close association between mesolimbic DA transmission and interest in addictive drugs (Marinelli and White 2000). Midbrain DA cell firing rates (Schultz 1998) and extracellular DA levels in nucleus accumbens (Gratton 1996) increase as animals approach rewards. Low to moderate doses of DA agonists increase drug-seeking behavior (Stewart 1984; Robinson and Berridge 1993). A larger DA response to acute stimulant drug administration predicts more rapid acquisition of drug self-administration (Zocchi, Orsini et al. 1998).

The magnitude of amphetamine-induced increases in extracellular DA in ventral striatum also correlated with the personality trait of novelty

seeking. Evidence of an association between TPQ novelty seeking and pharmaconeuroendocrine indices of DA neurotransmission has been reported (Gerra, Zaimovic et al. 2000). Since individuals at elevated risk for substance abuse have elevated Novelty Seeking scores (Howard, Kivlahan et al. 1997; Gabel, Stallings et al. 1999), this vulnerability might be related, in part, to an increased DA response to abused drugs. Novelty seeking, like risk for substance abuse, decreases with age (Jones and Meredith 1996).

Novelty seeking scores significantly predicted VAS drug wanting on the amphetamine but not placebo test day. One possibility is that amphetamine elicits a DA-mediated appetitive state that increases drug wanting. Susceptibility to drug-induced drug wanting might be related to a neurobiological trait predisposing to larger DA responses to stimulant drugs and to a personality trait of high novelty seeking. Animal studies suggest that novelty seeking behavior predicts the nucleus accumbens DA response to cocaine (Hooks, Jones et al. 1991) and propensity to self-administer amphetamine (Piazza, Deminiere et al. 1989).

Amphetamine-induced DA release did not correlate with self-reported mood-elevation or overt stimulant effects of the drug. Some evidence suggests that psychostimulant and mood-elevating drug effects

might be more closely related to increases in norepinephrine (Rothman, Baumann et al. 2001) and serotonin (Aronson, Black et al. 1995) than DA transmission. Previously reported correlations between euphorigenic and DA releasing effects of intravenous amphetamine (Laruelle, Abi-Dargham et al. 1995); (Drevets, Gautier et al. 2001) might be related to the ability of higher doses to release all three monoamines (Kuczenski and Segal 1989). The present study does not preclude a relation between DA and affect, though. Other evidence supports such an association (Murphy, Brodie et al. 1971; Depue and Iacono 1989). One possibility is that DA influences mood via its regulation of motivational states. The present study suggests that mesolimbic DA transmission might be more closely related to the appetitive component of emotion than providing a sufficient substrate of euphoric mood, *per se.*

The neuroanatomical pathways innervating ventral *vs.* dorsal striatum are well-described (Lynd-Balta and Haber 1994; Haber and McFarland 1999). In primates, the ventral striatum is interconnected with the limbic system and receives DA projections primarily from the dorsal tier of the midbrain tegmentum. Additional input to the ventral striatum comes primarily from limbic structures including amygdala, anterior cingulate and the medial and orbitofrontal cortex; these systems are thought to play important roles in associating behavior with reward,

attention to affectively relevant stimuli, and the initiation and inhibition of responses to rewards and punishments. In comparison, DA projections to the dorsal caudate-putamen come primarily from the ventral tier of midbrain DA cell bodies. Descending cortical inputs come primarily from sensory-motor cortices and the dorsolateral prefrontal cortex.

In the present study, *d*-amphetamine did not significantly decrease [^{11}C]raclopride BP in the dorsal caudate or putamen; however, substantial individual variability was apparent and the changes correlated positively and significantly with each other. These changes did not, however, correlate with changes in ventral striatum or with drug-induced drug wanting or novelty seeking. These observations are consistent with the evidence that DA release in dorsal *vs.* ventral striatum is differentially regulated, and suggest further that DA release in human striatal sub-compartments has different behavioral significance.

The conclusions suggested by the present study should be interpreted in light of the following considerations. The sample size is modest (n=8) though within the norms for assessing effects of pharmacological challenges within subjects. The identified correlations would not have withstood Bonferroni corrections, but they were replicated in ventral striatum as identified by two methods, parametric

mapping and manually drawn ROI. Second, as typically seen, only half of the subjects indicated that they wanted more of the drug (Uhlenhuth, Johanson et al. 1981). However, it was this individual variability that correlated with change in [^{11}C]raclopride BP. Third, the mechanisms mediating preferential increases in extracellular DA in the ventral striatum remain unclear. Some evidence, though, suggests that the effect might be related to slower DA release or uptake V_{max} (Cass, Gerhardt et al. 1992; Wu, Reith et al. 2001). Fourth, recent studies have questioned the mechanisms by which DA agonists decrease labeled D_2 ligand BP. The original model had proposed competition between the labeled ligand and endogenous DA for occupancy of the DA D2 receptors (Seeman, Guan et al. 1989) More recent work raises the possibility that increased synaptic DA levels might alter monomer to dimer ratios (Logan, Fowler et al. 2001)and / or induce receptor internalization (Laruelle 2000). Benzamide ligands, though, bind to both monomers and dimers (Zawarynski, Tallerico et al. 1998), and may not cross the cell membrane thereby restricting their access to cell surface receptor sites (Laruelle 2000; Seeman and Kapur 2000). Moreover, combination PET-microdialysis studies conducted in the same animal indicate that decreases in benzamide ligand BP have a strong linear association with increases in dialysate DA concentrations (Endres, Kolachana et al. 1997; Laruelle, Iyer et al. 1997).

These observations suggest that decreases in [^{11}C]raclopride BP in the same individual can be considered to reflect increases in extracellular DA.

In conclusion, the small sample size emphasizes the need for caution but the present results suggest that DA transmission in sub-compartments of human striatum is differentially affected by amphetamine and has different behavioral significance. First, a low oral dose of *d*-amphetamine increased extracellular DA with preferential effects in ventral striatum. Second, there was a close association between three variables, (i) the personality trait of novelty seeking, (ii) amphetamine-induced drug wanting, and (iii) individual susceptibility to amphetamine-induced DA release in ventral striatum. Both novelty seeking and drug-induced drug wanting might be related to susceptibility to drug seeking behavior and be predictive of vulnerability to substance abuse.

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Table 1 Subject characteristics.

Age	23.8±4.1 (range, 19 - 30
Current alcohol use (drinks / week):	1.97±1.4 (range, 0.25 - 5)
Lifetime drug use (ever used, mean±SD)	
Stimulants	5/8, 3.1±5.0 (range, 0-15)
Tranquilizers	0/8
Hallucinogens	5/8, 1.25±1.3 (range, 1-3)
Opiates	1/8, 0.12±0.4 (range, 0-1)
THC	7/8, 13.0±14.0 (range, 0-40)
Novelty Seeking	13.0±4.5 (range, 13 - 27)
Beck Depression Score	3.0±2.7 (range, 0 - 8)

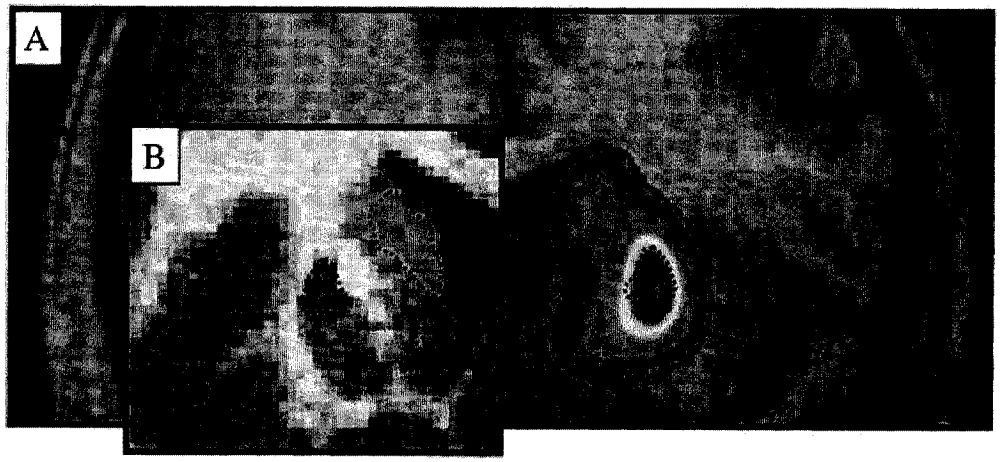

Figure 1 One subject's [^{11}C]raclopride binding potential map (right striatum) for the first scan day transformed to Talairach space and overlaid on his MRI in Talairach space. Regions of interest drawn on the same subject's MRI (left striatum) in Talairach space. *Red*: Ventral Striatum / Nucleus Accumbens (z: 8 to 4), *Blue*: Putamen (z: 2 to 10), *Green*: Caudate (z: 2 to 15).

Table 2 Effect of d-amphetamine (0.0, 0.3 mg/kg, p.o.) on Addiction Research Center Inventory (ARCI) Scores and Visual Analog Scales (max). Effects Assessed with 2-Tailed Repeated Measures t-tests.

	Placebo (Mean ± SD)	**Amphetamine** (Mean ± SD)	*p*-value
ARCI	4.6±4.2	11.8 ± 10.2	.02
Visual Analog Scale			
Alert	-1.5 ± 1.9	1.8± 2.2	.002
Mind Racing	0.25 ± 1.2	2.4± 2.0	.003
Energetic	-1.2 ± 1.5	2.0 ± 1.7	.01
Excited	0.0 ± 0.9	1.9 ± 1.7	.03
Euphoria	0.0 ± 0.8	1.4 ± 1.8	.09
Rush	-0.1 ± 0.6	1.6 ± 2.6	.13
Like Drug	-0.2 ± 1.8	1.4 ± 3.0	.17
High	0.2 ± 1.6	1.2 ± 2.1	.41
Want Drug	0.6 ± 1.1	1.4± 2.5	.46
Anxiety	0.2 ± 1.0	0.6 ± 1.4	.53

Table 3 [^{11}C]Raclopride binding potential values on test days with placebo or d-amphetamine (0.3 mg/kg, p.o.). Newman-Keuls post hoc tests.

Test Day	Ventral Striatum	Caudate	Putamen
Placebo	1.44 ± 0.5†	1.97 ± 0.2*	2.45 ± 0.3*†
d-Amphetamine	1.26 ± 0.4$^{\#\dagger}$	1.94 ± 0.2*	2.45 ± 0.3*†

#Different from placebo, p .05.
* Different from ventral striatum, p .001.
† Different from caudate, p .001.

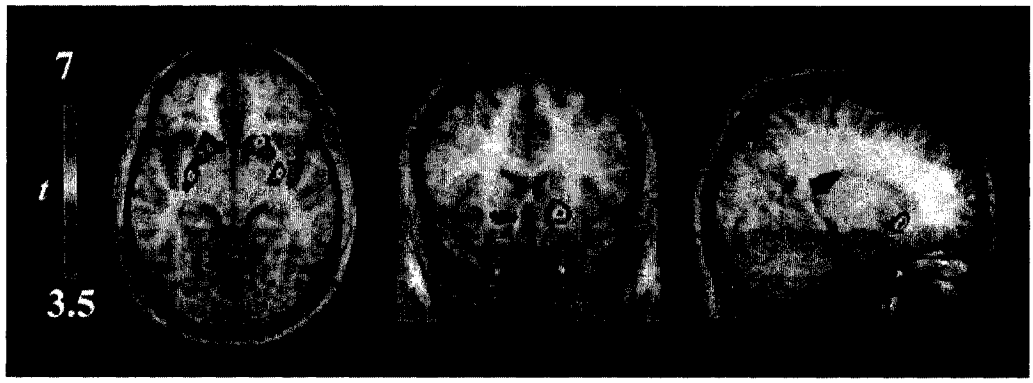

Figure 2 Statistically generated t-map of d-amphetamine-induced changes in [^{11}C]raclopride binding potential superimposed on average MRI. Right side on right.

Table 4 Pearson correlations with change in [^{11}C]raclopride binding potential.

ROI	*Want Drug*	NS	NS-1	HA	RD	RD-2
***t*-Map**	0.83**	0.43	0.79*	0.06	0.06	0.01
Ventral Striatum	0.62†	0.75*	0.74*	0.16	0.03	0.28
Caudate	0.14	0.13	0.08	0.25	0.13	0.24
Putamen	0.19	0.09	0.09	0.34	0.07	0.14

† p .10, * p .05, ** p .01. 'Want Drug': Self-reported drug wanting on the amphetamine administration test day. *t*-Map: statistically generated cluster of change; NS: Novelty Seeking; NS-1: Exploratory-Excitability; HA: Harm Avoidance; RD: Reward Dependence; RD-2: Persistence

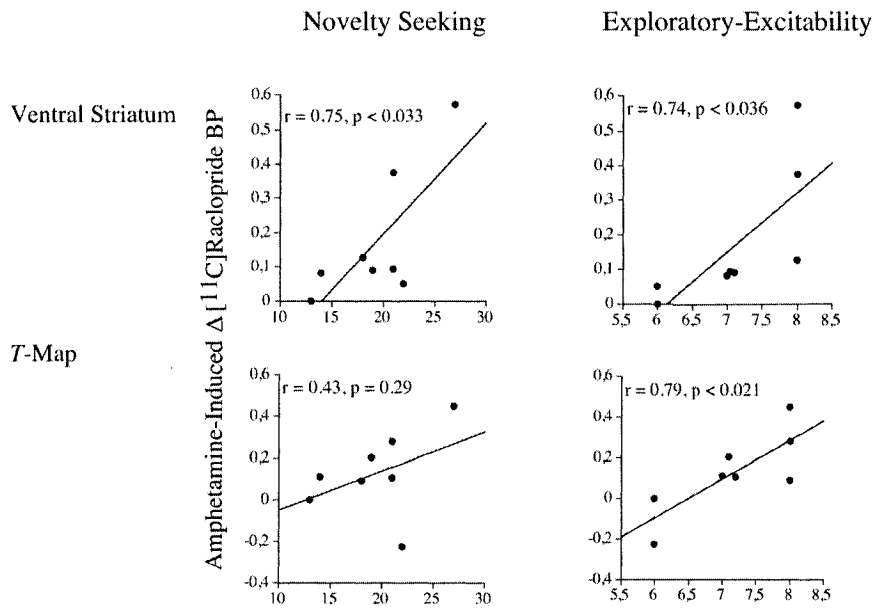

Figure 3 Correlations between d-amphetamine-induced increases in extracellular DA and Novelty Seeking and the Novelty Seeking subscale, Exploratory-Excitability. [^{11}C]Raclopride BP values were extracted from two regions, the manually drawn region of interest in ventral striatum and the statistically generated parametric t-map.

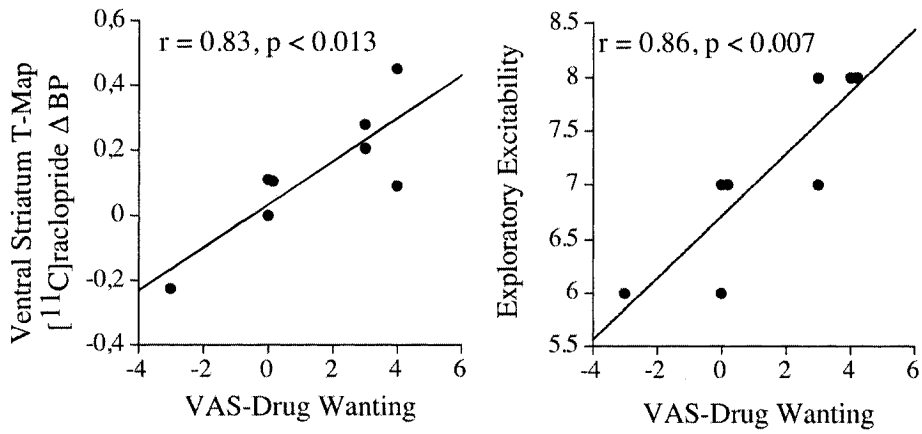

Figure 4 Correlation between d-amphetamine- induced drug wanting and (left) *d*-amphetamine-induced DA release, and (right) TPQ Novelty Seeking-Exploratory Excitability scores.

PREFACE CHAPTER V

The following section introduces the second manuscript (**study 2**) included in the present thesis. This manuscript entitled *"Alcohol promotes dopamine release in the human nucleus accumbens"* has been published in Synapse 2003; 49(4): 226-31.

A central concept in drug addiction research is that although drugs of abuse are chemically divergent with different initial molecular targets, their action in the brain must converge since addictions to most if not all drugs share strikingly similar features. The ability of addictive drugs to strongly activate DA neurotransmission in the limbic striatum is believed to be a key feature of this *common path* to addiction. In this regard, most drug commonly abused by humans have been shown to trigger a supra physiological increase in DA level in the nucleus accumbens. This event is (for the least) thought to initiate the process of addiction. Although neuroimaging studies from different groups have provided direct evidence that psychostimulants directly increase DA release in the striatum, evidence that this observation extends to drugs of abuse belonging to different pharmacological classes is still sparse - in fact results presented in the following manuscript provide the first evidence that alcohol, one of the most widely used and abuse drugs, promotes DA release in humans.

The general aim of the second study **(study 2)** included in the present thesis was to investigate DA release with neuroimaging, during the administration of an intoxicating dose of alcohol in moderate drinkers. In **study 2** of this thesis we report that alcohol promotes DA release in humans. Specific regional effects are strikingly similar to the effects of amphetamine reported in **study 1**, in that release appears to be preferential in the ventral striatum. A high heart-rate response to alcohol is believed to be a risk factor in the development of alcohol use. In line with the hypothesis that alcohol induced heart-rate increase represents a reinforcing state, which involves dopaminergic transmission, we report a positive relationship between DA release and this measure. In this study we also investigated individual differences in the responsiveness of the DA system to alcohol and found reactivity to alcohol was potentiated in those with high impulsivity trait.

Alcohol promotes Dopamine release in the Human Nucleus Accumbens.

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Key words: Alcohol, Dopamine, PET, [^{11}C]raclopride, Nucleus Accumbens, Addiction.

ABSTRACT

Microdialysis experiments in rodents indicate that ethanol promotes dopamine release predominantly in the nucleus accumbens, a phenomenon that is implicated in reinforcement by drugs of abuse. The aim of the present study was to test the hypothesis in humans that an oral dose of ethanol would lead to dopamine release in the ventral striatum, including the nucleus accumbens. Six healthy subjects underwent two [^{11}C]raclopride PET scans following either alcohol (1ml/kg) in orange juice or orange juice alone. Subjective mood changes, heart rate and blood-alcohol levels were monitored throughout the procedure. Personality traits were evaluated using the Tridimensional personality questionnaire. PET images were co-registered with MRI and transformed into stereotaxic space. Statistical parametric maps of [^{11}C]raclopride binding potential change were generated. There was a significant reduction in [^{11}C]raclopride binding potential bilaterally in the ventral striatum / nucleus accumbens in the alcohol condition compared to the orange juice condition, indicative of increased extra-cellular dopamine. Moreover, the magnitude of the change in [^{11}C]raclopride binding correlated with the alcohol-induced increase in heart rate, which is thought to be a marker of the psychostimulant effects of the drug. The present study is the first report that, in humans, alcohol promotes dopamine release in the brain,

with a preferential effect in the ventral striatum. These findings support the hypothesis that mesolimbic dopamine activation is a common property of abused substances, possibly mediating their reinforcing effects.

INTRODUCTION

Addiction is thought to result in large part from the reinforcing properties of drugs of abuse on brain reward systems, and in particular on mesolimbic dopamine (Wise and Rompre 1989; Koob, Sanna et al. 1998). Microdialysis studies in rats show that ethanol and other drugs of abuse, such as opiates, nicotine, amphetamine, and cocaine, acutely increase extracellular dopamine levels predominantly in the nucleus accumbens (NAc) (Di Chiara and Imperato 1988). The role of NAc dopamine in alcohol self-administration is further supported by reports of changes in consumption following alterations in mesolimbic dopamine neurotransmission (Rassnick, Krechman et al. 1993; Samson and Hodge 1993; Nowak, McBride et al. 2000) ethanol self-administration into the ventral tegmental area (VTA) (Gatto, McBride et al. 1994) and alcohol withdrawal-induced reductions in both dopamine neuron firing and NAc extra-cellular dopamine concentration (Diana, Gessa et al. 1992; Rossetti, Hmaidan et al. 1992)that are reversed by ethanol administration (Weiss, Parsons et al. 1996)

Alcohol is widely abused by humans; however no studies have directly investigated the implication of the dopamine system in mediating

some of its reinforcing effects. We now present, for the first time in humans, evidence that alcohol consumed orally promotes dopamine release specifically in the NAc.

We measured dopamine release in response to a single-dose administration of alcohol using positron emission tomography (PET) and the dopamine receptor ligand [^{11}C]raclopride. We used a two-scan method based on combined PET microdialysis evidence in primates that the binding of benzamides such as [^{11}C]raclopride is sensitive and proportional to extra-cellular dopamine concentration in the striatum (Endres, Kolachana et al. 1997; Laruelle, Iyer et al. 1997). This approach has been used in humans to measure the dopamine response to psychostimulants (Carson, Breier et al. 1997; Schlaepfer, Pearlson et al. 1997) (Volkow, Wang et al. 2001), and behavioural tasks (Koepp, Gunn et al. 1998). Two recent PET studies have also shown that dopamine release following amphetamine occurs mostly in the ventral striatum, and that the amount of dopamine released correlates with self-reported behavioural measures of euphoria or drug wanting (Drevets, Gautier et al. 2001; Leyton, Boileau et al. 2002).

MATERIALS AND METHODS

Seven healthy non-alcoholic, non-abstinent males, moderate drinkers (brief Michigan Alcoholism Screening Test; (Pokorny, Miller et al. 1972) aged 22 (± 0.6) were recruited from an existent longitudinal cohort (Tremblay, Pihl et al. 1994) and included in the study if they had experienced the alcohol dose required for participation in this study at least twice in a laboratory setting. Data from one of the seven subjects had to be excluded due to excessive motion during the scan. All subjects were free of active or past medical or psychiatric illness. Subjects fasted and abstained from caffeine or tobacco for a minimum of four hours before each test session. Five of the six subjects were non-smokers and one was a light smoker (1-2 cigarettes per day). They were also asked to refrain from taking drugs for seven days and alcohol for 24 hours prior to each experimental day. Before each scanning session, subjects underwent screening for drugs of abuse (Triage Panel for Drugs of Abuse, Biosite Diagnostics, San Diego, CA) including alcohol (Alcosensor III intoxicometer, Thomas Instruments, Montreal, QC). All subjects read and signed a consent form approved by the Research and Ethics Committee of the Montreal Neurological Institute.

Subjects participated in two [^{11}C]raclopride PET scans after consumption of alcohol in orange juice or orange juice alone (Figure 2).

Subject were told about drink content at the beginning of the session, however, to reduce the possible effect of alcohol associated olfactory cue, particular attention was made to avoid subject's contact with alcohol vapour before the beginning of consumption. PET data acquisition was performed at the same time of day (between 14:00 and 16:00) on separate days, one week apart, and counterbalanced for order of administration of alcohol (three out of six received alcohol on the first day, randomly chosen). Prior to scanning, a venous catheter was inserted in the subject's left arm. Oral consumption of alcohol (1ml/kg of 95% USP alcohol over 15 minutes) or alcohol-free mixture started 30 minutes prior to tracer injection. At the end of consumption, subjects were immediately positioned in the scanner and a 12-minute transmission scan was acquired using a ^{68}Ge source for the purpose of attenuation correction. Following the transmission scan, and 15 minutes after the end of alcohol consumption, [^{11}C]raclopride 10 mCi was injected as a bolus into the antecubital vein, after which PET dynamic acquisition (63 slices, 26 time frames of 60 minutes total duration) was performed.

Subjects were scanned on the CTI/Siemens ECAT HR+ PET camera with lead septa removed, with intrinsic resolution 4.2 mm FWHM. Blood samples, for plasma alcohol measurements, were withdrawn from the venous cannula before the initiation of drinking, at tracer injection (15

minutes after finishing drinking), and every 15 minutes thereafter. Subjective effects of alcohol, assessed with the Subjective High Assessment Scale (SHAS; Schuckit and Smith 1997) and heart rate were measured prior to alcohol consumption and throughout the procedure. The SHAS is a visual analog scale that assesses sensations such as feeling high, drunk, and drowsy. Prior to the first scan, all subjects completed the Tridimensional personality questionnaire (TPQ; Cloninger, Przybeck et al. 1991). This test assesses three dimensions of personality including novelty seeking (impulsive, excitable, exploratory temperament), which is thought to depend in significant part on activity in the dopamine pathways (Cloninger 1994). For the purpose of anatomical co-registration, subjects also underwent a 1x1x1mm anatomical T1-weighted MRI of the whole brain using a gradient echo pulse sequence (TR = 9.7 ms, TE = 4 ms, flip = 12°, FOV = 250, matrix = 256 x 256).

PET frames were summed across time, co-registered with the corresponding MRI (Woods, Mazziotta et al. 1993), and transformed into standardised stereotaxic space (Talairach and P 1988) by means of automated feature-matching to the MNI template (Collins, Neelin et al. 1994). Voxelwise [^{11}C]raclopride binding potential (BP) was calculated using a simplified reference tissue method (Lammertsma and Hume 1996; Gunn, Lammertsma et al. 1997) to generate statistical parametric images of

the change in binding (Aston, Gunn et al. 2000). BP values for each subject were extracted from regions of interest (ROI) manually drawn on the co-registered MRI on the left and right dorsal caudate (DC, drawn on transverse slices at Talairach-space *z* coordinate from +2 to +15 mm), dorsal putamen (DP, + 2 to + 10 mm), ventral putamen (-8 to -4 mm), NAc (-8 to -4 mm) and cerebellum, which was used as the reference region. BP values extracted from ROI during alcohol and control scans were analysed using a three-way ANOVA for dependent samples [Treatment x ROI x hemisphere]. Sphericity was assessed with the Mauchly test and, when indicated, corrections were made with Greenhouse-Geisser adjustments. When appropriate, Least Significant Difference *t*-tests, Bonferroni corrected, were applied to determine the significance of regional differences in BP between the alcohol and orange juice conditions. Heart rate during the ascending part of the blood alcohol curve was compared to a baseline taken just prior to the study session (▲max [15-30 min.]). Since one subject exhibited a change in heart rate during the test session with orange juice that was greater than 2 SD from the sample mean, magnitude of heart rate change was analysed with the non-parametric Wilcoxon matched pairs test. Maximum change in SHAS rating from baseline taken on the same day (▲ max [SHAS]) was used to evaluate the subjective effects of alcohol and control drinks. A t-test for paired samples was used to determine the difference between ▲ max [SHAS] for the

alcohol condition and **Δ** max [SHAS] for the control. Stepwise linear regression analysis was used to examine whether percent change in ROI BP could be predicted by changes in heart rate, change in SHAS scores, or TPQ personality ratings.

RESULTS

Screening for drugs of abuse was positive for only one subject (THC and trace cocaine prior to both scan conditions). Therefore, two different analyses were carried out, one excluding the data from this subject. In both cases, receptor parametric mapping identified significant reductions in [^{11}C]raclopride BP in bilateral ventral striatum in the alcohol compared to the alcohol-free condition (**Figure 1**). In the statistically generated t-map, [^{11}C]raclopride BP values were 16.8±16.3% lower on the test day with alcohol, compared to orange juice ($t(5)=2.54$, $p = 0.05$).

Analyses of [^{11}C]raclopride BP values in the a priori defined ROI supported the receptor parametric mapping analyses (Figure 3). A treatment x ROI x hemisphere ANOVA yielded a main effect of ROI ($F(3, 15) = 42.55$, $p<0.001$, Greenhouse-Geisser corrected) and a treatment X ROI interaction ($F(3, 15) = 3.21$, $p=0.05$). Bonferroni corrected pairwise comparisons confirmed that alcohol significantly reduced BP in the NAc ($p = 0.003$) and ventral putamen ($p = 0.001$) but not in the DC ($p = 0.98$) or

DP ($p = 0.84$). The percent change in [^{11}C]raclopride BP also varied with ROI ($F(3,15)=13,50$, $p = 0.001$). In both the nucleus accumbens (15.0±15.9%) and the ventral putamen (13.7±16.4%), the percent decreases in [^{11}C]raclopride BP were greater than those seen in either the dorsal putamen (5.2±17.5%) or caudate nucleus (4.0±16.4%) ($p < 0.01$).

The blood alcohol level reached a mean peak of 18.10 (±1.4) mmol/L (0.0833 gm %) at 30 minutes after drinking. During the expected ascending phase of the blood alcohol curve (15-30 minutes post drink) alcohol consumption resulted in small but consistent increases in heart rate (5.47 ± 6 beats/min; $t(5)=1.85$, $p=0.12$; 6/6 subjects higher during alcohol test, Wilcoxon matched pairs test, $z = 2.20$; $p = 0.028$) and self-reported feelings of "high" and "drunkenness" (paired t-test, Δ max [SHAS] alcohol vs. Δ max [SHAS] orange juice, $p < 0.01$). A stepwise linear regression showed that impulsiveness, one of the subscales on the novelty-seeking dimension of the TPQ, and heart rate increase recorded at 30 minutes (i.e. during the ascending phase of the blood alcohol curve) were the only predictors of BP change in the ventral striatum ($r = .985$; $p = 0.005$). Neither the subjective intoxication measures nor the peak blood alcohol level correlated with the change in [^{11}C]raclopride BP in any region.

DISCUSSION

The observed reduction in [^{11}C]raclopride BP confined to the ventral part of the striatum is indicative of dopamine release specifically in the NAc and ventral putamen in response to alcohol in humans. These results are similar to those of two other [^{11}C]raclopride PET studies, in which amphetamine was found to preferentially induce dopamine release in the ventral striatum in humans (Drevets, Gautier et al. 2001; Leyton, Boileau et al. 2002). In animals, in-vivo microdialysis studies have also shown a propensity for alcohol to induce dopamine release in the ventral striatum. Di Chiara and Imperato (1988) found that ethanol at rewarding doses had an almost ten-fold greater effect on dopamine release in the NAc than in the dorsal caudate. Moreover, low doses of ethanol produce a dose-dependent increase in the firing rate of A10 dopamine neurons in the ventral tegmental area, which project to the ventral striatum (Gessa, Muntoni et al. 1985). Activation of A9 dopamine neurons, which project to the dorsal striatum, only occurs at five-fold greater ethanol doses. It is unclear if results obtained in the present study reflect a purely pharmacological response. It is well accepted that drug associated cues also elicit DA cell firing (Carelli, Ijames et al. 2000) and autonomic reactivity (Stormark, Laberg et al. 1995) that could be in part responsible for the enhanced synaptic DA.

The dorsal and ventral striatum can be separated functionally and anatomically (Moore and Bloom 1978; Haber, Fudge et al. 2000). Their dopamine innervations originate in different cell groups in the midbrain, and their cortical connections likely account for their different functional roles. The ventral striatum, including the NAc and ventral putamen, belong to the "limbic" cortico-striatal loop that includes the amygdala, hippocampus, orbito-frontal cortex and cingulate cortex, structures involved in emotional behaviour and reward processing. There is much evidence for specific involvement of ventral striatal, or mesolimbic, dopamine in the reinforcing effects of addictive drugs (Wise and Rompre 1989; Koob, Sanna et al. 1998). It is thought to mediate associative learning, whereby drug-related cues acquire incentive value (Di Chiara, Tanda et al. 1999). Conditioned place preference, a laboratory test of conditioned incentive learning, is abolished by lesions or dopamine blockade of the ventral but not dorsal striatum (Everitt, Morris et al. 1991); (White, Packard et al. 1991). Our finding of dopamine release confined to the ventral striatum after oral ingestion of an intoxicating dose of alcohol may therefore account at least in part for its addictive properties in humans.

Ethanol most likely acts on dopamine neurons indirectly (Yim and Gonzales 2000). It potentiates $GABA_A$ receptor function (Weiner, Zhang et

al. 1994) to cause inhibition of GABAergic interneurons in the substantia nigra reticulata (Gessa, Muntoni et al. 1985), which leads to dishinbition and increased burst firing of dopamine neurons (Grace and Bunney 1985). As stated above, A10 neurons projecting to the ventral striatum appear to be more sensitive to these systemic effects of ethanol than A9 dopamine neurons projecting to the dorsal striatum (Gessa, Muntoni et al. 1985). Opioid peptides may also be involved in the dopamine releasing actions of ethanol (Acquas, Meloni et al. 1993; Benjamin, Grant et al. 1993)Gonzales et al., 1998).

The level of dopaminergic responsiveness in the NAc has been proposed as a marker of individual vulnerability to drug addiction. Numerous studies in rats have linked the propensity to self-administer drugs to enhanced dopamine release in the NAc in response to psychostimulants or stress (Piazza, Maccari et al. 1991; Hooks, Colvin et al. 1992; Zocchi, Orsini et al. 1998; Barrot, Abrous et al. 2001). In light of these studies, our finding of a correlation between the change in [^{11}C]raclopride BP in response to alcohol and two variables, the alcohol-induced increase in heart rate and the personality trait of impulsiveness, is interesting.

In humans, cardiac response has been hypothesized to be an index of the psychostimulant properties of alcohol and of dopamine activation (Conrod, Peterson et al. 2001)and therefore a marker of vulnerability to addiction. Our findings, although in a small number of subjects, lend support to this theory. Moreover, the personality trait of novelty-seeking, of which impulsiveness is one component, has also been linked to both dopamine function (Cloninger 1994) and to addictive propensity. High scores on the novelty-seeking scale of the TPQ predict later alcoholism (Cloninger, Sigvardsson et al. 1988) as well as relapse-rate in detoxified alcoholics (Meszaros, Lenzinger et al. 1999). Interestingly, in a previous PET study we found that amphetamine-induced dopamine release also targeted the NAc, and correlated with novelty-seeking scores (Leyton, Boileau et al. 2002).

In the current study, we found no correlation between alcohol-induced NAc dopamine release and subjective measures of intoxication. Previously, we similarly found no correlation between amphetamine-induced dopamine release and behavioural effects such as euphoria and excitation (Leyton, Boileau et al. 2002). The association between a drug's euphorigenic quality and dopamine release has not been established in humans; similarly, in rats the behavioural significance of increased DA remains a subject of debate. Our failure to find a correlation between

subjective effects and DA release likely reflects the fact that alcohol acts on multiple neurotransmitter systems. In particular, the SHAS mostly reflects the sedative effects of alcohol (Conrod, Peterson et al. 2001), which are probably not mediated by dopamine.

In conclusion, we showed that alcohol consumed by mouth in intoxicating doses promotes dopamine release in the ventral striatum. The observed relationship between the magnitude of change in [^{11}C]raclopride BP, personality and heart rate increase suggests that the paradigm we have developed could be used to investigate the factors that lead to vulnerability for alcohol dependence.

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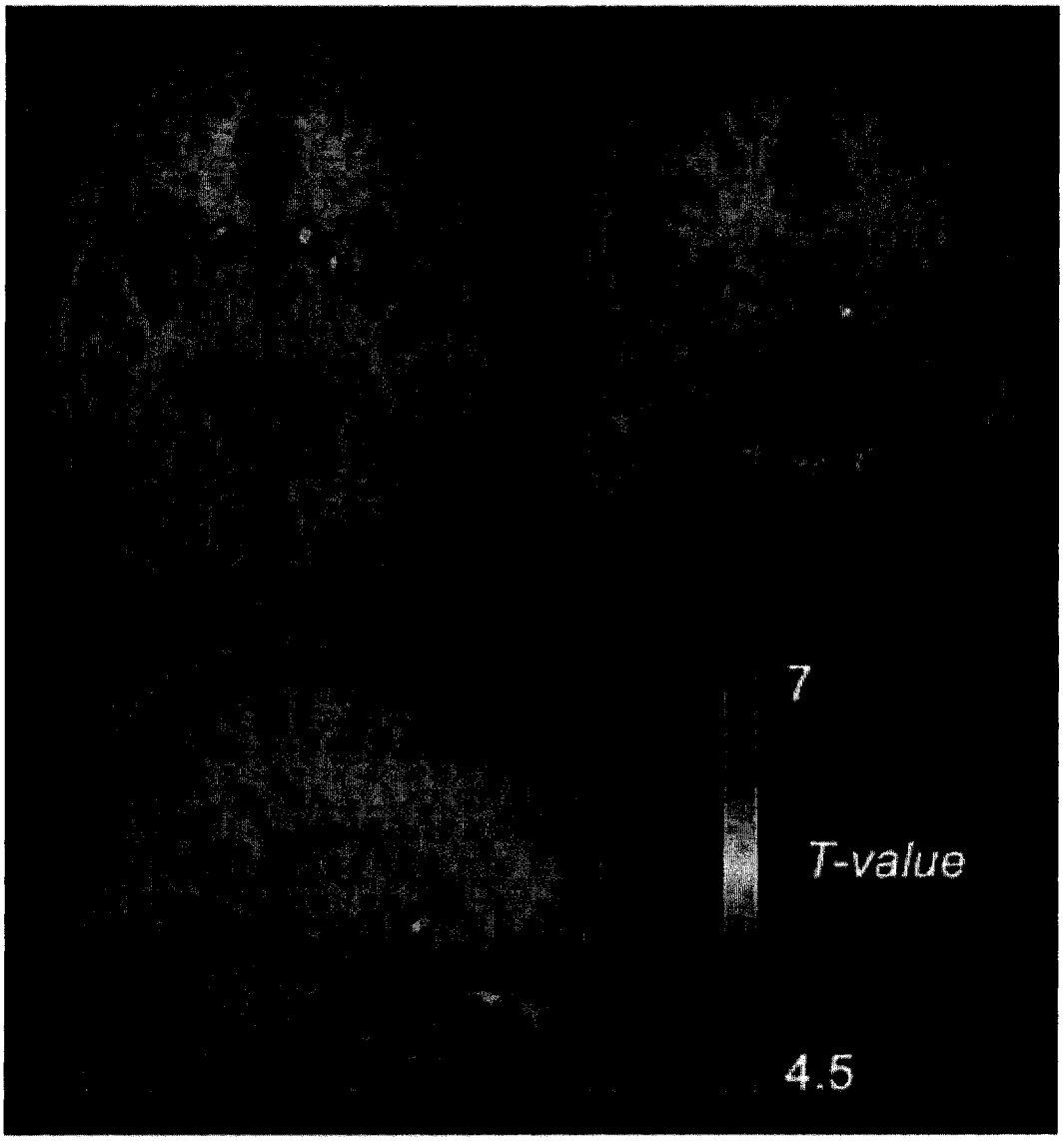

Figure 1 Statistical *t*-map of the change in [^{11}C]raclopride BP induced by an acute oral dose of alcohol (1 ml/kg) in healthy volunteers (n=6). Colour clusters superimposed on the average MRI from all subjects depict a significant change in BP in the ventral striatum.

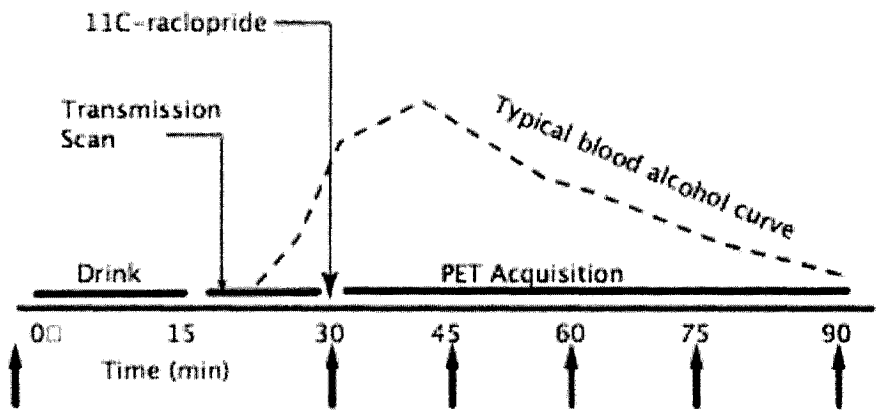

Figure 2 Study design. The vertical arrows indicate the time points of blood sampling, subjective mood assessments, and physiological measurements.

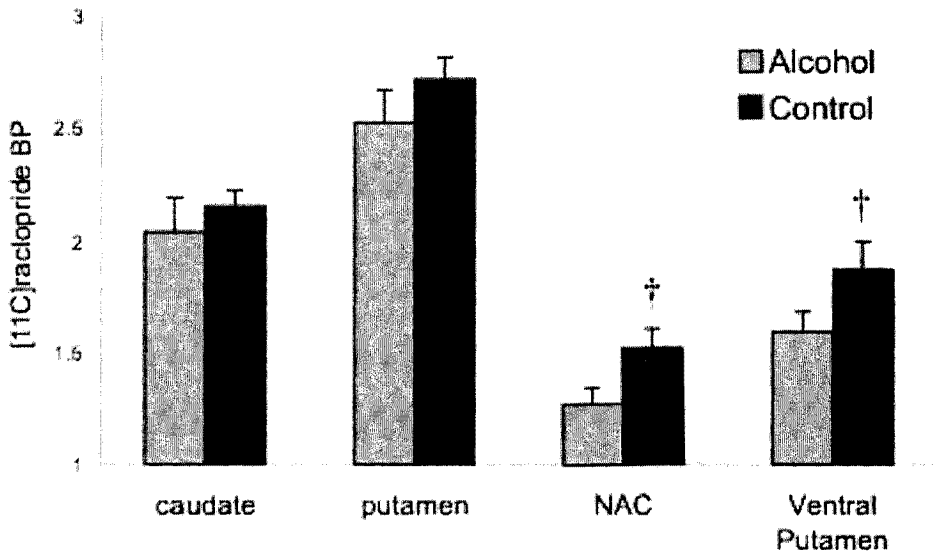

Figure 3 Mean [^{11}C] raclopride BP in the alcohol and control (orange juice) conditions. The data are extracted from manually drawn ROI on each subject's MRI. Bonferroni corrected pairwise comparisons; Differences between alcohol and control, $p<0.001$. Error bars represent the standard error of the mean.

PREFACE CHAPTER VI

The following section introduces the third manuscript (**study 3**) included in the present thesis. This manuscript entitled *"Modeling Sensitization to Stimulants in Humans: A [^{11}C]raclopride / PET Study in Healthy Volunteers."* Is in press in Archives of General Psychiatry 2006.

DA neurons are believed to undergo adaptive changes which are unmasked upon suspension of a chronic regimen of addicting drugs - one of these changes results in an increase psychostimulant-induced DA releasability i.e.: *sensitization*. While animal models have yielded an enormous amount of data in favor of the occurrence of behavioral and neurochemical sensitization after repeated administration of amphetamine, human studies have been surprisingly unsuccessful. In this regard PET studies of human cocaine abusers, which demonstrated a blunted DA response following the single dose administration of a psychostimulant have challenged the sensitization hypothesis (Volkow, Wang et al. 1997)

In the following manuscript we used PET and [^{11}C]raclopride to test the specific hypothesis that repeated amphetamine administration in healthy humans results in an increased DA release. Our results suggest that

neurochemical and behavioral sensitization to stimulants in humans can be achieved safely in the laboratory. In agreement with the prediction of sensitization, repeated amphetamine potentiated DA release in several sub-regions of the striatum, including the ventral striatum and posterior dorsal putamen. Notably, sensitization enhanced the psychomotor / arousing effects of the drugs. Finally, our findings also indicate that individuals vary in their propensity to sensitize and this vulnerability could be predicted by trait novelty seeking.

Volkow, N. D., G. J. Wang, et al. (1997). "Decreased striatal dopaminergic responsiveness in detoxified cocaine-dependent subjects." Nature **386**(6627): 830-3.

Modeling Sensitization to Stimulants in Humans: A [^{11}C]raclopride / PET Study in Healthy Volunteers.

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This work was presented at the following meetings:

1. Organization for Human Brain Mapping abstract, New York, US, June 13-17, 2003
2. Beyond the Nature/Nurture debate: Genes, environment and their interactions in psychiatry. Institute of Psychiatry, London 8-9 November 2004.

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ABSTRACT

Introduction: In animals, repeated exposure to stimulant drugs leads to an enhanced drug-induced psychomotor response and increased dopamine release. This phenomenon, known as sensitization, may confer vulnerability to drug addiction or drug-induced psychosis in humans. A similar phenomenon, referred to as endogenous sensitization, is also believed to play a role in the emergence of positive symptoms in patients with schizophrenia (Lieberman, Sheitman et al. 1997; Laruelle 2000).

Aim of the study: To determine whether behavioral and neurochemical sensitization occur in healthy volunteers following limited exposure to amphetamine in the laboratory.

Method: Ten healthy male human volunteers (age, 25.8 ± 1.8 years) received three single doses of amphetamine (0.3 mg/kg; by mouth) on days 1, 3 and 5. Using positron emission tomography (PET) and [^{11}C]raclopride, we measured dopamine release in response to amphetamine on first exposure (day 1) and 14 days and one year after the third exposure.

Results: The initial dose of amphetamine caused dopamine release in the ventral striatum (a reduction of [^{11}C]raclopride binding). Amphetamine 14 and 365 days after the third dose resulted in a greater psychomotor response and increased

dopamine release (a greater reduction of [^{11}C]raclopride binding), relative to the initial dose. Furthermore, this sensitized response to amphetamine was manifested not only as greater dopamine release in the ventral striatum but as an extension of dopamine release to the dorsal caudate and putamen. Novelty seeking personality and impulsivity predicted the degree of sensitization of the response to amphetamine induced by repeated amphetamine.

Conclusion: Sensitization to stimulants can be achieved in healthy humans in the laboratory. This phenomenon is associated with increased dopamine release and persists for at least one year.

INTRODUCTION

Altered dopamine (DA) neurotransmission is believed to play a critical role in the pathophysiology of psychosis and addiction (Robinson and Berridge 1993; Lieberman, Sheitman et al. 1997). The phenomenon of sensitization that occurs within the midbrain DA system when animals are repeatedly exposed to stimulant drugs or stress may help us understand how DA neurotransmission becomes dysregulated. Repeated intermittent exposure to stimulant drugs or stress results in heightened behavioral and neurochemical responses following re-exposure (Antelman, Eichler et al. 1980; Robinson, Jurson et al. 1988; Paulson and Robinson 1995). It is generally believed that, during the induction of sensitization, the repeated stimulation of DA receptors in the ventral tegmental area triggers a cascade of molecular events and changes in neuronal plasticity that, in turn, foster augmented DA release (Nestler 2001). In experimental animals, behavioral sensitization is an enduring (Robinson and Becker 1986; Hyman and Malenka 2001), time (Paulson and Robinson 1995) and context (Anagnostaras, Schallert et al. 2002) dependent phenomenon, which is associated with a long-lasting increase in stimulant drug-induced dopaminergic neurotransmission in the striatum (Kalivas and Stewart 1991; Kalivas 1993; Paulson and Robinson 1995; Pierce and Kalivas 1997). Sensitization is reported to cross-react with stress (Antelman, Eichler et al. 1980) and is affected by

individual differences (Hooks, Jones et al. 1991). A high locomotor response to novel environments in rats is a predictor of the development of sensitization (Hooks, Jones et al. 1991). Although widely described in experimental rodents, sensitization has seldom been investigated in humans (Rothman, Gorelick et al. 1994; Strakowski, Sax et al. 1996; Sax and Strakowski 1998; Wachtel and de Wit 1999; Richtand, Woods et al. 2001). Sensitization in humans is thought to confer vulnerability to drug addiction (Robinson and Berridge 1993; Robinson and Berridge 2001) and is invoked to account for psychosis recurrence in chronic methamphetamine users exposed to stimulant drugs or stress after long periods of drug-abstinence (Sato, Chen et al. 1983; Yui, Goto et al. 1997). In schizophrenia, endogenous sensitization might underlie the conversion to psychosis in prodromal and remitting patients (Lieberman, Sheitman et al. 1997; Laruelle 2000), a hypothesis that has gained partial validity from radioligand PET imaging studies which have demonstrated exaggerated amphetamine stimulated dopamine release and symptomatic exacerbation in patients with schizophrenia (Laruelle, Abi-Dargham et al. 1996) (Breier, Su et al. 1997) (Abi-Dargham, Gil et al. 1998).

PET neuroimaging with the D_2 receptor ligand [^{11}C]raclopride has been used to investigate striatal DA function non-invasively in humans. There is substantial evidence that an acute intra-synaptic rise in DA concentration

translates into a proportional reduction in the binding potential (BP) of [^{11}C]raclopride (Laruelle 2000), and that decreasing catecholamine neurotransmission increases BP (Leyton, Dagher et al. 2003). This imaging modality has been successfully used to demonstrate DA release in response to the administration of drugs of abuse in human subjects (Volkow, Wang et al. 1994; Drevets, Gautier et al. 2001; Leyton, Boileau et al. 2002). The mechanisms underlying the changes in BP in response to changes in synaptic DA concentrations are not fully elucidated. It is believed that rapid internalization of $D_{2/3}$ receptors in the endosomal compartment in the face of an agonist challenge may explain decreased [^{11}C]raclopride binding (Laruelle 2000).

The purpose of this study was to test and validate an experimental model of stimulant-induced sensitization of the effects of amphetamine on striatal DA release in humans in the laboratory, using the PET/[^{11}C]raclopride technique. The specific hypothesis was that amphetamine-stimulated DA release in the striatum is enhanced as a result of the repeated administration of oral d-amphetamine.

METHOD

Design Overview

Subjects completed an anatomical MRI scan and six experimental sessions (Table 1), receiving five oral doses of amphetamine (Dextroamphetamine sulfate, 0.3 mg/kg, p.o.) in the same physical setting, at the same time of day. During the sensitization phase, subjects received three doses of amphetamine (0.3 mg/kg, p.o.) with approximately two days between each dose (1.95 ± 0.6). A test dose was then given two weeks (17.2±3.2 days) after the last sensitization dose. PET [^{11}C]raclopride scans were conducted during (i) a drug-free session, (ii) the first exposure to amphetamine, and (iii) the test dose two weeks after sensitization. Seven of the 10 subjects returned for a final [^{11}C]raclopride amphetamine scan following a 12-month latency (407±60 days). Amphetamine doses 2 and 3 were administered during sham scans on days 3.1 ± 0.3 and 5.8 ± 0.7 in the course of which subjects underwent all aspects of the PET procedure except for the administration of radiotracer. Five of the subjects underwent the drug-free (control) session prior to receiving amphetamine; five had this session following the test dose (day 31.9 ± 6.5).

Day	Day	Day	Day		Day		1 year
0 or >22	1	3	5	14-day Latency	21	~ 1year	
PET*	PET	*Sham*	*Sham*		PET	Latency	PET
No drug	AMP	AMP	AMP		AMP		AMP

Table 1 Experimental design. **PET***: no-drug control scan performed in a counterbalanced order, either before (day 0, n=5) or after (day 22, n=5) the sensitization regimen. **PET**: amphetamine PET scan. Subjects received amphetamine (AMP: d-amphetamine, 0.3 mg/kg, p.o) one hour prior to being positioned on the PET couch. Behavioral and physiological data were gathered at 15-minute intervals. ***Sham***: Subjects received amphetamine one hour prior to being positioned on the PET couch; these sessions included all aspects of the PET procedure except tracer injection.

Subjects

Ten men (age, 25.8 ± 1.8 years) were recruited to participate in the sensitization study (approved by the Montreal Neurological Institute Research Ethics Board), seven of who returned for the follow-up scan one year later. All subjects scored above the normal population mean (13.7± 5.2) on the Novelty Seeking scale of the tridimensional personality questionnaire (TPQ)(Cloninger, Przybeck et al. 1991)(mean score and SD: 20 ± 4) which measures individual differences in response to novelty along four subscales on a scale from 0 to 35 ([1] Exploratory-Excitability vs. Stoic-Reserve, [2] Impulsiveness vs. Reflection, [3] Extravagance vs. Reserve, and [4] Disorderliness vs. Regimentation). Novelty Seeking subjects

were selected based on the hypothesis that trait Novelty Seeking is analogous in humans to the hyperactive motor response to a novel environment in rodents(Dellu, Piazza et al. 1996), which is a strong predictor of behavioral and neurochemical sensitization(Hooks, Jones et al. 1991). Exclusion criteria were as follows: current or previous personal history of significant medical illness; personal or first-degree relative history of psychiatric disorder, including, but not limited to schizophrenia, bipolar disorder and substance dependence (assessed using the Structured Clinical Interview for DSM-IV(Badiani, Browman et al. 1995)); regular use of tobacco (> 5 cigarettes/day); and positive urine toxicology for illicit drugs (Triage TM Panel for Drugs of Abuse, Biosite Diagnostics©, San Diego, CA, USA).

PET acquisition protocol

All subjects were asked to fast and abstain from caffeine or tobacco for a minimum of four hours prior to each experimental session. They underwent PET scans on a CTI/Siemens ECAT HR+ PET camera with lead septa removed (63 slice-coverage, with a maximum resolution of 4.2 mm full width half maximum in the centre of the field of view). A catheter was inserted into the subject's antecubital vein for the bolus injection of tracer and blood drawing. Attenuation correction was performed via a 10-minute ^{68}Ga transmission scan. Three of four PET acquisitions were performed following the administration of an oral dose of

0.3 mg/kg amphetamine ingested 60 minutes prior to the i.v. bolus injection of [^{11}C]raclopride (7 mCi), a drug-schedule previously shown by our group to reliably reduce [^{11}C]raclopride BP(Leyton, Boileau et al. 2002). Emission data were collected over 60 minutes in time frames of progressively longer duration. In addition, all subjects underwent high-resolution magnetic resonance imaging (MRI) on a Siemens 1.5 T Vision scanner for the purpose of anatomical co-registration.

Parametric Image generation and voxel-wise analysis

PET images were reconstructed using a 6 mm full width half maximum Hanning filter. Individual dynamic radioactivity PET data were averaged along the time dimension, co-registered to the individual's MRI, and transformed into standardized stereotaxic space(Evans, Marrett et al. 1992). The dynamic PET data were corrected for motion artifacts(Reilhac, Sechet et al. 2003). Parametric images were generated by computing [^{11}C]raclopride BP at each voxel using a simplified kinetic model that uses the cerebellum as a reference tissue devoid of DA $D_{2/3}$ receptors to describe the kinetics of the free and specifically bound ligand(Gunn, Lammertsma et al. 1997). The application of this kinetic model to[^{11}C]raclopride has been previously shown to be insensitive to changes in cerebral blood flow(Aston, Gunn et al. 2000).

Region of Interest Analysis and MRI Atlas-based Segmentation

The MRI volumes were corrected for image intensity non-uniformity(Sled, Zijdenbos et al. 1998) and linearly and non-linearly transformed into standardized stereotaxic space(Talairach and P 1988), using automated feature-matching to the Montreal Neurological Institute template(Collins, Neelin et al. 1994). Automated MRI tissue-type classification and segmentation (Collins DL 1995) were applied, to generate a binary representation of anatomical structures, including caudate, putamen and ventral striatum. Five bilateral areas from the segmented brains were selected for region of interest (ROI) analysis based on previous work (Mawlawi, Martinez et al. 2001; Martinez, Slifstein et al. 2003): ventral striatum (VS; limbic striatum), pre and post commissural dorsal caudate (DC; associative striatum), pre-commissural dorsal putamen (associative striatum), and post-commissural putamen (PDP; sensorimotor putamen). [^{11}C]Raclopride BP values from each ROI were then extracted and corrected for partial volume effects(Aston, Cunningham et al. 2002) .

Behavioral and physiological assessment of acute amphetamine effects

Mood and alertness were assessed at baseline and at 15-minute intervals throughout each experimental session, using visual analogue scales(Bond and Lader 1974) and the Bipolar Profile of Mood States(McNair, Lorr et al. 1992). The addiction research centre inventory (ARCI)(Haertzen, Hill et al. 1963) Benzedrine

Scale was administered at the end of every experimental session to measure the subjective effects of amphetamine. Physiological recordings including electro-oculogram for eye-blink rate and heart rate were carried out in 3-minute blocks at baseline and at regular intervals after amphetamine (45, 75, 90, 120 minutes) (F1000 system; Focused Technology, Ridgecrest, CA). Blood samples for cortisol, prolactin and plasma-amphetamine levels were drawn via the indwelling catheter at baseline, 45, 90 and 120-minutes post drug. Plasma cortisol and prolactin concentrations were measured by radioimmunoassay, using commercially available kits (Kodak Clinical Diagnostics Ltd., Amersham UK). Plasma amphetamine concentrations were analyzed with electron-capture gas chromatography, after extraction and derivatization of amphetamine(Asghar, Baker et al. 2001).

Statistical Analysis

t-Maps were generated(Aston, Gunn et al. 2000) to assess the contrasts between the drug-free control scan and amphetamine scans at doses 1, 4 and 5, as well as the profile of time-dependent changes in [^{11}C]raclopride BP as a function of repeated amphetamine administration (BP at dose 1 > BP at dose 4 > BP at dose 5). Voxel significance was set at $t \geq 4.2$, corresponding to $p < 0.05$ corrected for multiple comparisons across the entire striatum based on random field

theory(Worsley, Marrett et al. 1996). BP values extracted from ROI during amphetamine (dose 1 and 4) and control scans were analyzed using a three-way ANOVA for dependent samples (treatment x ROI x hemisphere). Sphericity was assessed with the Mauchly test and, when indicated, corrections were made with Greenhouse-Geisser adjustments. When appropriate, Least Significant Difference t-tests, Bonferroni corrected, were applied to determine the significance of regional differences in BP between the amphetamine dose 1, dose 4 and the drug-free baseline scans. A separate three-way ANOVA was conducted to assess difference between BP values extracted from ROI during amphetamine dose 1, 4, 5 (follow-up) and drug free-baseline for the 7 subjects who completed the follow-up study (one year later). VAS and POMS rating, plasma levels of amphetamine, cortisol, prolactin and physiological measures (heart-rate and eye-blink rate) where analyzed using two-way ANOVA for dependent samples (treatment x time). When appropriate these analyses included Greenhouse-Geisser adjustments. Pearson's product moment correlation was applied to [^{11}C]raclopride BP extracted from ROIs to assess whether the personality trait of Novelty Seeking could predict the extent of neurochemical sensitization ([^{11}C]raclopride BP at doses 4 and 5 minus [^{11}C]raclopride BP at dose 1) and whether the development of behavioral sensitization (behavioral response at doses 4 and 5 minus behavioral response at dose 1) correlated with the reduction in [^{11}C]raclopride BP. Voxel-wise linear correlation maps were also generated to

test the relationship between sensitization-induced decreases in [^{11}C]raclopride BP and Novelty Seeking personality score.

RESULTS

Behavioral and physiological measures

Subjective ratings: When compared to the first amphetamine administration (dose 1), re-exposure to a fourth amphetamine dose (dose 4) led to increased energy (POMS energetic: $F(4, 36) = 4.36$, $p = 0.029$; dose1 vs. dose 4: $p = 0.056$), alertness (VAS alert: $F(4, 36) = 11.6$, $p < 0.0001$; dose1 vs. dose 4: $p = 0.048$), clearheadedness (POMS clearheaded: $F(4, 36) = 3.02$, $p = 0.054$; dose1 vs. dose 4: $p = 0.009$) and positive mood (POMS agreeable: $F(4, 36) = 3.679$, $p = 0.04$; dose 1 vs. dose 4: $p = 0.002$) (Figure 1). Conversely, re-exposure to the fourth or fifth dose of amphetamine did not significantly affect amphetamine-induced euphoria (POMS Elated, VAS High, Euphoria, Rush) anxiousness (VAS Anxious) or drug-wanting (VAS Want-Drug), relative to first exposure. The "energy" response to amphetamine remained elevated after the one-year latency (POMS energetic: $F(5, 30) = 3.33$, $p = 0.056$; dose1 vs. dose 5: one-tailed, $p = 0.045$).

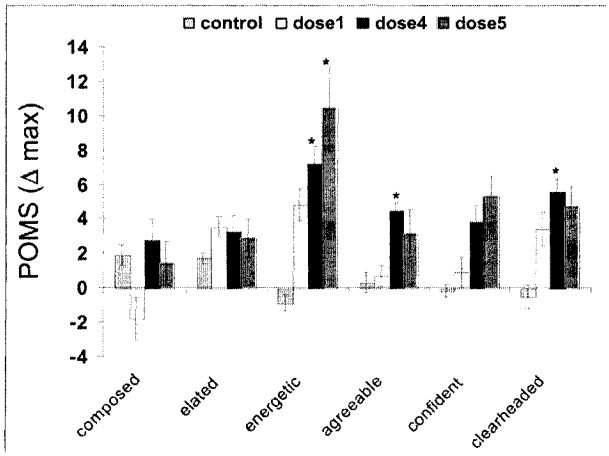

Figure 1 Behavioral effects of amphetamine. POMS scores (peak change from baseline; mean ± SEM) recorded during the drug-free control condition and after 0.3 mg/Kg of oral amphetamine (doses 1, 4 and 5). (*) Significantly increased compared to dose 1.

Physiological measures: Acute amphetamine yielded a time-dependent increase in the rate of eye-blinks per minute at every session (main effect of time: $F(4, 36) = 7.47$, $p = 0.005$). Relative to the first dose (dose 1), re-exposure to acute-amphetamine after the two-week latency period (dose 4), resulted in a small but significant increase in blinks per minute (1.1 ± 0.4 beats per minute) (main effect of session, $F(4, 36) = 7.47$, $p = 0.001$; dose1 vs. dose 4: $p = 0.021$) (Figure 2). This effect was still present upon amphetamine re-exposure one-year later although not statistically significant (Figure 2). Heart rate response to amphetamine was not significantly affected by pre-exposure to amphetamine ($F(4, 32) = 1.250$, $p = 0.31$).

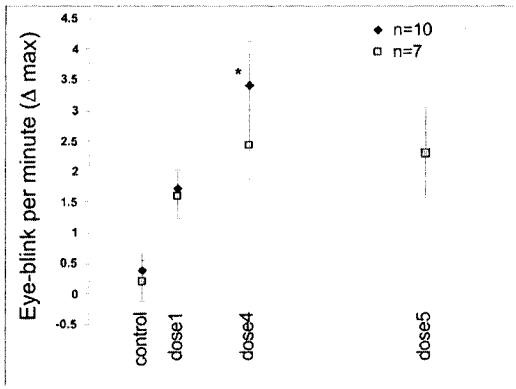

Figure 2 Eye-blink rate (blinks per minute change from baseline; Δ max ± SEM) during control and acute d-amphetamine conditions (doses 1, 4 and 5). (*) Significantly increased compared to dose 1.

Neuroendocrine measures: Relative to the control drug-free condition, amphetamine resulted in a significantly higher cortisol concentration ($F(1, 9) = 7.799$, $p = 0.021$), but not plasma prolactin levels ($F(1, 9) = 1.199$, $p = 0.30$). There were no differences in cortisol or prolactin levels between the different amphetamine sessions.

Plasma amphetamine: The plasma amphetamine concentrations rose in all sessions equally (main effect of time: $F(3, 15) = 64.91$, $p < 0.0001$) with plasma levels peaking on average at 120 min (20.5 ± 3.7 ng /ml). There was no difference between sessions (main effect of session: $F(3, 15) = 0.224$, $p = 0.734$). Amphetamine plasma levels at one-year follow-up were not analyzed.

PET [^{11}C]raclopride

Parametric Map t-statistical tests: Voxel-wise analysis over the whole brain, revealed significant ($t \geq 4.2$; $p < 0.05$) bilateral clusters of decreased [^{11}C]raclopride BP in response to amphetamine doses 1, 4 and 5 relative to the drug-free control scan (Figure 3 a-b-c). The clusters appear smaller in height and extent in the (drug-free - dose1) *t*-map compared to the (drug-free - dose 4) or (drug-free - dose 5) *t*-maps, suggestive of increased DA release in response to doses 4 and 5. The region of statistically significant reduction in [^{11}C]raclopride BP for dose 1 (relative to control) was confined to the ventral striatum and posterior dorsal putamen. However, with doses 4 and 5, there was progressive antero-dorsal extension of this region to include the dorsal parts of the caudate and anterior putamen. Figure 3d illustrates the trend towards decreased BP as a factor of repeated drug administration.

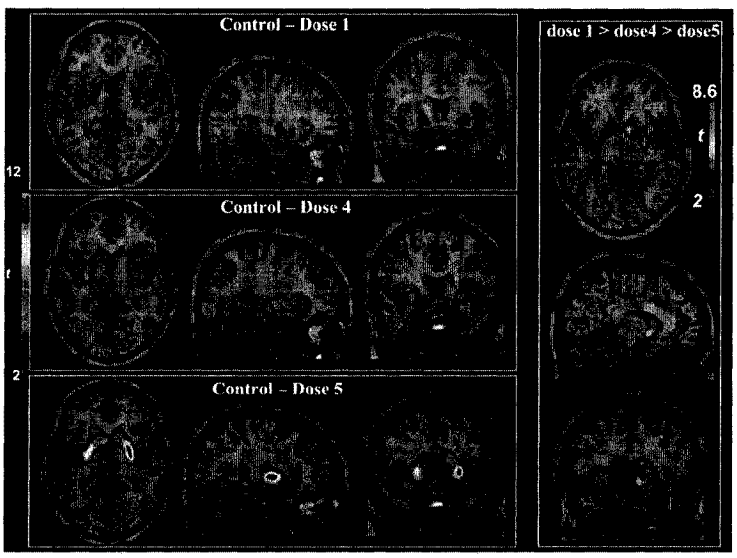

Figure 3 *t*-Statistical maps of [^{11}C]raclopride BP change illustrating a decrease of [^{11}C]raclopride BP following dose 1 **(A)**, dose 4 **(B)** and dose 5 **(C)** amphetamine administrations (0.3 mg/kg p.o.), relative to the drug-free control condition (x: 28; y:2; z: 0). **(D)** General linear model with dose as a regressor illustrating the progressive decrease in [^{11}C]raclopride BP as a factor of repeated amphetamine doses (x: 9; y:7; z: -6). Colored *t*-maps are overlaid on an average T1 MRI of all participants.

Region of interest analysis: Two-way repeated measure ANOVA of [^{11}C]raclopride BP, using ROI and session (drug-free, dose 1, dose 4) as factors, confirmed the *t*-map results (Figure 4 a-b). Both dose 1 and dose 4 of oral amphetamine, resulted in a decreased [^{11}C]raclopride BP, relative to the drug-free session, in two sub-compartments of the striatum (ROI x session interaction; $F(4, 36) = 3.887$, $p=0.01$). In bilateral VS and PDP, this effect corresponded to a significant decrease in the mean [^{11}C]raclopride BP of respectively -17.7 ± 9 % in the VS (Bonferroni corrected for one-tailed planned comparison; $p = 0.034$) and -7.3 ± 3 % in the PDP ($p = 0.031$), following dose 1 of amphetamine, and -28.4 ± 9 % in the VS ($p = 0.007$) and -14.3 ± 3 % in the PDP ($p = 0.001$), following dose 4. The first dose of amphetamine did not significantly reduce [^{11}C]raclopride BP in the anterior and posterior DC or in the anterior DP. Amphetamine dose 4 resulted in a greater [^{11}C]raclopride BP reduction than dose 1 in VS and PDP, corresponding to an additional -12.1 ± 5 % (VS; $p = 0.023$) and -7 ± 3.5 % (PDP; $p = 0.031$) reduction of [^{11}C]raclopride BP, but no difference in DC (-0.3 ± 2 %; $p = 0.99$). At one-year follow-up (dose 5, $n = 7$), amphetamine further reduced [^{11}C]raclopride BP relative to the drug-free session (-24.23 ± 12.5% in the VS, -7.84

± 4.5% in the DC and -20.10 ± 4.8% in the PDP). This effect corresponded to significant BP decreases from dose 1 (ROI x session interaction; $F(6, 36) = 2.483$, $p = 0.041$) (-15.40 ± 5.4% in the VS, $p = 0.02$; -7.38 ± 5.2% in the DC, $p = 0.09$; and -13.97±5.3% in the PDP, $p = 0.01$) and from dose 4 (-9.09 ± 2.5% in the DC and -9.05 ± 3.2% in the PDP).

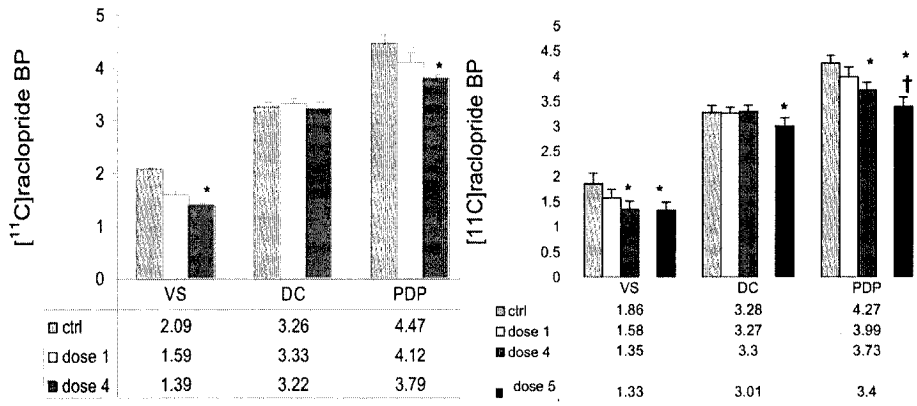

Figure 4 **(A)** Mean (and SEM) [^{11}C]raclopride BP in three sub-compartments of the striatum during the control drug-free scan (ctrl), and following amphetamine administration before (dose 1) and after repeated amphetamine (dose 4) (n = 10), and **(B)** at one-year follow-up (n=7). VS: ventral limbic striatum; DC: associative pre-commissural dorsal caudate; PDP: post-commissural dorsal putamen. (*) Significantly different from dose 1 ($p<0.05$), (†) significantly different from dose 4 ($p<0.05$).

To investigate whether repeated exposure to amphetamine affected baseline (drug-free) [^{11}C]raclopride BP, we compared the baseline striatal [^{11}C]raclopride

BP of those subjects who underwent the drug-free scan before first exposure (n = 5; mean striatal BP = 3.0 ± 0.2) to those who had it after the last exposure (dose 4) (n = 5; mean striatal BP = 2.9 ± 0.36) and found that there was no significant difference ($t = 0.469$, $p = 0.65$).

Brain-behavior Relationships

There were regionally specific correlations between DA release and various behavioral responses that sensitized to repeated amphetamine. Amongst those, the increase in eye-blink rate (dose 4 - dose 1; PDP: $r = -0.73$, $p = 0.015$), energy (dose 4 - dose 1; PDP: $r = -0.67$, $p = 0.03$; dose 5 - dose 1; VS: $r = -0.69$, $p = 0.04$) and alertness (dose 5 - dose 1; VS: $r = -0.75$, $p = 0.02$) correlated with the reduction in [^{11}C]raclopride BP (dose 4 - dose 1). Moreover, the magnitude of the reduction in [^{11}C]raclopride BP in DC was proportional to Novelty Seeking trait scores (dose 5 - dose 1; DC: $r = -0.73$, $p = 0.06$) (Figure 5) and impulsiveness (dose 5 - dose 1; DC: $r = -0.85$, $p = 0.014$).

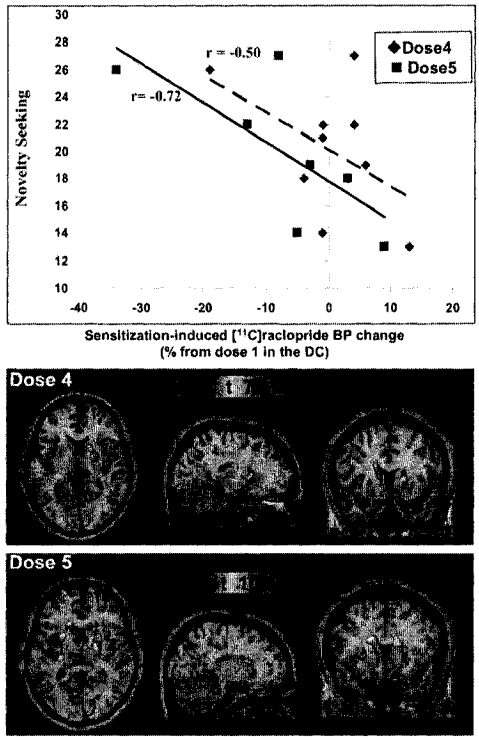

Figure 5 Relationship between novelty seeking personality and sensitization. a) Scatter plots and b) voxel-wise regression maps illustrating the relationship between Novelty Seeking personality trait and sensitization-induced changes in [^{11}C]raclopride BP (% decreases from dose 1) measured during amphetamine doses 4 (x=13, y=15, z=7) and 5 (x=11, y=15, z=15)

COMMENTS

Although widely described in experimental animals, sensitization of the effects of amphetamines has seldom been investigated in humans(Sax and Strakowski 1998; Richtand, Woods et al. 2001). Here, we report, using the [^{11}C]raclopride PET method, that repeated amphetamine administration in

humans led to persistent behavioral and neurochemical sensitization, characterized by increased psychomotor, energy, agreeableness and alertness responses upon re-exposure, together with a proportional increase in amphetamine-stimulated DA release, primarily observed in the limbic and sensorimotor striatum (PDP) and progressing to include the associative dorsal caudate (DC) at one-year follow-up. The dose 1 *t*-map and ROI analysis showing amphetamine-stimulated DA release confined to the ventral limbic (VS) and sensorimotor striatum (PDP) is consistent with previous reports (Leyton, Boileau et al. 2002; Martinez, Slifstein et al. 2003). Moreover, the progressive increase in DA response over time, from the initial amphetamine scan to the 14-day and one-year follow-up studies is compatible with reports indicating that sensitization is a delayed and enduring phenomenon in rats, occurring after withdrawal periods of two weeks or more and persisting for up to one year (Robinson and Becker 1986; Paulson and Robinson 1995). Our results suggest a regional disparity in the temporal emergence of sensitization in humans, with the DC only showing evidence of DA response to amphetamine at the one-year time point. This may reflect a difference between the DA projections to ventral and dorsal striatal regions in their ability to display sensitization, as suggested by some animal experiments(Paulson, Camp et al. 1991) (Porrino, Daunais et al. 2004) (Porrino, Lyons et al. 2004). However an alternative explanation for the lack of effect observed within the DC after the short latency might be the

presence of threshold effects. Effects of amphetamine on [^{11}C]raclopride BP being relatively modest in the DC(Drevets, Gautier et al. 2001; Leyton, Boileau et al. 2002; Martinez, Slifstein et al. 2003), it may be that sensitization-related changes are also present but undetected in the DC after the 14-day drug-free period.

Consistent with previous clinical reports indicating that sensitization of certain effects resulting from repeated amphetamine (e.g. vigor) may co-exist with tolerance to other effects (e.g. "liking")(Sax and Strakowski 1998; Richtand, Woods et al. 2001), repeated amphetamine increased the arousing effects of the drug (alertness, energy) but had little or no influence on drug-induced *high* and *euphoria*. The increase in amphetamine-stimulated DA release correlated with the progressive enhancement of the psychostimulant effect of amphetamine (energy, alertness, eye-blink rate), in line with the hypothesis that DA mediates only some of the behavioral components of sensitization (Paulson and Robinson 1995).

The finding that behavioral sensitization may be achieved experimentally in healthy volunteers and that it is associated with an enduring enhancement of striatal dopamine release in response to acute amphetamine, rests upon the following methodological and conceptual considerations: **(1)** Did the drug sensitization regimen affect D_2 receptor density (or affinity), hence modifying the D_2 baseline set point over time? **(2)** Are stimulant-induced changes in [^{11}C]raclopride BP stable over time and reproducible within-subject? **(3)** What is

the role of context or anticipation of drug effects, if any? **(4)** How generalizable is this finding? (5) Why is it that increased dopamine release in response to stimulant drugs has not been described in drug-dependent patients(Volkow, Wang et al. 1997)?

(1) The validity of the proposed interpretation, that the change in [^{11}C]raclopride BP during the last two amphetamine scans (doses 4 and 5), reflects a change in the extent to which amphetamine stimulates striatal DA release, rests upon the assumption that baseline BP is unaffected by the sensitization-inducing drug regimen. [^{11}C]raclopride BP represents a ratio between the concentration of binding sites (B*max*) and the affinity of [^{11}C]raclopride for $D_{2/3}$ receptors (K*d*). A change in B*max* or K*d* would render the study difficult to interpret. Although an abundant literature suggests that the development or expression of sensitization does not entail major changes in DA D_2 receptor density(Pierce and Kalivas 1997), reports of D_3 over-expression (Guillin, Diaz et al. 2001) and changes in D_2 receptor affinity have been made by some (Seeman, Tallerico et al. 2002), though not by others(Pierce and Kalivas 1997). In this study, half of the subjects underwent the drug-free scan (baseline) at the end, rather than at the beginning of the stimulant-inducing sensitization regimen, in effect, testing for a possible influence of repeated amphetamine on DA receptor density; indeed, [^{11}C]raclopride BP measurements obtained prior to versus following sensitization, were not significantly different. This was further

confirmed in a separate group of healthy volunteers (n = 6) in whom drug-free [^{11}C]raclopride BP was measured prior to, and 14 days after receiving the same amphetamine regimen described above (three administrations of oral amphetamine over five days). The mean striatal BP was respectively 2.35 ± 0.15 prior to, and 2.36 ± 0.23 following, repeated amphetamine (main effect of session: $F(1, 5) = 0.005$, $p = 0.94$). Voxel-wise analyses confirmed that baseline (drug-free) [^{11}C]raclopride BP was not significantly decreased by repeat amphetamine administration.

(2) In the present study, [^{11}C]raclopride BP was measured at several time points over the course of one-year. The stability and reproducibility of this method have previously been demonstrated in test-retest studies: both long-term stability of baseline [^{11}C]raclopride BP (11 months)(Hietala, Nagren et al. 1999) and within-subject reproducibility of amphetamine-induced decrease in ligand binding ([^{123}I]IBZM SPECT)(Kegeles, Zea-Ponce et al. 1999) have been demonstrated.

(3) In the present design, all drug administrations took place in the PET environment, in order to facilitate the expression of behavioral sensitization(Crombag, Badiani et al. 2001). It is not known what proportion of the behavioral and neurochemical effects described here are accounted for by responses to associative cues and anticipation. Indeed, DA release in anticipation of reward, has been shown both in humans during placebo administration and in

primates faced with cues that predict reward(Schultz, Dayan et al. 1997; de la Fuente-Fernandez, Phillips et al. 2002). Nonetheless, although the expression of sensitization can be modulated by context(Anagnostaras and Robinson 1996; Crombag, Badiani et al. 2001; Mead, Crombag et al. 2003), neuroadaptive changes thought to underlie neurochemical sensitization are known to occur in vitro(Robinson and Becker 1982; Vanderschuren, Schmidt et al. 1999) and independently of the drug-paired context (Battisti, Uretsky et al. 2000; Bradberry, Barrett-Larimore et al. 2000). Until further experiments specifically designed to test for conditioning are carried out, the possibility that this may have contributed to an enhanced response to stimulant cannot be entirely discarded.

(4) In this study, an enhanced behavioral and neurochemical response to amphetamine as a result of sensitization was observed in healthy male volunteers, scoring high on Novelty Seeking. Whether this finding can be generalized to males scoring low on Novelty Seeking, females or more generally, other non-clinical or clinical (drug dependence, PTSD, ADHD) populations, besides schizophrenia, is unknown, though plausible. Interestingly, neurochemical sensitization correlated with Novelty Seeking, an observation consistent with results from animal experiments, supporting theories linking this personality trait to vulnerability for substance abuse (Cloninger, Przybeck et al. 1991; Howard, Kivlahan et al. 1997): rodents with a high locomotor response to novel environments, when compared with low responders, show higher stress

and drug-induced firing in mesencephalic dopaminergic neurons, sensitize more readily to amphetamine, and show a higher propensity to self-administer drugs of abuse (Dellu, Piazza et al. 1996) (Deminiere, Piazza et al. 1989) (Piazza, Deminiere et al. 1989) (Hooks, Colvin et al. 1992).

(5) A report that detoxified cocaine-dependent patients exhibited an apparent tolerance or blunting of striatal DA responsiveness (and self-reported high) to a challenge dose of methylphenidate, relative to healthy controls, is somewhat surprising (Volkow, Wang et al. 1997). Based on their findings of decreased DA D_2 receptor levels in the striatum and decreased drug-induced DA release, Volkow and colleagues have argued that a malfunctioning dopamine system in chronic drug-users might be responsible for decreased sensitivity to non drug associated context and non drug reinforcers (Volkow, Fowler et al. 2004). Indeed, this theory is supported by fMRI studies showing decreased activation in cocaine abusers compared with controls when exposed to salient non-drug stimuli(Garavan, Pankiewicz et al. 2000); conversely, those subjects demonstrate large increases in activity in prefrontal cortex and nucleus accumbens, when attending to drug-cues. Thus, it is possible that in those studies, the absence of explicit pairing of the (PET) environment with the drug might have inhibited the expression of sensitization. Another plausible explanation for the blunted DA response to methylphenidate in chronic cocaine users could be that the decreased baseline [^{11}C]raclopride BP, interpreted as

decreased DA D_2 receptor levels, in fact, reflects elevated dopamine levels at baseline. Specifically, relative to stimulant naïve subjects, chronic cocaine users might be exhibiting cross-sensitization(Pani, Porcella et al. 2000; Stewart 2000; Barr, Hofmann et al. 2002; Nikulina, Covington et al. 2004; Kikusui, Faccidomo et al. 2005) to the stress related to the novel PET environment, or alternatively, be anticipating drug-reward(Schultz, Dayan et al. 1997) (de la Fuente-Fernandez, Ruth et al. 2001), in either case, releasing more DA at baseline, hence, making it difficult to detect further reductions in [^{11}C]raclopride BP.

In conclusion, the results presented in this study demonstrate that sensitization, expressed in the form of persistent changes in brain DA neurochemistry, may occur in humans following repeated intermittent exposure to stimulants. This finding has important clinical and pathophysiological implications. First, sensitization-like phenomena are believed to be central to the development of drug-seeking behavior(Robinson and Berridge 1993; Robinson and Berridge 2001). Animal studies show that sensitization increases the motivation to self-administer stimulant drugs, possibly via a mechanism that involves DA (Vezina 2004). It is thought that enhanced DA response to drugs may act to increase the incentive value of the drug (Robinson and Berridge 1993; Robinson and Berridge 2001), a hypothesis supported in humans by PET studies showing that the DA response to amphetamine correlates with desire for the

drug(Leyton, Boileau et al. 2002; Oswald, Wong et al. 2005). Our finding of a relationship between sensitization and novelty seeking personality provides a partial mechanism whereby drug addiction could result from the coupling of an inherent vulnerability and repeated drug exposure (Deroche-Gamonet, Belin et al. 2004).

Second, a phenomenon similar to sensitization induced by drugs may play a role in psychosis (Lieberman, Sheitman et al. 1997; Yui, Goto et al. 1999; Crombag, Badiani et al. 2001). It is hypothesized that repeated exposure to dopaminergic stimulation, whether from drugs or stress, results in a persistent hyperdopaminergic state which, in the presence of other risk factors (e.g. genetic vulnerability, perinatal lesion, altered brain development, stressful life events), could lower the threshold for acute psychosis. This may also explain why clinically stable remitted patients with chronic relapsing disorders such as psychosis or addiction, relapse in response to environmental stressors or drugs of abuse (Yui, Goto et al. 1999). Third, psychostimulants, such as methylphenidate and amphetamine are commonly prescribed to children with Attention Deficit Hyperactivity Disorder (ADHD). Although longitudinal studies have yielded no conclusive evidence that the therapeutic use of methylphenidate is unsafe (Spencer, Biederman et al. 1996; Volkow and Insel 2003), in particular during the course of treatment in ADHD, the present findings emphasize the

need to further investigate the behavioral and neurobiological consequences of long-term treatment with stimulant drugs.

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7.0. GENERAL DISCUSSION

7.1. OVERVIEW

Various imaging techniques can be used to study the effects of drug on the structure and function of the brain in living human. In this regard, PET, a functional imaging approach used to study the metabolism, physiology and neurochemical transmission in the brain, has yielded important empirical findings. Experiments reported in the present thesis focused on chemical (i.e., neurotransmitter) alterations following acute and repeated drug consumption. Specifically, we investigated the activation of DA $D_{2/3}$ receptors in the functional subdivisions of the human striatum after alcohol (**study 2**), and after acute (**study 1**) and repeated amphetamine administration (sensitization) (**study 3**). $D_{2/3}$ receptor availability (BP) was measured with PET and i.v. bolus [^{11}C]raclopride in healthy volunteers under control conditions and after the administration of amphetamine (0.3 mg/kg) or alcohol (1 ml/kg) by mouth. Main findings showed that **1)** both amphetamine and alcohol administration reduced $D_{2/3}$ receptor availability in limbic (VS) and sensorimotor (PDP) regions but not in the associative regions (DC, PDC, DP). **2)** Repeated administration of amphetamine yielded even greater BP reduction in the VS and this effect also expanded to the associative regions (DC, DP). Results of region-of interest analysis were confirmed by voxel-based analysis. **3)** Amphetamine or alcohol-related *drug-wanting* and cardiac response but not self-reported euphoria, *high, drug-liking*

were associated with changes in regional BP during acute challenges. These later subjective measures (euphoria, high, drug-liking) however consistently predicted BP changes at subsequent doses suggesting that anticipation of drug-reward (conditioning) may modulate DA response to later exposure. In addition inter-individual variability in response might represent vulnerability markers since we found that personality trait novelty seeking predicted drug-induced BP changes.

In the following sections we will firstly address **1)** methodological considerations relevant to the interpretation of radioligand displacement secondary to acute a repeated drug challenges. We will then discuss points that were raised by our main findings; **2)** How can we explain that the magnitude of effect seen secondary to alcohol is equal to that seen after amphetamine? **3)** What is the relevance of the higher sensitivity of the ventral striatum over the dorsal striatum, **4)** can the reduction in [^{11}C]raclopride BP be attributed to anticipation or conditioning? **5)** What is the clinical relevance of sensitization?

7.2. METHODOLOGICAL CONSIDERATIONS

As described in chapter III, changes in the binding ability of the radioligand [^{11}C]raclopride (BP) are used as a quantitative index of synaptic DA release to indicate alterations in neurotransmitter concentration in the striatum in

response to pharmacological or cognitive challenges (for review (Laruelle 2000). This method is being extensively applied in neuroscience and psychiatric research, however it does not provide a direct measurement of endogenous DA levels. The simplest framework for interpreting radioligand displacement studies is the competition paradigm. This theoretical model assumes that the exogenous radioligand compete with DA for the same DA receptor site. In this regard, the standard and well-replicated finding is that changes in [^{11}C]raclopride BP are dependent on endogenous transmitter levels, i.e.: DA-elevating challenges proportionally diminish [^{11}C]raclopride BP (see chapter III). Furthermore in line with the characteristic of a "true competition" model for reversible ligands, depleting DA stores by pre-treating with reserpine or α-methyl-paratyrosine, increases [^{11}C]raclopride BP by increasing the affinity of the receptor ($1/K_d$) but not receptor density (for example, through receptor upregulation) (B_{max}) (Ginovart, Farde et al. 1997; Laruelle, D'Souza et al. 1997). The interaction between binding of benzamide radioligands and DA is, as others have described, far more complex than a true competition (Laruelle 2000; Ginovart 2005). Indeed, some reservations have been expressed about the significance of the [^{11}C]raclopride BP method on the basis of a variety of technical points which are summarized from a critical review published by Laruelle (Laruelle 2000). **1)** One point of controversy regarding the validity of the occupancy model emerges from the observation that the principle seems to apply solely to benzamide compounds but not to butyrophenone such as spiperone. **2)** Another observation

that is inconsistent with the theoretical framework is the temporal discrepancy between microdialysis assessment of DA levels and changes in [^{11}C]raclopride BP. Specifically, whereas microdialysis reports that DA dissipates within two hours of amphetamine administration, [^{11}C]raclopride binding is still reduced for up to six hours after an equivalent dose (Cardenas, Houle et al. 2004). **3)** A finding that directly refutes the validity of a simple competition explanation comes from both *ex vivo* and *in vivo* findings that amphetamine treatment seem to affect B_{max} (Sun, Ginovart et al. 2003; Ginovart, Wilson et al. 2004). Thus suggesting the involvement of non-competitive mechanisms.

This being said it is likely that other factors actively contribute to [^{11}C]raclopride BP changes. Several proposals have been formulated in view of reconciling these discrepancies and to thoroughly characterize this commonly used index of DA release. Briefly, whereas the occupancy model posits that all receptors should be equally accessible to the ligand, the *two-state model* proposes the existence of a receptor state which is reserved for the agonist (Battaglia and Titeler 1982; George, Watanabe et al. 1985). Specifically, two inter-convertible $D_{2/3}$ receptor configuration exist, a high affinity state (G-protein-coupled; representing < 50% of $D_{2/3}$ receptors) and low affinity state (uncoupled with G-proteins; representing ≈ 50% of $D_{2/3}$ receptors); unlike antagonist that bind non-selectively to receptors in both states, agonist receptors ligands form a tight selective bond only with DA receptors in the high affinity state. This property

would result in the non-competitive occupation of the receptor by DA thus occluding the binding of [^{11}C]raclopride and reducing B_{max} (Seeman, Guan et al. 1989). For this reason, it has been suggested that one solution to resolve the flaws of the occupancy model and to obtain a more potent displacement, would be to use the advantage of full agonist ligands (for example: propyl-hexahydronaphtho-oxazin; PHNO and NPA) in which binding is theoretically expected to be "complete" (i.e.: 100% susceptible to competition by endogenous DA) (Cumming, Wong et al. 2002; Cumming, Gillings et al. 2003; Narendran, Hwang et al. 2004; Wilson, McCormick et al. 2005). Another interpretation to account for the inconsistencies in the initial model is that DA, upon receptor binding (to an allosteric site) causes a change in the biochemical conformation of the receptors which would influence apparent receptor density (*allosteric model*) (Ginovart, Wilson et al. 2004). The *internalization model* described in chapter III is yet another view - and probably the most accepted mechanism described to account for results that contradict the occupancy model. This mechanism suggests that in face of a pharmacological event that triggers massive DA release, the rate of endocytosis is increased dramatically, receptors are therefore redistributed to the cytosol in a compartment inaccessible to membrane-(quasi) impermeant antagonist radioligands (Laruelle 2000)(Figure 1). This could explain changes in B_{max} resulting from DA-elevating challenge and also explain the inconsistencies between ligands of different lipophillicity.

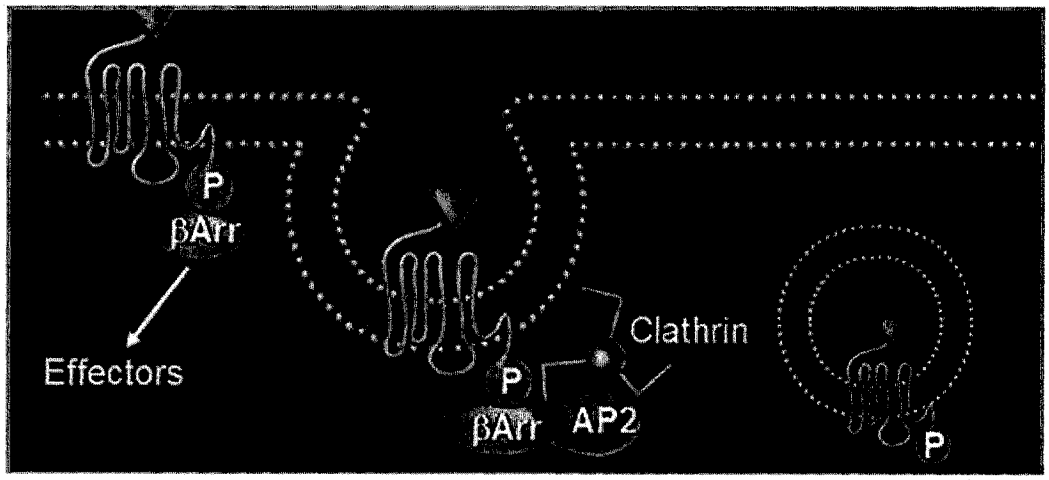

Figure 1 Schematic of the internalization of receptors. Loss of surface receptor number through endocytosis which occurs secondary to agonist treatment is a mechanism believed to explain changes in [^{11}C]raclopride binding.

The mechanism of non-competitive interaction might have some important implication when interpreting results, particularly those derived from clinical population in which receptor trafficking / internalization dysregulation has been reported (Bergson, Levenson et al. 2003); in the context of this thesis, some assumptions are necessary. The measurement of [^{11}C]raclopride BP is related to the total amount of neurotransmitter and when compared with a baseline scan, [^{11}C]raclopride BP measured during a pharmacological challenge reflects some sort of average alteration in the DA concentration over the course of the PET scan i.e.: PET measurements of [^{11}C]raclopride BP are valid estimates of DA release in humans. Based on published findings we can also make the assumption that tracer BP has a good within-subject reproducibility and reliability since both amphetamine-induced decrease [^{123}I]IBZM BP and baseline [^{11}C]raclopride BP were shown to be stable in a test/retest paradigm (Volkow,

Fowler et al. 1993; Kegeles, Zea-Ponce et al. 1999). We can also assume that the changes in regional cerebral blood flow (in regions of interest and reference regions) secondary to amphetamine or alcohol do not significantly affect the distribution volume of the ligand. Both experimentally and simulation studies explicitly looked at the impact of changing blood flow and found that the outcome measure (BP) was unaffected by physiologically possible changes in blood flow (Logan, Volkow et al. 1994).

As noted above, although [^{11}C]raclopride BP has a good within-subject reproducibility, remaining stable across (short) testing time, one issue that we were concerned with was whether this measure would be affected by the repeated amphetamine regimen. We addressed this issues in the discussion included in chapter IV; if repeated amphetamine causes a variations in B_{max} or k_d, the consequence would be a change in BP that may not reflect the same DA release accurately and could corrupt straightforward interpretations of differences in DA release between conditions. We therefore tested a group of subjects (n = 6) during a baseline scan prior to an after a repeated amphetamine regimen and found that baseline [^{11}C]raclopride BP (the ratio of B_{max} over k_d) was unaffected by the amphetamine pre-treatment. In light of the *two-state model* one might argue that these results do not discard the possibility that the repeated amphetamine treatment increased the proportion of $D_{2/3}$ in high affinity state. An increase in $D_{2/3}$ configured in high affinity state would lead to a greater

apparent reduction in [^{11}C]raclopride BP during a DA-elevating challenge. This idea has been supported by an *ex vivo* Scatchard analysis showing that in sensitized animals, the addition of guanine nucleotide (which converts the high-affinity state to low-affinity state) unmasks a 4-fold increase in $D_{2/3}$ receptors in the high affinity state (Seeman, Tallerico et al. 2002). *In vivo* studies using an agonist tracer such as PHNO or NPA would be valuable in teasing apart the contribution of possible changes in $D_{2/3}$ receptors affinity state from the dopaminergic response to sensitization.

7.3. THE QUESTION OF EQUAL MAGNITUDE

In the present dissertation we report that alcohol promotes a change in [^{11}C]raclopride BP in the VS (-13%) of equal magnitude to the changes in [^{11}C]raclopride BP induced by a low dose of amphetamine (-10% in the VS relative to a placebo and -17% relative to a drug-free condition). This adding to the general perplexing observation that DA elevating challenges (pharmacological and cognitive) measured *in vivo* with PET result in changes in [^{11}C]raclopride BP situated in the area of 15% (see chapter III). This observation however contrasts with microdialysis results which have shown that amphetamine is a much more potent DA-releasing agent. Specifically microdialysis studies which measure both synaptic and extra synaptic

concentration of neurotransmitter, have shown that amphetamine increases DA by a factor of ten in the VS whereas DA release secondary to alcohol is observed to be, in contrast, relatively weak representing approximately 1/4 of this effect (Di Chiara and Imperato 1988).

There are at least a few likely reasons to explain this discrepant finding. One of the reasons that have been evoked resides in a methodological limitation that is assumed to involve a *ceiling effect* occurring in binding studies *in vivo*. The *ceiling effect* entails that massive changes in DA release, as those triggered by amphetamine, do not reduce [^{11}C]raclopride binding by more than 50% (Laruelle 2000). The factors contributing to this phenomenon have been hypothesized to be due to the limited number of receptors on which changes can be assessed. The occupancy model claims that competition between DA and [^{11}C]raclopride is unlikely to be measured at extra synaptic site (Abi-Dargham, Martinez et al. 2000) mainly because synaptic DA receptors are not densely concentrated there and because extrasynaptic DA levels are low due to the very efficient DA reuptake system (Jones, Gainetdinov et al. 1998). In this case, both alcohol which increases DA mainly though phasic DA release and amphetamine which provokes increases in DA concentration via reverse transport through the DAT (in synaptic and extrasynaptic space) could be as potent in displacing [^{11}C]raclopride from synaptic sites. The low efficiency of DA, being an agonist, in occupying low affinity state receptors (50% of receptors are in high affinity

state) is also a factor contributing to the narrow window for measuring change. Lastly the portion of the receptor pool already occupied by DA in the baseline state (10%) also reduces the opportunity for displacement (Laruelle 2000). Under this model [^{11}C]raclopride binding would be susceptible to competition by endogenous DA on a small portion of receptors: those in high affinity state, located in the synapse and which are not occupied at baseline by DA i.e.: 44% (40/90) of receptors. In baboons, the increase in DA extracellular concentration following 1 mg/kg of amphetamine is believed to represent close to the maximal release that can be elicited in the brain; at this dose the displacement is approximately equal to the receptor population that is in fact susceptible to competition by endogenous DA (i.e.: 40%)(Laruelle, Iyer et al. 1997) (Figure 2). One conclusion is that changes in amphetamine induced DA might be greater however the method is not sensitive enough to detect those changes.

[^{11}C]raclopride BP merely measures relatively different amounts of neurotransmitter released and cannot distinguish between kinetically different neurotransmitter perturbations. One might think that the failure to find a magnitude difference between alcohol and amphetamine's ability to displace [^{11}C]raclopride is partly due to differences in timing and duration of the drugs i.e.: a difference in the pharmacokinetic of the drugs. Indeed it is recognized that the initial fast uptake of the drug into brain rather then its steady-state presence is key to its reinforcing effects (Seeman and Madras 1998). The finding that DA

changes induced by intravenously (peak brain uptake occurring about 10 min) and orally (peak brain uptake occurring about 60–90 min) -administered methylphenidate were comparable (-20%) suggests that pharmacokinetics of the drug could not explain equal magnitude (Volkow, Fowler et al. 2004).

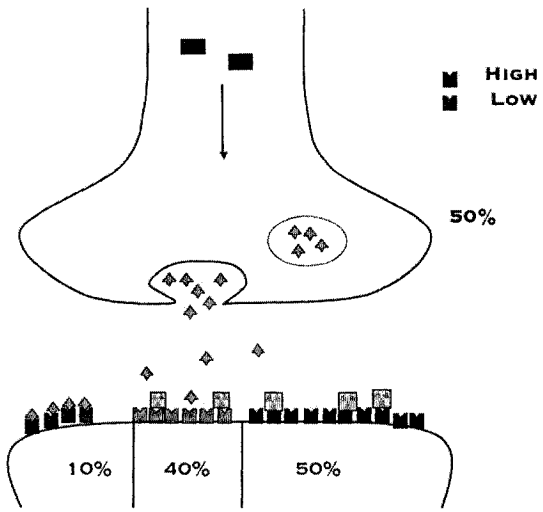

Figure 2 *Two-state model* of $D_{2/3}$ receptors affinity illustrating that $D_{2/3}$ receptors are divided into two states for agonists (high affinity and low affinity sites), with each site contributing to 50% of the population. Under baseline conditions (prior to the challenge), 10% of the sites are occupied by DA, and not available to [^{11}C]raclopride. Adapted from (Laruelle 2000).

7.4. WANTING OR LIKING?

In the studies reported in the present thesis no direct associations were observed between the subjective positive effects of amphetamine, i.e.: euphoria,

drug-liking, and acute changes in [^{11}C]raclopride BP. This contrasts with the work of others who have found that positive drug-induced mood was correlated with the supraphysiological changes in DA secondary to stimulant administration as measured by both $D_{2/3}$ receptor and DAT radioligand displacement (Drevets, Gautier et al. 2001) (Martinez, Slifstein et al. 2003) (Volkow, Wang et al. 1997). Our failure to find a relationship between drug-induced euphoria and changes in DA could in part be due to the different route of administration that was used in our studies. Whereas others have used mainly intravenous administration, subjects in our studies received the drug by mouth. In line with the views of Robinson and Berridge, we interpret our results as evidence that DA release secondary to drug reinforcement attributes incentive salience to cues associated with reward (Robinson and Berridge 2001) (see chapter I). Repeated administration of amphetamine lead to neurochemical and behavioral sensitization (see chapter VI), however in this paradigm we did not find that sensitization increased behavioral intention to use the drug, namely: *drug-wanting*. This finding is difficult to interpret. Unfortunately, the manner in which *drug-wanting* is measured in our studies, i.e.: by verbal self-report, is not optimal and might lack the precision to capture change. Furthermore, some have suggested that *drug-wanting* might exist even in the absence of awareness (Robinson and Berridge 1993). According to this perspective verbal assessments would be slightly inadequate measures and should be complement with behavioral measures such as drug-seeking (through progressive ratio schedule

for example). In addition it is possible that our failure to find changes in this index simply reflects subjects boredom in participating in a protocol that involves five sessions.

7.5. DORSAL AND VENTRAL MATTERS

We found that the acute effects of both alcohol and amphetamine on [^{11}C]raclopride BP were preferential in the ventral limbic striatum (VS) and sensorimotor striatum (PDP) but not in dorsal caudate (DC), and that after repeated doses of amphetamine the effect expanded to include the DC. In the context of DA activation by psychostimulants (and drugs of abuse in general), both PET and microdialysis studies report a higher sensitivity of the ventral striatum (nucleus accumbens) over the dorsal striatum (Di Chiara and Imperato 1988; Drevets, Gautier et al. 2001; Martinez, Slifstein et al. 2003). This anatomically selective pharmacodynamic effect has prompted the view that preferential ventral dopaminergic activation is one the main mechanisms of the reinforcing effects of psychostimulants (Di Chiara 2002).

The reason for this heterogeneous response is unclear. On one hand, several pre and postsynaptic factors have been suggested to contribute to this effect. The observation that amphetamine may induce larger DA release in the ventral striatum compared with the caudate-putamen might reflect features of

striatal and midbrain microscopic organization, i.e.: the distribution of receptor proteins and up-take sites for example. In this regard, it has been shown by both *post mortem* studies in humans (Piggott, Marshall et al. 1999; Hurd, Suzuki et al. 2001) and PET studies investigating pig as well as monkey brains (Rosa-Neto, Doudet et al. 2004) that there is a decreasing posterior to anterior gradient for D_2 receptors in the striatum. However D_3 receptors (which raclopride also bind to) are predominately distributed in the limbic striatum. Relative to the D_2 receptors, the D_3 have been shown to have a much higher affinity for the agonist (Sokoloff, Giros et al. 1992). The higher density of high affinity D_3 receptors in the VS may result in a higher potency for [^{11}C]raclopride displacement. In addition, midbrain *ventral tier* neurons, which project to dorsal parts of the striatum, express higher levels of mRNA for D_2 autoreceptors (Haber, Ryoo et al. 1995). This finding suggests a greater inhibition of DA neurons firing and release onto dorsal striatum. DAT are more densely distributed in the ventral caudate than in the putamen (Piggott, Marshall et al. 1999). This organization suggests an increased reuptake activity in the associative caudate. Although, since DAT blockade is the initial pharmacological effect of amphetamine it is difficult to estimate if a greater DAT density also increases the sites of action for amphetamine and in turn synaptic DA concentration.

On the other hand, the anatomically heterogeneous response could be in part related to methodological issues discussed earlier (see above). Specifically it

might be that dorsal and ventral striatum are differently affected by the *ceiling effect*. For example it is possible that synaptic vs extrasynaptic receptors or receptors in different configuration states (high affinity vs low affinity) are distributed unequally between ventral and dorsal structures (Martinez, Slifstein et al. 2003) (Laruelle 2000).

As described in chapter I, the striatum, is a heterogeneous structure that includes several anatomic subdivisions which have been functionally subdivided based on their connections (see chapter I) into *limbic areas*, involved in drive and motivation, *associative areas*, involved in cognition and *sensorimotor areas* involved in locomotion (Joel and Weiner 2000). PET [^{11}C]raclopride studies have now shown that distinct striatal compartments display a differential sensitivity to amphetamine. Specifically a greater effect is shown in the VS and PDP with no effect in the DC. Microdialysis studies do not systematically investigate the difference between DC and PDP, and therefore do not provide any reports of this specific effect. The pattern of change induced by DA-elevating drugs is believed to result from the non-reciprocal pattern of connection between these structures and the midbrain. Specifically, DA mediated information progresses from the VS limbic, to DC cognitive, to PDP sensorimotor areas in a feed-forward type inhibition (Martinez, Slifstein et al. 2003). Briefly increased DA release in the VS would lead to an inhibition of SNc through ascending VS GABAergic connections. This would in turn reduce DA release in the DC and the inhibitory

influence of DC on SNr. Cells of the SNr projecting to the PDP would thus be desinhibited, resulting in a larger DA release in the PDP (Martinez, Slifstein et al. 2003) (Figure 3).

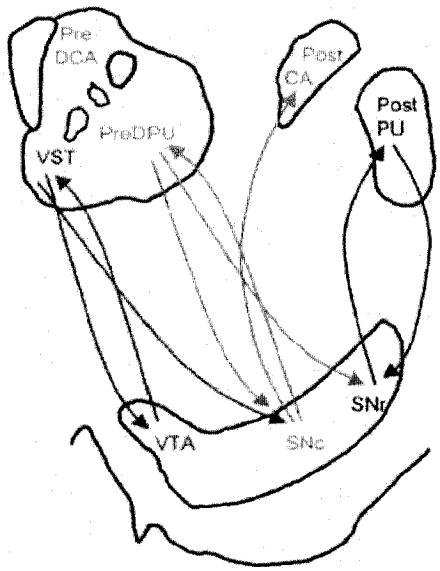

Figure 3 Schematic of the nonreciprocal striatonigrostriatal subcircuits, which provides a hypothetical model for the preferential effect of amphetamine in the VS and PDP. **VTA**: ventral tegmental; **SNc**: substantia nigra compacta, **SNr**: substantia nigra reticulata; **Pre DCA**: anterior dorsal caudate; **Pre DPU**: anterior dorsal putamen; **Post CA**: posterior dorsal caudate; **Post PU**: posterior dorsal putamen. The limbic loop is in *red,* the associative loop is in *green* and the sensorimotor loop is in *blue.* Ascending arrows represent DA projections from the midbrain descending arrows represent GABA projections from the striatum. Adapted from **(Martinez, Slifstein et al. 2003) (Haber, Fudge et al. 2000).**

In the present dissertation we report that the pattern of regional changes in BP induced by acute amphetamine was modified after repeated doses. The topography of the response shifted such that the DC was involved at the one-

year time point but not after a short latency period (14 days). Suggesting that changes in the functional activity of the DA system occupy larger portions of dorsal and ventral striatum with increasing exposure. This finding did not result from spill-over signal from other regions, since correction for partial volume magnified the difference. This regional disparity in the temporal emergence of sensitization may reflect a difference between the DA projections to ventral and dorsal striatal regions in their ability to display sensitization. Indeed, it has also been suggested that acute and sensitized behavioral responses to psychostimulant drugs involve activation of distinct neuronal circuits (Paulson and Robinson 1995; Vanderschuren and Kalivas 2000) and activate different populations of striatal neurons (Canales and Graybiel 2000). Specifically, it has been shown that initial exposure to drug involves mainly the limbic ventral striatum, an area that mediates motivational and affective functions, and as drug exposure is repeated the effect progressively spread dorsally to include most aspects of the dorsal striatum which mediate sensorimotor and cognitive functions (Porrino, Daunais et al. 2004; Porrino, Lyons et al. 2004). The mechanism involved in this reorganization and the nature of this adaptation remain to be clarified. It has been suggested however that the nigrostriatal circuits (includes the dorsal striatum) is involved in habit formation (White 1996). An alternative explanation for the lack of effect observed within the DC after the short latency might be the presence of threshold effects. As discussed above, the effects of amphetamine on [^{11}C]raclopride BP are relatively modest in

the DC it may be that sensitization-related changes are also present after the 14-day drug-free period but undetected in the DC.

7.6. DOES EXPECTATION MODULATE DOPAMINE RELEASE?

It is well known that contextual stimuli associated with stimulant drug administration acquire the ability to elicit conditioned psychomotor activation similar to that produced by the drugs themselves and become potent determinants of their effect. The DA system has been implicated in conditioning and reward prediction in both human and non-human primates (Schultz, Dayan et al. 1997) (de la Fuente-Fernandez, Phillips et al. 2002). It is possible those non-pharmacological variables, i.e.: drug-expectation, response to the PET environment, might have shaped the subject's responses to the drug. Indeed it has been shown that subjects self-rate response to drugs as more positive and have an increased metabolic reactivity to the drug when they are expecting to receive the drug (Wang, Volkow et al. 1999). Similarly animal studies have shown that response to drugs is larger when animal self-administer drugs in a drug-paired context (Duvauchelle, Ikegami et al. 2000). Perception of imminent drug consumption could in part explain the relatively large magnitude of [^{11}C]raclopride BP change during our alcohol vs orange juice paradigm when compared to the amphetamine vs placebo paradigm (see chapter IV & V). Similarly it can also partly explain the magnitude difference between the two

acute amphetamine studies presented in this thesis (see chapter IV & VI). Specifically, relative to **study 3** which was not placebo-controlled, **study 1** yielded a weaker magnitude of change in [^{11}C]raclopride BP (-17% and -10% respectively). In support of the role of anticipation in modulating subsequent drug response we found that positive response to drugs during the initial drug exposure (see chapter VI) predicted [^{11}C]raclopride BP response at following exposure (Figure 4). In as much as rewarding effects of a drug are a predictor of subsequent use (Davidson, Finch et al. 1993) these data lend support to the view that response of the DA system to initial drug exposure may constitute a risk factor for addiction.

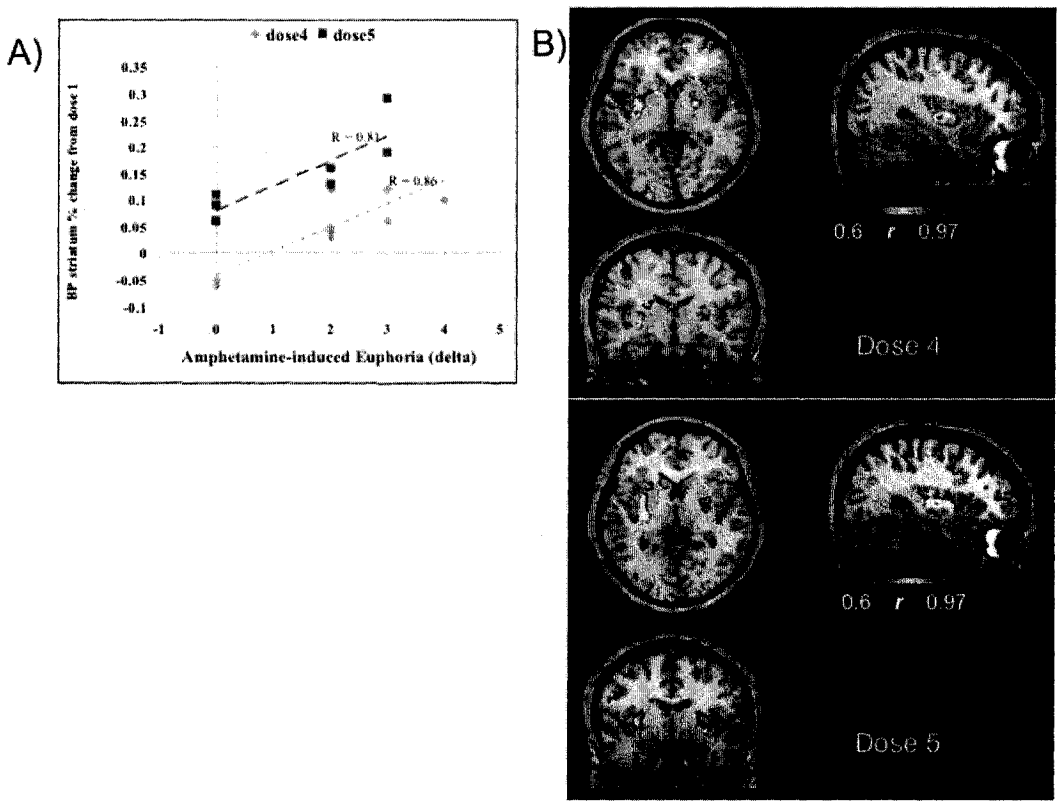

Figure 4 **a)** Scatter plots and **b)** voxel-wise regression maps illustrating the relationship between self-rated positive effects of amphetamine (*euphoria*) during initial dose and changes in [^{11}C]raclopride BP at subsequent exposure (dose 4 and dose 5).

In a recent study (n = 5; still in progress) healthy volunteers underwent the *sensitization-inducing* regimen described in chapter VI with the difference that the last challenge was with a placebo pill rather than with amphetamine. Results showed that the substitution of amphetamine by a placebo pill in the drug-paired context increased DA release in the VS to the same extend as an acute challenge of amphetamine (Boileau, Dagher et al. 2005) (Figure 5). This result allowed us to estimate that approximately 42% of the effect of sensitization on [^{11}C]raclopride BP (12% of the 29% observed) reported in **study 3** could be accounted for by response to associative contextual cues, placebo effect, sensitization to drug associated cues (associative sensitization) or to all of these factors combined. This suggesting that sensitization is powerfully modulated by context and conditioning.

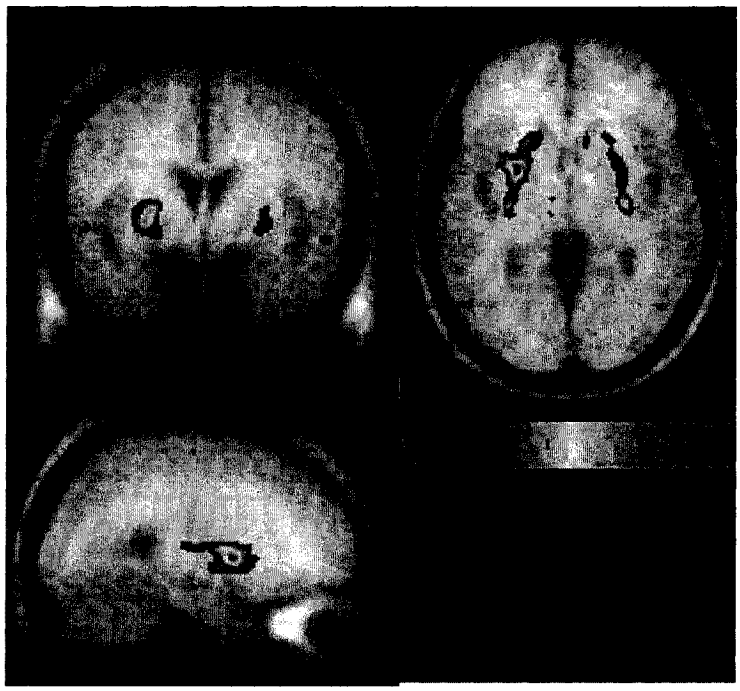

Figure 5 t-Map overlaid average MRI illustrating placebo induced changes in [^{11}C]raclopride relative to a control condition (no-pill). In this experiment, 5 healthy male volunteers received three doses of amphetamine (0.3 mg/kg, by mouth) in the same test environment (PET unit) on three separate days (every other day). Two weeks later, the amphetamine was substituted for a placebo pill.

7.7. IS NOVELTY SEEKING A RISK FACTOR FOR ADDICTION?

A plethora of vulnerability factors for drug-abuse have been identified in humans, including genetic, social (economic, familial), and personality indices. In this regard, novelty-seeking (NS) personality trait has not only been described as etiologically significant in addiction but it is believed that differences in this personality style reflect individual variation in mesolimbic DA

activity(Cloninger 1987). Individuals who scored high on Cloninger's Tridimensional Personality Questionaire (TPQ) NS scale (Cloninger, Przybeck et al. 1991) (see chapter III) are described as having a temperament related to impulsivity, investigatory behaviour and sensitivity to novelty (Eysenck 1953; Cloninger 1987; Jaffe and Archer 1987; Zuckerman and Kuhlman 2000). Furthermore they have been identified as more likely to be diagnosed with alcohol and drug dependence disorders (Cloninger, Sigvardsson et al. 1988). In support of this, a wealth of evidence has linked this measure with risk for drug misuse, relapse-rate in detoxified alcoholics and with sensitivity to reward (Meszaros, Lenzinger et al. 1999).

It is well know that DA function in laboratory animals modulates the predisposition to drug self-administration (Piazza, Deminiere et al. 1989). Although a considerable effort has been proffered in an attempt to understand the neurobiological basis of inter-individual responses to drugs in humans (specifically DA-related hormone levels, monoamine oxidase levels and $D_{2/3}$ receptor expression), evidence of neurobiological markers differentiating high and low responders is not entirely consistent (Koob 2003). One methodological approach, which greatly profits from the advent of PET technology, involves identifying putative vulnerability characteristics (for example personality style) that predict sensitivity of the DA system to the psychomotor stimulant (or rewarding) effects of drugs. With this in mind, one of the aims of this dissertation

was to investigate the relationship between DA transmission through radioligand-displacement studies and personality trait NS in otherwise healthy, "risk-free" individuals (assed through personal and family history of DSMIV axis I disorder). We found that subjects who scored high on the NS TPQ scale released more DA in the VS upon exposure to both amphetamine and alcohol (see chapter IV and V). They also seemed to be more susceptible to sensitize to a repeated amphetamine regimen or perhaps, more sensitive to environmental cues that predicted the administration of the drug since re-exposure to amphetamine after a drug-free latency, triggered a greater magnitude of DA release (see chapter VI). Furthermore these individuals demonstrated that the effect of the drug was likely reinforcing as they readily reported increased "drug-wanting" during acute administration of amphetamine (see chapter IV). Taken together these findings corroborate the relevance of using trait NS to predict DAergic response to drugs. The next relevant question then is whether these individuals are at higher risk for drug abuse?

NS have a distinct disposition to engage in behaviour motivated by positive incentive and are more sensitive to salient meaningful stimuli (Depue and Collins 1999). From an evolutionary perspective, a strong exploratory drive provides adaptive skills. It increases opportunities to achieve goal, novel resources, social cooperation and assertiveness. In as much as novelty-seeking reflects risk-taking, then by definition it carries with it some potential for

negative outcome - drug abuse being one of them. Though some argue that occasional drug experimentation is an "appropriate", normative, and perhaps adaptive developmental process (Shedler and Block 1990). One conclusion is that NS might have a higher primary appetitive motivation or sensitivity to salience by virtue of DA transmission, however, in the absence of other risk factors (emotional distress, bad coping styles, low self-esteem, poor parental bonding, socioeconomic status, genetic predisposition), this trait has been shown to be a poor predictor of response to the reinforcing-effects of amphetamine (Corr and Kumari 2000). In this regard, one factor that has been postulated to play a key role at rendering a brain more susceptible to disruption by drugs is a low level of $D_{2/3}$ receptors (Volkow, Fowler et al. 2004). Specifically, drug addicted individuals have been shown to have a markedly disrupted DA system (decreased $D_{2/3}$ receptors level) coupled with a diminished motivation and salience attribution to natural reinforcers (Volkow, Chang et al. 2001) (Figure 6). Under this hypothesis, it is believed that endogenous or (chronic) drug-induced diminished $D_{2/3}$ receptor expression puts individuals at greater risk for seeking drug stimulation in order to compensate for the *anhedonia* related to DA-deficiency.

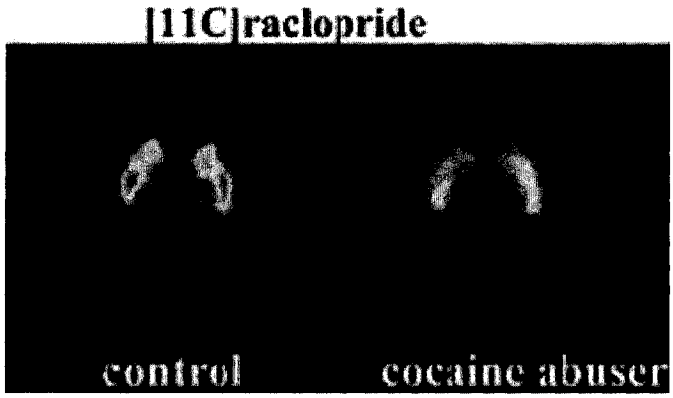

Figure 6 PET [^{11}C]raclopride measurements of DA $D_{2/3}$ receptor availability obtained in detoxified cocaine users and healthy control. The reduction in signal in (BP) in cocaine users is presumed to represent a reduced number of $D_{2/3}$ receptors. Adapted from (Volkow, Fowler et al. 2004).

Furthermore, it has been shown by the same group that non-addicted healthy individuals with a high striatal receptor density self-rate methylphenidate effects as negative (Volkow, Fowler et al. 2004). This suggesting that a high level of $D_{2/3}$ receptors can in fact act as a protective factor. Interestingly, studies in non-human primates have shown that a high level of $D_{2/3}$ receptors is associated with social dominance (Morgan, Grant et al. 2002). A correlation analysis including all subjects participating in the studies reported in the present thesis (n = 24) revealed that there was an association between the personality style exploratory-extraversion (NS1) and the density of $D_{2/3}$ in the striatum ($r = 0.54$; $p < 0.05$) (Figure 7). This does not mean that people with exploratory skills and high social dominance cannot become addicted. Rather more drugs might be needed to alter pre-existing brain chemistry (high $D_{2/3}$), decreasing $D_{2/3}$ expression and triggering DA deficiency and craving.

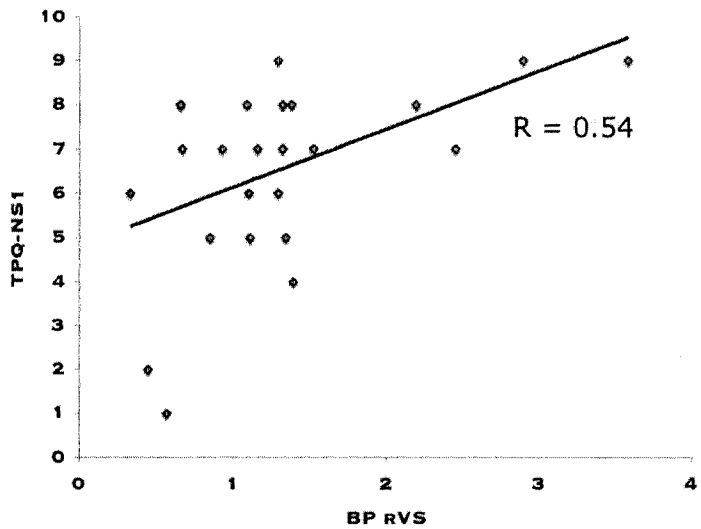

Figure 7 Scatter plots illustrating the relationship between exploratory-extraversion personality (NS1) and [^{11}C]raclopride measurement of baseline $D_{2/3}$ receptor density in a the right ventral striatum (rVS).

7.8. IS THERE EVIDENCE OF STIMULANT SENSITIZATION IN CHRONIC DRUG USERS?

Investigations from different laboratories have been unable to demonstrate psychostimulant hypersensitivity expressed in self-reported mood, cardiovascular, neuroendocrine (prolactin and cortisol) or DA release responses in chronic drug users re-exposed to cocaine, methylphenidate or amphetamine. Rothman and colleagues have in fact failed to observer augmented responses (behavioural, physiological or neuroendocine) to cocaine (25 mg i.v. and 96 mg i.n.) in habitual cocaine users after both a single dose administration (40 mg IV)

or 4 consecutive intranasal doses of cocaine (Rothman, Gorelick et al. 1994; Rothman, Henningfield et al. 1996). Additionally PET studies of stimulant-induced DA release using the neuroligand [^{11}C]raclopride have not shown neurochemical sensitization but rather blunting of striatal DA responsiveness in detoxified cocaine-dependent subjects and in alcohol abusers (reduction in [^{11}C]raclopride BP) relative to healthy control following administration of the DAT blocking agent methylphenidate (Martinez, Gil et al. 2005) (Volkow, Wang et al. 1997). In line with this, animal models of drug addiction have shown that mesolimbic DA transmission appears to be drastically reduced in its tonic activity (for a review see, (Melis, Spiga et al. 2005) (Di Chiara 2002). Other lines of studies have found that despite its reduced activity, the system remains hyper responsive to cues that predict drug. Specifically, it has been found that perceived drug availability (expectation) in detoxified cocaine abusers amplified the effects of psychostimulants, as well as viewing a video of drug paraphernalia increases activity in the OFC (Garavan, Pankiewicz et al. 2000) (Grant, London et al. 1996; Sell, Morris et al. 2000; Volkow, Fowler et al. 2004). The OFC is though to be involved in a circuit that encodes the motivational and emotional value of the drug. Dysfunction of the OFC related circuit, namely reduced basal metabolism in chronic drug-users is believed to contribute to the compulsive drug self-administration in addicted subjects (Volkow, Fowler et al. 1991). These and other results have been integrated in a model suggesting that in addiction, DA hypofunctioning increases the thresholds required for environmental events

to activate DA cells and in contrast this system remains hypersensitive to cues associated with drugs; thus conferring long-lasting vulnerability (Melis, Spiga et al. 2005).

At this point the source of this discrepancy remains unclear, and more human imaging studies are necessary to understand the neurochemical correlates of chronic abuse in humans. Specifically it would be interesting to investigate, in chronic drug users, DA response to the repeated amphetamine regimen described in chapter III. This would allow estimating if environmental cues associated with drug administration (PET environment) would magnify the effect of the drug, thus unmasking sensitization.

7.9. DOES SENSITIZATION HAVE A ROLE IN PSYCHOSIS AND SUBSTANCE ABUSE CO-MORBIDITY?

The association between psychosis and drug abuse is unquestionable. On one hand, a number of epidemiological studies of schizophrenia have documented that the use of drugs increasing DA transmission, for example psychostimulants such as amphetamine and cocaine, but also cannabis (Stefanis, Delespaul et al. 2004; Henquet, Krabbendam et al. 2005), increase the likelihood of developing psychosis. The psychomimetic effect of certain drugs has in fact been an argument in favor of the DA hypothesis of psychosis (Carlsson 1995).

On the other hand, there also happens to be an alarmingly high rate of substance abuse in patients suffering from schizophrenia: 26.5% of individuals with schizophrenia use or have used psychostimulants (methamphetamine, cocaine) in their lifetime, 70 to 90% smoke tobacco, 12 to 42% use or have used cannabis (for review (Murray and Fearon 1999). Furthermore, a retrospective report on cases of psychosis found that approximately one third (33-37%) of patients abused drugs prior to presentation to services (Hambrecht and Hafner 1996; Cantwell, Brewin et al. 1999). In summary, patients with schizophrenia engage in substance abuse to a greater extent than the general population. The nature of this association is a much-debated topic. Several factors have been proposed to interact in this association. One widely held explanation for substance use in schizophrenia includes the view that both disorders share a common etiology (genetic, neurodevelopmental) with common vulnerability factors (social, personality trait, biobehavioral, cognitive) and common premorbid signs (depression, anxiety, antisocial behavior) - and this is why both disorders are susceptible to drugs-use or attempt to self-medicate. Based on the idea of a common neuropathology, it has also been suggested that sensitization of the DAergic system could be a relevant vulnerability factor in the association between drug-abuse and schizophrenia. For example, progressive *endogenous* sensitization in schizophrenia, may initially lead to increased motivation to use drug, which would in turn elicit the onset of psychotic symptoms. Similarly, in individuals susceptible to drug-abuse, initial exposure to drugs might lead to

sensitization to the reinforcing properties of drugs and to the overwhelming compulsion to take drugs. Findings reported in the present dissertation reiterates the importance of conducting investigations aimed at looking at the central role of DA in both these disorders; this in view of developing pharmacotherapy, directed at the underlying pathologies linking addiction and mental illness.

7.10. IS THERE STIMULANT SENSITIZATION IN ADHD?

Five to ten percent of the school-age population, more than 1.5 million children in the United-States, eighty percent of them boys, are now medicated to treat ADHD (Wolraich, Hannah et al. 1996). Psychostimulants such as methylphenidate and d-amphetamine are the most efficacious treatment for ADHD (Santosh and Taylor 2000); they have a beneficial effect on behaviour and intellectual performance in school as well as social interactions with family and peers, and they reduce drop-out rate, substance use disorder, and antisocial behaviour apparently without producing long-term side effects (Solanto 2000). The hypothesis that dopaminergic mechanisms may play an important role in the pathophysiology of ADHD arises primarily from the clinical efficacy of the mixed DAT and norepinephrine transporter blocker methylphenidate. Converging evidence from neurocognitive investigations of ADHD deficits using DA related-tasks (for review see (Castellanos and Tannock 2002; Seidman, Doyle et al. 2004), from molecular genetics (for review, (Bobb, Castellanos et al. 2005)

and from both functional (Ernst, Zametkin et al. 1999; Rosa Neto, Lou et al. 2002; Ernst, Kimes et al. 2003; Lou, Rosa et al. 2004; Jucaite, Fernell et al. 2005; Volkow, Wang et al. 2005) and structural brain imaging studies (neuroanatomical volumetrics of the frontal cortex and striatal strucutres) (Giedd, Blumenthal et al. 2001; Seidman, Valera et al. 2004), strongly point towards a DA dysfunction in ADHD. Recent studies using PET and [^{11}C]raclopride have reported that adolescents with a current diagnosis of ADHD have an increased $D_{2/3}$ availability at baseline, possibly reflecting low resting synaptic DA. This measure of ligand binding was found related to baseline impulsivity and inattention and to positive treatment response to methylphenidate (Rosa Neto, Lou et al. 2002; Lou, Rosa et al. 2004). This suggesting that psychostimulant medication might correct decreased DA neurotransmission in ADHD thereby reducing inattention and impulsivity.

Given the extent of prescribed use of methylphenidate (Ritalin), the question of stimulant sensitivity in humans has been raised through longitudinal prospective studies of children who are diagnosed with Attention Deficit Hyperactivity Disorder (ADHD) and are treated daily with methylphenidate (or amphetamine). Most research looking at the development of sensitization in cases of ADHD has focused on assessing methylphenidate's potential to alter an individual's risk for drug addiction, this based on the fact that methylphenidate and amphetamine are known to interact with the dopaminergic system. Based on

animal findings of (cross-stimulant) sensitization, one would predict that methylphenidate exposure might increase sensitivity to the reinforcing effects of drugs. Some, but not all retrospective human data have supported this hypothesis. Elevated drug use in adults with prior ADHD diagnosis has been reported (Weiss and Hechtman 1979; Barkley, Fischer et al. 2003). As well, a high percentage of treatment-seeking cocaine abusers have been reported to have prior ADHD diagnosis (Cocores, Davies et al. 1987). Findings from other randomized trial studies have however contradicted the notion that stimulant treatment in childhood leads to substance use or abuse in later life (Mannuzza, Klein et al. 2003). Data examining other potential long-term behavioural consequences of early methylphenidate administration have shown that relative to vehicle-treated control animals, rats treated with methylphenidate during early-life (childhood) were significantly less responsive to natural rewards (sucrose, novelty-induced activity, and sex) and more sensitive to stressful aversive situations (increased anxiety-like behaviours and plasma levels of corticosterone) (Bolanos, Barrot et al. 2003; Carlezon, Mague et al. 2003).

To date, there are no studies directly looking at potential DA transmission changes (sensitization) that might result from methylphenidate treatment in ADHD. Neurobiological characterization of DA transmission in ADHD would be useful to determine neurochemical indexes of vulnerability to sensitization.

7.10. CONCLUDING REMARKS

In closing, the main findings of the present dissertation, drawn from modern clinical neuroimaging techniques, lend support to converging evidence in animals that drugs of abuse (amphetamine and alcohol) act through mechanisms involving the brain neurotransmitter DA. Specifically we have found that the acute administration of alcohol and amphetamine increases DA in the limbic and sensorimotor striatum. With repeated use this increase is potentiated or sensitized presumably through a presynaptic mechanism that facilitates release. The activation of the human ventral striatal dopaminergic system by acute amphetamine and alcohol correlated better with subjective ratings of drug-wanting, self-rated energy, alertness and with cardiac acceleration than with subjective affective reactions (drug-liking, euphoria). Behavioral sensitization also seems to selectively activate psychomotor activity and arousal and not subjective pleasure. Interestingly, we did find that subjective pleasure influenced DA neurotransmission since ratings of drug liking predicted the magnitude of DA release when the subject was re-exposed to amphetamine in the same drug-context. These observed correlations re-state the view that other functions than that of assigning hedonic value can be attributed to dopaminergic transmission in the mesolimbic pathway. Specifically our findings support the idea that VS DA appears to participate in the brain circuitry that regulates motivational arousal and detection of future potential drug-reward (salience).

The key issue remains: does the DA system and sensitization become less important when people become addicted, as the habit is learnt? Indeed, once addiction is established, drug self-administration remains relatively preserved despite manipulations of mesolimbic DA system. Addiction is a complex process and not surprisingly, it depends on a variety of factors and on neural adaptations within a widespread circuit. Drug dependence and addiction can be partly understood as gradual adaptations of the brain to chronic drug exposure. Specifically, in the early stage of addiction, sensitization would initially lead to a motivational conditioned response of the brain to drugs and to the subsequent maintenance of recreational drug taking. Repeated use of addictive drugs would then progressively induce a neural reorganization in the circuit responsible for addiction. In this regard, molecular neuroadaptations induced by chronic administration of drugs have been shown to occur downstream of DA receptors in the nucleus accumbens and elsewhere, possibly triggered by a drive to homeostasis. Several neural factors are increasingly seen as important for understanding the switch from motivated drug use (misuse) to addiction and craving i.e.: the switch to loss of control over drug-intake. A network of cellular adaptations leading to increased excitability of glutamate projections from the prefrontal, orbitofrontal and cingulate cortices to the VS (nucleus accumbens) has been identified as a potential mediator of the overwhelming desire to take drugs. Functional imaging evidence supports to this view by demonstrating that that detoxified drug-addicted individuals have a malfunctioning prefrontal cortex:

hypo-function under resting condition would presumably contribute to both reduced salience of non-drug stimuli and reduced decision-making ability and hyper-function during cues that predict drug availability presumably drive craving and relapse. This glutamate-dependent form of synaptic plasticity is believed to be initiated by chronic drug-induced adaptations in D_1 receptor signalling, which triggers compensatory changes in glutamate receptor function (facilitating LTP), and ultimately contributes to inappropriate plasticity. Unregulated DA release after repeated exposure to amphetamine (sensitization and withdrawal) may therefore play a role in this maladaptive glutamatergic plasticity and in the ensuing compulsive nature of addiction. To summarize sensitization might have a role in the initial stage of sensitization by maintaining interest in the drug and, through cellular adaptation, sensitization might gradually facilitate the neural reorganization necessary to complete the complex portrait of addiction. Many questions remain. For one, is the deficit in prefrontal glutamatergic transmission and in impulse control a cause or a consequence of addiction? Indeed, there may be a co-morbidity of drug-taking behaviour with impulsiveness because of genetic or developmental factors. Alternatively, drug taking could produce neural or neurotoxic side effect in the cortex, which facilitate sensitization. Combining magnetic resonance spectroscopy (to detect and quantify glutamate / glutamine tissue based content) with PET and novel DA ligands such as PhNO would be an interesting tool to get a better understanding of neural system changes that occur in the course of sensitization.

Research directed toward finding compounds that counteract the specific changes that drug use causes in the brain can profit from understanding the mechanisms of sensitization.

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APPENDIX

POSITRON EMISSION TOMOGRAPHY (PET) AND MAGNETIC RESONANCE IMAGING (MRI) CONSENT FORMS

As approved by the

Montréal Neurological Institute & Hospital Research Ethic Board

POSITRON EMISSION TOMOGRAPHY (PET) AND MAGNETIC RESONANCE IMAGING (MRI) CONSENT FORM

MONTREAL NEUROLOGICAL INSTITUTE & HOSPITAL
McConnell Brain Imaging Centre, Departments of Nuclear Medicine & Neuroradiology

MCGILL UNIVERSITY
Department of Psychiatry

Title of Project: **PET / [^{11}C]Raclopride Studies of Extracellular Dopamine: Effects of d-Amphetamine**

Principal Investigators: **Marco Leyton, Ph.D.**
Alain Dagher, M.D.

Co-Investigator: **Chawki Benkelfat, M.D.**

It is of the **utmost importance** for the subject that this questionnaire be filled out by a physician **and** the subject, as well the investigator.

1. REASON FOR THE STUDY

We are interested in how brain chemicals (especially a chemical called dopamine) are used by the brain. Abnormalities in dopamine function have been implicated in a large number of illnesses, including Parkinson's Disease, schizophrenia, and drug addiction. The objective of this study is to devise a new method of measuring the release of dopamine by brain cells. Dopamine release will be affected by the administration of the medication, dextroamphetamine (0.30 mg/kg, given orally).

2. PROCEDURES

Your participation in this study will involve three sessions of about three and a half hours each, and one session of about one hour. The first session will be an interview, the second two will be for the Positron Emission Tomography (PET) scans, and the final session for a Magnetic Resonance Imaging (MRI) scan.

a) Interview

On the first day, each potential subject will be interviewed by a research psychologist (Dr. Leyton) and will be asked about personal and family histories of depression and other psychological disorders. After this initial assessment, all those who will be participating in the study will have times scheduled for the two PET scans and one MRI scan.

b) Positron Emission Tomography Procedures (PET Scan)

1) The day before each PET scan, everyone will eat low protein meals and snacks. These will be delivered to every subject.

2) On the PET scan days, all participants will come to Montreal Neurological Institute at 12:00 noon, not having eaten or drunk anything except for water since the previous day. First they will fill in various paper and pencil tests to measure mood. Then they will have a blood sample taken to measure blood amino acid levels. The volume of blood taken will be 10 ml (2 teaspoons).

3) Immediately prior to the scan, a fine needle-catheter will be inserted into a vein in one arm for administration of a small amount of a radioactive substance: 10 mCi of ^{11}C-labeled raclopride (half life of 20 minutes).

4) You will be asked to lie on a couch in the scanner.

5) We estimate that each scan will take about two hours, during which time you will be requested to lie still on the couch in the scanner.

6) All procedures during the PET scan will be carried out by a qualified nuclear medicine technician, and supervised by a qualified nuclear medicine physician.

7) Shortly before one of the PET studies, you will be asked to take a placebo whereas before the other PET scan you will be asked to take a medication called dextroamphetamine. This is a drug used to treat narcolepsy, attention deficit disorder, and depression. It acts by stimulating the release of dopamine from brain cells. You will receive a dose of dextroamphetamine of 0.30 mg/kg, and both it and the placebo will be given orally.

c) **Magnetic Resonance Imaging Procedures (MRI Scan)**

You will be asked to lie on a couch that will be moved into a cylindrical opening where pictures of your head will be taken during a period of 30 to 40 minutes. The MRI machine will be quite noisy during the scan. To reduce the noise, you will be given earplugs.

3. CONTRAINDICATION

a) **For PET Scan**

The following are contraindications for this procedure.

1) Under 18 years old
2) Previous radiation doses received within the past year (over 5 mSv)

b) **For MRI Scan**

The following are contraindications for this procedure.

1) Pacemaker
2) Aneurysm Clip
3) Heart/Vascular Clip
4) Prosthetic Valve
5) Metal Prosthesis

c) **For Receiving Dextroamphetamine**

The following are contraindications for this procedure.

1) A history of heart or lung disease.
2) A history of psychiatric illness

4. ADVANTAGES OF THE PROPOSED STUDIES

Both PET and MRI studies are tests, not treatments. It is hoped that the information obtained will help our understanding of the function of the human brain. This may, in the long term, help the diagnosis and treatment of neurological disorders.

5. DISADVANTAGES OF THE PROPOSED STUDIES

a) **PET**

(i) Some discomfort may be caused by insertion of the fine needle-catheter into the vein, as well as immobility on the couch.

(ii) The main **Risk** of participating in this study is exposure to radiation from the short-lived tracer substances injected into your body: ^{11}C-Raclopride (two times 10 mCi) will result in less than 5 mSv of radiation to the whole body. This level of radiation dose is about twice that which you receive annually from natural background radiation (0.9 - 2.2 mSv) in various regions of North America. This is also 25% of the annual dose limit allowed for those who work in a high radiation environment, such as nuclear medicine technicians. The degree of **Risk** associated with exposure to an additional 5 mSv of radiation is thought to be very low. This amount of additional radiation increases the risk of fatal cancer by about 2 in 10,000 during a lifetime, whereas the current overall risk of fatal cancer is about 2,300 in 10,000. Similar risks, equivalent to those from the dose you are receiving, are associated with:

a)	smoking 2 packs of cigarettes during a lifetime.	(cancer, heart disease)
b)	driving 2,000 miles by car (Montreal to Calgary).	(accident)
c)	flying 20,000 to 60,000 miles by air.	(accident)
d)	living 100 days in New York or Boston	(air pollution)

Additional information available upon request

b) **MRI**

During this study, you will be exposed to a strong magnetic field. No long-term negative side-effects have been observed from this type of study. As mentioned above, the MR is very noisy and you will be given earplugs to reduce this effect.

c) Dextroamphetamine

Side-effects of this drug include palpitations, mildly elevated blood pressure, restlessness, headache and dizziness. In some cases, anxiety, euphoria, or agitation may occur. It may also cause psychosis but this is very rare at the dose you will receive. All of these effects are transient, and wear off after 3 hours. Participants will be asked to remain for observation during these 3 hours. If an adverse response were to develop, the participant would be treated by the one of the study's physicians as deemed necessary.

6. EFFECTS OF PARTICIPATION IN THIS STUDY ON YOUR TREATMENT

Positron emission tomography or magnetic resonance imaging do not interfere with any treatment or other diagnostic tests.

7. CONFIDENTIAL NATURE OF THIS STUDY

The results of the testing will be kept confidential. No personal information will be released to third parties without your written approval. Your name, date of birth, address and telephone number may have to be forwarded to the Atomic Energy Control Board of Canada upon their request.

8. INCIDENTAL FINDINGS

Any incidental findings regarding your own health will be communicated to you and/or to your physician at your request.

9. DISCONTINUATION OF THE STUDY BY THE INVESTIGATOR

At any time during the testing, the investigators have the right to terminate the study for purely scientific reasons.

10. COMPENSATION

Upon completion of both MRI and PET studies you will receive $100.00 as compensation for your time and inconvenience. If studies have to be terminated for scientific reasons, compensation will be adjusted according to the fraction of the studies completed.

11. SUBJECT'S STATEMENT CONCERNING WITHDRAWAL FROM THE STUDY

I understand that my participation in this research project is voluntary and I may withdraw at any time, including during the procedure, without prejudice to myself.

Montreal Neurological Hospital
MAGNETIC RESONANCE

Patient Name: _____

Medicare #: _____

QUESTIONNAIRE AND CONSENT FORM

It is of the ultimate importance for the patient safety that this questionnaire is filled out by the physician and the patient, and attached to the request for service.

1. Previous surgery (type and date)_____.

2. Does the subject have any of the following?

	YES	NO
Cardiac pacemaker	____	____
Surgical clip on an aneurysm or other vessel	____	____
Surgical clip or valve on the heart	____	____
Prostheses (please specify type and location) _____	____	____
Implants (please specify type and location) _____	____	____
Metal or metallic fragments in any part of the body (please specify)_____	____	____

3. Is the subject pregnant? ____ ____

Is the subject currently taking prescription medication? ____ ____

All of my questions regarding this exam have been satisfactorily answered. I hereby give consent to Magnetic Resonance examination.

_____ _____ _____

Signature of Patient Signature of Physician
Date

SUBJECT'S DECLARATION OF CONSENT

I, _____, have read the above description with one

of the above investigators, _____.

I fully understand the procedures, advantages and disadvantages of the study which have been explained to me. I freely and voluntarily consent to participate in this study.

I hereby certify that I have not participated in a PET investigation anywhere before.

Further, I understand that I may seek information about each test either before or after it is given, that I am free to withdraw from the testing at any time if I desire, and that my personal information will be kept confidential.

SIGNATURE _____	_____	_____	
SUBJECT	**DATE**	**CONTACT NO.**	
SIGNATURE _____	_____	_____	
INVESTIGATOR	**DATE**	**CONTACT NO.**	
SIGNATURE _____	_____	_____	
WITNESS	**DATE**	**CONTACT NO.**	
SIGNATURE _____	_____	_____	
PHYSICIAN	**DATE**	**CONTACT NO.**	

TOMOGRAPHIE PAR ÉMISSION DE POSITONS (TEP) ET IMAGERIE PAR RÉSONANCE MAGNÉTIQUE (IRM) - FORMULAIRE DE CONSENTEMENT

INSTITUT ET HÔPITAL NEUROLOGIQUES DE MONTRÉAL
Centre McConnell d'imagerie cérébrale,
départements de médecine nucléaire et de neuroradiologie

Titre du projet : La libération de dopamine par l'alcool.

Chercheurs : **Alain Dagher MD, Jean-Marc Assaad, Robert Pihl PhD, Chawki Benkelfat MD, Marco Leyton PhD.**

1. MOTIF DE L'ÉTUDE

L'objet de l'étude est de mesurer la libération transitoire de la dopamine dans le cerveau. La dopamine, molécule produite naturellement dans le cerveau, facillite la transmission de l'influx nerveux entre les cellules. Il a été démontré que la dopamine est impliquée dans le contrôle des activitées motrices, dans la régulation des émotions et dans les procéssus d'attention. La libération de dopamine a lieu au cours de situations plaisantes. La maladie de Parkinson, la schizophrénie et les problèmes de dépendance aux drogues ont été associés à un dysfonctionnement du système dopaminergique. Nous nous intéressons donc la libération de dopamine dans le cerveau humain après la consommation d'alcool. Nous voulons aussi voir s'il y à un lien entre le pouls et la libération de dopamine.

2. PROCÉDURES

Pour participer à cette étude, vous devrez nous consacrer trois sessions. Les deux dernières seront réservées à la tomographie par émission de positons (TEP), et dureront à peu près quatre heures chaque, et la première à la séance d'imagerie par résonance magnétique. L'étude TEP consistera de deux scans, d'une durée de 60 minutes chaque. Immédiatement avant chaque scan TEP on vous donnera soit du jus d'orange, soit du jus d'orange et de l'alcool. Le dose d'alcool sera identique à celle que vous avez reçu durant l'étude préliminaire. Pendant la durée de l'étude votre pouls sera mesuré automatiquement, ainsi que votre taux d'alcool sanguin. Vous devrez rester sur les lieux sous observation jusqu'à ce que votre taux d'alcool soit inférieur à 0.04%.

a) Tomographie par émission de positons
Procédures (étude TEP)

1) Vous serez peut-être invité(e) à ne rien manger ou boire sauf de l'eau pendant les trois heures qui précèdent le début de cette étude.
2) Un cathéter à aiguille fine sera introduit dans une veine de votre bras pour l'administration de petites quantités (à préciser) d'une substance radioactive à vie courte: ^{11}C-raclopride, 10 millicuries chaque injection (demi-vie du ^{11}C : 20 minutes).
3) Vous serez pesé, et on vous installera des électrodes afin d'enregistrer votre pouls. Une première mesure de votre taux d'alcool sanguin sera prise à l'aide d'un intoxocomètre.
4) On vous demandera ensuite de boire sur une période de 15 minutes trois consommation contenant soit du jus d'orange, soit du jus d'orange et de l'alcool.

5) Vous serez invité(e) à vous allonger sur la couchette de la caméra TEP.
6) La durée de cette étude est d'environ deux heures pendant lesquelles vous devez rester immobile sur la couchette de la caméra.
7) Toutes les modalités de l'étude TEP seront exécutées par un technicien qualifié en médecine nucléaire, sous la surveillance d'un médecin qualifié en médecine nucléaire.
8) Après le scan vous resterez dans l'unité jusqu'a ce que les effets de l'alcool aient diminué au point où vous pourrez quitter les lieux.

b) Imagerie par résonance magnétique
Procédures (étude IRM)

Vous serez invité(e) à vous allonger sur la table de l'appareil que l'on fera glisser dans une ouverture cylindrique pour prendre des images de votre tête pendant 30 à 40 minutes. La machine IRM fait beaucoup de bruit durant cette opération. Pour atténuer ce bruit, on vous donnera des bouchons pour vos oreilles. Vous pourrez communiquer avec le technicien par microphone pendant le scan.

c) Administration d'alcool

Avant l'un des deux scans TEP vous pourrez recevoir une quantité d'alcool pour élever votre taux d'alcool au dessus de la limite légale au Québec (0.08% d'alcool dans le sang). Ainsi on vous demandera de ne pas conduire pour vous rendre aux lieux d'expérimentation. Votre rhytme cardiaque sera enregistré constamment pendant l'étude. Votre niveau d'alcool sera mesuré trois fois à l'aide de prise de sang et deux fois par intoxicomètre (i.e. : par expiration)

3. CONTRE-INDICATIONS

a) Contre-indications à l'étude TEP
1) Grossesse ou allaitement
2) Être âgé(e) de moins de 18 ans
3) Exposition à des doses radioactives dans les 12 derniers mois (plus de 5 mSv).

b) Contre-indications à l'étude IRM
1) Stimulateur cardiaque
2) Clip d'anévrisme
3) Clip cardiaque ou vasculaire
4) Valve prothétique
5) Prothèses métalliques
6) Grossesse
7) Claustrophobie aigüe

4. AVANTAGES DES ÉTUDES PROPOSÉES

La tomographie par émission de positons et l'imagerie par résonance magnétique sont des examens, pas des traitements. Nous espérons que les renseignements glanés nous aideront à mieux comprendre le fonctionnement du cerveau humain. Cela pourrait à long terme contribuer au diagnostic et au traitement de certains troubles neurologiques.

5. INCONVÉNIENTS DES ÉTUDES PROPOSÉES

TEP

1) Il se peut que vous ressentiez un léger inconfort au moment où le cathéter à aiguille fine sera introduit dans la veine, et à rester immobile sur la couchette.

2) Le **RISQUE** principal de votre participation à cette étude est une exposition aux radiations des traceurs à vie courte qui seront injectés dans votre organisme ou que vous devrez inhaler. La substance radioactive administrée exposera votre organisme à une dose maximale de 5 mSv. Cette dose équivaut á peu prés au double des radiations auxquelles vous êtes exposé(e) annuellement dans le cadre des rayonnements naturels (0,9 - 2,2 mSv) dans les diverses régions d'Amérique du Nord. Elle équivaut également à 25 % de la dose annuelle moyenne autorisée pour les personnes qui travaillent dans un milieu à fortes radiations, notamment les techniciens en médecine nucléaire. Le niveau de **RISQUE** qui se rattache à une exposition à 5 mSv supplémentaires est jugé très faible. Cette quantité de rayonnement augmente les risques de cancer d'environ 2 pour 10 000 durant votre vie, alors que le risque global de contracter un cancer fatal est d'environ 2 300 pour 10 000. Pour donner une idée de ce niveau de risque, voilà à quoi il correspond :

   a) fumer 2 paquets de cigarettes durant sa vie (cancer, maladie cardiaque)
   b) parcourir 2 000 milles en voiture (accident)
   c) parcourir 20 000 à 60 000 milles en avion (accident)
   d) vivre 100 jours à New York ou à Boston (pollution atmosphérique)

*** Renseignements supplémentaires sur demande**

IRM

Pendant l'examen IRM, vous serez exposé(e) à un champ magnétique puissant. Aucun effet secondaire à long terme n'a été observé à l'issue de ce type d'étude. Comme nous l'avons indiqué ci-dessus, la machine est très bruyante et on vous donnera des bouchons pour atténuer le bruit.

6. EFFETS DE VOTRE PARTICIPATION SUR VOTRE TRAITEMENT

La tomographie par émission de positons et l'imagerie par résonance magnétique ne nuisent à aucun autre traitement ou test diagnostique.

7. CARACTÈRE CONFIDENTIEL DE L'ÉTUDE

Les résultats de cette étude resteront confidentiels. Aucune donnée vous concernant ne sera transmise à un tiers sans votre autorisation écrite. Vos nom, date de naissance, adresse et numéro de téléphone pourront être transmis à la Commission de contrôle de l'énergie atomique du Canada, si cette dernière en fait la demande.

8. CONSTATATIONS FORTUITES

Toute constatation fortuite sur votre santé sera portée à votre connaissance ou à celle de votre médecin, si vous en faites la demande.

9. INTERRUPTION DE L'ÉTUDE PAR LE CHERCHEUR

Le chercheur a le droit de mettre fin à cette étude à tout moment, pour des raisons purement scientifiques.

10. COMPENSATION

À l'achèvement des études IRM et TEP, vous toucherez $150 à titre de dédommagement. Advenant qu'il faille mettre fin à ces études pour une raison scientifique, votre compensation sera fonction de la fraction des études terminées.

11. DÉCLARATION DES SUJETS QUI SOUHAITENT SE DÉSISTER

Il est entendu que votre participation à ce projet de recherche est purement volontaire et que vous pouvez vous en désister à tout moment, y compris durant son déroulement, sans que cela soit préjudiciable à vous-même ou à votre traitement. Si vous désirez annuler l'étude après avoir reçu l'alcool, vous devrez tout de même rester dans l'unité jusqu'à ce que votre taux d'alcool soit inférieur à 0.04%.

QUESTIONNAIRE POUR L'IMAGERIE PAR RÉSONANCE MAGNÉTIQUE

1. Chirurgie antérieure (type et date) _____

2. Le sujet porte-t-il l'un ou plusieurs des éléments suivants?

	OUI	NON
Stimulateur cardiaque	____	____
Clip d'anévrisme ou clip sur un autre vaisseau	____	____
Clip chirurgical ou valve cardiaque	____	____
Prothèses (veuillez préciser type et site)		____
____		
_____		
Implants (veuillez préciser type et site)		____
____		
_____		
Métal ou fragments métalliques dans le corps (veuillez préciser)	____	____
_____		
3. Le sujet est-elle enceinte?	____	____
Le sujet prend-il des médicaments sur ordonnance?	____	____

DÉCLARATION DE CONSENTEMENT DU SUJET

Je soussigné(e) _____ ai pris connaissance de ce qui précède en présence de l'un des chercheurs suivants, _____.

J'ai parfaitement compris les procédures, les avantages et les inconvénients de cette étude. Je consens volontairement et librement à y participer.

Je certifie par la présente que je n'ai jamais participé à une étude TEP auparavant.

Il est par ailleurs entendu que je peux demander des renseignements à propos de chaque examen avant ou après son déroulement, que je suis libre de me désister de ce protocole à tout moment si je le souhaite et que toute donnée me concernant restera confidentielle.

SIGNATURE _____	_____	_____	
	SUJET	**DATE**	**N CONTACT**
SIGNATURE _____	_____	_____	
	CHERCHEUR	**DATE**	**N CONTACT**
SIGNATURE _____	_____	_____	
	TÉMOIN	**DATE**	**N CONTACT**
SIGNATURE _____	_____	_____	
	MÉDECIN	**DATE**	**N CONTACT**

POSITRON EMISSION TOMOGRAPHY (PET)
MAGNETIC RESONANCE IMAGING (MRI) CONSENT FORM
MONTREAL NEUROLOGICAL INSTITUTE & HOSPITAL

McConnell Brain Imaging Center, Departments of Nuclear Medicine & Neuroradiology

Title of Project: Sensitization to Psychomotor Stimulants: A PET study with 11C-Raclopride.
Investigators: Dr Alain Dagher, M.D. Dr Benkelfat, M.D., Marco Leyton, Ph.D., Mirko Diksic, Ph.D., Isabelle Boileau.

1. REASON FOR THE STUDY

The purpose of this study is to identify the chemical mechanisms in the brain that lead to the development of sensitization to drugs. Sensitization, sometimes called reverse tolerance, is an enhanced behavioural response to drugs like cocaine or amphetamine that occurs after repeated exposure. Studies done in animals suggest that sensitization to drugs results from changes within a brain chemical system called the dopamine (DA) neurotransmitter system. Dopamine is naturally produced by the brain. It facilitates transmission between nerve cells and has been implicated in motor control, in the regulation of emotions and in processes related to learning and attention. Dysfunctions in DA neurotransmission are associated with disorders such as schizophrenia and drug addiction. It is believed that sensitization might be related to these problems. In the following (2) studies we are looking at short and long term (12-months) dopamine changes in the brain in response to four or five doses of dextroamphetamine (0.3 mg/kg given orally). This drug is widely prescribed for the treatment of attention deficit disorder and can be safely given to healthy humans.

2. PROCEDURES

Participants will take part in ***one*** of the two studies described below. Both of these studies include 6 (approximately 3-hour long) experimental sessions taking place on separate days at the Montreal Neurological Institute PET unit (MNI) and / or Allan memorial Institute department of psychiatry (AMI) (Figure 1). In either study, you will receive four low doses of amphetamine by mouth (0.30 mg/kg per session), given every few days, over approximately three weeks. If you are participating in ***Study1*** you will also be asked to come back to the lab 12 months later at which time you would receive a 5th dose of amphetamine.

The amphetamine given in ***Study1*** will be administered while participants lie inside of the PET camera. For comparison purposes, one PET scan will be conducted without amphetamine. If you are available to come back 12 months later, a final PET scan will be conducted then also. Two of the sessions in ***Study 1*** will be "pretend scans"; that is, the procedure will be identical but you will not actually receive the PET tracer injection. ***Study2*** is quite similar to ***Study 1***. It will also involve PET scans, but the amphetamine will be given on separate days in a different room.

For all sessions of both studies, electrodes will be placed on your skin and we will record heart activity, eye-blink rate and other physiological measures. Blood samples will be drawn every 15 minutes from an intravenous line, and mood will be assessed by questionnaires. For both studies you will also participate in two other sessions: the first will be an interview (see a) and the second will be reserved for a Magnetic Resonance Imaging (MRI) study that lasts about one hour (see c). Hence the studies are composed of 8 sessions (6 of which are illustrated in the following figure. The interview takes place before these 6 days and the MRI will be scheduled at your convenience.

Figure1: Schedule for study 1 and 2.

	Day 0 or 22	Day 1	Day 3	Day 5		Day 21	12 months
STUDY 1	^{11}C-raclopride No drug MNI	^{11}C-raclopride ⊗ 0.3 mg/kg MNI	No scan ⊗ 0.3 mg/kg MNI	No scan ⊗ 0.3 mg/kg MNI	*Latency 14 days*	^{11}C-raclopride ⊗ 0.3 mg/kg MNI	^{11}C-raclopride ⊗ 0.3 mg/kg MNI
	Day 0	**Day 1**	**Day 3**	**Day 5**		**Day 21**	**Day 22**
STUDY 2	^{11}C-raclopride No drug MNI	No scan ⊗ 0.3 mg/kg AMI	No scan ⊗ 0.3 mg/kg AMI	No scan ⊗ 0.3 mg/kg AMI		^{11}C-raclopride No drug MNI	No scan ⊗ 0.3 mg/kg AMI

a) Interview

On the first day, you will be interviewed about personal and family histories of psychological disorders, you will complete a personality assessment, blood will be drawn for routine blood analysis and an EKG (to monitor your heart) will be done. After this initial assessment, if you are eligible to participate in the study you will have times scheduled for the PET and MRI scans.

b) Positron Emission Tomography Procedures (PET Study)

1) You will be asked to avoid excessive fluid intake.
2) On your arrival at the MNI PET unit you will be asked to fill in questionnaires.
3) On three days in ***Study1***, you will receive an oral dose of amphetamine one hour prior to the injection of ^{11}-C raclopride. In ***Study 2***, the PET scans will always be conducted without amphetamine.
4) Prior to scanning, a fine needle-catheter will be inserted into an arm vein for the administration of small amounts of a radioactive substance. In your instance, this will be Raclopride which is labeled with the short-lived radioactive atom ^{11}-C (physical half-life = 20 minutes). The total dose administered to you will be less than 7 millicuries per scan. On the "pretend" scan days, the catheter will also be inserted but the raclopride tracer will not be injected PET images will not be collected.

5) Blood will be drawn for hormonal measurements via the catheter in your arm vein. The total amount of blood will be 28ml (2 tbsp) 7 ml per sample (1 tsp).
6) Electrodes will be placed on your skin in order to record your heart rate and muscle tone.
7) The length of the study is approximately two hours (60 minutes preparation + 60 minutes of PET scanning). During this time you will be requested to lie still on the couch in the scanner.
8) All procedures during the PET study will be carried out by a qualified nuclear medicine technician, and supervised by a qualified nuclear medicine physician.

c) Magnetic Resonance Imaging Procedures (MRI Study)

You will be asked to lie on a couch that will be moved into a cylindrical opening where pictures of your head will be taken during a period of 30 to 40 minutes. The MRI machine will be quite noisy during the scan. To reduce the noise, you will be given earplugs. You will be able to communicate with the technician during the procedure.

3. CONTRAINDICATION

a) For PET Study

1) Pregnancy or Breast Feeding
2) Under 18 years old
3) Previous radiation doses received within the past year (over 5 mSv / 25 mCi)

b) For MRI Study

1) Pacemaker
2) Aneurysm Clip
3) Heart/Vascular Clip
4) Prosthetic Valve
5) Metal Prosthesis
6) Claustrophobia

c) Dextroamphetamine

1) Heart problems.
2) High Blood Pressure.
3) History of drug abuse.
4) Glaucoma.

4. ADVANTAGES OF THE PROPOSED STUDIES

The PET study is not a treatment. It is hoped that the information obtained will help our understanding of the function of the human brain. This may, in the long term, help the diagnosis and treatment of neurological disorders.

5. DISADVANTAGES OF THE PROPOSED STUDIES

a) PET

Some discomfort may be caused by insertion of the fine needle-catheter into the vein, as well as immobility on the couch. The main **RISK** of participating in this study is exposure to radiation from the short-lived tracer substance injected in your body or

inhaled. The administered radioactive material will expose your body to a maximal dose of 5 mSv. This level of radiation dose is about twice that which you receive annually from natural background radiation (0.9 - 2.2. mSv) in various regions of North America. It is also 25% of the current average annual dose limits allowed for those who work in a high radiation environment, such as nuclear medicine technicians. The degree of **RISK** associated with exposure to an additional 5 mSv of radiation is thought to be very low. This amount of additional radiation may increase the risk of fatal cancer by about 2 in 10,000 during a lifetime, while the current overall risk of fatal cancer is about 2,300 in 10,000. Similar risks, equivalent to those from the dose you are receiving, are associated with:

- Smoking 2 packs of cigarettes during a lifetime (cancer, heart disease)
- driving 2,000 miles by car (accident)
- flying 20,000 to 60,000 miles by air (accident)
- living 100 days in New York or Boston (air pollution)

**Additional information available upon request.*

b) MRI

During this study, you will be exposed to a strong magnetic field. No long-term negative side effects have been observed from this type of study. As mentioned above, the MR is very noisy and you will be given earplugs to reduce this effect.

c) Dexedrine, dextroamphetamine

d-Amphetamine is currently in clinical use in Canada for the management of attention deficit hyperactivity disorder (ADHD) and sleep-disorder (narcolepsy). Side-effects of this drug include palpitations, mildly elevated blood pressure, restlessness, headache and dizziness. In some cases, anxiety, euphoria or agitation may occur. Sustained high doses of amphetamine (>100mg /day) can lead to dependence or cause psychosis but this is very rare at the dose you will receive. The long term effects of sensitization are not known. All of these effects are transient and wear off after three hours. Participants will be asked to remain in the McConnell Brain Imaging Center of the Montreal Neurological Institute for observation during these three hours. If an adverse response were to develop, the participant would be treated by one of the study's physicians as deemed necessary.

6. EFFECTS OF PARTICIPATION IN THIS STUDY ON YOUR TREATMENT

Positron emission tomography imaging does not interfere with any treatment or other diagnostic tests.

7. CONFIDENTIAL NATURE OF THIS STUDY

The results of the testing will be kept confidential. No personal information will be released to third parties without your written approval. Your name, date of birth, address and telephone number may have to be forwarded to the Canadian Nuclear Safety Commission, upon request.

8. INCIDENTAL FINDINGS

Research scans are not subject to clinical review. However, any incidental findings will be communicated to you and, upon your request, to your physician.

9. DISCONTINUATION OF THE STUDY BY THE INVESTIGATOR

At any time during the testing, the investigators have the right to terminate the study for any reason.

10. COMPENSATION

Upon completion of both MRI and PET studies you will receive, as compensation for your time and inconvenience: ***Study1,*** 230.00$, for the first part of the study (7 sessions) and 80.00$ for the follow-up study (12 months later); ***Study2***, 260.00$. If studies have to be terminated for any reason, compensation will be adjusted according to the fraction of the studies completed.

11. SUBJECT'S STATEMENT CONCERNING WITHDRAWAL FROM THE STUDY

I understand that my participation in this research project is voluntary and I may withdraw at any time, including during the procedure, without prejudice to myself or my treatment.

IT IS ESSENTIAL FOR THE SUBJECT THAT THIS QUESTIONNAIRE BE FILLED OUT BY A PHYSICIAN, THE SUBJECT AS WELL AS THE INVESTIGATOR.

QUESTIONNAIRE FOR MAGNETIC RESONANCE IMAGING

1. Previous surgery (type and date)

_____.

1. Does the subject have any of the following?

(a) Cardiac pacemaker
(b) Surgical clip on an aneurysm or other vessel
(c) Surgical clip or valve on the heart
(d) Prostheses (please specify type and location)
(e) Implants (please specify type and location)
(f) Metal or metallic fragments in any part of the body (please specify)

2. Is the subject pregnant?
3. Is the subject currently taking prescription medication?

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IT IS ESSENTIAL FOR THE SUBJECT THAT THIS DECLARATION OF CONSENT BE FILLED OUT BY A PHYSICIAN, THE SUBJECT AS WELL AS THE INVESTIGATOR.

SUBJECT'S DECLARATION OF CONSENT

I, _____, have read the above description with one

of the above investigators, _____.

I fully understand the procedures, advantages and disadvantages of the study, which have been explained to me. I freely and voluntarily consent to participate in this study.

Further, I understand that I may seek information about each test either before or after it is given, that I am free to withdraw from the testing at any time if I desire, and that my personal information will be kept confidential.

SIGNATURE	_____	_____	_____
	SUBJECT	DATE	CONTACT NO.
SIGNATURE	_____	_____	_____
	INVESTIGATOR	DATE	CONTACT NO.
SIGNATURE	_____	_____	_____
	WITNESS	DATE	CONTACT NO.
SIGNATURE	_____	_____	_____
	PHYSICIAN	DATE	CONTACT NO.