BEHAVIOURAL AND NEOUROCHEMICAL CORRELATES OF PSYCHOSTIMULANT WITHDRAWAL AS AN ANIMAL MODEL OF DEPRESSION

by

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ABSTRACT

Previous studies in humans and animals have shown that withdrawal from high doses of psychostimulant drugs can lead to a number of aversive psychological symptoms. One of the more prominent symptoms is anhedonia, a core symptom of major depression, manifested behaviourally as decreased interest in normally rewarding stimuli. Several preclinical studies have shown that withdrawal from a chronic schedule of psychostimulant administration can produce discrete disturbances in psychological and affective processes, such as decreases in rodents’ responding for rewarding electrical brain stimulation, and disruption in responding for natural rewarding stimuli (i.e. a sucrose solution, or a sexually receptive rat). These effects are likely due to a long-lasting reduction in the efflux of dopamine (DA) in limbic nuclei, such as the nucleus accumbens (NAc), a brain area that is involved in mediating reward processes.

The main purpose of this thesis was to further investigate the relationship between psychostimulant withdrawal and anhedonia in the rat. In the first series of experiments the brain microdialysis technique, coupled with HPLC, was used to evaluate in freely-moving rats the effect of withdrawal on stimulus-induced changes in DA efflux in the NAc following a 4-day escalating-dose schedule of D-amphetamine administration (1-10 mg/kg, i.p., at ~8 hr intervals). In drug-withdrawn rats, the DA efflux in the NAc was significantly blunted in response to both pharmacological (a 5 mg/kg D-amphetamine injection i.p.), and natural rewards (4% sucrose), compared to vehicle-treated rats. The second series of experiments, investigated whether the negative affective state of drug-withdrawn rats could affect their response to the positive contrast paradigm, in which rats shifted from a 4% to 32% sucrose condition respond significantly more than rats always
maintained on 32% sucrose. Drug-withdrawn rats failed to display successive positive contrast, suggesting that incentive contrast is a particularly sensitive measure to detect changes in motivation and emotion. These data are consistent with many previous reports that withdrawal from a binge-like regimen of psychostimulant drug administration disrupts responding for natural reward stimuli, and provide important support for the hypothesis that withdrawal following exposure to escalating doses of psychostimulant drugs produces depressive-like symptoms, and that this model of psychostimulant drug withdrawal may be used as an animal model of depression.
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CHAPTER I INTRODUCTION

Major depressive disorder (MDD), commonly known as depression, is one of the most serious psychiatric disorders in today's society. Severe forms of depression affect 2%-5% of the U.S. population, while milder forms of the illness affect a further 20% (Blazer, 2000). The World Health Organization has predicted that depression will be the second main cause of disability by 2020 (Murray and Lopez, 1997). Much of the burden of this disability is attributable to ubiquity and chronicity. Depression is manifested with heterogeneous symptoms at the psychological, behavioural and physiological levels, and is influenced by a variety of factors. It is a complex disorder that causes immense suffering for those afflicted, as evidenced by a rate of suicide attempts that is 11 times greater than that of the general population (Kessler, 2000). Depression is almost twice as common in females than in males, beginning in puberty and continuing to menopause (Kessler, 2000). In addition, the postpartum period increases the risk of first onset of the illness and a recurrence in those already ill (Depression Guideline Panel, 1993).

Depression has been described by mankind for several millennia. Hippocrates first used the term “melancholia” (which means black bile in Greek) to express a feeling of sadness, around 400 B.C. (Akiskal, 2000). Most of the major symptoms of depression observed today were recognized in ancient times, as well as the contributions of innate predispositions and external factors in causing the illness. The ancients also recognized a large overlap of depression with anxiety and excessive alcohol consumption, both of which are well established today. However, despite the similarities between ancient descriptions of depression and those of the modern era, it is only in the second part of the
20th century that the brain became the focus of efforts to understand the pathophysiology of this disorder.

Before the purpose of this thesis is presented, some key background on symptoms, causes, neural circuits and neurotransmitter abnormalities involved in depression, and a review of related animal models is provided. Particular emphasis is given to the use of psychostimulant withdrawal as an inducing condition in animal models of depression, as this model has been the focus of the present research.

1.1 Diagnosis of Depression

Since 1960s, MDD has been diagnosed based on symptomatic criteria according to the Diagnostic and Statistical Manual of Mental Disorders, currently in its fourth edition (DSM-IV; American Psychiatric Association, 1994). According to the DMS-IV, MDD is defined as a chronic state (≥ 2 weeks) of a patient suffering from at least one core symptom and five or more of the following secondary symptoms (Table 1). The core symptoms are: 1) depressed mood, characterized by lack of motivation, and 2) inability to experience pleasure during normally rewarding activities (anhedonia). The secondary symptoms are: 1) significant weight loss or weight gain; 2) insomnia or hypersomnia; 3) psychomotor agitation or retardation; 4) fatigue or loss of energy; 5) feelings of worthlessness or guilt; 6) diminished ability to think or concentrate; 7) recurrent thoughts of death. In addition, the symptoms must cause clinically significant occupational or social impairment, and cannot be due to a general medical condition (i.e. hypothyroidism), or substance abuse (consumption of street drugs such as cannabis). The
episodes can be further specified as mild, moderate, or severe; with or without psychotic features; in full or partial remission; chronic (at least 2 years in duration); with catatonic, melancholic, or atypical features; or with postpartum onset. Seasonal variation and interepisode recovery may also be specified. To be considered a recurrent illness, two or more episodes separated by at least 2 consecutive months below symptom criteria must be evident. It is clear from these criteria that the diagnosis of depression, as opposed to most diseases of other organ systems (diabetes, cancer, etc) is not based on objective diagnostic tests (serum chemistry, or biopsies), but rather on a highly variable set of symptoms. Accordingly, depression should not be view as a single disease, but as a heterogeneous syndrome comprised of numerous factors with distinct causes and pathophysiologies.

1.2 Etiology of Depression

Medical research has contributed much to our understanding of depression. However, scientists still do not know the exact mechanism that triggers depressive illness. Hence, there is no single cause of depression. In the past, it was believed that depression was the result of thoughts or emotions that were troubling for a person. Today, scientists realize that there can be several factors working together that lead a person to become depressed. Environmental, genetic, and biological causes are three of the most important of these factors (Nestler et al., 2002).
Table 1. Similarities between major depressive disorder and psychostimulant withdrawal in humans\(^a\) (Table taken from: Barr AM, Markou A, and Phillips AG 2002. A ‘crash’ course on psychostimulant withdrawal as a model of depression. Trends Pharm Sci 23: 475-482)

<table>
<thead>
<tr>
<th>Major depressive disorder</th>
<th>Psychostimulant Withdrawal</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Behavioural (DSM-IV criteria)</strong></td>
<td></td>
</tr>
<tr>
<td>Depressed mood and/or irritability</td>
<td>Severely depressed mood and/or irritability</td>
</tr>
<tr>
<td>Diminished interest or pleasure in daily activities</td>
<td>Loss of interest or pleasure in daily activities</td>
</tr>
<tr>
<td>Large increase or decrease in appetite</td>
<td>Increase in appetite</td>
</tr>
<tr>
<td>Insomnia or excessive sleepiness</td>
<td>Excessive sleepiness</td>
</tr>
<tr>
<td>Psychomotor agitation or retardation</td>
<td>Psychomotor retardation</td>
</tr>
<tr>
<td>Fatigue or loss of energy</td>
<td>Fatigue and/or loss of energy</td>
</tr>
<tr>
<td>Diminished ability to think or concentrate</td>
<td>Poor ability to concentrate or confusion</td>
</tr>
<tr>
<td>Feelings of worthlessness and/or guilt</td>
<td>Unknown</td>
</tr>
<tr>
<td>Recurrent thoughts of death or suicide</td>
<td>Significant suicidal ideation</td>
</tr>
<tr>
<td><strong>Behavioural (non-diagnostic)</strong></td>
<td></td>
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<tr>
<td>Feelings of restlessness</td>
<td>Restlessness</td>
</tr>
<tr>
<td>Comorbid anxiety</td>
<td>High levels of anxiety</td>
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<tr>
<td>Carbohydrate craving</td>
<td>Increased craving for carbohydrates</td>
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<tr>
<td>Elevated drug self-administration</td>
<td>Greater drug-seeking and drug-taking behavior</td>
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<td><strong>Physiological</strong></td>
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<td>Disrupted HPA axis</td>
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<tr>
<td>Disrupted sleep architecture</td>
<td>Decreased REM latency; higher REM density</td>
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<tr>
<td>Changes in regional brain metabolism</td>
<td>Elevated metabolic activity in orbitofrontal cortex</td>
</tr>
</tbody>
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\(^a\)Abbreviations: DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition; HPA, hypothalamic-pituitary-adrenal; REM, rapid eye movement
1.2.1 Environmental Factors

Stress and emotional traumas are among the most potent environmental factors that may induce depressive episodes (Nestler et al., 2002). Emotional traumas include early life traumas such as birth trauma, or loss traumas such as the death of a parent in childhood, or the experience of being neglected (lack of loving parents) or abused. Stressful stimuli may also precipitate depression, especially in vulnerable persons. Some examples are: extreme stress such as a serious loss, loss of a job, financial problems, changes in life pattern; unhealthy social conditions, such as poverty, homelessness, and community violence, experiences that undermine self-confidence like social or work related failures.

However, stress per se is not sufficient to cause depression. Some people do not become depressed after serious stressful experiences, whereas others become depressed after stresses that for most people are quite mild (Nestler et al., 2002). This underlies the view that depression is triggered by the interactions of several different factors, which makes the mechanisms of such interactions an important focus of investigation.

1.2.2 Genetic Factors

Epidemiologic studies show that roughly 40%-50% of the risk of depression is genetic (Sanders et al., 1999; Fava and Kendler, 2000). This makes depression a highly heritable disorder, at least as heritable as several common complex medical conditions (type II diabetes, hypertension, asthma, certain cancers), which are often thought of as
genetic. The strongest evidence for genetic factors comes from studies of the incidence of depression in adoptive versus biological relatives of depressed patients. Among adopted people who developed depression, biological relatives were found to be eight times more likely than adoptive relatives to suffer from depression (Wender et al., 1986). In addition, comparison of concordance rates in identical and fraternal twins reinforces the conclusion that genetics are involved. Identical twins have a concordance rate of approximately 67% compared with a rate of only 15% for fraternal twins (Gershon et al., 1989). However, the search for specific genes that confer this risk has been frustrating, and to date no genetic abnormalities have been identified with certainty. There are many reasons for this difficulty, including the fact that depression is a complex phenomenon with many genes possibly involved. Thus, any single gene might produce a relatively small effect and would, therefore, be difficult to detect experimentally.

1.2.3 Biological Factors

Biological causes of depression are related to changes in the chemistry of the brain. These changes include imbalances in neurotransmitters, and fluctuations in hormonal levels. The observation that pharmacological manipulation of monoamines influenced depressive symptoms led to the hypothesis that depression results from reduced availability or functional deficiency of serotonin (5-HT), norepinephrine (NE), and dopamine (DA; Bunney and Davis, 1965; Schildkraut 1965; Schildkraut et al., 1968). This view is supported by the pharmacological action of both tricyclic antidepressants
(TCAs), and monoamine oxidase inhibitors (MAOIs), antidepressant drugs that increase synaptic levels of monoamines in the brain.

A role for acetylcholine in depression has also been proposed (Janowsky et al., 1972). Cholinergic agonists, cholinesterase inhibitors, and acetylcholine precursors have all been shown to worsen mood in depression (Janowsky et al., 1983; Dube 1993). Depressed patients show a heightened response to muscarinic cholinergic agonists as evidenced by worsening of mood, anergia, pupillary constriction, and sleep (Dilsaver and Coffman, 1989). We can conclude that no single neurotransmitter abnormality underlies depression, and that therefore, it may be that an imbalance in monoaminergic/cholinergic neurotransmission characterizes depression.

Another key biological system disturbed in depressive illness is the hypothalamic-pituitary-adrenal (HPA) system. Thus, many depressed patients have higher blood levels of cortisol than do healthy subjects, and a dysregulated circadian rhythm of cortisol secretion (Gibbons, 1964; Deakin et al., 1990; Wong et al., 2000). Corticosteroids have been further implicated in the mechanisms of depression by the hypothesis that antidepressants may act through normalization of the HPA system (Holsboer and Barden, 1996). Indeed, elevated cortisol levels observed in depressive patients usually return to normal under antidepressant treatment (Bhagwagar et al., 2002), or once depression disappears (Steckler et al., 1999).
1.3 Neural circuitry of depression

While for some neuropsychiatric disorders such as Parkinson's disease, Huntington's disease, or Alzheimer's disease, pathological lesions have been identified in specific regions of the central nervous system, there is still a very rudimentary understanding of the neural circuitry underlying normal mood and the abnormalities in mood that are a hallmark of depression.

Most research has focused on the hippocampus and its related circuits (i.e., HPA axis) as the site involved in the generation and treatment of depression (Nestler et al., 2002). However, in recent years, there has been increasing recognition of the role played by particular subcortical structures, such as the nucleus accumbens, the amygdala, and certain hypothalamic nuclei that are critical in regulating motivation, eating, sleeping, energy level and responses to rewarding and aversive stimuli, which are all abnormal in depressed patients (Drevets, 2001; Liotti and Mayberg, 2001) (Fig. 1). In the following paragraphs the role of the hippocampus and HPA axis, and the role of the brain reward pathways will be discussed briefly to illustrate current approaches to understanding the neurobiology of depression.

1.3.1 Dysregulation of the Hippocampus and the HPA axis

Stress-related events are considered primary causes of depression. The brain reacts to stress by activating the HPA axis. Neurons in the paraventricular nucleus (PVN) of the hypothalamus secrete corticotropin-releasing factor (CRF), which stimulates the
**Fig 1. Neural circuitry of depression.** A simplified summary of a subset of neural circuits in the brain that may contribute to depression. Structures implicated in depressive symptoms include hippocampus (HP), prefrontal cortex (PFC), nucleus accumbens (NAc), amygdala, and hypothalamus. The figure also shows the monoaminergic pathways originating in the ventral tegmental area (VTA), which provides dopaminergic input to the NAc, amygdala, PFC, and other limbic structures, the locus coeruleus (LC), that contains the noradrenergic cell bodies, and the dorsal raphe (DR) that contains serotonergic cell bodies.

Fig. 1

GABAergic
Glutamatergic
Dopaminergic
Peptidergic
NEergic/5HTergic
synthesis and release of adrenocorticotropin (ACTH) from the anterior pituitary. ACTH then stimulates synthesis and release of glucocorticoids (cortisol in humans and corticosterone in rodents) from the adrenal cortex. Glucocorticoids exert profound effects on general metabolism and significantly affect behaviour, acting directly on numerous brain regions (for a review see Nestler et al., 2002). The hippocampus exerts an inhibitory influence on hypothalamic CRF-containing neurons, while the amygdala exerts a direct excitatory influence. Under normal physiological circumstances, glucocorticoids regulate hippocampal neurons, enhancing hippocampal inhibition of HPA activity. However, a prolonged increase of glucocorticoids, observed under severe stress, may damage hippocampal neurons (Sapolsky, 2000), reducing the inhibitory control that the hippocampus exerts on the HPA axis. The result is a further increase in glucocorticoid levels, and, therefore, an excessive activation of the HPA axis (Nestler et al., 2002). Dysfunction of the HPA axis has been involved in depression. Abnormal, excessive activation of the HPA axis is observed in approximately half of individuals with depression, and these abnormalities are corrected by antidepressant treatment (De Kloet et al., 1988; Arborelius et al., 1999; Holsboer, 2001a). Postmortem studies of depressed patients have reported increased levels of CRF in the PVN of the hypothalamus, whereas levels of CRF receptors are downregulated perhaps as a response to elevated CRF transmission. At the preclinical level, studies in rodents separated from their mothers early in life show abnormalities in HPA axis function, similar to those seen in depressed humans. These abnormalities can persist into adulthood and be controlled by antidepressant treatment (De Kloet et al., 1988; Francis and Meaney, 1999; Heim and Nemeroff, 2001).
How may hyperactivity of the HPA axis lead to depression? One hypothesis is that high levels of cortisol may be toxic to hippocampal neurons, whose functionality may become impaired (Nestler et al., 2002). Antidepressant treatments would work, then, by reversing these abnormalities, although the molecular and cellular mechanisms by which prolonged enhancement of monoamine transmission would produce such actions are not known. A second hypothesis proposes that hyperactivity of the HPA axis may contribute to depression by enhancing CRF transmission in the hypothalamus and other brain regions innervated by these neurons (Nestler et al., 2002). This hypothesis is suggested by the fact that centrally administered CRF decreases appetite, decreases sexual behaviour, and increases heart rate and blood pressure (Arborelius et al., 1999; Holsboer, 2001a), symptoms often observed in depressed patients.

These observations have helped in the development of new antidepressant drugs. There is growing evidence that glucocorticoid receptor antagonists, such as mifepristone (RU486), may be useful in treating some cases of depression (Belanoff et al., 2001). Similarly, antagonists of the CRF1 receptor, the major CRF receptor in the brain, have proven helpful in several stress-based rodent models of depression (Arborelius et al., 1999; Holsboer, 2001). These drugs may treat depression by limiting hypercortisolism through actions on the HPA axis (Nestler et al., 2002).

1.3.2 Impairment of Brain Reward Pathway

Recent brain imaging and autopsy studies have suggested abnormalities in several brain areas of depressed individuals well beyond the hippocampus (Zhu et al., 1999;
Rajkowska, 2000; Drevets, 2001; Liotti and Mayberg, 2001; Manji et al., 2001). An example is provided by the brain’s reward pathway, which arises in dopaminergic neurons in the ventral tegmental area (VTA) of the midbrain and projects to the ventral striatum in the nucleus accumbens (NAc; mesolimbic system) (Fig. 1). These VTA neurons also innervate several other limbic structures, including the amygdala and limbic regions of the cortex, especially anterior cingulate cortex (Berger and Gaspar, 1994).

The VTA→ NAc pathway plays a critical role in reward. Several studies have indicated that under normal conditions these structures regulate an animal’s response to natural rewards, such as food, sex, and social interaction (Fibiger and Phillips, 1988; Koob et al., 1998; Wise, 1998; Everitt et al., 1999). Moreover, virtually all drugs of abuse increase DA transmission in the NAc, which partly mediates their rewarding effects (Koob et al., 1998; Wise, 1998). Recent work in non-human primates has suggested that the firing patterns of VTA dopamine neurons are sensitive to the reward expectations: new rewards activate the cells, whereas the absence of an expected reward inhibits the cells (Schultz, 2000).

While the role of the VTA→ NAc pathway has been largely studied in reward and addiction, its possible involvement in mood regulation and depression has received much less attention. However, given the prominence of anhedonia as well as changes in appetite and sexual behaviour seen in many patients with depression, Nestler and colleagues (2002) hypothesized a role for the brain’s reward circuitry in mediating these symptoms. These authors argue that during early phases of withdrawal from many types of drugs of abuse some patients present aversive emotional symptoms that are similar to those seen in depression, and these symptoms are thought to involve the brain’s reward
pathways and the amygdala. From these theoretical and empirical rationales emerges the need to systematically examine the role of the VTA→NAc reward pathway in mood regulation. How to achieve this goal? Nestler and colleagues (2002) proposed using behavioural tests that measure different types of reward (i.e. natural reward such as food or sex, or pharmacological reward such as drug of abuse) in depression research. One example is a paradigm called intracranial self-stimulation (ICSS), where an animal works to electrically stimulate certain brain areas, including the mesolimbic dopamine system. Drugs of abuse decrease the threshold for self-stimulation, whereas aversive conditions such as drug withdrawal states, or severe stress, increase the thresholds. Therefore, changes in ICSS threshold may provide a novel measure of the affective state of an animal that cannot be inferred from more traditional animal models of depression. Consistent with this hypothesis, several studies have shown that withdrawal from a chronic schedule of psychostimulant administration is associated with a reduction in rodents’ responding for rewarding ICSS (Kokkidinis et al., 1980; Markou and Koob, 1991; Lin et al., 1999).

Another approach suggested by Nestler and colleagues (2002) is to examine the molecular and cellular changes that occur in the VTA-NAc pathway after exposure to drugs of abuse, in the context of depression models. For example, recent studies have shown that drugs of abuse activate cyclic AMP response element-binding protein (CREB, one of the transcription factors regulating the synthesis of new proteins) in the NAc (Berke and Hyman, 2000; Nestler, 2001; Shaw-Lutchman et al., 2002). Moreover, it has been shown that increased CREB function in the NAc decreases rewarding responses to drug of abuse, as measured by conditioned place preference, whereas decreased CREB
function has the opposite effect (Carlezon et al., 1998; Pliakas et al., 2001). CREB-mediated transcription is also induced in the NAc in response to acute and chronic stress (Pliakas et al., 2001). In addition, an increased CREB function in the NAc decreases an animal’s sensitivity to several types of aversive stimuli, including anxiogenic and nociceptive stimuli, whereas decreased CREB function increases that sensitivity (Barrot et al., 2002). Therefore, it appears that CREB in the NAc controls the behavioural responsiveness of an animal to emotional stimuli in general, such that the increase in CREB seen after stress or drug exposure may contribute to symptoms of emotional numbing or anhedonia, observed in some forms of depression, and in drug withdrawal states. It has been proposed that the opioid peptide dynorphin may be one target gene through which CREB produces this behavioural phenotype (Carlezon et al., 1998; Pliakas et al., 2001).

These data implicate CREB within the mesolimbic dopamine system in the regulation of mood, motivation and possibly depression, and underline the need to examine neural circuits outside the hippocampus for a complete understanding of these phenomena.

1.4 Neurotransmitters in depression

Virtually every neurotransmitter that has been discovered has been studied for its potential role in the pathophysiology of depression. However, two neurotransmitters have been most consistently shown to be altered in depression: 5-HT and NE. Pharmacological manipulation of these monoamine levels can either induce or alleviate symptoms of depression. For example, early clinical studies showed that treatment with reserpine, a
drug that interferes with storage of monoamines and thus depletes their levels, could cause depression in a small percentage of individuals (Freis, 1954; Muller et al., 1955). In the 1960s, this observation led to the hypothesis that depression results from reduced availability of 5-HT and/or NE (Schildkraut, 1965; Bunney and Davis, 1965; Coppen, 1967). The monoamine depletion hypothesis was further supported by the discovery that the prototypical antidepressant drugs, the TCAs and MAOIs, increase synaptic levels of monoamines. The TCA drugs (i.e. imipramine) inhibit transporter-mediated reuptake of 5-HT and NA, one of the primary mechanisms for inactivation of monoamines. The MAOI antidepressants (i.e. phenelzine) block monoamine oxidase, one of the primary enzymes responsible for the degradation of the monoamines inside the neurons, and thus increase levels of monoamines.

The original monoamines hypothesis, initially based on NE and 5-HT deficiency, is now extended to DA. The implication of DA in depression is suggested by clinical observations, biochemical and preclinical studies. In the following paragraph the relationship between DA and depression is further discussed.

1.5 Dopamine and Depression

Depression is a common disturbance in schizophrenia and Parkinson's disease, which are pathologies related to a dysfunction in the central DA systems (Merschdorf et al., 2003; Kulhara and Avasthi, 2003). There are similarities between symptoms of schizophrenia and Parkinson's disease and depression. Some symptoms of depression such as anhedonia and decreased motor activity are also observed in schizophrenia
Juckel et al., 2003). Symptoms of Parkinson’s disease such as psychomotor retardation and diminished motivation are common in depressed patients (Brown and Gershon, 1993). Treatment of Parkinson’s disease patients with L-DOPA has been reported to be associated with an antidepressant effect which can precede the improvement in the physical symptoms of the disease (Murphy, 1972). These observations support the hypothesis that the depressed mood experienced by many patients with Parkinson’s disease is the result of decreased DA availability.

Further evidence of a role for DA in the biology of depression emerges from biochemical studies. Presynaptic dopaminergic function can be assessed by measuring levels of homovanillic acid (HVA), a major metabolite of DA, in the cerebral spinal fluid (CSF). Several studies have shown decreased CSF HVA levels in depressed patients as well as in depressed subjects that attempted suicide (Brown and Gershon, 1993; Lambert et al., 2000). Low CSF HVA levels have been also reported in depressed subjects with prominent psychomotor retardation (Post et al., 1978). The implication of DA in depression is also supported by the technique of acute tyrosine depletion (McLean et al., 2004). Tyrosine is the precursor of DA synthesis. Results of neuropsychological tests of healthy volunteers with a reduction in tyrosine availability to the brain paralleled those reported in previous investigations of depressed patients.

Animal models of depression also suggest a role for DA in the pathophysiology of depression. For example, the forced swimming test is an animal model used to predict the antidepressant activity of drugs. With this model it has been shown that the mesolimbic dopaminergic system has a permissive role in the effect of desipramine, as the antidepressant-like effect of desipramine was reduced after the administration of sulpiride.
(a D2 receptor antagonist) bilaterally into the NAc (Cervo et al., 1990). An indirect effect of the dopaminergic system in the antidepressant-like activity of selective 5-HT re-uptake inhibitors was also shown in the mice forced swimming test, as the antidepressant-like effect of selective 5-HT re-uptake inhibitors was modulated by agonists and antagonists of DA receptors (Renard et al., 2001). The chronic mild stress-induced anhedonia is another animal model of depression (Willner, 1997). The biochemical and behavioural changes associated with the chronic mild stress are a decrease in D2/D3 receptor binding in the NAc (Papp et al., 1994) and a functional subsensitivity to the rewarding and locomotor stimulant effects of the D2/D3 agonist quinpirole administered systemically or within the NAc (Willner et al., 1992). Together all these results are consistent with the hypothesis of a role of DA in depression.

1.5.1 Dopamine Pathways and Receptors in the Central Nervous System

Four main dopaminergic pathways have been identified within the central nervous system (CNS). DA neurons that project to forebrain structures originate mainly from two areas in the midbrain, the substantia nigra (SN) and the VTA. Neurons from the SN project primarily to the dorsal striatum (caudate and putamen; the nigrostriatal pathway), and are classically associated with motor functions. The degeneration of this pathway is implicated in Parkinson’s disease. Neurons from the VTA project predominantly to the NAc (both shell and core regions; the mesolimbic pathway), and to the medial prefrontal cortex (mPFC; the mesocortical pathway) as well as other cortical areas, and are associated with limbic functions (Nauta et al., 1978; Groenewegen et al., 1991).
Moreover, the hypothalamus is the place of origin of a projection towards the pituitary gland, which controls prolactin secretion (the tubero-infundibolar pathway).

DA receptors are classified into two sub-families: the D1-like receptor subtypes (D1, D5), coupled with the Gs protein, which activate adenylcyclase, and the D2-like family of receptors (D2, D3 and D4) coupled with G proteins, which inhibit adenylcyclase (Missale et al., 1998). D1 and D2 receptors are the most abundant subtypes in the CNS, but D1 dopamine receptor is the most widespread. D1 mRNA has been found in the striatum, NAc, olfactory tubercule, hypothalamus and thalamus. In other areas, such as the SN pars reticulata, numerous binding sites for the D1 receptor have been found, but no mRNA, suggesting that in these areas the D1 receptor is present in projections only (Missale et al., 1998). The D2 dopamine receptor is located mainly in the striatum, olfactory tubercule, NAc, the SN pars compacta, the VTA and the pituitary gland. D2 receptors are pre- and post-synaptic receptors, contrary to D1-like receptors, which are mainly post-synaptic (Missale et al., 1998).

1.6 Animal models of depression

Animal models have proven to be indispensable tools to investigate biological mechanisms underlying human diseases, and in the search to identify new treatments. Ideally, an animal model should mimic the human condition of interest with respect to its etiology, symptomatology, treatment, and biological basis (McKinney and Bunney, 1969). Clearly, meeting such requirements is difficult, especially for depression. The wide spectrum of symptoms that characterize depression highlight the difficulty of
researchers face in reproducing the disorder in the laboratory. For instance, many symptoms of depression (e.g. depressed mood, feelings of worthlessness, suicidiality) cannot be measured in laboratory animals. In addition, the lack of known depression vulnerability genes means that genetic causes of depression cannot be replicated in animals. Most models using nonhuman primates or rodents have exposed healthy animals to adverse experience, in most cases a stressor, for prolonged periods of time. The resulting phenotype accounts only for experience-related behavioural changes bearing a resemblance to depression. In such models, the fact that the development of depression is strongly influenced by genetic factors is ignored.

In light of these difficulties, the development of a perfectly homologous model, reproducing as closely as possible all aspects of depression, seems out of reach. Willner (1985) offered an approach for the evaluation of animal models of depression on the basis of how well they satisfy three distinct validity criteria. These criteria are 1) face validity, how well the model resembles the human condition; 2) construct validity, how well the model is consistent with our understanding of the etiology of depression in humans; and 3) predictive validity, how well the model responds favourably to the same antidepressant drugs as humans with depression. To date numerous animal models have been developed to mimic human depression, and many studies have been directed at assessing and validating the different models of depression (for a review see Nemeroff, 1998). These models are instrumental in detecting the antidepressant-like potential of novel compounds in preclinical settings. The models commonly used are diverse and were developed originally based on the behavioural consequences of stress, pharmacology (drugs), or genetic manipulations.
1.6.1. Models based on stress

There is general agreement that depression is more likely following the occurrence of stressful events (Dura et al., 1990; Monroe and Simons, 1991), and, by far, the largest number of animal models of depression relies on this premise. The validity of this approach is supported by one of the most significant effects of stress on animals: reduced sensitivity to reinforcement, both positive and negative. That is, uncontrollable stress impairs the seeking of positive reinforcers as well as the avoidance of negative reinforcers (Nestler et al., 2002). Three rats model of depression will be discussed: learned helplessness, chronic mild stress, and prenatal/neonatal stress.

**Learned Helplessness**

In the learned helplessness paradigm animals are exposed to aversive stimuli, such as inescapable electric footshocks (Seligman and Beagley; 1975). Exposure to such stimuli results in a long-lasting deficit in escape performance, which has been termed learned helplessness. This model has good face validity because rats with learned helplessness exhibit signs of human depression such as loss of appetite and weight (Weiss, 1968), exhibit decreased locomotor activity (Wagner et al., 1977), and perform poorly in both appetitively- and aversively-motivated tasks (Roselini and DeCola, 1982; Zacharko et al., 1982). These behavioural characteristics are considered to correspond to the loss of appetite and weight, psychomotor retardation, and anhedonia, demonstrated by depressed humans (DMS-IV, 1994). Pharmacological treatments that are clinically
effective in treating depression, such as tricyclics, MAOIs, atypical antidepressants, and electroconvulsive shock therapy, are effective in reducing the behavioural and physical abnormalities of rats exposed to uncontrollable stress (Willner, 1984; Petty et al., 1992). Thus, the learned helplessness model appears to have good predictive validity for identifying potentially useful pharmacotherapies for depression in humans. The construct validity of the learned helplessness model of depression rests on the assumptions that animals exposed to uncontrollable aversive events do become helpless, that a similar state is induced in people by uncontrollable aversive events, and that helplessness in people is a symptom of depression.

**Chronic Mild Stress**

The chronic mild stress (CMS) model was developed by Willner (1997) to mimic anhedonia, a core symptom of depressive disorders. In the CMS paradigm, animals are subjected for several weeks to a series of mild stressors (intermittent food and water deprivation, overnight illumination, cage tilt, noise, etc; Willner et al., 1992; Willner, 1997). This treatment induces a reduction of sucrose preference, decreased intracranial self-stimulation, altered sexual and aggressive behaviour, loss of body weight, overactivation of the HPA system, decreased locomotor activity (Willner, 1997), and disrupted sleep (Cheeta et al., 1997). Nearly all clinically effective antidepressant tested in this model reversed the induced reduction in consumption of sweetened solution (Willner et al., 1992; Monleon et al., 1995). Moreover, sucrose preference returned to baseline levels only after a 3- to 5-week delay, paralleling response time to antidepressant
medications in those with depression. Thus, face and predictive validity of the CMS model appear to be respected. Recently, neurochemical changes caused by CMS have been studied. In rats exposed to CMS, the 5-HT2A and beta-adrenergic receptors in the cortex increase, and these increases are normalized by chronic, but not acute, antidepressant administration (e.g., imipramine) (Papp et al., 1994). Such increases have been reported in presumably depressed suicide victims (Arango et al., 1990). Similarly, in rats exposed to CMS, dopamine D2 and D3 receptors were decreased and the decrease was reversed after chronic imipramine treatment (Papp et al., 1994). CMS also causes a decrease of dopamine release in vivo in rats (Willner, 1997). The observed monoamine changes in the CMS model are related to hedonic responses and reward, which further supports the CMS model as a valid model of depression (Willner, 1997).

*Prenatal/neonatal stress*

Several models involving manipulation of early life environment have been used, including prenatal stress, and maternal separation (Francis et al., 1999; Caldji et al., 2000; Ladd et al., 2000). Prenatally stressed rats (when the dam is subjected to repeated footshock during the early stage of pregnancy) exhibit elevated activity of the HPA axis and defensive behaviour before and after weaning, as well as exaggerated behavioural, physiological, and neuroendocrine responses to stressful stimuli that persist into adulthood (Takahashi et al., 1992; Ward et al., 2000; White and Birkle, 2001). On the other hand, neonatal stress in rats, such as maternal separation, might serve as a suitable paradigm for the effects of aversive early experience on behavioural responsiveness to
stress later in life, as an animal model of behavioural responses associated with mood disorders in human depressives (construct validity; Ladd et al., 2000; Heim et al., 2001). Several classes of antidepressant drugs attenuate or reverse the behavioural and endocrinological effects of maternal separation, providing this model with a good predictive validity (Ladd et al., 1996).

1.6.2. Models based on Genetics

As described earlier, human depressive disorders are well known to have a strong genetic component. Although the specific genes conferring the risk for depression have not been identified, several models have been developed that have a genetic basis. For instance, the Flinders Sensitive Line (FSL) of rats is one of the earliest and best studied line of animals specifically bred as a genetic animal model of depression (Overstreet and Russell 1982). Based on the observation that human depressives are hypersensitive to cholinergic agonists (Janowsky et al., 1972), the original FSL rats were developed by selective breeding for high and low sensitivity to the anticholinesterase diisopropylfluorophosphate. The FSL rats share many similarities with depressed humans. For example, FSL rats have elevated REM sleep (Shiromani et al., 1988), appetite and weight changes (Overstreet, 1993), reduced locomotor activity (Overstreet and Russell, 1982), decreased sensitivity to rewarding stimuli, as indicated by decreased intake of a sucrose solution after exposure to CMS (Pucilowski et al., 1993), exaggerated immobility in the Porsolt forced swimming test (Overstreet et al., 1994), and learning difficulties (Yadid et al., 2000). Moreover, both FSL rats and depressed individuals have
similar changes in serotonergic and dopaminergic systems as a result of chronic treatment with antidepressant (Overstreet 1993). Thus, this model has potential etiological validity and some construct validity as indicated by anhedonia and REM sleep abnormalities seen in these animals (Yadid et al., 2000).

Very recently, transgenic models of depression have been developed. Mouse mutants with altered HPA system activity are candidate strains for a murine depression-like syndrome, because HPA system imbalances have been shown to be a key biological marker for depression in humans. In particular, alterations of corticosteroid receptors (glucocorticoid receptors, and mineralcorticoid receptors) appear promising in modeling affective disorders (Urani and Grass, 2003). Moreover, current techniques even allow a conditional gene disruption in specific brain regions, and sometimes at a specific time point (Kellendonk et al. 1999; Rossant and McMahon, 1999).

1.6.3. Models based on pharmacology

Most of the pharmacological models of depression are based on the monoaminergic hypothesis of depression, which is based on the deficiency in one or several biogenic monoamines (5-HT, NE, or DA). All currently available antidepressant medications work by enhancing the transmission of one or more monoamines, and this class of models attempts to induce depression by doing the opposite- antagonizing one or more monoamine systems. One of the first animal models of depression involved reducing catecholamine levels in the brain, using drugs such as reserpine and tetrabenazine. Reserpine depletes the aminergic pools in the presynaptic nerve terminals,
imitating the pathophysiology postulated for depressive patients. The reversal of some effects of reserpine administration (e.g., ptosis and hypothermia) is used to predict the antidepressant activity of drugs (Bourin, 1990; Almeida et al., 1998). In animals treated with reserpine, a decrease in the preference for sucrose was also observed (Skalsiz et al., 2002). Antidepressant treatment, including lithium and electroconvulsive therapy (ECT), has been shown to reverse behavioural and biochemical deficits induced by reserpine (Redrobe and Bourin, 1999; Vetulani et al., 1986). Based on the theory that depression can also result from excessive serotonergic neurotransmission, the 5-hydroxytryptophan model of depression has been developed. The administration of this 5-HT precursor produces depression-like symptoms in rodents, and this effect is attenuated by antidepressant treatment (Meyers, 2000).

1.7 Psychostimulant Drug Withdrawal in Humans

Psychostimulant drugs are a class of psychoactive compounds that have the common property of activation of the central nervous system, and subsequently, behaviour, inducing symptoms such as euphoria, increased energy, hyperactivity, increased arousal and self-confidence, when taken at high doses (Breiter et al., 1997; Drevets et al., 2001). Commonly used psychostimulants, legally available in most countries, include caffeine and nicotine, whereas illegal psychostimulants include cocaine and amphetamine. Physiologically, psychostimulants typically increase the functional activity of central monoaminergic and cholinergic systems (Fibiger and Phillips, 1988; Coury et al., 1992; Nestby et al., 1997; Mark et al., 1999). In particular, cocaine and
amphetamine act primarily as indirect monoamine agonists, and alter the reuptake and release of monoamines by binding to plasmalemmal and vesicular monoamine transporters (Brown et al., 2001). This property results in substantial increase in synaptic and extrasynaptic levels of dopamine, noradrenaline and serotonin (Rothman et al., 2001), which are generally considered to mediate the euphorigenic effects of the drugs (Drevets et al., 2001; Verhoeff et al., 2003), although the relationship between the levels of these neurotransmitters and subjective mood is not completely understood (Volkow et al., 2000).

A feature common to psychostimulant drugs is the phenomenon of the withdrawal syndrome that results from termination (or discontinuation) of drug administration. The withdrawal syndrome appears because tolerance quickly develops to the reinforcing properties of the drug (Brauer et al., 1996; Mendelson et al., 1998). However, the nature and intensity of the withdrawal syndrome vary depending on the specific drug and dose. For example, caffeine and nicotine abstinence symptoms are not severe, and include headaches and irritability, while withdrawal symptoms from high doses of cocaine and amphetamine induce a number of psychological symptoms such as anhedonia, depressed mood, loss of energy, that are manifestly similar to those of MDD (table 1).

1.7.1 Behavioural Effects

A comparison of the effects of psychostimulant withdrawal with the DMS-IV diagnostic criteria for MDD, independent of symptom duration, shows that almost all of the indications of MDD are observed during psychostimulant withdrawal (table 1).
Although the duration of psychostimulant withdrawal in humans is generally less than the two-week period necessary for diagnosis of MDD, both depressed mood and anhedonia are consistently evident during psychostimulant withdrawal (Weddington et al., 1990; Pathiraja et al., 1995). Table 1 shows that also the remaining diagnostic criteria for MDD are widely present during psychostimulant withdrawal. For example, changes in appetite (Dackis et al., 1987; Srisurapanont et al., 1999), hypersomnia (Thompson et al., 1995), feelings of fatigue, often combined with psychomotor retardation (Uslaner et al., 1999), suicidal thoughts and ideation (Lowenstein et al., 1987), impaired concentration and confusion (Roberts and Bauer, 1993), represent symptoms common to both MDD and psychostimulant withdrawal.

### 1.7.2 Physiological effects

Psychostimulant withdrawal in humans and MDD are similar not only at the behavioural level, but also at the physiological level. MDD is associated with several physiological markers, such as hormonal, electrophysiological, and metabolic indices, many of which are present during psychostimulant withdrawal. Dysfunctions of the HPA axis frequently observed in MDD include elevated levels of cortisol, decreased dexamethasone-mediated negative feedback, and increased cerebrospinal levels of CRF (Plotsky et al., 1998; Holboer, 2000). In abstinence, cocaine addicts have been shown elevated diurnal levels of ACTH and β-endorphin, as well as the glucocorticoid hormone cortisol, in a pattern that is similar to depressed patients (Goodwin et al., 1993; Deuschle et al., 1997). In addition, neuroimaging studies and EEG techniques have shown other
similarities between the two disorders. For example, there is a remarkable similarity between the EEG activity of depressives and abstinent psychostimulant abusers during sleep (Herning et al., 1997). MDD is characterized by a reduced REM latency and increased REM density during the night (Kupfer et al., 1976; Landolt and Gillin, 2002). Abstinence from both amphetamine (Watson et al., 1972) and cocaine (Thompson et al., 1995) mimics the effects of MDD on these parameters.

1.7.3 Treatment of psychostimulant withdrawal in human

Because of the similarities between MDD and psychostimulant withdrawal symptoms, the majority of treatments for psychostimulant withdrawal include drugs that have antidepressant properties and that stimulate monoamine pathways. Because of the crucial role of the mesolimbic dopamine system in the rewarding properties of psychostimulant drugs (Wise and Rompre, 1989; Volkow et al., 2002), treatment therapies have often focused on pharmacological manipulation of the dopamine system. For example, earlier reports indicated that the dopamine D2 receptor agonist bromocriptine could provide rapid relief of the dysphoria of cocaine withdrawal (Tennant and Sagherian, 1987; Giannini et al., 1987). However, more recent studies have failed to reproduce the same beneficial effects (Eiler et al., 1995; Handelsman et al., 1997). But, when bromocriptine was used in combination with the tricyclic antidepressant desimipramine, a noradrenergic reuptake inhibitor, symptoms of cocaine withdrawal were attenuated (Giannini et al., 1987), suggesting that bromocriptine may be more useful as an adjunct therapy. Lisuride, a dopamine D2 receptor agonist, and pergolide, a
dopamine D1/D2 receptor agonist, have been shown to be effective in alleviating sleep-related deficits (Gillin et al., 1994). The dopamine D2 receptor agonist apomorphine produced a rapid reversal of depressive symptomatology during cocaine withdrawal (Malcolm et al., 1991). In patients with severe withdrawal symptoms the indirect dopamine agonist amantadine has also proven to be more successful than direct agonists at treating the symptoms of psychostimulant withdrawal (Tennant and Sagherian, 1987; Giannini et al., 1989).

Several antidepressant drugs have been also used in the treatment of psychostimulant withdrawal. The TCA amitriptyline was successful in reversing the dysphoria of amphetamine withdrawal (Tuma, 1993). The antidepressant amineptine, which acts primarily at the dopaminergic system, has been shown repeatedly to provide a rapid attenuation of the symptoms of amphetamine withdrawal (Srisurapanont et al., 1999; Jittiwutikan et al., 1997). Venlafaxine, an antidepressant with selectively high affinity for the noradrenergic and serotonergic reuptake sites reduced symptomatology of depression during cocaine withdrawal in patients with comorbid MDD (McDowell et al., 2000).

The variety of pharmacological compounds that provide at least some amelioration to the symptoms of psychostimulant withdrawal suggests that there are several neurochemical substrates involved in the symptomatology of this disorder. Noteworthy is the fact that almost all of these treatments act on the monoamine systems, like most of the antidepressant drugs. Moreover, many drugs that alleviate the symptoms of psychostimulant withdrawal also show antidepressant properties in humans, suggesting
that psychostimulant withdrawal and MDD share some degree of pharmacological isomorphism, as they respond to similar psychoactive compounds.

1.8 Psychostimulant withdrawal in animals

The strong similarities between psychostimulant withdrawal and MDD in humans provided the impetus for the development of an animal model of depressive symptomatology, where comparisons can be made between the behavioural, physiological and pharmacological aspects of psychostimulant withdrawal in animals and humans (Seltzer and Tonge 1975; Leith and Barrett, 1980; Kokkidinis et al., 1986; Geyer and Markou, 1995).

1.8.1 Behavioural effects

As mentioned above, animal models of depression are designed to induce quantifiable behavioural alterations that parallel a specific symptom of this disorder. Anhedonia, one of the core symptoms of both depression and psychostimulant withdrawal, manifested behaviourally as decreased interest in normally rewarding stimuli, has proven to be easily modeled in rodents. Reduced motivation to obtain various reinforcers appears to be the best characterized of the behavioural effects of psychostimulant withdrawal in animals. Several studies have examined the anhedonia associated with psychostimulant withdrawal by assessing animals’ responding for ICSS (Leith and Barrett, 1976, 1980; Kokkidinis et al., 1980; Cassen et al., 1981; Lin et al.,
1999; Markou and Koob, 1991). As mentioned in a previous paragraph, ICSS is a well-validated technique that consists in brief electrical self-stimulation of specific brain areas, such as the VTA, which leads to neurochemical changes in other brain structures, such as the NAc, in which reward processes are hypothesized to occur (Phillips and Fibiger, 1990; Wise, 1996; Ivanova and Greenshaw, 1997). Both the rate of responding for ICSS and the thresholds for ICSS have been used as measures of the reward value of the stimulation (Barr et al., 2000). Lowering of threshold, or increases in ICSS response rates are interpreted as an increase in the reward value of the stimulation, whereas elevation of thresholds or decreases in ICSS response rates is interpreted as a decrease in the reward value of the stimulation. For instance, when rats were withdrawn after being allowed to self-administer cocaine for prolonged periods of time, their thresholds for ICSS reward were increased when compared to the amount of cocaine consumed during the cocaine “binge” (see Koob, 1992 for review). Similar results have been obtained after repeated treatment with experimenter-administered cocaine (see Koob, 1992) or amphetamine (Borowsky and Kokkinidis, 1992), confirming the reliability of the phenomenon. Moreover, post-cocaine elevations in thresholds were reversed by administration of either the dopamine agonist bromocriptine or the tricyclic desmethylimipramine (Markou and Koob, 1992), drugs used in the treatment of psychostimulant withdrawal and dependence.

Rats withdrawn from an escalating-dose schedule of D-amphetamine also exhibit a reduced motivation to obtain natural reinforcers, including responding for a sucrose solution on a progressive ratio schedule of reinforcement (Barr and Phillips, 1999), and access to a sexually receptive conspecific (Barr et al., 1999). Interestingly, in both the sexual behaviour and progressive ratio studies, rats exhibited deficits in appetitive aspects
of the tasks, whereas consummatory responses remained unaffected. The reasons for this selective effect are unclear, but may be related to the selective dysregulation of specific neural substrates, or a greater sensitivity of the neurochemical pathways involved in appetitive responding versus substrates involved in consummatory behaviour.

Furthermore, it has been recently shown that rats withdrawn from an escalating-dose schedule of D-amphetamine display both increased magnitude and duration of successive negative contrast, providing compelling evidence of a meaningful and sustained change in affect in this model of anhedonia (Barr and Phillips, 2002). Successive negative contrast, a phenomenon observed in many species including rodents, primates and humans, occurs when the incentive property of a rewarding stimulus is devalued unexpectedly (Schnorr and Myers, 1967; Flaherty, 1991; Specht and Twining, 1999).

With the exception of symptoms such as “feeling of worthlessness” and “suicidal ideation” that are not possible to model in rodent paradigms, the remaining diagnostic criteria for major depression, can all be modeled to a greater or lesser degree in psychostimulant withdrawn rodents. For example, changes in homeostatic behaviours, such as sleeping and eating, can be readily modeled in rodents (Jones and Caul, 1989; Touret et al., 1995; Dugovic et al., 1992), as well as decreased locomotion in cocaine and amphetamine withdrawn rats (Seltzer and Tonge, 1975; Herman et al., 1971; White et al., 2003). In addition rats display increased anxiety during postdrug withdrawal, as measured by increased acoustic startle (Barros and Miczek, 1996), open arm exploration on an elevated-plus maze, and defensive burying (Basso et al., 1999).
1.8.2 Physiological effects

The strong behavioural analogies between the effects of psychostimulant withdrawal and the symptoms of depression give a high degree of construct validity at the psychostimulant withdrawal model of depression. In addition, there are also a number of important physiological similarities between psychostimulant withdrawal in rodents and MDD in humans. Neuroendocrine changes, such as elevated levels of cortisol, and increased cerebrospinal levels of CRF, consistently observed in depressed patients, are reflected by elevated levels of corticosterone in rats withdrawn from a “binge”-like dose of cocaine (Sarnyai et al., 1998), while ambiguous results have been observed when examining corticosterone levels during amphetamine withdrawal (Swerdlow et al., 1991). Several studies examining the neurochemical alterations associated with cocaine withdrawal, using in vivo techniques such as microdialysis, have shown reduced extracellular levels of DA in limbic nuclei (i.e. NAc), during cocaine withdrawal (Weiss et al., 1992), while mixed results have been found for amphetamine withdrawal (Paulson and Robinson, 1996). Moreover, changes in pre-and post-synaptic 5-HT-mediated activity have been observed in cocaine withdrawn rodents (Baumann and Rothman, 1998), with decreased 5-HT-mediated neurotransmission in the NAc (Parson et al., 1995).

Since evidence suggests that MDD is related to deficits in the mesolimbic dopamine system (Fibiger, 1995; Drevets et al., 2001) these findings provide a degree of etiological validity between the physiology of this model of depression and the human disorder.
1.8.3 Treatment of Psychostimulant Withdrawal in Animals

Several studies have reported effects of treatment with different compounds on the behavioural symptoms of psychostimulant withdrawal in rodents, providing evidence for a predictive validity of this model of depression. Research in the 1970's showed that rats subchronically treated with the antidepressants pargyline and mianserin presented diminished effects of amphetamine withdrawal on locomotor deficits in an open field task (Lynch and Leonard, 1978), although the tricyclic antidepressant amitriptyline was found ineffective. However, the tricyclic drug imipramine reversed the behavioural depression associated with methamphetamine withdrawal, showing that certain tricyclic drugs are effective in this model (Selzer and Tonge, 1975).

The anhedonia of psychostimulant withdrawal in animals has also been modulated by treatment with various antidepressant drugs. It has been recently shown that the dopamine partial agonist terguride reversed the decrease in responding for a sucrose solution under a progressive ratio schedule of reinforcement during amphetamine withdrawal (Orsini et al., 2001). Moreover, the tricyclic antidepressant desmethylimipramine shortened the effects of cocaine withdrawal on ICSS responding, while both imipramine and amitriptyline reduced the effects of amphetamine withdrawal (Kokkidinis et al., 1980). Rats pretreated with lithium, which has therapeutic properties for MDD (Bauer et al., 2000), did not show the post-amphetamine depression of ICSS responding (Predy and Kokkidinis, 1981). Harrison and colleagues (2001) determined the capacity of the selective serotonin reuptake inhibitor (SSRI) fluoxetine to attenuate the effects of amphetamine withdrawal on ICSS responding. The authors observed that
fluoxetine attenuated the effects of drug withdrawal by shortening the duration of its effects on ICSS responding. Moreover, when the selective 5-HT$_{1A}$ receptor antagonist p-MPPI was co-administered with fluoxetine, the duration of drug withdrawal were even further shortened, implying that the psychostimulant withdrawal model of depression might be able to differentiate fast-acting antidepressant treatments from standard antidepressant treatments. Because there is evidence that the 5-HT$_{1A}$ receptor might be a valid substrate for the development of rapidly acting antidepressants (Andree et al., 1999; Blier and Ward, 2003), the results presented by Harrison and colleagues suggest that the psychostimulant withdrawal model of depression might be an excellent tool to identify detecting fast-acting antidepressant.

1.9 Hypotheses and Objectives of This Study

The effects of psychostimulant withdrawal in humans bear remarkable similarity to MDD (table 1). This has been further explored through the development of animal paradigms, which allow for the accurate measurement of several prominent symptoms of drug withdrawal, such as anhedonia. Evidence of a state of anhedonia in drug-withdrawn animals has been determined with the refined use of rodent models of reinforcement. It has been well demonstrated that rats in withdrawal from chronic psychostimulant treatment display decreased hedonic capacity by way of reductions in responding for rewarding electrical brain stimulation reward (Kokkinidis et al., 1980; Markou and Koob, 1991; Lin et al., 1999). These findings suggest that chronic administration of psychostimulant drugs can induce adaptations in the neuronal substrates mediating ICSS
reward that are expressed as withdrawal symptomatology after discontinuation of drug administration.

Recent findings from our laboratory have shown that rats exhibit reduced motivation to obtain natural reinforcers, such as a sucrose solution (Barr and Phillips, 2002), or a sexually receptive animal of the same species (Barr et al., 1999), for up to 5 days following the termination of an escalating-dose schedule of D-amphetamine administration. Moreover, rats withdrawn from the same regimen of D-amphetamine show increased successive negative contrast, providing compelling evidence of a meaningful and sustained change in affect in this model of anhedonia (Barr and Phillips, 2002). Taken together, these studies demonstrate how psychostimulant withdrawal can produce discrete disturbances in psychological and affective processes, hence providing important support for the hypothesis that withdrawal following exposure to escalating doses of psychostimulant drugs produces depressive-like symptoms.

Recent brain imaging and autopsy studies have shown abnormalities in the brain reward pathway of depressed patients (Zhu et al., 1999; Rajkowska, 2000; Drevets, 2001; Liotti and Mayberg, 2001; Manji et al., 2001). As noted in Section 1.3.2, the brain reward pathway arises from dopaminergic neurons in the VTA of the midbrain and projects mainly to the ventral striatum in the NAc. The VTA$\rightarrow$NAc pathway plays a critical role in reward, mediating not only an animal's response to natural reinforcers, such as food, sex, and social interaction (Mogenson and Phillips 1978; Koob et al., 1998; Wise, 1998; Everitt et al., 1999), but also pharmacological rewards, such as drugs of abuse (Koob et al., 1998; Wise, 1998). Using in vivo techniques, studies in rats have demonstrated increases in DA efflux in the NAc during appetitive and consummatory phases of feeding.
behaviours (Phillips et al., 1993; Wilson et al., 1995). Furthermore, virtually all drugs of abuse increase DA transmission in the NAc, which partly mediates their rewarding effects (Koob et al., 1998; Wise, 1998). Therefore, it is hypothesized that some of the depressive-like symptoms observed during psychostimulant withdrawal in rodents, such as anhedonia and decreased motivation, are likely due to a reduced responsiveness of the brain reward system, inducing a long-lasting reduction in the efflux of DA in limbic nuclei, such as the NAc. Since withdrawal from psychostimulant drugs in both humans and animals leads to an attenuation of reward, the psychostimulant withdrawal syndrome in animals may prove a valuable tool for studying the neurobiological substrates underlying depression.

Despite a large body of evidence that supports psychostimulant withdrawal as a promising means to model depression in animals, important questions remain unanswered. This thesis will address several outstanding questions. For example, how are the behavioural changes discussed above related to corresponding neurochemical changes in the mesolimbic DA pathway? Also, given the critical role that the dopaminergic VTA→NAc pathway plays in reward, can we assess changes in the DA system during the administration of a binge-like regimen of D-amphetamine, and, more importantly, during withdrawal? Moreover, how would animals that are in a state of anhedonia induced by psychostimulant withdrawal, respond to a rewarding stimulus when its incentive properties are increased unexpectedly, as in the positive contrast paradigm?
1.9.1 Specific aim 1

To further investigate psychostimulant withdrawal as means of inducing depression in animals, the first purpose of this thesis will be to examine if the behavioural changes observed following repeated administration of D-amphetamine are correlated with neurochemical changes in the NAc.

In the first experiment, DA efflux in the NAc was monitored with microdialysis at two different time points during the administration of an escalating dose schedule of D-amphetamine, previously shown to affect motivated behaviour (see table 2 for a summary). Subsequently, in order to study the response of the mesolimbic dopaminergic system during withdrawal, the rats will be challenged with a 5-mg/kg injection of D-amphetamine 72-hr after the last D-amphetamine injection.

It is hypothesized that the anhedonia experienced by drug-withdrawn animals will be reflected at the neurochemical level as a blunted NAc DA response to a pharmacological reward.

The second experiment, will examine the response of the NAc dopaminergic system to a natural reward (4% sucrose solution). DA efflux will be monitored during the preparatory and the consummatory phases of feeding behaviour before and 72-hr after the escalating-dose D-amphetamine regimen. Using in vivo techniques, several studies in rats have demonstrated increases in DA efflux in the NAc during appetitive and consummatory phases of feeding behaviours (Phillips et al., 1993; Wilson et al., 1995).
However, it is hypothesized that the anhedonia experienced by drug-withdrawn animals will be reflected at the neurochemical level as a blunted NAc DA response to a natural reward.

1.9.2 Specific aim 2

As discussed above, several experiments have shown that in animals, psychostimulant withdrawal may induce aversive affective states, such as anhedonia. In particular a recent study in our laboratory, which employed the negative contrast paradigm, has provided further evidence towards this result. This study demonstrated enhanced successive negative contrast in rats withdrawn from a binge-like regimen of D-amphetamine, indicating greater sensitivity to unanticipated changes in reward value. The negative contrast occurs when the incentive property of a rewarding stimulus is devalued unexpectedly. However, it still remains to be determined whether animals that are in a state of anhedonia induced by psychostimulant treatment have a generalized inability to respond to unexpected gains in reward value, as well as losses.

Accordingly, the purpose of the experiment, presented in Chapter 3, was to determine the effect of withdrawal from an escalating-dose schedule of D-amphetamine on the consumption of a 32% sucrose solution, in rats subjected to a positive contrast paradigm, switching them from a familiar 4% sucrose solution to a novel 32% solution.
It is hypothesized that the negative affective state induced by psychostimulant withdrawal would also disrupt the positive affect induced by an unexpected gain in incentive value in the successive positive contrast paradigm.
CHAPTER II  Attenuated nucleus accumbens dopamine efflux in response to rewarding stimuli during withdrawal from an escalating-dose schedule of D-amphetamine.

2.1 Introduction

Amphetamines are psychostimulant drugs that potently activate the CNS. Amphetamines are chemically synthesized in two chimeric forms, an “L” form, which acts more on the cardiovascular system, and a “D” form, which acts more at the CNS level and is more commonly used in behavioural studies. The catecholamine terminal is the primary site of action of D-amphetamine. Catecholamines, such as DA and NA, are normally released from synaptic terminals by an impulse-dependent process, achieved via action potential discharge in DA and NA cell bodies. This action potential then propagates to the axon terminal to trigger calcium-dependent release of neurotransmitters into the synaptic cleft. D-amphetamine increases the extracellular concentration of DA by interfering with the reuptake process by competing with DA for the uptake carrier, as well as causing DA release from the terminal via reversal of this transport process (Kuczenski, 1983). Therefore, amphetamine induced DA release is calcium and impulse-activity independent.

As noted in the introduction, previous studies in humans and animals have shown that withdrawal from high doses of psychostimulant drugs leads to a number of aversive psychological symptoms. One of the more prominent symptoms is anhedonia, a core symptom of major depression, manifested behaviourally as decreased interest in normally rewarding stimuli. Several studies have shown that withdrawal from a chronic schedule
of psychostimulant administration is associated with a reduction in rodents' responding for rewarding electrical brain stimulation (Kokkinidis et al., 1980; Markou and Koob, 1991; Lin et al., 1999), and an increased immobility in both the forced swim test, and the tail suspension test (Cryan et al., 2003). Moreover, rats withdrawn from an escalating-dose schedule of D-amphetamine exhibit a reduced motivation to obtain natural reinforcers, such as a sucrose solution (Barr and Phillips, 2002), or a sexually receptive animal of the same species (Barr et al., 1999). Interestingly, the latter experiment reports that drug-withdrawn rats exhibit decreases in certain preparatory components of sexual behaviour (e.g. anticipatory locomotor activity in the 5 min period preceding the presentation of an estrous female), whereas their copulatory behaviours were left fundamentally unaltered. These results are in agreement with previous research in our laboratory that showed that rats exposed for three weeks to chronic mild stress (an animal model of depression) exhibit a significant reduction in appetitive responses for sucrose, rather than for their sucrose consumption, compared to control subjects (Phillips and Barr, 1997). These effects are likely due to a long-lasting reduction in the efflux of DA in limbic nuclei, such as the NAc, the brain area that is thought to be involved in mediating reward.

In the light of the above assertions, the experiments presented in this chapter employed the microdialysis technique to evaluate, in freely-moving rats, the effect of withdrawal from a 4-day escalating-dose schedule of D-amphetamine (1-10 mg/kg, i.p. at ~ 8 hr intervals) on stimulus-induced changes in extracellular DA levels in the NAc. The D-amphetamine escalating-dose regimen used is a recently modified version (Barr and Phillips, 2002) of that described by Leith and Barrett (1976).
In the first experiment, DA efflux in the NAc was measured at two time points during the escalating-dose schedule of D-amphetamine: the 5th, and the last injection (table 2). In addition, in order to study the response of the accumbal dopaminergic system during withdrawal, rats were challenged with a 5-mg/kg injection of D-amphetamine (i.p.) 72 hr after the last D-amphetamine injection. In the second experiment, NAc DA efflux in response to a natural reward (4% sucrose solution), during the preparatory and the consummatory phases of feeding behaviour, was evaluated before and after the escalating dose D-amphetamine regimen.

It has been demonstrated that all drugs of abuse increase DA transmission in the NAc, which partly mediates their rewarding effects (Koob et al., 1998; Wise, 1998). Furthermore, using in vivo techniques, such as microdialysis, several studies in rats have demonstrated increases in DA efflux in the NAc during appetitive and consummatory phases of feeding behaviours (Phillips et al., 1993; Wilson et al., 1995).

However, it is hypothesized that the anhedonia experienced by drug-withdrawn animals will be reflected at the neurochemical level as a blunted NAc DA response to a pharmacological and natural reward.

2.2 General Materials and Methods

2.2.1 Subjects

Thirty-eight male Long-Evans rats (Charles River, Quebec), weighing 250-300 g on arrival in the laboratory, were pair-housed for at least a week prior to surgery and
singly afterwards, in a temperature-controlled colony (21°C) under reverse light/dark cycle conditions (lights on at 0400 hours). Food and water were available ad libitum, unless otherwise indicated. All experimental procedures were performed in accordance with the standards of the Canadian Council on Animal Care and were approved by the Committee on Animal Care, University of British Columbia.

2.2.2 Surgery

All rats were anesthetized with ketamine hydrochloride (100 mg/kg, intraperitoneally, i.p.) and xylazine (7 mg/kg, IP), and implanted bilaterally with stainless steel guide cannulae (19 gauge, 15 mm long) positioned 1 mm below dura, directly over the NAc (+1.7 AP and ± 1.1 ML from bregma, -7.8 DV from dura). Co-ordinates were determined according to Paxinos and Watson brain atlas (1997). Animals were allowed to recover for one week before being assigned to experiment I (n=31), or experiment II (n=7).

2.2.3 Drug administration

Escalating doses of the drug D-amphetamine sulfate (Sigma, St. Louis, MO, USA) were administered following this protocol: rats were injected 3 times per day (8 am, 3 pm, 10 pm; intraperitoneally, i.p.) for 4 days. For the first three days rats received a starting dose of 1.0 mg/kg of D-amphetamine, which escalated by 1 mg/kg with each subsequent dose. On the fourth day, rats received three doses of 10 mg/kg. Animals, therefore, received a total of 12 injections over a 4-day period. The incremental dose
regimen is used to minimize the chance of acute toxicity associated with high D-amphetamine doses.

2.2.4 Microdialysis

The microdialysis technique consists of the implantation of a dialyzing probe in the target area, in this case the NAc. The insertion of the probe is possible because of the presence of two tubes of silica: the inlet, through which flows an artificial cerebro-spinal fluid, and the outlet where the dialyzate is collected. The principle of this technique is that molecules with a molecular weight (MW) lower than 65000 Daltons present in the extracellular space diffuse passively down the concentration gradient, while molecules with a higher MW remain outside the probe.

2.2.5 High pressure liquid chromatography

DA levels in NAc dialysates were analysed by HPLC with electrochemical detection. The HPLC system was equipped with an ESA 582 pump (ESA Inc., Bedford, MA, USA), an Antec Leyden (Leyden, The Netherlands) electrochemical detector, a Tosoh Bioscience Super ODS TSK column (2μm particle, 2.0 mm x 10.0 mm; Montgomeryville, PA), and a Valco (Houston, TX) Cheminert C1 microbore injector (5 μl loop). The mobile phase (70 mM Sodium Acetate, 40 mg/L EDTA, and 50 mg/L of sodium octyl sulfate, pH 4.0, 12% methanol) flowed through the system at a rate of 0.18 ml/min.
2.2.6 Histology

At the end of experiments I and II, rats were deeply anaesthetized with sodium pentobarbital (100 mg/ml) and perfused intracardially with 0.9% NaCl. Brains were promptly removed and stored in formalin with 20% (w/v) sucrose for a week, sliced into coronal sections (50 μm thick) using a cryostat, and then stained with cresyl violet. The placement of probes was verified according to the atlas of Paxinos and Watson (1997). Only animals with tracts in the NAc were included in the statistical analysis (details are discussed in the following sections).

2.2.7 Data analysis

The average concentration of DA in the last three stable samples (less than 10% variation across three consecutive samples in experiment I and less than 5% in experiment II) before treatment, was considered as the basal level (100% baseline) and subsequent values were expressed as % changes from basal level ± SEM. Statistical significance of the neurochemical data was evaluated by two-way analysis (Group x Time) of variance (ANOVA) tests for repeated measures, and followed, when appropriate, by Dunnett’s (within group comparisons against the control mean) and Dunn’s (between group comparisons) methods of multiple comparisons.
2.3 Methods Experiment I

The purpose of this experiment was to evaluate the response of the dopaminergic system to a pharmacological reward. To facilitate microdialysis sampling, in the drug-treated group (n=14), the first D-amphetamine injection (1 mg/kg) was administered at 10 pm, and the following doses at the times indicated in table 2. The drug dose was dissolved in isotonic saline (1 ml), and the injection volume was adjusted appropriately to the animals’ weight, so that any change in body weight would be compensated by adjusting the dose. Control rats (n=7) were treated with isotonic saline under the same schedule as rats in the D-amphetamine group.

A separate group of rats was used as control for acute studies of the drug (n=10).

2.3.1 Microdialysis

To allow the rats to acclimate to the test environment and to ensure adequate equilibration of the dialysis probes, rats were placed in a test chamber 14-16 hr before the start of each experiment, and concentric microdialysis probes (2 mm active membrane length) with silica inlet-outlet lines were unilaterally implanted in the NAc. An artificial cerebrospinal fluid (10mM sodium phosphate, 1.2 mM CaCl2 3.0 mM KCl, 1.0 mM MgCl2, 147 mM NaCl, pH 7.4) was perfused through the probes continuously, from the time of probe implantation until the end of the experiment, at a constant rate of 1 µl/min with a microinfusion pump (Harvard, model 22). Samples were collected every 30 min and analyzed for DA immediately.
Table 2. Protocol indicating injection and withdrawal time for experiment I

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Withdrawal
There were three microdialysis sessions: one during the fifth D-amphetamine injection (5 mg/kg), one during the last D-amphetamine injection (10 mg/kg), and the third beginning 72 hr later. Because the rats were bilaterally implanted with guide cannulae, 7 rats from the drug-treated group and 3 rats from the saline-treated group were used in the first microdialysis section; 7 rats from the drug-treated group and 4 rats from the saline treated group were used in the second microdialysis section (data from one rat of each group were not included in the statistical analysis because of probe mispalacing); and finally all rats from the drug-treated group and the saline treated group were used in the 3rd microdialysis session (data from 4 rats in the drug-treated group were not included in the statistical analysis because of probe mispalacing).

2.4 Methods Experiment II

The purpose of this experiment was to evaluate the response of the dopaminergic system to a natural reward following withdrawal from an escalating dose regimen of D-amphetamine. Again to facilitate microdialysis sampling, the first D-amphetamine injection (1 mg/kg) was administered at 3 pm, and the following doses at the times indicated in table 3.

2.4.1 Training, drug administration and microdialysis

One week after surgery, rats were trained to drink a 4% sucrose solution for a 10-min period in the testing chambers. A removable plastic-mesh screen divided each
chamber into a smaller and a larger compartment. A removable drinking tube was attached to the outer side of the wall of the smaller compartment. The sucrose solution was made available via a drinking spout protruding into the cage through a hole in the wall of the cage. During the training session each rat was confined to the large compartment of the chamber for 10 min. During this period (preparatory phase) rats were prevented from gaining access to the sucrose by the screen. The screen was then removed and drinking was allowed for 20 min (consummatory phase). Rats were food deprived 12 hr before each training session. All the training sessions were performed between 9 am and 10 am. Once rats had reached a constant level of sucrose consumption (training day 3), the behavioural test with concurrent microdialysis sampling was performed. Samples were collected every 5 min and immediately analyzed for DA by HPLC. Data from one rat were not included in the statistical analysis because of lacking of responding during the behavioural test. On the next day, rats were subjected to a 4-day escalating dose schedule of D-amphetamine administration, as described above.
Table 3. Protocol indicating injection and withdrawal time for experiment II

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Amphetamine injections (mg/kg)    Withdrawal

52
Finally, rats were tested for their sucrose consumption with concurrent microdialysis sampling 72 hr after the last D-amphetamine injection. Training and testing occurred under the dark cycle.

2.5 Results Experiment I

In the first microdialysis session (Fig. 2), a two-way repeated measures ANOVA of DA efflux in the NAc indicated a significant Group x Time interaction \[ F_{(18,108)} = 35.547, \ p < 0.001 \]. In drug-naïve rats \( (n=6) \), an acute administration of D-amphetamine \( (5 \text{ mg/kg, i.p.}) \) resulted in a significant simple main effect of Time on DA efflux \[ F_{(9,108)} = 27.591, \ p < 0.001 \]. Post hoc analyses revealed a significant increase above baseline that remained elevated for more than 3 hr (Dunnett's, \( p < 0.05 \)). In rats that were pretreated with the first four doses of the escalating dose schedule \( (1, 2, 3 \text{ and } 4 \text{ mg/kg, i.p.; } n=7) \), there was a significant simple main effect of Time on DA efflux \[ F_{(9,108)} = 40.5, \ p < 0.001 \] following a 5 mg/kg injection of D-amphetamine. Although the D-amphetamine-evoked DA response was significantly increased above baseline (Dunnett’s, \( p < 0.05 \)), this effect was significantly blunted throughout the experiment compared to that in the acute D-amphetamine condition (e.g., maximal increase of +3400% vs. +1000% observed 60 min post injection, respectively; Dunn’s \( p < 0.05 \)).

In the second microdialysis session (Fig. 3), a two-way repeated measured ANOVA of DA efflux in the NAc indicated a significant Group x Time interaction \[ F_{(18,99)} = 33.580, \ p < 0.001 \]. The acute administration of D-amphetamine \( (10\text{mg/kg}) \) to
drug-naïve rats (n=5) again resulted in a significant simple main effect of Time on DA efflux \(F(9,99)=27.906, p<0.001\). Post hoc analyses revealed a significant increase above baseline that remained elevated for more than 3 hr (Fig 3; Dunnett’s, p<0.05). In rats that were pretreated with the first 11 doses of the escalating dose schedule of D-amphetamine (1-10, and 10 mg/kg, i.p.; n=6), there was a significant simple main effect of Time on DA efflux \(F(9,99)=32.308, p<0.001\) following a 10 mg/kg injection of D-amphetamine. Although the D-amphetamine-evoked DA response was significantly increased above baseline (Dunnett’s, p<0.05), as with the lower dose of drug, this effect was significantly blunted throughout the experiment compared to that in the acute D-amphetamine condition (e.g., maximal increase of +3200% vs. +1000% observed 60 min post injection, respectively; Dunn’s p<0.05).

Fig. 4 shows the effect of a 5mg/kg D-amphetamine challenge on drug-withdrawn rats. The average basal concentration of DA in the NAc, uncorrected for probe recovery, was 1.9 nM ± 0.21 per 9 µl sample in the saline treated rats. A two-way repeated measured ANOVA of DA release indicated a significant Group x Time interaction \(F(126,9)=33.814, p<0.001\). The administration of an escalating-dose schedule of D-amphetamine (1-10 mg/kg) did not significantly affect this parameter, because the average concentration of DA in drug-treated rats of 2.5 nM ± 0.23 per 9 µl sample did not differ from values observed in saline-treated rats (Dunn’s p>0.05). In order to study the response of the accumbal dopaminergic system rats were challenged with a 5-mg/kg injection of D-amphetamine (i.p.) 72 hr after the last D-amphetamine or saline treatment. Figure 4 shows that the escalating dose schedule of D-amphetamine (1-10 mg/kg)
induced tolerance to the ability of the drug to increase the efflux of DA in the NAc. D-amphetamine treated rats showed a ~ 900% increase in NAc DA efflux, in contrast to a ~ 2200% increase in DA efflux in saline treated animals (Dunn’s p<0.05).
Fig. 2. Effect of D-amphetamine (5 mg/kg, i.p.) on DA efflux in the NAc of drug-naïve (blue circles, n=5), repeated D-amphetamine treated (red circles, n=7), and repeated saline treated (green circles, n=9) rats. Data are presented as means ± SEM.

* indicates p< 0.05 (Dunnett’s test) vs basal values

† indicates p< 0.05 (Dunn’s test) vs drug-treated group
Fig. 3. Effect of D-amphetamine (10 mg/kg, ip) on DA efflux in the NAc of drug-naïve (blue circles, n=5), repeated D-amphetamine treated (red circles, n=6), and repeated saline treated rats (green circles, n=9). Data are presented as means ± SEM.

* indicates p< 0.05 (Dunnett’s test) vs basal values

† indicates p< 0.05 (Dunn’s test) vs drug-treated group
Fig. 4. Effects of 72 hr withdrawal from an escalating-dose schedule of D-amphetamine (1-10 mg/kg i.p., n=9; red circles), or from a saline treatment (n=7; green circles) on a challenge administration of D-amphetamine (5 mg/kg, i.p.) on DA efflux in the NAc. Data are presented as means ± SEM, and are expressed as a percentage of basal values.

* indicates p< 0.05 (Dunnett’s test) vs basal values
† indicates p< 0.05 (Dunn’s test) vs drug-treated group
Figure 5 shows the percentage changes in dialysate concentrations of DA obtained from the NAc during the preparatory and the consummatory phase of a 4% sucrose solution. Both the anticipation and consumption of a 4% sucrose solution increased the DA efflux in the NAc. A two-way repeated measures ANOVA of DA efflux indicated a significant Group x Time interaction \[F(2,32)=5.73, p<0.001\]. In drug-naïve rats (n=6), the presentation of 4% sucrose resulted in a significant simple main effect of Time on DA efflux \[F(2,60)= 7.866, p<0.001\]. Notably, the maximal increase (+45\%) was observed during the first 5 min of the preparatory phase preceding the sucrose intake, while only a ~25\% increase in DA efflux was observed in the first 5 minutes of sucrose consumption. In contrast, in rats withdrawn from an escalating-dose schedule of D-amphetamine (n=7) the preparatory phase of the behavioural test induced only a 20\% increase of the DA efflux in the NAc, while the DA increase during the consummatory phase was ~ 30\% (Fig. 6). ANOVA revealed a significant simple main effect of Time on DA efflux \[F(2,72)= 2.834, p<0.005\].

This experiment shows a significant attenuation of the NAc DA efflux (45\% before vs. 20\% the D-amphetamine regimen) during the preparatory phase preceding a period of sucrose intake in drug-withdrawn rats (Dunn’s p<0.05).
Fig. 5. Changes in dopamine efflux in the nucleus accumbens in drug-naïve rats (n=6; blue circles) in response to a natural reward (4% sucrose solution).

Data are presented as means ± SEM, and are expressed as a percentage of basal values.

* indicates p< 0.05 (Dunnett’s test) vs basal value
Fig. 6. Effects of 72 hr withdrawal from an escalating-dose schedule of D-amphetamine (1-10 mg/kg i.p., n=7; red circles), in response to a 4% sucrose solution. Data from Fig. 5 are also shown for comparison.

Data are presented as means ± SEM, and are expressed as a percentage of basal values.

* indicates p< 0.05 (Dunnett’s test) vs basal value

† indicates p< 0.05 (Dunn’s test) vs drug-treated group
2.7 Discussion

The results of the present study indicate that the response of the NAc dopaminergic system to rewarding stimuli in rats withdrawn from an escalating-dose schedule of D-amphetamine is significantly blunted compared to control subjects, reflecting the development of tolerance to the drug. The first experiment indicates that the adaptations in the evoked release of DA in the NAc were evident after the 5th injection (5 mg/kg D-amphetamine), and the last injection (10 mg/kg D-amphetamine) of the drug regimen, as the increase in DA efflux was significantly attenuated compared to the acute condition (5 and 10 mg/kg D-amphetamine to drug-naïve rats). Remarkably, this tolerance was maintained for 72 h following withdrawal from the drug.

The second experiment shows that drug-naïve rats display a significant increase in DA efflux during both the preparatory and consummatory phases of sucrose intake. Remarkably, the maximal increase in DA efflux was observed during the first five min of the preparatory phase preceding the sucrose intake (+45% vs. +27% in the consummatory phase), consistent with the hypothesis that anticipatory aspects of feeding behaviours are preferentially associated with changes in DA metabolism and/or release in the NAc (Blackburn et al., 1989). However, the first 5 min of the preparatory phase of a 4% sucrose solution in rats withdrawn from an escalating-dose schedule of D-amphetamine induced only a 20% increase of the DA efflux in the NAc, while the consummatory phase induced an increase in DA efflux similar to drug-naïve rats (+31%). Examination of the DA efflux across the entire 10 min preparatory phase indicates that similar amounts of DA were released during the drug-naïve condition and the drug-treated condition. Therefore, it could be argued that the administration of the escalating-dose regimen of D-
amphetamine produced a change in the temporal profile of the DA efflux in the NAc during the preparatory phase. Interestingly, the magnitude of DA efflux during consumption of sucrose did not significantly differ between groups. These data are in agreement with previous research in our laboratory. Phillips and Barr (1997) reported that rats exposed for three weeks to chronic mild stress (CMS) exhibit a significant reduction in appetitive responses for sucrose, as measured by the number of nose pokes into a niche during a 10-min period prior to the insertion in the cage of the spout of a bottle with a 1% sucrose solution. These findings suggest that the motivation to search for a sweet solution may in fact be a more sensitive measure of the effects of CMS than the level of sucrose consumption. A substantial body of data indicate that one of the neural substrates affected by CMS is the mesolimbic DA system (Papp et al., 1991; Willner et al., 1992): alterations in the stimulated release of DA in the NAc have been reported as well as decreased numbers of D2 receptors in the same brain region (Papp et al., 1991). Several experiments have shown that either 6-hydroxydopamine lesions of dopaminergic neurons or systemic doses of dopaminergic antagonists tend to preferentially disrupt those behaviours linked to incentive motivation (Fibiger and Phillips, 1988; Robinson and Berridge, 1993).

Similar results have been obtained in a study on sexual behaviour in the male rat (Barr et al., 1999). The authors showed that withdrawal from an escalating dose schedule of D-amphetamine decreases certain preparatory components of sexual behaviour in sexually experienced male rats (e.g. anticipatory locomotor activity in the 5 min period prior to the presentation of an estrous female), reflecting a decrease in anticipatory search behaviours, but leaves their copulatory behaviors fundamentally unaltered. Furthermore,
post-ejaculatory intervals were significantly longer in D-amphetamine treated rats, demonstrating a reduction in an additional component of motivated sexual behaviour. In vivo microdialysis studies have supported a role for the mesoaccumbens dopamine in sexual motivation, by showing that DA levels in the NAc rise significantly above baseline in sexually experienced male rats when they are exposed to a receptive but not accessible female during a 5-min anticipatory period (Fiorino et al., 1997; Fiorino and Phillips, 1999). Copulatory behaviour has been more closely linked to dopaminergic activity in the medial preoptic area of the hypothalamus (Hull et al., 1995), and this may explain the dissociation between the effects of psychostimulant withdrawal on preparatory and consummatory behaviours.

The escalating-dose schedule of D-amphetamine used in the present study has been previously used to demonstrate that withdrawal from a psychostimulant can generate anhedonia and a consequent decrease in rats' motivation to obtain a rewarding sucrose solution, as measured by decreased breakpoints on a progressive ratio schedule of reinforcement, without affecting their free consumption of the same solution (Barr and Phillips, 1999). The results of the present study are therefore, consistent with those of previous studies, and allow us to refine the definition of anhedonia in drug-withdrawn animals reflected at the neurochemical level as a blunted NAc DA response to both pharmacological and natural rewards.
CHAPTER III Inhibition of successive positive contrast in rats withdrawn from an escalating-dose schedule of D-amphetamine

3.1 Introduction

Negative affective states are a cardinal symptom of mood disorders, including unipolar depression, bipolar illness, and dysphoria resulting from psychostimulant drug withdrawal (Markou et al., 1998). Accordingly, in order to gain a better understanding of the biological correlates of mood disorders, there is an urgent requirement for animal paradigms that provide objective measures of these dysphoric states (Geyer and Markou, 1995; Willner, 1990). One model of anhedonia in rodents that is gaining increased support utilizes withdrawal from repeated exposure to the psychostimulant drugs cocaine and the amphetamines (Barr et al, 2002; Markou and Koob, 1991; Cryan et al., 2003). Prominent amongst the effects of withdrawal from these drugs are numerous reports of reduced responding for brain-stimulation reward (Cassens et al, 1981; Kokkinidis et al, 1980; Leith and Barrett, 1976; Markou and Koob, 1991). Rats also show reduced motivation to obtain natural rewards, including responding for a sweet sucrose solution on a progressive-ratio schedule (Barr and Phillips, 1999), and access to a sexually-receptive conspecific (Barr et al, 1999). In addition to this amotivational state, rats display increased anxiety during postdrug withdrawal, as measured by increased acoustic startle (Barros and Miczek, 1996), open arm exploration on an elevated-plus maze, and defensive burying (Basso et al, 1999).
A recent report from our laboratory of increased successive negative contrast in rats withdrawn from a binge-like regime of D-amphetamine provides particularly compelling evidence of a meaningful and sustained change in affect in this model of anhedonia (Barr and Phillips, 2002). Successive negative contrast occurs when the incentive property of a rewarding stimulus is devalued unexpectedly and is observed in many species including rodents, primates and humans (Flaherty, 1982, 1996; Schnorr and Myers, 1967; Specht and Twining, 1999). Several theoretical explanations have been offered to account for successive negative contrast, including the induction of negative affective states such as frustration and disappointment (Amsel, 1958; Crespi, 1942; Flaherty, 1982, 1996). The fact that animals (shifted to a reward of lesser value than received previously) decrease their running speed as they approach the devalued reward, as well as consume a smaller amount, is also consistent with reduced incentive motivation (Berridge and Robinson, 1998).

A particularly compelling feature of the incentive contrast phenomenon is its bivalent nature. When animals are trained to respond for a reward of a constant value and then unexpectedly receive one of higher incentive value, they often consume greater quantities or run faster in a runway than animals rewarded consistently with the stimulus having greater incentive value (Crespi, 1942; Flaherty, 1996). This elation effect may reflect an elevated mood akin to euphoria consistent with increased incentive motivation. Given the recent finding that withdrawal from an escalating dose regimen of D-amphetamine significantly enhanced both the magnitude and duration of successive negative contrast in rats (Barr and Phillips, 2002), we hypothesize that the negative affective state induced by psychostimulant withdrawal would also disrupt the positive
affect induced by an unexpected gain in incentive value in the successive positive contrast paradigm. The fact that this effect may occur against a background of increased responding, as a consequence of a shift in sucrose concentration from 4% to 32% would provide important evidence that any changes in responding during drug-withdrawal are not simple performance deficits. Instead, significant results would support the hypothesis that interactions may occur between the negative affective state produced by drug-withdrawal and the emotional correlates of positive or negative incentive contrast. As such, these data may provide further support for the use of incentive contrast procedures to examine the neurobiological and pharmacological correlates of mood disorders as suggested by Flaherty (1996).

3.2 Materials and Methods

3.2.1 Subjects

Thirty-two male Long-Evans rats (Charles River, Quebec), weighing 275–300 g on arrival in the laboratory, were housed individually in a temperature-controlled colony (21°C) under reverse light/dark cycle conditions (lights on at 0400 hr). Training and testing occurred during the dark phase. Water was always available ad libitum in the home cage. All procedures were conducted in accordance with the Canadian Council on Animal Care guidelines and were approved by the UBC Animal Care Committee.
3.2.2 Behavioural apparatus

Subjects were trained and tested in four Plexiglas chambers (42×38×38 cm) fitted with wire grid floors. Each test cage was fitted with a lick-activated solenoid valve that provided rats with a drop of sucrose solution each time their tongue contacted the tip of a metal drinking spout, located 4 cm above the chamber floor. The solenoid valve regulated the volume of the drops of sucrose (either 32% or 4% wt/vol) to 0.01 ml. A small light (1.1 W) attached to the roof of the chamber was turned on to designate the start of each training and test session, and was turned off when the session finished; the activation/termination of the valve coincided with light onset/offset. Recording of lick data was computer-controlled, with a sampling frequency of 10 ms (100 Hz).

3.2.3 Drug administration

Escalating doses of the drug D-amphetamine sulfate (Sigma, St. Louis, MO, USA) were administered following the same protocol described in Chapter 2. Subjects were not exposed to the test chambers at any time during administration of the drug. For the first day of injections, the rats generally displayed elevated locomotor activity and exploratory types of behavior, and thereafter exhibited increasing levels of stereotypy. The D-amphetamine was dissolved in isotonic saline (1 ml/kg), and subjects were weighed each morning before the 8 am injection; body weights were also recorded before the five testing session. Control subjects were injected with isotonic saline under the same schedule as rats in the D-amphetamine group.
3.2.4 Training and testing

Ten days after their arrival in the colony, rats were placed on a deprived feeding schedule, which maintained their body weight at approximately 85%-90% free-feeding weight, for the duration of the experiment. Rats were weighed and fed daily (~18 g Rat Diet 5012; PMI Feeds, Delta, British Columbia, Canada) in their home cages. After subjects had attained the desired body weight, they were randomly assigned to two different groups (n=16 per group), which received either 32% sucrose (unshifted group) or 4% sucrose (shifted group). Animals were given access to their respective sucrose solutions for a 5-min period once per day in the testing apparatus. Daily training sessions continued until rats had reached an asymptotic level of consumption of the sucrose solutions (~ training day 10). Subsequently, each of the two groups of animals was subdivided into two further groups (n=8 per group), with respect to the number of licks that they exhibited in the final 5-min training session. One group from each of the 32% and 4% sucrose solution exposed animals was then subjected to the 4-day regimen of D-amphetamine injections described above, while the remaining groups received injections with the vehicle solution. Following the conclusion of the drug/vehicle treatment, all groups were tested for their consumption (measured as the number of licks) of a 32% sucrose solution for 5 additional days, tested once per day. For the two groups of animals trained with the 4% sucrose solution, the presentation of the 32% solution represented an unexpected increment in the rewarding value of the stimulus.
3.2.5 Data analysis

All data were initially tested for statistical significance using ANOVA, and where appropriate, further analyzed using the Dunn’s or Dunnett’s method of multiple comparisons. Statistical analyses were performed using the Systat statistical package.

3.3 Results

Prior to the treatment with D-amphetamine or vehicle and the shift from 4% to 32% sucrose, there was a clear difference in lick-rate in the groups responding for 4% sucrose (825±64; 872±69) as compared to those responding for 32% sucrose (1410±69; 1340±69) (Fig. 7). A two-way repeated measures ANOVA of lick-rates indicated that there was a significant Group x Time interaction \( [F(5,140)=5.271, p<0.001] \). Post hoc analyses revealed that vehicle-treated rats switched from 4% to 32% sucrose exhibited significantly higher lick-rates than those vehicle-treated rats maintained on 32% sucrose. This significant effect persisted throughout all 5 days of post-switch testing. Strikingly, successive positive contrast was completely inhibited in the drug-treated group shifted from 4% to 32% sucrose, for up to 108h following withdrawal from the escalating-dose schedule regimen of D-amphetamine. The post-hoc analyses of data from the two shifted groups confirmed that the D-amphetamine-treated group exhibited significantly lower levels of sucrose consumption during the 5 post withdrawal trials than the vehicle treated group. Furthermore, withdrawal from drug treatment had no effect on lick rates in the group maintained on 32% sucrose.
Fig. 7. Effects of withdrawal from a 4-day regimen of D-amphetamine, or vehicle on successive positive contrast in rats following an unexpected switch from 4% to 32% sucrose. Sucrose consumption was measured as number of licks per 5 min. session. Lick rates are shown from trials conducted 24 and 48 hrs before drug or vehicle treatment and for 12 to 108 hrs following withdrawal from drug administration.

*Significantly different from 32%-32% vehicle group, p<0.05.

**Significantly different from 32%-32% D-amphetamine group, p<0.05.

†Significantly different from 4%-32% D-amphetamine group, p<0.05.
In addition to lick rate, latencies to approach the spout dispensing sucrose solution were measured on every trial (see Table 4). A two-way repeated measures ANOVA of latencies indicated that there was a significant Group x Time interaction \([F(18,168)]=3.418, p<0.001\). Examination of the pre-treatment latencies revealed significantly shorter response latencies in both groups given access to 32% sucrose, relative to 4% sucrose. The other significant finding was a slight increase in latency scores (mean=27.6 s) in the drug-treated group when tested 12 hr following withdrawal. Importantly, drug-withdrawal had no effect on response latencies at the same time point in the unswitched group maintained on 32% sucrose. Furthermore, response latencies in the switched group subjected to drug-withdrawal decreased to control values on all subsequent trials. A comparison of daily body weight prior to and during treatment with either the escalating dose regimen of D-amphetamine or vehicle revealed small but significant reductions in body mass of 5 g \((F_{(14,60)}=3.85, p<0.042)\) and 7 g \((F_{(14,60)}=9.71, p<0.001)\) respectively across the 4-day injection period (see Table 5).
Table 4. Latencies to approach the drinking spout, 24 hr before and 12-108 hr after vehicle or D-amphetamine treatment, expressed in seconds. Data are expressed as means ± SEM.

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>32%-32% Veh (s)</th>
<th>4%-32% Veh (s)</th>
<th>32%-32% Drug (s)</th>
<th>4%-32% Drug (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>-24</td>
<td>5.0 (2.4)</td>
<td>10.8 (2.4) **</td>
<td>6.8 (2.4)</td>
<td>11.7 (2.4)*</td>
</tr>
<tr>
<td>12</td>
<td>5.1 (4.5)</td>
<td>4.8 (4.4)</td>
<td>10.0 (4.5)</td>
<td>27.6 (4.5)* †</td>
</tr>
<tr>
<td>36</td>
<td>3.8 (1.7)</td>
<td>6.5 (1.7)</td>
<td>5.9 (1.7)</td>
<td>8.0 (1.7)</td>
</tr>
<tr>
<td>60</td>
<td>4.1 (0.9)</td>
<td>6.6 (0.9)</td>
<td>5.4 (0.9)</td>
<td>4.5 (0.9)</td>
</tr>
<tr>
<td>84</td>
<td>4.6 (1.0)</td>
<td>5.7 (1.1)</td>
<td>6.2 (1.0)</td>
<td>4.8 (1.0)</td>
</tr>
<tr>
<td>108</td>
<td>4.6 (1.0)</td>
<td>4.7 (1.0)</td>
<td>5.1 (1.0)</td>
<td>4.1 (1.0)</td>
</tr>
</tbody>
</table>

† Significantly different from 4%-32% Veh (p<0.05)

* Significantly different from 32%-32% Drug (p<0.05)

** Significantly different from 32%-32% Veh (p<0.05)
Table 5. Body weights before and after the vehicle or D-amphetamine treatment, expressed in grams. Data are expressed as means ± SEM.

<table>
<thead>
<tr>
<th>Time (days)</th>
<th>Drug (g)</th>
<th>Vehicle (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pretreatment</td>
<td>330 (2.5)</td>
<td>340 (2.6)</td>
</tr>
<tr>
<td>Treatment 1</td>
<td>329 (2.5)</td>
<td>341 (2.8)</td>
</tr>
<tr>
<td>Treatment 2</td>
<td>326 (3.7)*</td>
<td>340 (3.8)</td>
</tr>
<tr>
<td>Treatment 3</td>
<td>327 (3.6)*</td>
<td>344 (3.6)*</td>
</tr>
<tr>
<td>Treatment 4</td>
<td>325 (3.5)*</td>
<td>333 (2.8)*</td>
</tr>
</tbody>
</table>

*Significantly different weight from pre-treatment (p < 0.05)
3.4 Discussion

The present experiment provides a convincing demonstration of the sometimes elusive phenomenon of successive positive contrast in rats, as evidenced by significantly higher lick rates in vehicle-treated rats switched from 4% to 32% sucrose relative to those maintained on 32% sucrose. This effect was robust and still evident 108 h after the switch in sucrose concentration. Furthermore, successive positive contrast is completely inhibited for an extended period during withdrawal from an escalating-dose regimen of D-amphetamine. Several features of these data are noteworthy. Rats in the shifted condition, which were also experiencing drug withdrawal, increased their lick response rate at the 36 hr time point compared to those maintained by rats in the 32% sucrose unshifted group, thereby confirming their capacity for normal consummatory responding. Response latencies, which were significantly longer under the 4% sucrose preshift condition were also reduced following access to 32% sucrose, but did not differ from latencies displayed by rats maintained on 32% sucrose throughout the experiment. Withdrawal from D-amphetamine had no significant effect on sucrose consumption or lick-rate in rats maintained on 32% sucrose throughout the experiment, nor were response latencies affected by drug withdrawal. Although response latencies in the switched group, when measured 12 hr following withdrawal, were marginally longer than those maintained on 32% sucrose during withdrawal, it is highly unlikely that this slight delay in initiation of drinking could account for the nearly 30% difference in lick-rate observed between these two groups. The inhibition of successive positive contrast following D-amphetamine withdrawal parallels the enhancement of successive negative contrast under
a comparable state of drug withdrawal (Barr and Phillips, 2002) and together these data provide further support for the use of a withdrawal from binge-like exposure to psychostimulant drugs as a valid model of the negative affective states that define idiopathic and drug-induced depression.

Associative generalization decrements have been postulated to account for the phenomenon of negative contrast (Capaldi, 1971; Flaherty, 1982; Spear and Spitzner, 1966). This hypothesis proposes that a change in either the rewarding environment or the value of the rewarding stimulus results in a reduced association between the two and a commensurate decrease in consumption of the reward. Furthermore, response generalization may be exacerbated in the novel state of withdrawal from D-amphetamine (Grilly, 1975). However, it is highly unlikely that this proposal can explain the disruption of successive negative contrast following withdrawal from D-amphetamine because in our previous study, animals maintained on 4% sucrose during withdrawal did not reduce their consumption of 4% sucrose (Barr and Phillips, 2002). The same situation pertains to the present study, as rats withdrawn from D-amphetamine and given 32% sucrose throughout the experiment also maintained responding at pre-drug levels. The same data also make it unlikely that amphetamine-induced anorexia can explain the absence of successive positive contrast observed in the present study. Previous studies have reported reduced motor activity following amphetamine withdrawal (Paulson et al, 1991; Pulvirenti and Koob, 1993) but again, this cannot explain the present findings, as psychomotor deficits should have affected sucrose consumption in the unshifted group subjected to drug withdrawal.
Many studies have employed treatment with a wide variety of drugs in an attempt to identify the neurochemical correlates of incentive contrast (see Flaherty, 1991), but acute treatment with amphetamine was ineffective in reducing negative contrast. To the best of our knowledge, the effects of either acute or chronic treatment with amphetamine on successive positive contrast have not been studied. Benzodiazepines have been shown to be a particularly effective class of drugs in disrupting successive negative contrast (Flaherty et al., 1986), suggesting an important role for GABA-containing neurons in the inhibition of other neural systems subserving incentive contrast. The role of brain dopamine systems is less clear as treatment with chlorpromazine or the more specific dopamine receptor antagonist haloperidol both failed to disrupt successive negative contrast (Flaherty et al., 1992). Using a different procedure to demonstrate both positive and negative contrast with brain-stimulation reward, Phillips and Lepiane (1986) have observed a selective effect of the dopamine receptor antagonist pimozide on positive but not negative contrast, suggesting that dopamine may be particularly important in mediating positive incentive contrast.

As reported in Chapter 2, employing the same escalating dose regimen of D-amphetamine, we observed tolerance to the drug-induced increase in DA efflux in the NAc, measured by brain microdialysis and HPLC with electrochemical detection (Vacca, et al., 2004). This tolerance was maintained for 72hr following withdrawal. In the second experiment presented in Chapter 2, we showed that drug-naïve rats displayed a significant increase in DA efflux during both the preparatory and consummatory phases of sucrose intake, whereas drug-withdrawn rats failed to show increased DA efflux in the preparatory phase but did show the previously observed increase in DA efflux in the NAc.
during consumption of sucrose. Furthermore, DA efflux in the NAc is also attenuated during successive negative contrast (Genn et al., 2004). Collectively, these findings suggest that the failure to observe successive positive contrast in the present study following withdrawal from D-amphetamine may be directly related to dysfunction of the mesolimbic dopamine pathway.

Currently there is a great deal of interest in the neurobehavioral economics of drug addiction (Bickelet al., 1995; Heyman, 2003; Rachlin, 2003), from both the perspective of behavioral economics and the effects of prolonged exposure to psychoactive drugs on assessment of reward value and choice, essential aspects of decision making. The present data provide clear evidence that withdrawal from a binge-like episode of D-amphetamine treatment, which has much in common with the anhedonia experienced by humans in a psychostimulant–withdrawal state, has a profound effect on the relative value of natural rewards. It will be important in future studies to examine the effects of acute treatment with psychostimulants on successive positive contrast to see if they may further distort the relative value of an unexpected gain in reward, thereby biasing choice towards short-term gain represented by stimuli with high incentive value.
CHAPTER IV GENERAL DISCUSSION

Despite decades as a “hidden illness”, depression has emerged as one of the most significant public health problems in today’s society. Although animal models of human disease have proven to be of considerable value in elucidating basic pathophysiological mechanisms and in developing novel and more effective treatments, the goal of modeling human mental disorders such as depression appears particularly challenging because of the phenotypic heterogeneity of symptoms that characterizes this disorder. Moreover, depression is associated with multiple primary pathologies such as Parkinson’s disease and Huntington’s disease (Matthews et al., 2005).

Given these observations, how would one then begin to develop a valid animal model of such a complex human disease? Harro (2004) has suggested that “it may be overstretching to develop an animal model with the underlying principle that the more symptoms present, the better”, and indeed, the aim of several animal models is to mimic only one symptom of a human disease. While this approach may be seen as a limitation, it should be noted that not all symptoms of depression need to be present in order to make a diagnosis of depression, and it has been suggested that for the advancement of valid animal models of depression “animal studies must focus on modeling very specific clinical features, mindful of any controversy around the presence or significance of the phenomenon under scrutiny” (Matthews et al., 2005).

An animal model in rodents that follows these principles and is gaining increased support is the subject of the present thesis and employs withdrawal from repeated exposure to psychostimulant drugs, such as amphetamines, to mimic anhedonia, a core
symptom of human depression, which is defined as reduced hedonic reaction to pleasurable rewards. This model takes advantage of the fact that, in humans, withdrawal from psychostimulant drugs induces a condition that bears remarkable similarity to the symptoms of major depressive disorder. It should be noted that the purpose of administering such a schedule of psychostimulant drugs is not to mimic the phenomenon of drug abuse per se, but rather to induce behavioural deficits that may have relevance to both drug-induced and non-drug induced depression.

Anhedonia is believed to reflect a decrease in the sensitivity/activity of the brain reward system (Wise, 1989; Willner, 1997). To date, several animal studies have reported signs of anhedonia during withdrawal following exposure to sustained psychostimulant administration (Markou and Koob, 1991; Barr and Phillips, 1999; Barr et al, 2002; Cryan et al., 2003). Barr and Phillips (1999) showed that withdrawal from an escalating-dose schedule of D-amphetamine, similar to that used in this thesis, induces decreased break points under a progressive ratio (PR) schedule for a sucrose solution. Under this schedule, rats increase their operant responding for a fixed reward until they reach a break point that reflects the maximal amount of effort an animal will expend to receive a reward. Thus, the break point provides an objective measure of the animal’s motivation. These data were confirmed by Orsini and coworkers (2001). However, recently, Feldon’s group (Russig et al., 2003) failed to observe any significant difference between D-amphetamine and saline treated rats using the PR responding for sucrose reward. This discrepancy is probably due to differences between the two protocols, since the doses that these authors used on the last injection day were half of those used by Barr and colleagues.
Amphetamine withdrawal has also been associated with elevations in brain reward thresholds in rats (Geyer and Markou, 1995), as measured by ICSS. ICSS leads to neurochemical changes in brain areas, such as the NAc, in which reward processes are hypothesized to occur (Wise, 1996). This well-validated technique allows investigators to quantify the amount or frequency of current required to maintain responding by animals, providing a measure of the sensitivity of the reward system. Generally, psychostimulants reduce the current necessary to maintain threshold of responding. However, during psychostimulant withdrawal, animals require higher intensity electrical brain stimulation to maintain responding, indicating deficits in reward function.

On the first day of D-amphetamine injections, rats generally displayed elevated locomotor activity and exploratory types of behaviour, and thereafter exhibited increasing levels of stereotypy. In humans, psychostimulant drugs such as cocaine and amphetamines produce mood elation and increased motor activity, which are considered to be characteristic of mania. Given that one of the most validated animal model of mania utilizes amphetamine or other psychostimulants to affect locomotor activity in animals (Machado-Vieira et al., 2004), it may be possible to conjecture that during the first day of D-amphetamine administration, we might be able to model the manic phase of bipolar disorder, whereas during withdrawal from the same treatment we can model the depressive state. However, this hypothesis still needs to be tested. At present, models of bipolar disorders can replicate specific aspects of mania or depression, however, their main limitation is not being able to account for changes between these two mood states, which are the hallmarks of MDD. The protocol presented in this thesis clearly mimics the
depressive phase of bipolar disorder and may indeed be extended as a model of bipolar illness.

Given the consistency and large amount of behavioural data that exist on the psychostimulant withdrawal model of anhedonia, a primary aim of this thesis was to further investigate the relationship between psychostimulant withdrawal and anhedonia in the rat using behavioural paradigms and integrating this data with neurochemical studies. The first part of our study investigated the effect of D-amphetamine withdrawal on stimulus-induced changes in extracellular DA levels in the NAc following an escalating-dose schedule of D-amphetamine. Results show that the response of the NAc dopaminergic system to rewarding stimuli, such as a pharmacological reward or a natural reward, in rats withdrawn from an escalating-dose schedule of D-amphetamine, was significantly blunted compared to saline treated rats, reflecting the development of tolerance to the reinforcing properties of the drug. Interestingly, the tolerance to D-amphetamine was already present when the rats were injected with the fifth dose of the escalating-dose regimen (5 mg/kg, i.p.), and was then maintained through the administration of the subsequent doses, as demonstrated by the significantly attenuated increase in DA efflux measured after the administration of the last D-amphetamine dose (10 mg/kg, i.p.), as compared with the control rats. Several studies examining the neurochemical alterations associated with cocaine withdrawal, using in vivo techniques, have shown reduced extracellular levels of DA in limbic nuclei, such as the NAc, during cocaine withdrawal (Weiss et al., 1992). However, in an earlier study, equivocal findings in this region were reported for amphetamine withdrawal (Paulson and Robinson, 1996). The experiments presented in this thesis show clear and unambiguous evidence of
neuroadaptations within the mesolimbic DA system following treatment with escalating-doses of D-amphetamine, adding an important dimension to the large body of evidence supporting the psychostimulant withdrawal as a model of depression. Similar results have been reported by Di Chiara and colleagues (1999). Using the chronic mild stress model of depression, these authors have shown blunted mesolimbic DA responsiveness to appetitive rewarding stimuli, as measured by microdialysis. These findings suggest that the effects of D-amphetamine withdrawal mimic those seen in alternate animal models of depression, and therefore provide additional support for the use of psychostimulant withdrawal as a rodent model of depression.

Given the evidence indicating that depression is related to deficits in the mesolimbic DA system (Fibiger, 1995; Drevets et al., 2001), these findings suggest a high degree of etiological validity between the physiology of the psychostimulant model of depression and the human disorder. It is also worth mentioning that during cocaine withdrawal changes in pre- and postsynaptic 5-HT mediated activity (Baumann and Rothman, 1998), and decreased 5-HT mediated neurotransmission in the NAc (Parsons et al., 1995) have also been observed, whereas to date few studies have examined the role of NA during psychostimulant withdrawal (Paulson et al., 1991).

In the experiments presented in this thesis, D-amphetamine was administered in escalating doses (starting from 1 mg/kg to 10 mg/kg), in order to minimize the chance of toxicity associated with repeated high D-amphetamine doses. A protocol of this kind allows rats to receive a limited number of high doses of D-amphetamine without the risk of inducing overdose and the death of the animal. However, numerous studies indicate that when amphetamines are administered continuously or in high doses, they may exert
neurotoxicity, causing long-lasting toxic effects on dopaminergic and serotonergic neurons in the central nervous system. For example, following administration of D-amphetamine continuously for three days via osmotic minipumps, Ryan and colleagues (1990) found evidence for neurotoxicity at doses between 20 and 60 mg/kg/day, with dopaminergic structural (neuronal cell body) and axonal degenerations, particularly in the striatum. No significant neurotoxicity was observed at lower doses. In our 4-day protocol, rats were injected for the first two days with presumably non-toxic doses (6 mg/kg/day 1; 15 mg/kg/day 2). However, on the third and fourth day of D-amphetamine administration, rats received cumulative doses of 24 mg/kg and 30 mg/kg on the respective days, which fall in the range of neurotoxicity proposed by Ryan and coworkers (1990). Therefore, according to previous studies it appears that on the last two days of drug administration rats may have been subjected to neurotoxic doses of D-amphetamine. While, to date there have been no experiments designed to find out if that is the case, it can be argued that a) the potentially neurotoxic doses were administered for only two days, and they never reached the high concentration reported by Ryan and colleagues (60 mg/kg/day), b) that in the experiment of Ryan and colleagues the doses were not escalating, therefore they did not allow for the development of tolerance, c) D-amphetamine was administered continuously and not at 8 hr intervals.

Another issue that deserves attention is the possibility that the protocol of D-amphetamine injections used in this thesis may induce sensitization in the weeks following the end of the drug administration. This possibility cannot be excluded, and future studies will address this issue, although it should be noted that protocols that aim to induce sensitization in animals involve intermittent administration of the drug (see for
an example Weinstein et al., 1997), and that an incubation period (when no drug is administered) is crucial for the development of sensitization.

The studies in the present investigation also show that DA efflux in the NAc is attenuated during the preparatory phase of feeding behaviour only in rats withdrawn from an escalating-dose schedule of D-amphetamine, whereas in the consummatory phase we observed a similar increase in the DA efflux in the NAc in both drug-withdrawn and drug-naïve rats. These are very significant results because they confirm previous work from our laboratory. Barr and colleagues (1999) reported that, following exposure to an escalating-dose schedule of D-amphetamine, rats exhibit decrements in anticipatory and motivational measures for sexual reinforcement. In these animals copulatory behaviour was fundamentally unaltered by the drug treatment, however, during the anticipatory phase there was a decrease of certain preparatory behaviours, such as reduced locomotor activity in the 5 min period prior to the presentation of an estrous female. Moreover, longer post-ejaculatory intervals were also noted. These results reflect a decrease in anticipatory search for rewarding stimuli. Using the chronic mild stress (CMS), an animal model of depression discussed earlier, Phillips and Barr (1997) reported that rats exposed for three weeks to the CMS exhibit a significant reduction in appetitive responses for sucrose, as measured by the number of nose pokes into a niche during a 10 min period prior to the insertion in the cage of a bottle containing a 1% sucrose solution, compared to control animals. Taken together, these results suggest that the motivation to search for a reward, such as a sweet solution, or a sexual partner, may be a more sensitive measure of the decreased motivation and anhedonia in animal models than the actual consumption of the reward. These observations allow us to refine the
definition of anhedonia, from the traditional concept of decreased interest in normally rewarding stimuli, to decreased motivation to seek, or to work for, natural rewards.

In a recent review of the role of the brain reward system in depression, Naranjo and colleagues (2001) expanding on an earlier proposal by Blackburn and colleagues (1992) argue that the mesolimbic DA pathway, rather than mediating reward (i.e. pleasure), serves to induce approach behaviours for consumption, positive reinforcement, and learning, all of which lead the organism to adapt to the environment. The authors hypothesize that DA neurons may be detectors and encoders of positive characteristics of events, such as the positive value of an object, behavioural act, or internal physical state. This hypothesis is corroborated by electrophysiological studies showing that DA neurons discharge in response to conditioned stimuli predictive of food reward to a greater extent than when animals actually eat the meal (Schultz, 1997; 1998). Furthermore, Garris and colleagues (1999) showed that during experimenter-delivered stimulation to the VTA, but not during ICSS, the DA signal was always detected, suggesting that NAc DA is involved in the novelty rather than the pleasure itself. Taken together, these studies suggest that the dopaminergic neurons of the mesolimbic system respond to stimuli that have alerting (i.e. predictive) and arousing properties (Blackburn et al., 1992; Naranjo et al., 2001). The present thesis shows increases in the DA efflux in the NAc during both the preparatory and consummatory phases of feeding behaviour, in drug-naïve rats, as expected (Phillips et al., 1999; Wilson et al., 1995). However, in drug-withdrawn rats, although we measured an increase of DA efflux in the NAc in both the phases, the increase in the preparatory phase was significantly lower than in the drug-naïve rats. These data suggest
that the escalating-dose schedule of D-amphetamine is able to induce changes in the activity of the mesolimbic system, affecting DA responsiveness to rewarding stimuli.

Chapter 3 provides a clear demonstration that rats treated with an escalating-dose regimen of D-amphetamine failed to display successive positive contrast when shifted from 4% to 32% sucrose, whereas rats in a vehicle treated control condition displayed successive positive contrast by responding at a significantly higher lick rate in a 5 min testing session. The inhibition of successive positive contrast following D-amphetamine withdrawal parallels the enhancement of successive negative contrast observed in rats withdrawn from the same drug treatment (Barr and Phillips, 2002). Genn and colleagues (2004) have recently shown that DA efflux in the NAc was significantly attenuated when rats unexpectedly experience a reward of a lesser value in a negative contrast paradigm. In future studies, it would be interesting to assess changes in DA efflux in rats experiencing negative and positive contrast paradigms during withdrawal from an escalating-dose schedule of D-amphetamine. Results of the present thesis are consistent with many previous reports that withdrawal from a binge-like regimen of D-amphetamine disrupts responding for natural reward stimuli, and indicate that psychostimulant withdrawal can induce an anhedonic state that is a robust phenomenon detectable across a variety of behavioural tasks. This consistency in the data suggests that this experimental design and method of delivering the psychostimulants produces a robust and reliable reward deficit. In addition, incentive contrast appears to be a particularly sensitive measure of these changes in motivation and emotion and our data provide further support for the use of psychostimulant withdrawal as a model of the negative affective states that define both idiopathic and drug-induced depression.
As Flaherty noted in the epilogue of his important synthesis of research on the effects of reward magnitude on animal behavior entitled *Incentive Relativity*, successive negative contrast "provides a model for the characterization of the neurobiology and psychopharmacology of disappointment (Flaherty, 1996, p173). To this, we would add that both successive negative and positive contrast may provide measures of affect that are specifically disturbed in mood disorders such as anxiety and depression and as such have much to offer in studying basic processes related to these disorders. Later in the epilogue, he also notes that anticipatory contrast may enhance our understanding of drug addiction (Flaherty, 1996). The present data support this conjecture and further emphasize the relevance of incentive contrast to many of the most current topics in behavioral neuroscience and psychopharmacology.
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