THE DEVELOPMENT OF AN ANIMAL MODEL OF DEPRESSION: A FOCUS ON ANHEDONIA

by

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Abstract

Depression is one of the more frequent psychiatric disorders, that continues to exact a tremendous cost to society, in both human and financial terms. Novel therapies for the treatment of this disorder will originate from preclinical research, which is based upon the use of animal models. In the current dissertation, we describe the development of two animal models of depression. The first three experiments describe in detail a series of studies that were conducted to provide further validation of the Chronic Mild Stress (CMS) model of depression. Despite initially promising results in Experiment 1, in which CMS-treated rats exhibited significant decreases in appetitive investigations for a sucrose solution, the finding of Experiments 2 signified that there were fundamental flaws in this model, as CMS-treated rats failed to display reduced motivation to obtain a similar solution under a progressive ratio schedule of reinforcement. In Experiment 3, *in vivo* microdialysis in the ventral striatum was used to demonstrate that CMS-treated rats failed to exhibit the hypodopaminergia that is hypothesized to lead to depressogenic behaviours in these animals. The results of Experiments 2 and 3 rendered the CMS paradigm unsuitable for further research and questioned its validity as a legitimate model of depression. In Experiments 4-7, we describe the extensive research that we undertook to validate an alternate rodent model of depression, namely the Psychostimulant Withdrawal model. The results of Experiments 4 indicated a reduced motivation of rats to obtain a sucrose solution under a progressive ratio schedule, while Experiment 5 indicated that this model induced sexual deficits in rats comparable to those seen in unipolar depression; Experiment 6 demonstrated protracted negative contrast effects in

drug withdrawn animals, hence suggesting this paradigm is amenable to modeling subtle psychological processes. In Experiment 7, we demonstrate that the model responds to an appropriate therapeutic strategy as rats exhibited an earlier recovery of rewarding brain self-stimulation following repeated electroconvulsive shock. The dissertation commences with a general review of depression and animal models of this disorder; it concludes with an evaluation of the Psychostimulant Withdrawal model of depression, integrated with the findings of the current dissertation.

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Acknowledgements

The better parts of this thesis are dedicated to the memory of my father, Douglas Maxwell Barr:

"By Jove, I am not covetous for gold,
Nor care I who doth feed upon my cost;
It yearns me not if men my garments wear;
Such outward things dwell not in my desires:
But if it be a sin to covet honour,
I am the most offending soul alive".

Henry V, Act 4, Scene 3.

Introduction:

As new cures for old diseases are constantly sought, the generation of novel and improved therapies for the treatment of human disorders is a process that is fundamentally based upon the use of animal models. These models have a variety of different functions, sometimes being used to test drugs with putative therapeutic qualities, and other times they are used to provide insight into the disease itself. The modeling of psychiatric disorders poses a particular challenge, given the disparate development of the brain between humans and lower mammals, such as rats and mice. Consequently, especial care and attention must be given to the propagation of such models. With a disorder such as depression, in which some of the most "human" of experiences are affected, there must be an attempt to span both biological and psychological boundaries. It is not an easy task.

The purpose of this dissertation has been the development and validation of an animal model of depression. The dissertation begins with a review of Major Depressive Disorder (MDD), encompassing its prevalence and possible causes. After the importance of this disorder has been described, there is a brief summary of the "rules" by which animal models of psychiatric should be developed. Following this is a review of some of the strengths and weaknesses of the more popular animal models of depression that are currently in use. At this point, the dissertation then describes in detail the experiments that were conducted to validate the psychostimulant withdrawal model of depression.

Economic Cost of Depression:

MDD is one of the leading causes of worldwide disease burden. According to several recent studies of premature mortality and worldwide disability, MDD currently ranks in the top five disease burdens (Murray and Lopez 1997, Holden 2000, Hyman 2000). The World Health Organization (WHO) predicts that MDD will be second only to heart disease by 2020 in terms of economic burden, as mortality from infectious disease declines, and will be the leading cause of worldwide disability in terms of the number of people affected (Holden 2000). The economic impact of MDD is particularly pervasive, due to the chronic nature of the illness and the relatively low rate of mortality when compared to other major diseases, such as cardiovascular disease and cancer. Unlike these other two diseases, MDD afflicts people mostly in the age group 25-44, which often coincides with the most highly productive years of employment (Lane and McDonald 1994). For women, who are diagnosed with MDD approximately three times more often than men, these years represent important childbearing years, suggesting future generational costs to society.

MDD is associated with greater morbidity from almost all physical illness, including respiratory problems and cardiovascular disease (Sims 1988). One study showed that medical inpatients with clinically significant depression had more procedures performed, stayed in hospital for 40% longer, and incurred 35% greater hospital costs (Levenson et al. 1990). The majority of the economic burden of depression, however, arises from lost human productivity. The true economic impact of MDD has only become apparent more recently, as emphasis has shifted away from mortality as being the traditional measure of disease burden (Hyman 2000). The WHO, amongst others, has pioneered the use of a measure called the Disability Adjusted

Life Year (DALY), which corresponds to healthy years of life lost to disability and premature mortality (Murray and Lopez 1996). Using this measure, MDD ranks second only to cardiovascular disease in terms of lost DALYs, and far ahead of other psychiatric and neurological conditions such as schizophrenia, Alzheimer's disease and alcohol abuse (Hyman 2000). Actual dollar estimates of the financial burden of MDD are difficult to obtain, due to the many "hidden" costs of the disorder. Greenberg et al. (1993) reported that the direct costs for the USA resulting from MDD in 1990, including hospital and primary care costs, were US$12.4 billion per year. The indirect costs in the same year, which reflect lost earnings, were at least US$43.7 billion per year. These numbers also fail to account for other indirect costs of MDD, such as higher rates of substance abuse amongst clinically depressed individuals (Markou et al. 1998) that may incur societal costs of many additional billions of dollars. Similar proportional costs have been observed in other countries, such as Australia, where MDD was found to be the top-ranking cause of non-fatal disease burden (Mathers et al. 2000). In Britain, an estimated 80 million working days per year are lost through mental illness (Creed 1993), of which the majority result from MDD. The relative economic impact of MDD may be even higher in some countries, such as in areas of intense conflict; for example, extremely high rates of depression were reported in Lebanon during the early 1990s (Karam et al. 1998). The staggering economic worldwide burden of MDD underscores the importance of understanding this disorder, and generating effective pharmacological compounds for its treatment.

Epidemiology of Depression

MDD is probably the most common of the psychiatric disorders (Fava and Kendler 2000). Estimates for lifetime prevalence of the disorder, based upon large, community-based surveys, have yielded figures in the range of 15-17% for the United States (Kessler et al 1994). These figures appear to be consistent for most western countries, including Canada (Bland et al. 1988), New Zealand and Australia (Joyce et al. 1990; Mathers et al. 2000) and Western Europe. Slightly lower figures have been obtained for certain Asian countries, such as Taiwan (Hwu et al. 1989) and South Korea (Lee et al. 1987), although it has been suggested that this may reflect cultural idiosyncrasies in the expression of MDD or the use of different screening devices (Holden 2000). A large, worldwide study conducted by the WHO plans to complete a multinational survey of over 150,000 people in North America, Western Europe, Mexico, Chile, Cuba, Columbia, Ukraine, South Africa, India, China, Japan, Indonesia and New Zealand by mid-2001 (Holden 2000). As the survey seeks to conduct interviews in a local, culturally valid manner, it is hoped that accurate statistics will be obtained for worldwide prevalence of the disorder.

The annual prevalence of major depression in North America remains higher than for any other psychiatric disorder, and the National Comorbidity Study noted that nearly 5% of the population reported meeting criteria for MDD in the previous 30 days (Blazer et al. 1998). Of those who are diagnosed with MDD, approximately 15% will eventually commit suicide (APA 1994). MDD is also a chronic, relapsing disorder and it is believed that almost 80% of people who are diagnosed with depression will experience a second or further episode (Mueller and Leon 1996). In addition, evidence from several sources suggests that the average age of onset of

MDD has decreased, and the overall risk of suffering from a depressive disorder has increased, over each of the past several generations (Klerman and Weissman 1989; Cross-National Collaborative Group 1992).

The epidemiological risk factors associated with depression are extensive (Fava and Kendler 2000). Clearly, one of the most important factors is the gender of the individual; in the National Comorbidity Survey, the lifetime prevalence rate of depression was estimated to be 21.3% in women, and only 12.7% in men (Blazer et al. 1994). These figures are supported by several other recent North American studies (Regier et al. 1993; Kessler et al. 1994), as well those in other western countries (Piccinelli and Wilkinson 2000). While there are undoubtedly artifactual determinants that may increase the likelihood of gender differences in the measurement of the prevalence of MDD, there is a general consensus that the overall trend of these differences is genuine (Piccinelli and Wilkinson 2000).

Aside from gender, there are three other epidemiological risk factors that stand out in the consistency of their association with MDD (Kessler 1997). The first of these, which consists of exposure to stressful life events, is associated with a substantial increase in risk for the onset of MDD. Current data indicate that the nature of the relationship between stress and depression is largely causal (Kendler et al. 1999), as opposed to other interpretations such as simply an increased perception of stress. The most influential stressors include the loss of personal relationships, marital difficulties, job loss and major health problems (Kessler 1997). The second major risk factor for the development of MDD is the experience of childhood adversity (Fava and Kendler 2000). Early childhood experiences, such as family instability, poor mothering, overcrowding and dependence on social welfare are all important factors in determining future vulnerability to MDD (Sadowski et al. 1999; Fergusson et al. 2000). The

remaining major risk factor for vulnerability to MDD is certain predisposing personality traits. Psychodynamic theorists, such as Aaron Beck, have long postulated that particular personality types are more vulnerable to the effects of environmental stressors, and current research tends to support this claim. The personality construct "neuroticism" has been shown repeatedly to predispose individuals to subsequent episodes of depression (Roberts and Kendler 1999), particularly in women. Several other risk factors have been associated with MDD, such as low levels of social support and urban residence, but the causal association of these factors remains weaker than for the factors described above (Fava and Kendler 2000).

Genetics of Depression

While environmental factors appear to play a prominent role in the etiology of MDD, it is becoming increasingly clear that genetic factors may be equally as important in the development of the disorder. To date, there have been relatively few studies that have been able to provide accurate estimates of the genetic influence on the development of MDD. The use of large, registry-based twin studies has allowed for the comparison of concordance rates of MDD between monozygotic and dizygotic twins. A large study by Kendler et al. (1993) determined that in women, MDD (when diagnosed using DSM-III-R criteria) was moderately heritable, reflected in a heritability estimate of 0.42. However, the authors of the study concluded that the influence of genetic factors may have been underestimated due to the unreliability of lifetime reports of MDD, and that the true estimate of heritability was closer to a value of 0.70. In support of these data, a more recent study of male twins from the Vietnam Era Twin Registry reported that the additive genetic influence on the development of MDD provided a heritability estimate of 0.47 (Lyons et al. 1998), although this was only for early-onset depression.. It is of interest that the same study determined that late-onset MDD had a much lower heritability estimate (0.10), suggesting the possibility of genetically distinct sub-types of major depression.

A recent meta-analysis of twin and adoption studies that included all sub-types of MDD concluded that the heritability of liability to MDD was 33% (Sullivan et al. 2000). This is comparable to the genetic contribution of other important biomedical traits, such as blood pressure and serum cholesterol (Fava and Kendler 2000). The manner of the expression of these genetic factors is likely to be quite complex, though, and unlike that of many other non-psychiatric conditions. For example, it has been hypothesized that genes and environment

interact partially to influence the overall risk of illness, but also influence the sensitivity of individuals to the depressive effects of environmental stressors (Kendler et al. 1995).

It appears that the manner of inheritance of MDD does not fit models of simple Mendelian inheritance. This suggests that a relatively large number of individual genes are likely to be involved, none of which may themselves have a major impact on the risk of developing MDD (Fava and Kendler 2000). Moreover, these genes could interact with each other in complex interactions, such as during particular developmental phases of the individual. Currently, there is a paucity of data regarding the nature of those genes implicated in the etiology of MDD. This is unlike the situation for Bipolar Depression, in which numerous genetic loci, including 4p16, 12q23-q24, 16p13, 21q21 and Xq24-q26 have been indicated as possible regions of linkage (Craddock and Jones 1999). While a recent study provided evidence for a minor genetic contribution to MDD by an allelic variation of the dopamine D3 receptor (Dikeos et al. 1999), most studies have failed to identify regions of genetic linkage to MDD (Balciuniene et al. 1998). Indeed, there has been a surprising inability to provide genetic linkage to genes that would be expected to be involved in the etiology of MDD, such as those involved in the neuroendocrine system (Neiswanger et al. 1998) or the serotonin transporter (Seretti et al. 1999).

Non-Biological Theories of Depression

While the focus of the current dissertation involves the modeling of various biological attributes of MDD, it is recognized that non-biological theories of the etiology of depression have provided invaluable theoretical frameworks for present views of the disorder. As early as the days of Hippocrates (460-357 BC), references were made to the condition of "melancholia", which was described as a state of "aversion to food, despondency, sleeplessness, irritability and restlessness" (Freeman 1994). Later Greco-Roman physicians emphasized the balance of the four "humors", and the Arab scholar Avicenna speculated that black bile, mixed with phlegm, would lead to illness that was "coupled with inertia, lack of movement, and quiet" (Okasha 1999). Galen's temperamental types have often been considered as forerunners of current personality dimensions, which (in certain combinations) can lead to various mood-states (Brink 1979).

Late nineteenth and early twentieth century European psychologists, such as Sigmund Freud and Karl Abraham, revolutionized the field of psychiatry by emphasizing the role of psychodynamic, subconscious forces in the individual's mind. Thus, depression was a result of internalized thanatotic impulses that were incompatible with the person's psyche. This inward-turned aggression and ensuing guilt could often be observed as an increased hostility to others, according to these theorists (Wallace 1976).

More recent non-biological theories of MDD have tended to focus on cognitive and learning factors as etiological bases for the development of the disorder. In particular, Aaron Beck has emphasized the importance of attributional styles, and how they can lead certain individuals to think with a strongly negative bias (Beck 1971). This cognitive bias leads to a

downward spiral of self-reinforcing negativity, and eventually results in a severe mood disturbance (Alloy et al. 1999). Learning theorists, such as Martin Seligman, have preferentially emphasized the role that previous disappointments and failures have on future reward expectancy. The cumulative inability to attain desired goals in certain individuals finally leads to the adoption of a "learned helplessness," whereby action and contingency are no longer associated, and the person simply "gives up trying" (Seligman 1972; Abramson et al. 1978).

Biological Theories of Depression

Monoamine Theory of Depression:

As with many other medical disorders, some of the earliest insights into the biological basis of MDD were provided through serendipitous discovery. The observation that sub-groups of depressed patients who were being treated for tuberculosis with the drug iproniazid showed improvement in mood led researchers to discover that the drug had potent properties as a monoamine oxidase inhibitor (MAOI) (Pletscher 1991). At the same time, it was also noted that approximately 15% of patients who were being treated for hypertension with the drug reserpine (Serpasil), which causes depletion of catecholamines in the brain, went on to develop clinical depression (Muller et al. 1955; Goodwin and Bunney 1971). On the basis of these and other data, Joseph Schildkraut proposed the catecholamine hypothesis of MDD in the mid-1960s (Schildkraut 1965). The essence of this theory is that depression arises from abnormally low levels of catecholamines in the brain, with norepinephrine (NE) being the most important of the biogenic amines (Schatzberg and Schildkraut 1994). Almost 40 years later, a strong body of evidence still exists that alterations in the central noradrenergic system play an important, if not always essential, role in the etiology of MDD (Anand and Charney 2000), although it is now less clear whether central NE is deceased or actually increased in unipolar depression. Levels of 3-methoxy-4-hydroxyphenylglycol (MHPG), one of the major metabolites of NE, can be quantified to provide a measure of central NE turnover. However, urinary drug-free measures of MHPG have provided mixed results, with more consistent findings for bipolar disorder, and equivocal results for MDD (Leonard 1997). Nevertheless, plasma measures of NE and its

metabolites suggest that this monoamine is reduced in the brain of the majority of unipolar depressives (Grossman and Potter 1999; Lambert et al. 2000); it should be noted, though, that a recent study observed increased in CSF levels of NE in patients with melancholic-type depression (Wong et al. 2000). In support of increased central levels of NE in MDD, several studies have found that patients with MDD exhibit an upregulation of the α_2-adrenoceptor autoreceptor, presumably as a homeostatic compensation to reduce NE neuotransmission (Gonzalez et al. 1994). Additionally, others have reported that β-adrenoceptors in unipolar depressives exhibit a decreased adenylate cyclase response to specific NE agonists, which would be consistent with a desensitization arising from increased circulating levels of NE (Potter et al. 1993). Given that many antidepressants act to increase levels of NE, primarily through the NE transporter (Frazer 2000; Kent 2000), it appears that the role of NE in MDD is a complex one, and much remains to be resolved in this field of research.

In contrast to NE, it is apparent that decreased levels of central serotonin, or 5-hydoxytryptamine, (5-HT) are a major factor in the development of MDD. Multiple independent lines of research support this conclusion. Firstly, imaging and post-mortem studies have indicated a reduced density of the 5-HT transporter (5-HTT) in the brain, consistent with lower levels of central 5-HT (Perry et al. 1983; Malison et al. 1998). Reduced levels of 5-HTTs are also observed in the platelets of unmedicated depressives (Owens and Nemeroff 1994), although recent studies have called into question the relevance of these findings to level of central 5-HTTs (Yatham et al. 2000b). Imaging studies have also reported decreased levels of the 5-HT2 receptor in cortical regions in depressives (Yatham et al.1999,2000a), but the relationship of this finding to central levels of 5-HT remains undetermined. Levels of 5-hydroxyindoleacetic acid (5-HIAA), the major metabolite of serotonin, are lower in CSF measurements of medication-free

unipolar depressives (Csernansky and Sheline 1993), and suggest that central levels of 5HT are lower in MDD. Lower concentrations of 5-HT and 5-HIAA have been reported in postmortem brain tissue of depressive who committed suicide, in agreement with studies that have noted decreased levels of the 5-HT precursor tryptophan in the plasma of depressed patients (Anderson et al. 1990). Hence, tryptophan depletion studies in humans have demonstrated that sub-groups of depressives can be induced to relapse into depression when subjected to acute tryptophan depletion (Leyton et al. 2000), emphasising the importance of physiologically normal levels of 5-HT for stable mood. Finally, many of the most efficacious antidepressant treatments in use today work through increasing serotonin levels in the brain, either directly or indirectly (Mann 1999; Kent 2000).

Dopamine (DA), in contrast to NE and 5-HT, has not traditionally been considered an important neurochemical substrate in the etiology of MDD. The reasons for this oversight remain unclear, as ever-increasing data implicate a potentially critical role for DA in unipolar depression (Willner 1995). Given the large volume of evidence obtained from animal studies that emphasises DA's role in reinforcement and reward, as well as the capacity of many direct and indirect DA agonists to increase mood in humans, there is a sound theoretical basis for proposing a major role for DA in the etiology of MDD. Many newer antidepressants, including bupropion and amisulpride, exhibit a pharmacological profile that suggests a primary mode of action through the dopaminergic system. Studies of human depressives have shown conclusively that levels of homovanillic acid (HVA), a metabolite of DA, are decreased in the CSF of these patients (Willner 1983), especially those with psychomotor retardation.

Neuropeptide and Neurotrophin Theories of Depression:

A number of different neuropeptides have generated recent interest as potential factors in the etiology and treatment of depression. While the entire list is exhaustive, and includes, amongst others, β-endorphin, neuropeptide Y, cholecystokinin, arginine vasopressin, galanin and somatostatin (Nestler 1998), certain neuropeptides have received particular attention. Primary amongst these neuromodulators is corticotropin-releasing factor (CRF), which is currently the focus of many novel antidepressant drug strategies. CRF, which is secreted by neurons within the hypothalamus, exerts powerful control over the hypothalamic-pituitary adrenal (HPA) axis (Nemeroff 1998), by controlling the release of pituitary neuropeptides. This axis is disrupted in one third to one half of all depressives, and several recent studies have shown that CSF levels of CRF are elevated in sub-groups of patients with MDD, particularly those with a history of early childhood trauma (Plotsky et al. 1998; Heim et al. 2000). Animal studies have shown that central administration of CRF induces depressive-like symptoms, and recently-developed CRF antagonists display an antidepressant-like profile in various pre-clinical paradigms (Macey et al. 2000).

Alternate neuropeptides that have recently become a popular focus of research include substance P and a variety of different neurotrophic factors. The discovery that substance P antagonists exhibit antidepressant properties (Kramer et al. 1998) has led to a large number of recent studies that have sought to understand how neurokinin receptors might be involved in MDD. The importance ascribed to various neurotrophic factors in the etiology of MDD is derived largely from animal studies, where factors such as BDNF (and TrkB, its receptor) and NT-3 induce regeneration of 5-HT fibers in the brain (Mamounas et al. 1996), and also display

an antidepressant profile in pre-clinical tests (Siuciak 1997). However, there is clearly a need for postmortem studies of the levels of these neurotrophins in the brains of depressed individuals before firm conclusions can be drawn about the role of these factors in the etiology of depression.

Neuroimmunological Theories of Depression:

Despite being a relatively new field of neuroscience, psychoneuroimmunology has already contributed a large body of evidence that implicates the immune system in the etiology of MDD. Numerous findings have converged to suggest that the excessive release of proinflammatory cytokines in both humans and animals can lead to depressive-like behaviour. In humans, the treatment of cancer patients with cytokines such as interleukin-2 (IL-2) and interferon-alpha (IFNα) can lead to depressive-like symptoms (Meyers 1999), while these same cytokines lead to anhedonia in rodent paradigms of reward-related responding (Anisman and Merali 1999). Depressed humans exhibit numerous signs of immune activation and suppression (Dantzer et al. 1999), and there is consistent evidence for a disturbance in the balance of IL-1β and IL-1 receptor antagonist in MDD (Licinio and Wong 1999). It is also of interest that antidepressants, such as clomipramine and sertraline, are able to inhibit the stimulated production of the inflammatory mediator interferon-gamma (IFN-γ), and concurrently increase the production of the anti-inflammatory cytokine IL-10 (Maes et al. 1999).

Alternate Theories of Depression:

Since the discovery of the first effective therapeutic treatments for depression, there have been many theories put forward to explain the biological origin of MDD. A review of all of these theories is certainly beyond the scope of the current dissertation, but it is pertinent to mention a few of them. One of the foremost theories is that alterations in the hypothalamic-pituitary-thyroid axis (HPT) may contribute to unipolar depression in certain individuals, and it is well established that treatment with triiodothyronine (T_3) can increase the efficacy of tricyclic antidepressants (Joffe and Levitt 1993). Similarly, there is some convincing evidence for a disruption of the hypothalamic-pituitary-growth hormone (HPG) axis in MDD, which includes a decreased GH response after a challenge with the adrenergic drug clonidine.

With the advent of neuroimaging techniques, more recent theories have sought to emphasise that MDD may be expressed as a result of imbalances in regional brain function. For example, it has been hypothesised that a disruption in the balance of the two halves of the frontal cortex may lead to changes in mood and psychiatric symptoms (Goldberg and Podell 1995). PET and fMRI studies indicate that unipolar depression is frequently associated with regionally increased glucose metabolism in the orbitofrontal cortex, amygdala and possibly the anterior cingulate, with corresponding decreases in the dorsolateral prefrontal cortex and basal ganglia (Kennedy et al. 1997). Many of the regional brain alterations in metabolic activity are reversed by treatment with conventional antidepressants or electroconvulsive therapy (ECT), which suggests that such changes may be state-dependent.

Molecular theories of MDD have become prominent in the last decade, based largely upon the improvement of various laboratory assay techniques. Instead of focussing upon

specific neurotransmitters, some have hypothesised that the primary biological deficit in MDD may lie at the level of signaling pathways, and that antidepressants exert their heterogeneous effects through the transcription factor cyclic adenosine 3', 5'-monophosphate (cAMP) and cAMP-dependent response-element-binding protein (CREB) (Baker and Greenshaw 1989; Duman et al, 1997). Other groups have applied an interdisciplinary approach and sought to combine recent advances in the understanding of chronobiology with the body of knowledge concerning disruption of circadian rhythms in MDD. To date, this novel field of research has failed to identify specific genes or proteins that are implicated in the etiology of MDD, but there are solid theoretical grounds for further research.

The recent discovery that specific regions of the adult primate brain continue to produce post-mitotic neurons has led to a novel theory of depression. The essence of this theory is that sustained stress may lead to decreased neurogenesis in the hippocampus, with resulting cognitive deficits and HPA-axis abnormalities (Jacobs et al. 2000); serotonergic antidepressants are able to increase levels of hippocampal neurogenesis and therefore help to reverse these effects.

Future theories of the biological basis of MDD are likely to evolve, based upon the large amount of data that are certain to be obtained from genomic and proteomic studies (Flanigan and Leslie 1997). The almost overwhelming quantity of information from these studies will require the use of sophisticated animal models of depression to assist in the interpretation of these data.

Animal Models of Depression

The term "animal model of depression" is frequently a misnomer, for several reasons. The first of these is that many so-called "animal models" of depression are not modeling depression at all, but rather fulfil entirely another function. In addition, if these animal paradigms do attempt to simulate major depression, then they tend only to focus on a specific symptom (and by definition, MDD is recognised as a syndrome that consists of multiple co-presenting symptoms).

The list of "animal models of depression" has grown to include more than 20 experimental paradigms that are currently in use (Willner 1991). The most common use of animal models of depression is in the pharmaceutical industry, where they are used to screen for novel compounds with antidepressant properties. In these cases, the models may or may not be required to mimic depression, but whether they do so or not is often considered irrelevant, as it is only their capacity to discriminate putative antidepressants that is considered pertinent. For example, two of the most frequently used rodent models of depression are the forced swimming test (FST) and the tail suspension test (TST) (Dalvi and Lucki 1999). The FST, also known as the "behavioural despair test" and the "Porsolt test", is probably the most well known and ubiquitous antidepressant screen in use (Lucki 1997). The test consists of placing either rats or mice in a cylindrical container that is filled with lukewarm water to a depth that requires the animal to swim to maintain its head above the surface of the water. The animal is placed in the container for approximately ten minutes and its behaviour is monitored and scored, according to certain criteria (Lucki 1997). Preadministration of the antidepressant compound will typically increase the amount of time that the animal engages in "active behaviors", compared to vehicle-

treated subjects. The FST is a highly effective screen for antidepressants (particularly when combined with an open field test), with over 90% accuracy, yet this paradigm provides us with next to no information about the etiology of MDD in humans. Similarly, the tail suspension test has been used successfully in the detection of numerous antidepressant compounds (Dalvi and Lucki 1999). The TST is simple to administer, and is readily reproducible in numerous laboratories. However, the limitations of this model do not extend beyond a capacity to suggest potential antidepressant properties in a drug. While there is clearly an essential need for these types of pharmacological screens, the use of such paradigms is unlikely to increase our knowledge of the biochemical basis of MDD.

Pharmacological Validity:

It has therefore been suggested that before an animal paradigm can be considered a true "model" of a psychiatric disorder, it should be able to meet certain criteria (McKinney and Bunney 1969; Willner 1986; Geyer and Markou 1995). The most frequently applied criterion to an animal model of MDD is that it be able to detect drugs with the relevant therapeutic potential. Typically, a drug is administered to an animal either acutely or sub-chronically, which should lead to quantifiable, objective behavioural alterations in the animal. This is frequently referred to as "pharmacological validity" or "pharmacological isomorphism" (Matthyse 1986). Ideally, the animal model should be able to detect antidepressant compounds of different chemical classes, such as tricyclics, SSRIs and MAOIs, as well as respond to non-pharmacological therapies for MDD, including ECT, REM sleep deprivation and transcranial magnetic stimulation (TMS). As approximately 30% of severely depressed humans are unresponsive to antidepressant drugs, the

animal model clearly has stringent standards applied to it. In addition, the time course of response to antidepressant therapies should be commensurate with the time course of therapeutic effects that is observed in humans. Thus, animal models in which subjects respond acutely to antidepressant drugs do not closely resemble the human situation, in which therapeutic effects normally take 2-3 weeks to begin (Leon 2000). It has been argued by proponents of acutely-responding models that this may simply reflect differences in metabolism between rats or humans, such as in the rate that neuroactive metabolites are created (Porsolt et al. 1978); alternately, it has also been stated that the dose of drug that rodents receive is almost an order of magnitude greater than humans receive, and that if animals are given lower doses over a longer period, then similar effects are observed (Borsini and Meli 1988). As it is unlikely that experiments with humans will be undertaken to determine the effects of extremely high doses of antidepressant drugs, this issue may remain unresolved for a while.

When applying the criterion of pharmacological validity to a model, it is almost as important that the animal does not respond to certain drugs as that it does respond to others. An efficacious animal model of MDD should respond exclusively to antidepressants, and not to other classes of psychoactive drugs, such as anxiolytics or neuroleptics. This type of validity, often referred to as "discriminant validity" (Campbell and Fiske 1959), assures that the model is specifically one of depression, and not of other psychiatric disorders. Discriminant validity is also essential for models that are used as pharmacological screens, as it prevents the detection of "false-positive" compounds (Willner 1991). However, given that the therapeutic drugs of one class often have therapeutic effects in another disorder, such as the with use of neuroleptics for the treatment of depression (O' Neal et al. 2000) or the use of ECT for schizophrenia (Kupchik et al. 2000), and that a specific symptom (such as anhedonia) may appear in different mental

disorders (Sax et al. 1996), a more relaxed attitude towards discriminant pharmacological validity is sometimes allowed, depending upon the behaviour involved in the animal model and the symptom that is being modeled (Willner 1986).

Face Validity:

A second type of validity that is essential for any animal model of psychiatry is "face validity". This refers to the capacity of the animal model to mimic the human condition in a number of important aspects (Mosier 1947). In a seminal paper, McKinney and Bunney (1969) suggested that an animal model of MDD should model depression in etiology, biochemistry, symptomatology and treatment. In practice, though, it is often the case that the animal model is being used to help to understand the etiology and biochemistry of the disorder that it is modeling. Thus, in more recent years, face validity has been applied more as a measure of the capacity of the model to mimic the symptomatology of the human disorder (Geyer and Markou 1995). Without a phenomenological and behavioural isomorphism between the model and the disorder, there can be little confidence that any biochemical changes that may be observed in the animal model bear any relationship to the human condition. It is evident that there are clearly some aspects of MDD that are uniquely human, and could never be modeled in rodents, such as "feelings of worthlessness" or "excessive and inappropriate guilt". However, the phylogenic development of the human brain has allowed for the conservation of most of the major neuroanatomical regions that are present in rodents, with correlated function (Passingham 1985). The expansion of much of the telencephalon has therefore resulted in differences that are largely quantitative as opposed to qualitative (Kaas 1995). As these more primitive regions of the

"triune" brain exert a powerful modulatory control over higher cortical centers (MacLean 1985), the behavioural repertoire that is displayed by rodents may be used to model many of the behavioural deficits that are exhibited in depression. In theory, many of the behavioural symptoms of depression may be reflections of physiological changes in subcortical regions of the brain, such as the hypothalamus, basal ganglia or hippocampus (Soares and Mann 1997; Plotsky et al. 1998), that are highly conserved in rodents and humans. Thus, a brief perusal of the criteria that are used to assist in the diagnosis of MDD (see below) indicates that the majority of observable symptoms can be modeled in animals.

- depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad or empty) or observation made by others (e.g., appears tearful). (In children and adolescents, this may be characterized as an irritable mood.)
- markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day
- significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day.
- insomnia or hypersomnia nearly every day
- psychomotor agitation or retardation nearly every day
- fatigue or loss of energy nearly every day
- feelings of worthlessness or excessive or inappropriate guilt nearly every day
- diminished ability to think or concentrate, or indecisiveness, nearly every day
- recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

Clearly, depressed mood, feelings of guilt and suicidal ideation are beyond the capacity of an animal model to mimic. In contrast, the remaining symptoms can be (and have been) modeled (Willner 1991). Additionally, there are numerous other behavioural alterations that occur in MDD which are amenable to objective simulation in animals, such as decrements in sexual and grooming behaviour. Thus, for an animal model of MDD to have a high degree of face validity, it should be able to induce at least several of the major behavioural disturbances that accompany MDD.

Construct Validity:

An important remaining criterion for any animal model of psychiatry to fulfil is that of "construct validity". The essence of this criterion is that the animal model be based on a sound theoretical rationale, and that it measures what it purports to measure (Willner 1986; Geyer and Markou 1995). In the case of an animal model of MDD, construct validity is often reflected in how accurately the dependent measure represents a cognitive or affective trait. For example, the forced swim test was originally hypothesised to measure "behavioural despair" in animals; by the mid-to-late1980s, however, it was apparent that this interpretation was no longer valid, as it became evident that alternative explanations, such as shifts in ergonomic response strategies, became more plausible (Thierry et al. 1984; Borsini et al. 1986). Hence, the FST, despite its high degree of pharmacological validity and apparent face validity, failed to meet the criteria for an effective animal model of MDD, largely because of its low level of construct validity. Although construct validity is often a difficult aspect of an animal model to determine, it can be increased with the use of well-established animal protocols. For instance, if the animal model is to be used to measure the effects of a depressogenic manipulation on a behavioural trait such as memory or attention, construct validity can be maximised with the use of standardised tasks that have been subject to rigorous testing in their own field, independently of the field of animal models of psychiatry.

Reliability and Reproducibility:

Even if an animal model of a psychiatric disorder meets the three main criteria for being valid, there are practical considerations of the model that will determine its general usefulness and popularity. The first of these factors is the reliability of the model (Geyer and Markou 1995). The "reliability" of the model refers to the consistency and the stability with which the variable of interest that is being observed can be generated. It is suggested that this consistency should be evident in the ability to measure the variable objectively, so that the same behaviour is being measured consistently over repeated experiments. In addition, the model should exhibit a small within-subject and small between-subject variability, so that the power to detect statistical differences is maximised. The "reproducibility" of the model refers to the capacity of multiple different research groups to reproduce the behavioural phenomena of the model, under essentially identical conditions (Markou et al. 1993). A model of MDD that meets the three validation criteria, yet cannot be reproduced in different laboratories, must have the generalizability of its results brought into question. Would such a model truly represent an animal model of MDD, or is it merely an artifact of a particular strain of animals that are bred by a particular animal supplier? Although such misgivings may appear trivial and unlikely, there has been widespread concern about exactly this kind of issue in several other animal models of psychiatric disorders.

Current animal models of depression

With more than 20 animal models of MDD that are currently in use (Willner 1991), there would appear to be little need for the development of additional models. However, unlike the situation for many other areas of medicine, such as in the study of diabetes or cancer for which there are satisfactory animal models available, there is a lack of substantive models in psychiatry. Given the vastly greater complexity of an organ such as the brain compared to the somatic organs, it is not surprising that animal models of mental disorders are constantly being reviewed and revised, as more is being learned in the fields of neuroscience and psychiatry. Many of the earlier animal models of MDD are no longer in use: paradigms such as muricidal behaviour (Mann and Enna 1982) and exposure to severe physical stressors (Katz 1982) are no longer considered to be valid models of MDD. At present, there are between 5-10 models that are used widely for research into the etiology and neurobiology of major depression. The following is a brief review of the most popular of these models, with a consideration of their relative strengths and weaknesses.

Leaned Helplessness:

The learned helplessness phenomenon was originally described by Seligman and his co-workers in dogs, and extended to other species (Seligman 1972). The basis of this model is that exposure to uncontrollable stress leads to behavioural deficits that are not seen in subjects that are exposed to an identical, controllable stressor. With the use of a "yoked"-design experiment, Seligman showed that dogs that could control the termination of an electric shock maintained the

ability to escape from a subsequent stressor; in contrast, approximately two thirds of yoked animals failed to learn to escape (Overmeier and Seligman 1967). This failure to escape was interpreted as learning by the animal that its earlier attempts to escape were useless, i.e. the animal was "helpless".

While this model has provided reams of valuable data about the effects of stressor controllability on psychological variables, its contribution to the etiology of MDD is less clear. The learned-helplessness model shows a high degree of pharmacological validity, with positive responses to tricyclic antidepressants (Sherman et al. 1982), ECS (Dorworth and Overmeier 1977), MAOIs and atypical antidepressants (Petty and Sherman 1980). In addition, at first glance the model would also appear to display a high degree of face validity, by mimicking the "hopelessness" that is apparent in many depressed humans. However, additional studies have demonstrated that the primary effect of exposure of animals to this paradigm is to generate a high level of anxiety (Willner 1986). There is strong evidence that the learned "helplessness" actually reflects cognitive deficits that arise from very high levels of anxiety, impairing the animal's ability to plan executive behaviours effectively (Geyer and Markou 1995). In agreement with this theory, pre-treatment of animals with the anxiolytic drug lorazepam can prevent the induction of learned helplessness (Sherman et al. 1979). The construct validity of the model rests on three assumptions: (i) animals become helpless after exposure to uncontrollable stress, (ii) a similar state is induced in humans by lack of control over stressors and (iii) this helplessness can lead to depression in humans (Willner 1986). A detailed analysis of each of these assumptions is beyond the scope of the current dissertation, but data suggest that the validity of all are open to question (Willner 1986). Hence, the learned-helplessness model of MDD coalesces into a model with high pharmacological validity, but with uncertain levels of

face and construct validity; furthermore, learned-helplessness is often difficult to demonstrate in rats, suggesting a low level of reproducibility (Freda and Kline 1976).

Flinders Sensitive and Resistant Lines:

As part of an attempt to develop a genetic animal model of depression, the Flinders Sensitive and Resistant Lines (FSL and FRL) were generated. Unlike the development of many other "genetic" models in different areas of psychiatry, such as in schizophrenia (Mohn et al. 1999), the FSL and FRL are a product of selective breeding, rather than genetic manipulation through knockout strategies. The FSL are a strain of outbred Sprague-Dawley rats that were originally bred for sensitivity to the anticholinesterase agent diisopropyl flurophopshate (Russell et al. 1982), compared to their controls, the FRLs. An increased sensitivity to cholinergic drugs is frequently observed in depressed humans (Janowsky et al. 1994), and early studies with these strains demonstrated that the FSL rats displayed numerous behavioural deficits compared to the FRL rats that were indicative of depressive symptomatology (Overstreet 1992).

Measures of pharmacological validity of this model have generally been positive. The results of studies with tricyclic antidepressants have indicated a selectively greater effect of these drugs on FSL rats compared to FRL rats (Schiller et al. 1992). The SSRI sertraline has also been shown to be effective in this model (Overstreet et al. 1995), and discriminant validity has been demonstrated through the absence of effects of other classes of drugs, such as anxiolytics. In addition, the model exhibits pharmacological isomorphism as it requires chronic treatment with antidepressants before effects are observed. At the level of face validity, the FSL rats exhibit numerous behavioural differences from FRL rats. These effects include sleep deficits,

psychomotor retardation and anhedonia (Overstreet 1992), all of which are symptoms of MDD. Also, several physiological differences in FSL rats have been reported that parallel changes noted in MDD, including alterations in the HPA-axis (Owens et al. 1991).

The validity of the FSL model of depression is questionable, though, when its construct validity is assessed. Firstly, the pharmacological validity it displays is only apparent when both strains of animals are tested in the forced swim test, rather than a more ethologically valid test of natural behaviour. As the FST is itself criticised for a lack of construct validity, the use of the FST with these strains suggests a degree of circularity. Additionally, the evaluation of other aspects of face validity are often determined with tasks that are sometimes ambiguous. For example, FSL rats were concluded to be "anhedonic" in a sucrose preference test after exposure to stress (Pucilowski et al. 1993); the reduced sucrose preference, though, could be a result of alternative factors (Forbes et al. 1996), and in more sophisticated tests of reward related function, such as with rewarding electrical brain stimulation, no effects were observed in this model (Matthews et al. 1996). Practical issues are also of importance in this model, as it requires the development of careful breeding programs, and it may be lengthy and expensive to initiate studies with this model.

Neonatal exposure to REM-suppressing drugs:

Developmental factors may represent important variables in the etiology of MDD (Kaufman et al. 2000). Hence, several animal models have been generated that emphasise the role of disruption of early development in rodents. The most successful and foremost amongst these is the exposure of neonatal rats to REM-suppressing drugs, such as clomipramine (Vogel et

al. 1990). In this model, neonatal rats are injected with drugs that suppress REM sleep between days postnatal 8 to postnatal 21. No differences are observed in these animals until adulthood (approximately 4 months of age), when affective differences start to emerge (Hartley et al. 1990). The current list of behaviours that has been altered by neonatal exposure to REM-suppressors includes increased immobility in the FST, changes in REM sleep patterns, decreased aggressiveness, reward-seeking and sexual activity, as well as increased voluntary alcohol consumption (Dwyer and Rosenwasser 1998).

The pharmacological validity of this model remains largely undetermined. Although a wide range of behaviours has been shown to be disrupted by this model, indicating a relatively high degree of face validity, there have been few studies that have attempted to reverse the behavioural sequelae of this model. In one such study, it was demonstrated that the deficits in sexual responding that occurred in male rats after neonatal exposure to clomipramine could be reversed by sub-chronic treatment with imipramine and REM sleep deprivation (Vogel et al. 1990). A substantially greater body of research is required to demonstrate the ability of different classes of antidepressant therapies to reverse behavioural aberrations before the pharmacological validity of this model can be fully determined. The construct validity of the model appears to have several shortfalls too, as the model may be more representative of an animal model of chronic dysthymia than MDD, per se. Unlike MDD, in which periods of depression are interspersed with periods of remission (Simon 2000), animals in this model are chronically and unrelentingly "depressed", which does not accurately simulate the situation with humans. The limitations of this model also include practical considerations, as animals must be raised from birth until at least 4 months of age before depressogenic effects are observable; furthermore, it is

impossible to develop a "within-subjects" experimental design with this model to measure baseline levels of non-depressed behavior.

Olfactory Bulbectomy:

The olfactory bulbectomy (OB) model of depression is currently one of the most widely used of all animal models of depression. By removing the olfactory bulbs of adult rats, a syndrome of behavioural deficits develops approximately 1-3 weeks later. Typically, OB rats exhibit hyperlocomotion in a novel environment, and this effect can be reversed by treatment with chronically administered antidepressant treatment (Leonard and Tuite 1981). The OB model is relatively simple and fast to use, and has been widely reproduced with a high degree of reliability in numerous laboratories throughout the world. The widespread popularity of the model derives in large part from its high degree of pharmacological validity (Kelly et al. 1997). All major classes of antidepressant drugs have been shown to be active in this model, including tricyclics (amitryptyline, imipramine), SSRIs (paroxetine, sertraline, fluvoxamine but not fluoxetine), MAOIs (moclobemide) and atypicals (mianserin, nomifensine and trazodone) (Kelly et al. 1997). Most importantly, reversal of OB-altered behaviour requires chronic, as opposed to acute, administration of antidepressant drug treatment.

The face validity of the OB model has been moderately well established. The majority of studies have focused on hyperlocomotion, which is of questionable relevance to MDD, but several interesting studies have indicated that cognitive processes are also disturbed. Tasks such as maze learning and impulse control are reduced in OB rats, and some of these effects have

been reversed with antidepressant treatment (Redmond et al. 1994). Deficits have also been observed in tasks such as step-down passive avoidance (Joly and Sanger 1986).

The construct validity of the OB model is based upon the assumption that lesioning the olfactory bulbs in adult rats can lead to behavioural deficits that reflect an underlying affective disturbance. There is clearly no human correlate for the etiology of this model, and in this respect the model's validity is low. Proponents of the model claim that lesions are secondary to alterations in limbic activity, particularly within the amygdala. However, many of the behavioural effects of the OB model may be attributable in part to increased anxiety, and axiolytics have potent effects in this model. The potential utility of this model is also seriously curtailed by the nature of the inducing manipulation. The removal of the animal's olfactory bulbs precludes measuring responding for most primary rewards, such as for food and sexual partners, because these are primarily olfactory stimuli to rats. Thus, measures of depression based on "anhedonia" or motivation are impractical.

Animal Models of Depression– Synthesis:

With numerous animal models of depression available to researchers, there are several key issues facing this field of study. It is worth considering that animal models of depression have many different purposes, and in some regards, these purposes have already been met. For instance, there are several highly effective pharmacological screens for the detection of putatively therapeutic drugs, such as the FST, TST and the OB model. A limitation of these tests, though, is that none of them is clearly selective for the effects of rapidly acting antidepressant treatments, which is a major issue in the field of psychiatry.

A second concern in the study of animal models of MDD is the development of a rodent model that simultaneously has both high face validity and high construct validity. For those models that are used to provide insight into the etiology of MDD, it is essential that they have somewhat realistic inducing conditions, and that they produce behavioural phenomena that parallel the symptoms of MDD. In order to maximise the construct validity of the model, the behavioural "symptoms" in the animal model should clearly represent the core trait that they are supposed to. Through the use of well-established animal paradigms, this validity of these traits can be determined.

Finally, the need for a model with high pharmacological, face and construct validity must be balanced by its reproducibility in other locations. The model must be reliable and provide consistent results, regardless of which particular experimental group performs the study. An ideal model will also take into consideration other factors such as duration, cost and ease of use.

Chronic Mild Stress

The link between stress and depression is well-established (Lloyd 1980; Brown and Harris 1988). Less clear is the nature of the link between stress and MDD; for example, do high levels of stress precipitate depression, or do depressed individuals simply have a cognitive and affective bias that makes them recall stressful events more clearly? In addition, what types of stress cause depression, and are there particular symptoms that are brought on by these types of stress?

The chronic mild stress (CMS) animal model of depression has been developed, in part, to determine the answers to these questions. Based upon a series of earlier studies by Katz et al. (1981; 1982), in which rats were exposed to a series of severe stressors (such as intense footshock, cold water immersion and 48hr food/water deprivation) with ensuing behavioural alterations, Willner and his colleagues modified the nature of this protocol (Papp et al. 1991; D'Aquila et al. 1994). Instead of using severe stressors, the paradigm was modified to utilise only mild stressors, and rather than measure the effects of stress on locomotor activity, the dependent variable became the hedonic state of the animals. In a typical experiment, animals are exposed to a variety of mild stressors (e.g. cage tilting, change of cage mate, overnight illumination and intermittent food and/or water deprivation), that change every few hours over a period of weeks or months. The hedonic state of the animals is determined by tracking, over repeated weekly tests, a decrease in the consumption of and/or preference for a weak (1-2%) sucrose solution. Alternate measures of anhedonia have also been assessed through the use of place preference conditioning (Papp et al. 1991) and rewarding electrical brain self-stimulation (Moreau et al. 1992).

The predictive validity of the model is high, with antidepressant drugs from all major classes, including ECT, having therapeutic effects (Willner 1997a). The model also has a high degree of discriminant pharmacological validity, as anxiolyics (chlordiazepoxide), neuroleptics (haloperidol, risperidone) and psychostimulants (amphetamine) all failed to reverse the CMS-induced anhedonia (Willner 1997a). The most desirable aspect of the CMS model, pharmacologically speaking, is that the onset of therapeutic effects closely mimics the profile of effects in humans. Most antidepressant drugs begin to reverse the CMS-induced consummatory deficits within 2-3 weeks, which reflects the typical duration required to observe effects in patients with MDD. The face validity of the CMS model is high too, as a wide range of normally "rewarding" behaviours in rats is attenuated with this model (Willner 1997b), which resembles the "markedly diminished interest or pleasure in all, or almost all, activities" that demarcates one of the two core symptoms of depression (American Psychiatric Association 1994). In addition to decreasing responsiveness to rewards, the CMS model induces the appearance of other symptoms of MDD, including alterations in sexual behaviour and aggression (D'Aquila et al. 1994). Various biological markers of MDD have also been reported in the CMS model, such as advance shifts in diurnal rhythms (Gorka et al. 1996), fractionation of the normal sleep architecture (Cheeta et al. 1996) and hyperresponsiveness of the HPA axis (Ayensu et al. 1995). The construct validity of the CMS model must be considered amongst the highest of all animal models of depression. Extensive research with the CMS model has determined that the CMS-induced anhedonia is reflected in numerous different measures of "reward", most of which generate concordant results. The assertion that reduced consumption of a mildly rewarding sucrose solution is indicative of anhedonia is supported by the following observations:

- Food and water intake remains unaffected in the CMS model (Papp et al. 1991). This suggests that the reduced consumption of a 1% sucrose solution is not part of a general trait of the animal to consume less fluids or food (e.g. as a result of reduced thirst or hunger), but rather a selective reduction in their consumption of mildly rewarding stimuli.
- Similar effects are observed in animals consuming calorie-free saccharin solutions (Willner et al. 1987). Again, these data indicate that CMS animals are not showing an aversion to caloric solutions.
- Effects of CMS are evident in both single-bottle tests and two-bottle (sucrose-water) preference tests (Willner et al. 1987), refuting the possibility that the animals are rejecting fluid consumption in a "taste-aversion" manner.

However, several recent studies suggested that the decrease in consumption of the 1% sucrose solution may have an alternate explanation. When rats are exposed to CMS, they typically exhibit a decrease in weight compared to control subjects (Willner et al. 1997a). Others have hypothesised that this decrease in weight, of a magnitude between 10-20% of control animals, may explain the decreased consumption of the sucrose and saccharin solutions (Matthews et al 1995; Forbes et al. 1996). These groups have shown that consumption of the sucrose solution is linearly related to body weight, suggesting that lighter, i.e. stressed, animals will consume less. This explanation might also account for the alterations in intracranial self-stimulation (ICSS) with rewarding brain stimulation seen in CMS-exposed rats and mice (Forbes et al. 1996). It is not obvious, however, if this hypothesis provides a valid explanation of why CMS-exposed animals reduce their consumption of a 1% sucrose solution without affecting their overall consumption of food and water. Clearly, it would therefore be of interest to determine if the rewarding or motivational properties of the 1% sucrose solution could be shown to be altered in

CMS-exposed rats, independent of their actual consumption of the fluid (and therefore independent of potential confound of reduced body weights).

EXPERIMENT 1:

EFFECTS OF CHRONIC MILD STRESS ON AN APPETITIVELY MOTIVATED TASK

Synopsis:

The Chronic Mild Stress paradigm is an animal model of depression, based upon the exposure of rodents to a series of chronic mild stresses, which results in a decrease of the hedonic capacity of these animals. However, several recent studies have suggested that the CMS model may be confounded by weight loss in stressed animals. We therefore evaluated the effects of exposure of rats to CMS in an appetitively motivated task. Rats were trained to expect the presentation of a 1% sucrose solution after a 10 minute cued anticipatory period. Half of the animals were then exposed to CMS. The number of nose-poke investigations into a niche that subjects made for the sucrose solution during the 10 minute period differed significantly between stressed and non-stressed animals by the second week. In contrast, differences in the consumption of the sucrose solution did not appear until the fourth week of exposure to CMS. These results are discussed with reference to differential effects of stress on appetitive vs consummatory behaviours.

Introduction:

The CMS animal model of depression focuses upon and successfully emulates the core depressive symptom of anhedonia (a reduced interest and/or pleasure in normally rewarding activities) (Willner et al. 1987). By subjecting animals to a prolonged series of mildly uncomfortable and unpredictable stressors, a significant reduction is observed in subjects' consumption of rewarding stimuli, such as a weak sucrose solution or ICSS of natural reward sites in the brain (Papp et al. 1991; Moreau et al. 1992).

While there is a strong body of evidence that supports the hypothesis that CMS leads to a reduced hedonic capacity in rodents, several recent studies have provided evidence which suggests that these "consummatory" changes may be more closely linked to changes in body weight (Matthews et al 1995; Forbes et al. 1996). The capacity of the CMS model to affect an animals' interest in obtaining the sucrose solution, independent of its free consumption of the solution, may provide an opportunity to assess anhedonia without the confound of stress-induced weight loss. The majority of studies to date have investigated the effect of CMS on "consummatory" behaviours. However, it has been well established that "appetitive" or "preparatory" behaviours (i.e. those instrumental behaviours that lead to the availability of the rewarding stimulus) are more susceptible to behavioural disruption by various experimental manipulations. In particular, dopamine (DA) antagonists and 6-OHDA lesions of the mesoaccumbens DA pathway have been shown to disrupt preferentially conditioned or instrumental responses before affecting consummatory behaviours (Fibiger 1993; Salamone 1994).

Given the large body of evidence implicating the role of DA in anhedonia and the CMS procedure (Muscat et al. 1990; Papp et al. 1994), the current experiment was undertaken to determine if an appetitive task in the CMS model of depression would be affected preferentially compared to the standard consummatory measure. Furthermore, it was predicted that CMS-induced changes in an appetitive task could provide an operational measure of anhedonia that would not be confounded by changes in the weights of stressed animals.

Methods:

Subjects

Animals were male Sprague-Dawley rats (2 groups; n=9 per group), weighing 300-350g at the start of the experiment. The animals were housed individually in a temperature and light controlled colony (12 hr light/dark cycle; lights on at 06:30) with food and water available ad libitum, except when specified as otherwise. All procedures were performed under the guidelines of the Canadian Council on Animal Care and the University of British Columbia Committee on Animal Care.

Training

The testing chambers consisted of Plexiglas boxes (26 × 25 × 29 cm) with a hole in one wall (4.5 cm above the floor) through which the spout of a Richter drinking tube could be placed. Inset 2.5 cm from this wall was an opaque, " false" aluminium wall, which in turn had a 7.5 × 9 cm section removed in the center to allow the animal free access to the Richter tube. Another

aluminium panel (10 × 13 cm) was placed over the smaller cut-out section, firmly supported between two parallel guide strips, which could be manually lowered or raised to deny or allow the animal access to the Richter tube. This guillotine door was clearly painted white with a large black "+" sign to make it highly visible to the subject. By using a chamber with this design, it was possible to control access by the rat to the Richter tube, and to observe when the subject poked its head through the " false" aluminium wall to investigate the presence the Richter tube and/or drink from it.

Subjects were given two 60 minute habituation sessions in the test chambers on consecutive days, during which the Richter tube was not presented and the aluminium panel remained lowered. All animals were then given two 1 hour presentations of the Richter tube in their home cages, also on consecutive days, when the tubes were filled with a 1% sucrose solution. Following these habituation sessions to the test chambers and sucrose solution, subjects were given a twelve session training schedule. The animals were placed individually in the test chambers at approximately 16:00 , and deprived of both food and water. On the ensuing day, at approximately 12:00 (always 20 hours after food and water deprivation), the animals were exposed to white noise (≈ 65 dB) and the aluminium panel was raised to provide access to the Richter tube. During the first two training sessions, the Richter tube (filled with a 1% sucrose solution) was presented immediately upon the initiation of the two above cues; the radio was then turned off after 60 seconds, but the aluminium panel remained open for 1 hour, and subjects were able to drink freely during this period. At the end of the session, the panel was lowered and 10 minutes later subjects were returned to their home cages, where they were given free access to both food and water for 48 hrs. Training continued according to this schedule.

For the first two training sessions, Richter tubes were presented at the same time as the auditory cue and the raising of the panel, while for the next two sessions, the tube was presented 2 minutes after cue onset. In these and subsequent training sessions, the white noise was always terminated exactly 60 seconds after presentation of the Richter tube, regardless of the time between the initiation of the cues and the presentation of the sucrose. Two training sessions with a 5 minute interval, two sessions with 8 minute intervals, and four sessions with 10 minute intervals completed the twelve session training schedule. During the last two 10 minute interval sessions, four separate behavioural measures were manually recorded. The first of these measured the time from when the auditory cue began and the aluminium panel was raised to when the subject first placed his head or nose past the " false" aluminium wall to investigate the presence of the Richter tube (this was termed as " latency to door"). The second measure recorded the " number of investigations" that the rat made during the 10 minute period between cue initiation and sucrose presentation - defined as the number of times that the subject placed any part of his head past the " false" aluminium wall. Thirdly, the latency to consume sucrose was measured from when the Richter tube was presented, after the 10 minute cued anticipatory period. Finally, the total amount of sucrose solution that the rat consumed was measured by weighing the preweighed Richter tube at the end of the 60 minute consummatory period.

Testing

Subjects were divided into two groups of nine rats each, matched on their sucrose consumption from the final two training sessions. One group served as controls, while the other was subjected to the chronic mild stress regimen. The testing procedure was similar to the final four training sessions: briefly, subjects were food and water deprived at 16:00h and placed into

the test chambers, and at 12:00 the next day, the auditory cue was turned on and the aluminium panel raised. A 10 minute anticipatory period followed, during which the rat typically made a number of investigations of the niche that would contain the spout of the Richter tube. After 10 minutes the sucrose solution was presented, and the rat was allowed to consume freely for 60 minutes. During the test sessions, the four separate behavioural measures were recorded as described above; 10 minutes after testing, subjects were returned to their home cages and given food and water ad libitum. All subjects were tested once per week, on the same day. The results were analysed by analysis of variance (ANOVA) and supplemented with tests of simple main effects.

Stress

The weekly stress regime used was similar to that used by Willner et al. (1987), and consisted of one period of overnight illumination, two periods of 45° cage tilt (7 and 8 hours), two periods of soiled housing (18 hours) and two periods of paired housing (22 hours).

Results:

The latencies for subjects to investigate the niche at the start of each test session did not differ significantly [$F(1,16) = 3.476$, $p < 0.081$], although there was a trend towards longer latencies in the experimental group.

Nor were there significant differences between groups in the latencies the groups took to consume from the Richter tube upon its presentation, which were normally within the range of 3

- 7 seconds. The CMS procedure, however, produced a dramatic effect on the number of investigations made in the 10 minute period prior to the availability of sucrose [$F(1,16) = 18.84$, $p < 0.001$], as well as a significant group × time effect [$F(9,144) = 2.03$, $p < 0.04$]. A strongly significant effect of CMS was observed, in accordance with previous studies, on the total mount of 1% sucrose solution that was consumed [$F(1,16) = 13.04$, $p < 0.002$] [Figure 1.1].

Post-hoc tests revealed that CMS-exposed animals exhibited significantly fewer niche investigations after only three weeks of stress, whereas decreases in sucrose solution consumption did not appear until after the fifth week of exposure to CMS.

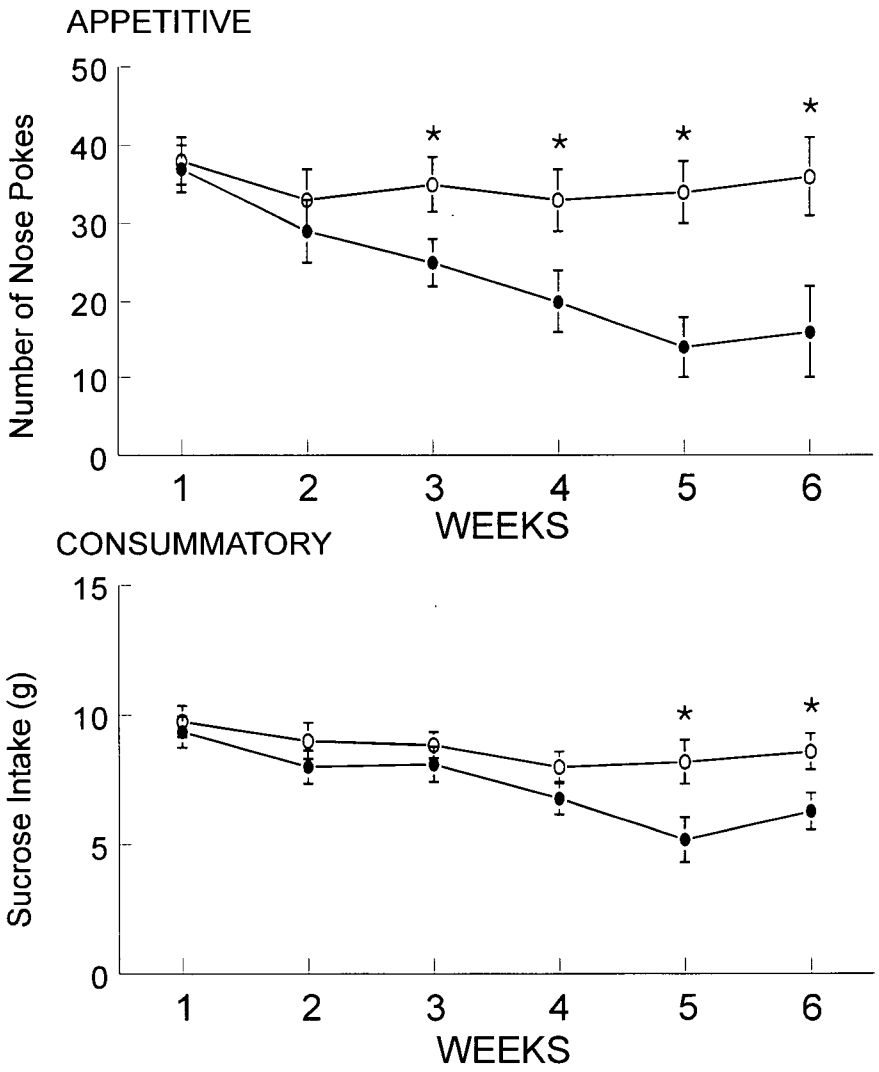


Fig. 1.1. Effects of CMS upon levels of levels of appetitive and consummatory responding for a 1% sucrose solution. The duration of the appetitive and consummatory phases are 10 and 60 min respectively. * Denotes significantly different at $p < 0.05$ level. ○ Control, ● CMS

Discussion:

The data from this experiment provide further new evidence that the CMS animal model of depression accurately resembles a number of different symptoms of human endogenous depression. The major finding of this study was that an appetitive behaviour could provide a more sensitive measure of the effects of CMS than a consummatory one, in that the appetitive behaviour was disrupted earlier and more significantly than the consummatory behaviour.

While few studies have measured the effects of chronic mild stress on concurrent appetitive and consummatory behaviours, the results of the present experiment are consistent with the observation of decreased sexual mounting activity (considered an appetitive behaviour) by stressed as compared to non-stressed male rats (D'Aquila et al. 1994). In this study, measures of sexual consummatory behaviour (i.e. intromission, ejaculations) were not recorded. A number of studies have demonstrated place- preference conditioning deficits to rewarding stimuli in stressed animals (Papp et al. 1991; Muscat et al. 1992), which may be seen as a disruption of anticipatory/ appetitive behaviour; however, these studies did not evaluate the longitudinal development of behavioural effects of chronic exposure to CMS, unlike the present study.

Evidence that appetitive behaviours may be disrupted more easily than consummatory ones comes from numerous studies which have observed the effects of a wide range of dopamine antagonists on such behaviours (for reviews see Fibiger 1993; Salamone 1994). One earlier study performed in the author's laboratory used the D_2 antagonist pimozide on unstressed rats and measured its consequences on a number of different appetitive and consummatory behaviours, similar to those used in this study (Blackburn et al. 1987). The results from that study demonstrated a significant effect on the number of investigations rats made into a niche in

anticipation of a cued meal, which resembles the effect of CMS in this experiment. A significant effect was also seen on the latencies taken for subjects to enter the niche for the first time, typically being 2 - 3 times longer in drugged rats than controls; a result of comparable magnitude was found in this study too, but the large variance in individual latencies meant that this effect barely missed significance. This latter result may emphasise the importance of the role that individual differences in response to stress play when using the CMS procedure.

The second appetitive latency measured in this study was not significantly different between groups, as both groups of rats took equal time to drink the sucrose solution upon its presentation. Thus, conditional or instrumental responding may be more severely disrupted when the associative link between CS and UCS is weaker. It would therefore be of interest to investigate the effects of CMS on other types of conditioned responding when compared to consummatory behaviour, such as in the acquisition of a new response in the absence of the primary reward, or on second-order schedules of reinforcement (Robbins et al. 1989).

Given the large literature demonstrating the ability of stress to affect DA release in various limbic structures, including the nucleus accumbens (Carlson et al. 1993; Kalivas and Duffy 1995), it is likely that the similarity between the effects of CMS and low doses of DA antagonists on appetitive behaviours is not coincidental. Previous studies have established that CMS causes subsensitivity of postsynaptic D_2 receptors in the nucleus accumbens (Willner et al. 1991; Papp et al. 1994), and that this in turn may be partly responsible for rats' decreased sensitivity to reward. As the nucleus accumbens is also critical for many appetitive behaviours (Salamone 1994), it is possible that these subsensitive D_2 receptors also disrupt appetitive behaviours. Typically, appetitive responses produce a smaller *in vivo* DA efflux in the nucleus accumbens than consummatory behaviours (Fiorino et al. 1997), and this may explain why, in a

subsensitive system, appetitive behaviours are disrupted more easily than consummatory ones (Fibiger 1993.

The use of an appetitive paradigm that measures an animal's interest in obtaining a natural reward should theoretically be independent of stress-induced changes in body weight. Although it has been hypothesised that decreased consumption of a weak sucrose solution may be related more to changes in body weight than a state of anhedonia (Forbes et al. 1996), the appetitive measures used in the present study suggest that anhedonia (i.e. a reduced interest in normally rewarding stimuli) may be apparent by the third week of exposure to CMS.

In conclusion, anhedonia - the core symptom of depression that the CMS model replicates - is often defined as a " loss of interest or pleasure" (American Psychiatric Association 1994) and currently nearly all studies involving the CMS procedure have focused on the loss of "pleasure" rather than loss of "interest". By using the ethologist's distinction of dividing goal-directed behaviours into both appetitive and consummatory behaviours (i.e. interest versus pleasure), results from this experiment demonstrate that these two types of behaviour are differentially sensitive to the CMS. This observation may help to provide further insight into the progressive development of the anhedonia which the CMS animal model of depression duplicates, and even help in the creation of antidepressant drugs designed to be more effective in the alleviation of appetitive than consummatory behaviours in humans.

EXPERIMENT 2:

CHRONIC MILD STRESS HAS NO EFFECT ON RESPONDING BY RATS FOR SUCROSE UNDER A PROGRESSIVE RATIO SCHEDULE

Synopsis:

Exposure of rats to chronic unpredictable mild stress (CMS) has been shown to produce a syndrome in which a wide range of consummatory behaviours are attenuated, resembling a state of anhedonia, which may be reversed by treatment with antidepressant drugs. The aim of the present study was to determine if CMS would also affect a rat's motivation to respond for a sucrose solution, as assessed by its performance under a progressive ratio (PR) schedule of reinforcement. Control studies demonstrated that break points in non-stressed rats were sensitive to both the concentration of sucrose solution used, as well as the period of food and water deprivation used prior to testing. Exposure of rats to CMS had no effect upon break points when responding under a PR schedule for either a 1% or 7% sucrose solution, although subjects did display the typical reduction in consumption of a freely consumed 1% sucrose solution. These results are not readily understood within the theoretical framework of the CMS model of anhedonia, and imply instead that both the neural and psychological correlates of motivation may be less susceptible to modulation by the effects of CMS than the free consumption of sweet solutions.

Introduction:

Currently, few animal models are available which provide adequate face and construct validity for the symptoms of depression in humans. One paradigm that addresses this issue is the Chronic Mild Stress (CMS) model as developed by Willner et al. (1987; 1991), which is purported to model the core human depressive symptom of anhedonia (i.e., a loss of interest in or pleasure from normally rewarding activities). An impressive body of evidence has been gathered to support the hypothesis that animals subjected to a daily series of relatively innocuous stressors begin to show anhedonic-like behaviour after two to three weeks of CMS. Typically, rats exposed to sufficient periods of CMS demonstrate a decrease in their consumption of a weak sucrose solution (Willner et al. 1987), and also an increase in the stimulation frequency required to support threshold intracranial self-stimulation (ICSS) of the ventral tegmental area (Moreau et al. 1992). Both of these effects imply that there is a dysregulation of the neural substrates involved in normal hedonic behaviour.

A substantial body of data also indicates that one of the neural substrates affected by CMS is the mesolimbic dopamine (DA) system (Papp et al. 1994; Willner et al. 1991); alterations in the stimulated release of DA in the nucleus accumbens (Stamford et al. 1991) have been reported, as well as decreased numbers of D2 receptors in the same region of the brain (Papp et al. 1994). Numerous experiments have shown that either 6-OHDA lesions of dopaminergic neurons or systemic doses of dopaminergic antagonists tend to disrupt preferentially behaviours linked to incentive motivation (Fibiger 1993; Salamone 1994). Appetitive/preparatory and complex instrumental responses are particularly sensitive to

modulation of the mesolimbic DA system, and doses of neuroleptics that inhibit lever-pressing for either food or water leave consummatory responses intact (Salamone 1996).

In recent years, the Progressive Ratio (PR) procedure has seen increased use as a means to measure an animal's motivation to respond for rewarding stimuli (Richardson and Roberts 1996). Specifically, by training rats to lever-press for a fixed reward, and progressively increasing the number of presses required to obtain each subsequent reward, a measure can be obtained of the total amount of effort that animals will expend, with the final ratio achieved (i.e. the "break point") providing an objective evaluation of the subject's motivation to work for a particular reward. Reduced motivation is an important symptom of melancholic-type depression, and therefore, the present experiment was undertaken to determine if a state of anhedonia induced by CMS model of anhedonia could be inferred from a reduction in an animal's motivation to work for a sucrose reward, as measured by break point values obtained with the PR procedure. Furthermore, it was also of interest to determine whether performance on a PR schedule would provide a more sensitive measure of anhedonia than the traditional consummatory measures (Willner et al. 1987; 1991).

Methods:

Subjects

Twenty-four male Long-Evans rats (Charles River, Quebec) weighing 250-300g at the start of the experiment, were housed individually in a temperature controlled colony (21±1°C) under a 12-hr light-dark cycle (lights on at 07:00 hrs). One subject had to be sacrificed during

the experiment, and its data were not included. All experiments were conducted in accordance with the Canadian Council on Animal Care guidelines for work with laboratory animals.

Training

Subjects were maintained on a constant 20-hr food and water deprivation schedule (in which food and water were available for only 4 hrs per day), whereby their weight was reduced to approximately 90% of their free feeding weight and maintained at this level by carefully controlling food intake (subjects were limited to 21 grams of food per day). Pilot studies had demonstrated that this procedure generated more stable patterns of responding on the PR schedule than intermittent deprivation. All subjects were tested daily between 09:00 and 13:00 hours, individually, in four Plexiglas test cages (25 x 25 x 25 cm) enclosed within sound attenuating chambers, and fitted with a removable response lever and lickometer.

Based upon previously published experiments (Montgomery and Suri 1996) which reported that consumption of different concentrations of a sucrose solution is described by an inverted ∪-shaped curve, animals in the present study were trained with a 7% sucrose solution, the most preferred concentration. Initially, subjects were given a 48-hr exposure period to the 7% sucrose in their home cages, and were then allowed two 30 min habituation sessions in the test chambers, during which both the response lever and fluid spout were withdrawn. For the next four days, subjects were given daily 1-hr sessions during which they had free access to the sucrose solution in the test chambers, which they could obtain by licking a metal spout connected to a lickometer (all subjects learned this readily). Response levers were then introduced into the chambers and subjects were placed on a 1-hr session fixed ratio (FR) schedule of responding as

follows: 3 days at FR1/0.05 ml of 7% sucrose solution, 2 days at FR2/0.10 ml sucrose, 1 day at FR3/0.15 ml sucrose and 2 days at FR10/0.40 ml sucrose per reinforcement.

All rats learned to barpress on an FR schedule and were subsequently placed on a PR schedule of reinforcement. Response requirements were based on the following schedule, whereby successive reinforcements could be earned according to the following number of barpresses: 1, 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145. The final ratio achieved represented the "break point" value, and the session was terminated when subjects failed to reach the next barpress criterion within 1 hour. The size of the reward was set at 0.5 ml of 7% sucrose solution per reinforcement, as this was shown in a pilot study to sustain high levels of responding, without allowing rats to consume quantities of sucrose near to their free consumption values, thus minimizing the effect of satiety on the PR data.

Animals received six PR sessions, and levels of responding were stable by the fourth session (mean performance over the last three sessions varied by approximately ± 1 break point per subject). To verify that rats responding on a PR schedule were responsive to alterations in the hedonic value of the sucrose solution, subjects were split into two groups (n = 11; 12) which were matched on their break points over the last three training sessions. Each group then received two 1-hr free consumption sessions in the test chambers over two days, while the response levers were removed: one group received 0.7% sucrose while the other was given 30% sucrose. Subjects were returned to their home cages for 24-hrs and on the following day animals were tested on a PR schedule with one of the two different concentrations of sucrose solution. After this test, all rats were rehabituated to the 7% solution with two 1-hr free consumption sessions, followed by one further PR session.

Chronic Mild Stress

Following rehabituation to the 7% sucrose solution, subjects were again divided into two groups, matched on break point values from their last three training sessions (it should be noted that these two groups did not correspond to the groups formed previously to examine hedonic shifts with the 0.7% and 30% sucrose solutions). One group (n = 12) remained as control subjects, while the other group (n = 11) was transferred to a separate colony and exposed to the CMS procedure as described by Willner et al. (1987; 1991)

The basic CMS protocol was as follows: two 18-hr periods of paired housing; two 20-hr periods of 40° cage tilt; two 18-hr periods of damp bedding; one period of overnight illumination and four 2-hr sessions of white noise (> 75 dB). All stressors were applied pseudo-randomly throughout the week to make them less predictable, and all stressors were terminated at least two hours before any behavioural testing occurred. It is important to note that the stressor of acute intermittent food and water deprivation used routinely by Willner and colleagues was not employed, as all subjects were already on a 20-hr deprivation schedule with limited access to food.

Behavioural Testing Following CMS

Behavioural testing commenced four days after the initiation of CMS (see Table 2.1), and thereafter animals were tested on the PR schedule for 7% sucrose every four days. This procedure continued for seven test sessions (i.e. until 28 days after CMS began), by which time animals submitted to CMS are reported to exhibit significant changes in their hedonic responses relative to control subjects (Willner et al. 1991). As no effects on break point values were evident at this time, it was hypothesized that the magnitude of the reward may have been

overshadowing any differences in motivation between both groups. Therefore, the 7% sucrose solution was replaced with a 1% sucrose solution and the experiment was repeated as follows. Four days after the last PR session (i.e. day 32), the response levers were removed and subjects were allowed to consume 7% sucrose freely for one hour. On days 35 and 36, all subject were habituated to the 1% solution with 1-hr free consumption sessions, and on day 40 both groups were tested for their free consumption of 1% sucrose solution during a 1-hr session (the normal consummatory measure used in CMS studies). Testing continued with the PR schedule on a weekly basis for the next three weeks, while standard 1-hr free consumption tests with 1% sucrose were conducted every three days after a previous PR session.

The final phase of this study examined the effect of minimal food and water deprivation on the rewarding value of the sucrose solution. Animals were now given ad lib access to food and water for 12-hr a day and tested after a 12-hr deprivation period (in comparison with the previous 20-hr restriction). Subjects were given one week for their weight to stabilize under this new regimen, and were then tested on days 65 and 72 for their free consumption of 1% sucrose. They were tested on days 69 and 76 with the PR schedule. After the final PR test on day 76, rats were rehabituated to the 7% sucrose solution used previously with two 1-hr free consumption session on days 79 and 80, and were finally tested for their free consumption of 7% sucrose on day 83.

The weights of all animals were recorded weekly, after subjects had been fed and watered, and at least four hrs after subjects had been exposed to any type of stressor.

TABLE 2.1

SCHEDULE OF EVENTS

Day(s)	Event(s)
0	Subjects matched into 2 groups
1-83	One group exposed to CMS regimen
4,8,12,16,20,24,28	All subjects tested under PR schedule for 7% sucrose
32	1 Hr test of free consumption of 7% sucrose, 20 Hr dep.
35,36	Habituation to 1% sucrose in test chambers
40,47,54	1 Hr test of free consumption of 1% sucrose, 20 Hr dep.
44,51,57	Tested under PR schedule for 1% sucrose, 20 Hr dep.
58-64	Ad lib access to food and water
65,72	1 Hr test of free consumption of 1% sucrose, 12 Hr dep.
69,76	Tested under PR schedule for 1% sucrose, 12 Hr dep.
79,80	Rehabituation to 7% sucrose solution in test chambers
83	1 Hr test of free consumption of 7% sucrose, 12 Hr dep.

***Results*:**

The effects of testing animals under the PR procedure with three different concentrations of sucrose solution are shown in Fig. 2.1. Analysis with paired t-tests revealed that when the concentration of the sucrose solution was increased from 7% to 30%, there was a small increase in the value of the break point which was only marginally significant, [$t(1,21) = 3.86$, $0.05 < p < 0.10$]. However, a reduction in the sucrose concentration from 7% to 0.7% was accompanied by a significant decrease in the break point, [$t(1,21) = 88.0$, $p < 0.001$]. This result demonstrated that a 7% sucrose solution may represent an optimal concentration for investigating decreases in a rat's motivation to respond for a sweet reward. This responsiveness to alterations in the reward value of the sucrose solution was seen again during the main testing phase of the experiment, as shown in Fig.2.2. A two-factor ANOVA demonstrated a significant effect of testing condition, [$F(2,42) = 108.136$, $p < 0.001$], but no interactive effects. Initially, subjects in both CMS and control groups were deprived of food and water for 20 hrs and responded for 7% sucrose; when this solution was replaced with a 1% solution, a significant reduction in break points was observed in both groups. Additionally, when the concentration of the sucrose solution (and hence its hedonic value) remained at 1%, and drive level was decreased by reducing deprivation from 20-hr to 12-hr, a further significant drop in break points was observed. In contrast to these changes in break points in both the experimental and control groups to alterations in hedonic value and drive level, CMS had no significant effect on break points with any of the three different conditions, [$F(1,21) < 1$, NS].

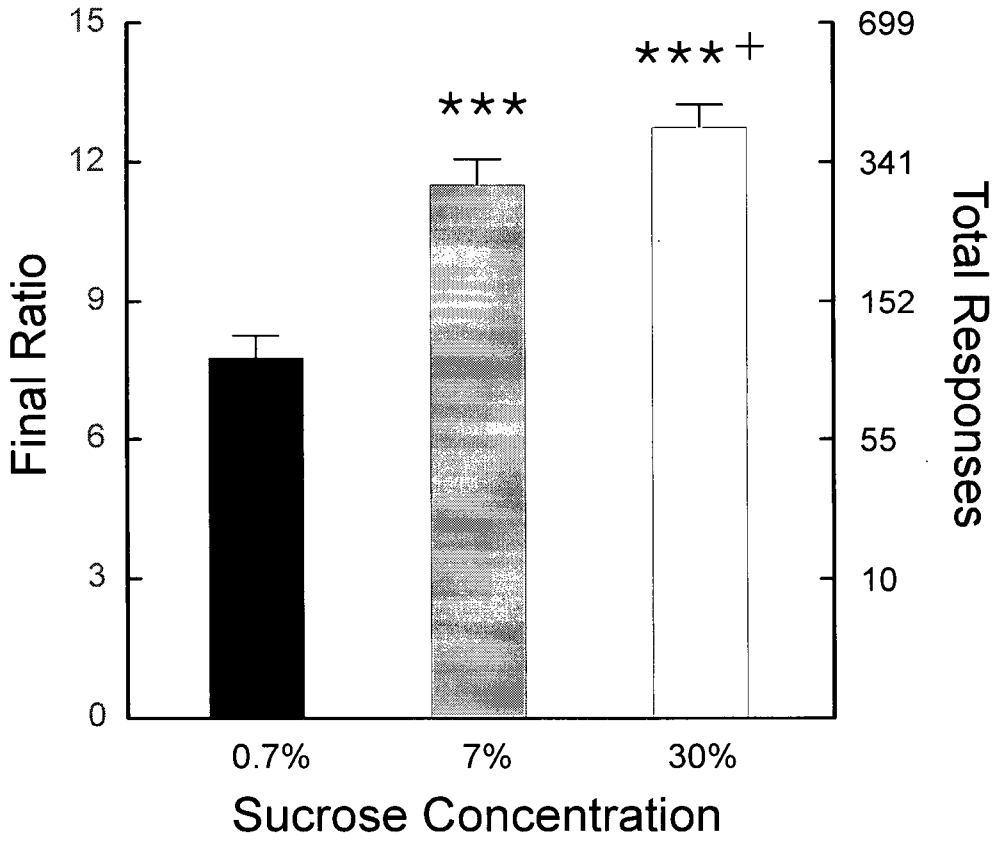


Fig. 2.1

Effects of changes in sucrose concentration on final ratios (i.e. break points) attained under a progressive ratio schedule by control rats. *** denotes $p<0.001$ compared to 0.7% sucrose; + denotes $p<0.10$ compared to 7% sucrose solution.

When subjects were tested on the standard measure of hedonia (free consumption of a 1% sucrose solution for 1-hr) after 20-hr of food and water deprivation on days 40, 48 and 54, a one factor ANOVA showed that animals in the CMS condition consumed significantly less sucrose than control subjects, [$F(1,21) = 8.31$, $p < 0.01$], (Table 2.2). There was no significant interaction. Similarly, when animals were tested with the 1% sucrose but after only 12-hr of deprivation, on days 65 and 72, CMS clearly reduced consumption again, [$F(1,21) = 11.88$, $p < 0.005$]. The same subjects displayed no effect of CMS when given a 1-hr free consumption test of 7% sucrose, after 20-hrs of deprivation on day 32, [$F(1,21) = 0.92$, NS], or after 12-hrs of deprivation on day 83, [$F(1,21) = 0.43$, NS]. A one factor ANOVA of body weights measured weekly (Fig. 2.3) demonstrated a significant effect of CMS on this measure, [$F(1,21) = 36.27$, $p < 0.001$], as well as a significant effect of test day, [$F(11,231) = 142.50$, $p < 0.001$]; and an interactive effect of group × test day, [$F(11,231) = 10.43$, $p < 0.001$]. A simple main effects post-hoc comparison between groups demonstrated that the body weights of rats in the CMS and control groups differed significantly by the second week of testing, which lasted until the end of the experiment.

TABLE 2.2

FREE SUCROSE CONSUMPTION VALUES (g) FOR 1HR TEST SESSIONS

	Control	CMS
1% SUCROSE, 20 HR DEP.	13.88 ± 1.03	9.58 ±1.08 *
1% SUCROSE, 12 HR DEP.	11.19 ± 0.70	7.68 ± 0.74 **
7% SUCROSE, 20 HR DEP.	34.33 ± 2.24	36.46 ± 2.34
7% SUCROSE, 12 HR DEP	29.33 ± 1.73	31.73 ± 1.80

Values are mean ± SEM

* CMS and control groups differ significantly, $p<0.01$

** CMS and control groups differ significantly, $p<0.005$

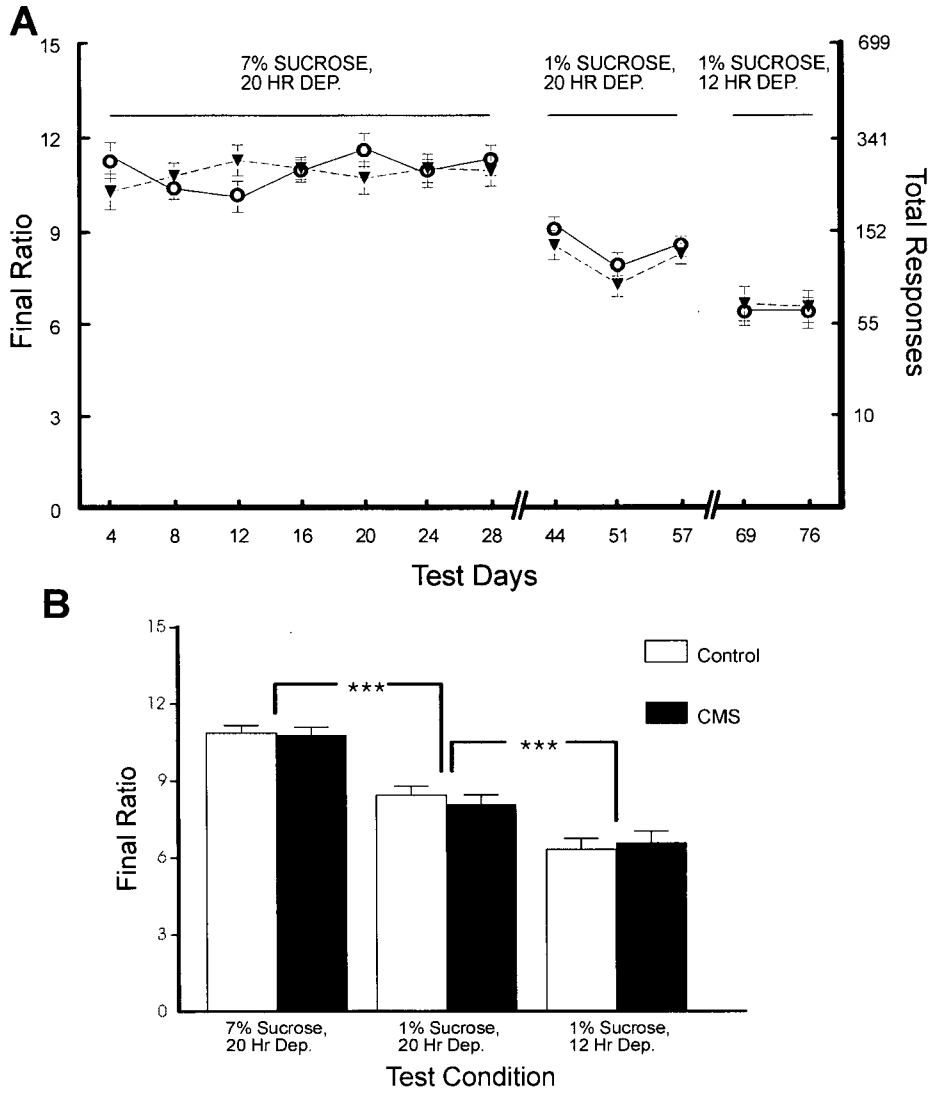


Fig. 2.2 Effects of chronic mild stress on final ratios attained under a progressive ratio schedule, as a function of sucrose concentration (7% or 1%) or hours of food and water deprivation (20 Hr or 12 Hr) for both CMS (▼) (n=11) and control (o) (n=12) groups.

A: Final ratio (i.e. break point) values and corresponding cumulative total lever presses for individual daily test sessions. No significant difference was found between CMS and control groups.
B: Final ratio values averaged over the test sessions for different test conditions. Data are presented as (mean ± SEM). *** within brackets denotes significantly different ($p<0.001$) between testing condition, but with no between group difference.

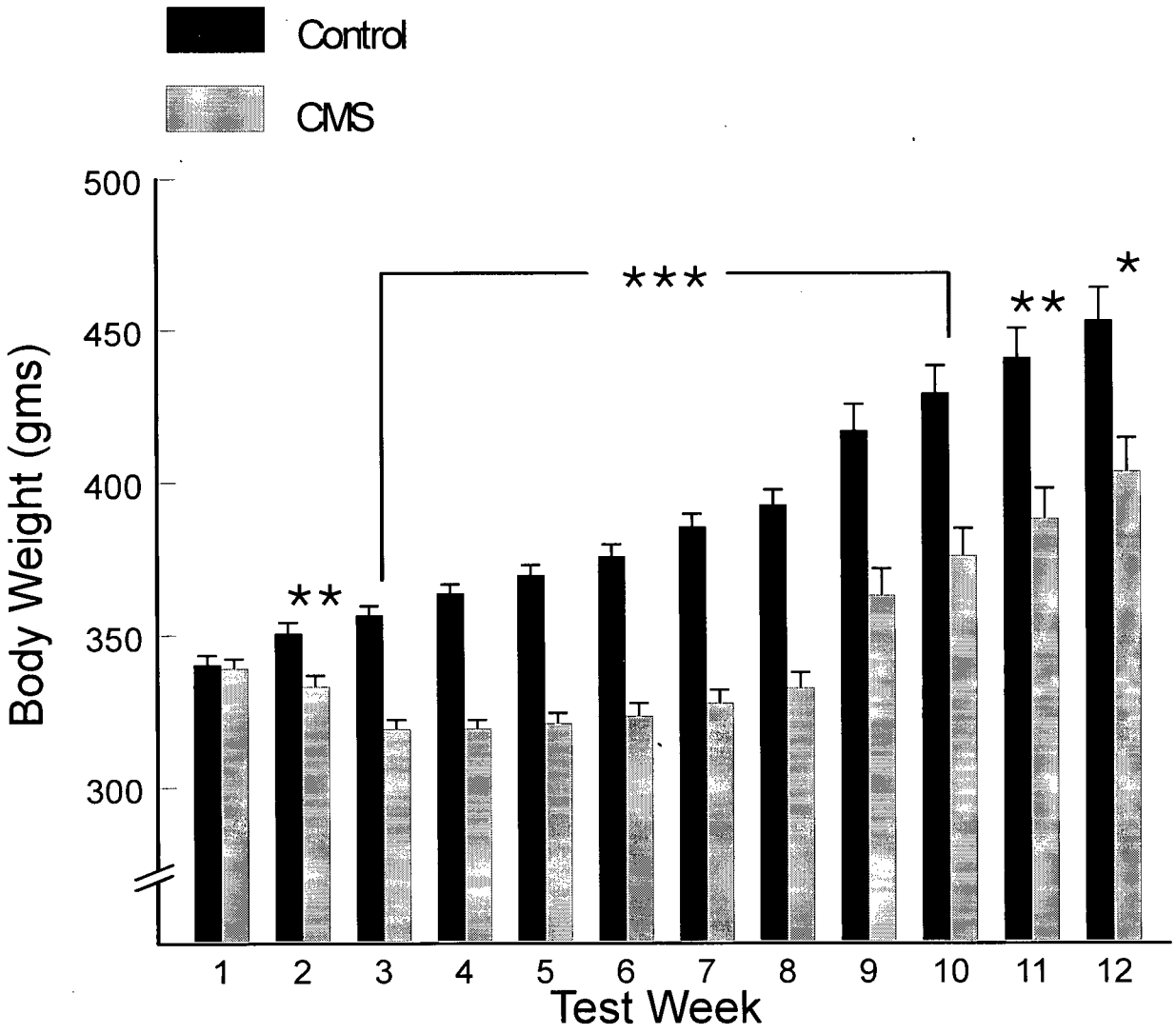


Fig.2.3 Effects of chronic mild stress on body weight, measured weekly. Control subjects were maintained at 90% of free feeding body weight from weeks 1-8, after which subjects were deprived of food and water for 12 Hr per day. * denotes a significant difference ($p<0.01$) from controls, ** denotes a significant difference ($p<0.005$) from controls, *** denotes a significant difference ($p<0.001$) from controls for all weeks enclosed by brackets.

Discussion:

The present results confirm one of the main findings of the CMS model of depression, namely that rats subjected to this treatment consume smaller quantities of 1% sucrose solution than control subjects (Willner et al. 1987; 1991). Paradoxically, the same rats do not differ with respect to the amount of work they are willing to perform to receive a fixed quantity (0.5 ml) of a 1% sucrose solution, as measured by the break point achieved under a PR schedule of reinforcement. Control experiments confirmed that this procedure was sensitive to the effect of reducing the concentration of sucrose from 7% to 0.7%, as reflected in a significant reduction in the value of the break point. This effect was replicated within the main experiment of this study following a reduction from 7% to 1% sucrose solution. Furthermore, when drive level was reduced by subjecting the rats to only 12 hrs as compared to 20 hrs of deprivation, we again observed a significant reduction in the break point in both the CMS and control groups. Two conclusions may be drawn from the pattern of results: first that the PR procedure is sensitive to changes in motivation induced by a reduction in the value of the sucrose reinforcer or drive level; and second, that rats subjected to CMS are as motivated as control subjects to work for different concentrations of sucrose solution. This pattern of results would appear to run counter to an important prediction of the CMS model of depression, namely that this treatment reduces the reward value of sucrose solutions because of anhedonia (Papp et al. 1994).

One explanation for the differential effects of CMS on free-consumption of sucrose solution as compared with operant responding for this reinforcer under a PR schedule, may be related to selective effects of CMS on the hedonic property of a sweet solution, as distinct from the desire or willingness to work for the same stimulus. Berridge and his colleagues refer to the

psychological correlates of these two conditions, as "liking" versus "wanting" (Berridge et al. 1989; Berridge 1996). These researchers draw a further distinction between these two constructs, in terms of the underlying neural substrates. The motivation to respond for a sweet solution is linked to activity in the mesotelencephalic dopamine system. Berridge et al. have shown that 6-OHDA lesions of the nucleus accumbens cause aphagia without affecting hedonic reactions to a sweet solution (Berridge et al. 1989). More recently, Ikemoto and Panksepp (1996) demonstrated that DA antagonists can reduce "wanting" a sucrose solution by rats as assessed by various appetitive measures, whereas measures of consumption of the solution were unaltered. Complementary to this work, Agmo et al. (1995) have established that specific doses of neuroleptics can block the formation of a place preference while leaving sucrose consumption unchanged; conversely, certain doses of the opioid antagonist naloxone reduce consumption but do not affect place preference conditioning. Therefore it appears that "liking" may be partially subserved by separate neural systems from those involved in "wanting". The neural systems involved in "wanting" probably include the mesolimbic DA system and regulate most appetitive behaviours, whereas those circuits concerned with "liking" may regulate consummatory behaviours (Berridge 1996). If this explanation is correct, then it follows that the present finding of no change in break points after CMS poses a challenge to the role of the mesotelencephalic dopamine system in depression as inferred from studies with the CMS procedure (Willner et al. 1991).

The question remains as to whether the break point value is a valid measure of the anhedonia characteristic of human depression. One study has addressed this issue, in which depressed human subjects were tested for their performance on a PR schedule, using a monetary reinforcer (Hughes et al. 1985). While the results showed that depression caused a significant

reduction in break points, a number of methodological concerns, such as the small sample size and absence of a control group, emphasize the need for a well-controlled study applying the PR paradigm to depressed human volunteers, in order to test its efficacy as an instrument for measuring melancholic-type depression. At present, there are no other reported studies with animal models of depression in which the motivation of animals to obtain primary rewards has been determined.

Recently, the CMS model of anhedonia has been criticized for failing to take significant reductions in body weight into account as a factor in reduced sucrose intake. When sucrose consumption was adjusted for the difference in body weights between stressed and control animals, consumption deficits between these groups were no longer significant (Forbes et al. 1996). In the present experiment, differences in body weight between CMS-exposed rats and controls were apparent by the second week of behavioural testing. When sucrose consumption was recalculated according to the method of Matthews et al. (1995; Forbes et al. 1996), and consumption per gram of body weight was determined, the sucrose intake did not differ significantly between the two groups. However, the issue is complicated by the fact that despite the notable difference in weight between stressed and control animals (approximately 10-15%), similar volumes of a 7% sucrose solution were consumed when subjects of each group were allowed to drink freely for 1 hr: these data indicate that sucrose consumption is not rigidly determined by body weight. Nevertheless, these data raise another note of caution in interpreting the effects of CMS on hedonic processes.

EXPERIMENT 3:

CHRONIC MILD STRESS MODIFIES EXTRACELLULAR LEVELS OF DOPAMINE AFTER CONSUMPTION OF A SUCROSE SOLUTION

***Synopsis*:**

A large body of evidence suggests that alterations in neurotransmission within the mesocorticolimbic dopamine system may contribute towards the development and expression of MDD. The chronic mild stress (CMS) animal model of depression represents a well-tested rodent paradigm that emulates anhedonia, one of the two core symptoms of unipolar depression. Indirect evidence exists for changes in dopaminergic neurotransmission in the CMS model. The purpose of the present study was therefore to determine if these hypothesised changes could be detected *in vivo*, with the use of *in vivo* microdialysis. After 6 weeks of exposure to CMS (or control conditions), rats were implanted with microdialysis probes directed at the nucleus accumbens. Subjects were then allowed to consume a fixed volume of a 1% sucrose solution over 1 hr, and dopamine and its metabolites were measured. The results indicated an unexpectedly greater relative increase of dopamine release in CMS compared to control animals. These results call into question the validity of CMS as an animal model of hypodopaminergic-related melancholic-type depression.

***Introduction*:**

The monoamine theory of depression is perhaps one of the most established theories in the field of psychiatry (Hyman 2000). For over thirty years, there has been an ever-increasing body of evidence which suggests that alterations in monoamine neurotransmission can contribute to the expression of depressive disorders (Schildkraut 1965; Delgado 2000). Although the majority of research has focused upon norepinephrine and serotonin as neural substrates of MDD, there remains a convincing argument that changes in dopamine neurotransmission may be an important factor in the etiology of MDD. A wide range of data have provided support for the hypothesis that reduced neurotransmission within the mesocorticolimbic dopamine system may contribute towards depressive symptomatology (Willner 1995); in particular, it is posited that lower levels of endogenous dopamine release may lead to anhedonia and vegetative symptoms. Furthermore, recent preclinical studies with rodents have indicated that different classes of antidepressant drugs may act through a common dopaminergic pathway to generate their therapeutic effects (Santiago et al. 1998; Zhang et al. 2000)

The CMS model of depression is an animal paradigm of MDD that seeks to simulate the core depressive symptom of depression (Willner et al. 1987). An impressive body of data indicate that after rodents are exposure to chronic levels of mild, unpredictable stress, they begin to exhibit a decrease in their hedonic capacity for rewarding stimuli. This effect is typically measured by the reduced consumption of a weak sucrose solution in CMS-exposed animals (Papp et al. 1991). In a related manner, a number of studies using the CMS model have found evidence for changes in dopaminergic neurotransmission. Both behavioural and receptor binding studies implicate changes in dopaminergic neurotransmission specifically within the nucleus

accumbens (Papp et al. 1993; 1994). Given that mesoaccumbens dopamine is hypothesised to play an important role in motivated behaviour (Fibiger and Phillips 1988; Ikemoto and Panksepp 1999; Schultz et al. 2000), it is clearly of interest to determine whether or not there are *in vivo* differences in dopaminergic neurotransmission in CMS-exposed rats.

The development and use of *in vivo* microdialysis in recent years has provided an invaluable research tool for the measurement of levels of neurotransmission in awake, freely-moving animals (Tossman et al. 1986; Stamford and Justice 1996). When the technique is combined with sample analysis by high performance liquid chromatography with electrochemical detection (HPLC-ED), it allows for the specific and sensitive measurement of levels of monoamines and their metabolites in discrete brain regions (Di Ciano et al. 1995). The purpose of the present experiment was thus to determine if rats that are exposed to CMS for a chronic duration exhibit changes in basal levels of dopaminergic transmission, as well as in response to a rewarding stimulus. As a caveat, however, several recent studies have shown that dopamine release within the nucleus accumbens is linked not only to the incentive qualities of a consumed stimulus, but also the amount of the stimulus that is consumed (Wilson et al. 1995; Martel and Fantino 1996). Because we are interested in the former rather than the latter index of dopamine function, all animals were allowed to consume only a small, fixed quantity (5ml) of 1% sucrose, to avoid a potential confound of reduced fluid consumption in CMS-exposed animals.

Methods:

Subjects

Twenty male Long-Evans rats (Charles River, Quebec) weighing 250-300g at the start of the experiment, were housed individually in a temperature controlled colony (21±1°C) under a 12-hr light-dark cycle (lights on at 07:00 hrs). All experiments were conducted in accordance with the Canadian Council on Animal Care guidelines for work with laboratory animals.

Surgery

Rats were anaesthetised with ketamine hydrochloride (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.), and placed in a standard stereotaxic apparatus. The dorsal surface of the skull was exposed and holes were drilled bilaterally over the nucleus accumbens (AP = +1.7mm from bregma, ML = ±1.2mm from midline), and two guide cannulae (19 guage, 15mm) were implanted bilaterally to a DV depth of –1.0mm from dura. The guide cannulae were secured to the skull with surgical stainless steel screws and dental acrylic. The guide cannulae were permanently obdurated until the insertion of the microdialysis probes. Animals were allowed to recover from surgery for at least one week before behavioural manipulation began.

Chronic Mild Stress

Animals were randomly divided into two groups (n=10 per group). One group remained as control subjects, while the other group was transferred to a separate colony and exposed to the CMS procedure as described by Willner et al. (1987; 1991). Three animals from the CMS-exposed group eventually had to be excluded from the study, as the guide cannulae of these

subjects became irreparably damaged by the daily exposure to CMS. The basic CMS protocol was as follows: two 18-hr periods of paired housing; two 20-hr periods of 40° cage tilt; two 18-hr periods of damp bedding; one period of overnight illumination and four 2-hr sessions of white noise (> 75 dB). All stressors were applied pseudo-randomly throughout the week to make them less predictable, and all stressors were terminated at least two hours before any behavioural testing occurred.

Behavioural Training and Testing

Following the second week of exposure to CMS, both groups of animals were given a 24 hr exposure period to a 1% sucrose solution in their home cages. The following day, animals were then allowed a 30 min habituation session in the microdialysis test chambers, during which the fluid spout was withdrawn. From this time hence, animals were given once-weekly exposure to a small (5 ml), fixed volume of 1% sucrose solution in the microdialysis test chambers, which they were allowed to consume over a 1 hr period. This volume represents an amount of liquid which is less than that normally drunk by either CMS-exposed or control animals, as determined by results obtained from either our or others' laboratories (Willner 1987; Phillips and Barr 1997; Barr and Phillips 1998). The purpose of restricting the animals' consumption to a small fixed volume was to ensure that any observable differences in neurotransmission would not simply reflect different volumes consumed by the two groups (Wilson et al. 1995; Martel and Fantino 1996). After the sixth week of exposure to CMS, both groups of animals were implanted with microdialysis probes and left overnight in the microdialysis test chambers. The following day, after stable levels of dopamine had been obtained, rats were allowed to consume a fixed (5 ml) volume of 1% sucrose solution over a 1 hr period. Dialysate samples were collected for nine

successive samples (each sample was collected every 15 minutes) subsequent to the presentation of the sucrose.

Microdialysis Procedure

The microdialysis probes used in this procedure were constructed according to the design of Fiorino et al. (1993). In brief, the probes were of a concentric style, consisting of a stainless-steel cannula (24 gauge, 34mm), polyethylene tubing (PE 50, Intramendic, Boston MA), fused silica tubing (PolymicroTechnologies Inc., Phoenix AZ) and a semi-permeable hollow-fiber membrane at the distal end (65kDa cutoff weight, 340 μm o.d., 2mm exposed membrane, Filtral 12, Germany). Probes were perfused at 1.0 μl/min with a modified Ringer's solution (0.01 M sodium phosphate buffer, pH 7.4, 1.3 mM $CaCl_2$, 3.0 mM KCl, 1.0 mM $MgCl_2$, 147 mM NaCl) using a gastight syringe (Hamilton, Reno NV) and a syringe pump (Harvard Apparatus, South Natick MA). Probes were secured by a guide collar to the guide cannula, and a steel coil that was attached to a liquid swivel (Instech, PA) was used to protect the probe.

Microdialysis analytes, which typically consisted of a volume of 15μl, were separated by reverse-phase chromatography (Ultrasphere column, Beackman, Fullerton CA, ODS 5μm, 15cm, 4.6mm, inner diameter). The mobile phase consisted of 6g/L of sodium acetate, 70 mg/L of sodium octyl sulfate, 10 mg/L EDTA, 35mL/L of glacial acetic acid, which was pH adjusted to 3.5. 10% degassed methanol was added to the mobile phase prior to use. Analyte concentrations were quantified by electrochemical detection, using a Coulochem II EC detector (ESA, Bedford MA); the working potentials were +450mV (electrode 1), -300mV (electrode 2) and +450mV (guard cell). Chromatograms were recorded on a dual-pen chart recorder (Kipp and Zonen,

Bohemia NY). The typical probe recoveries were 22% for dopamine and 18% for DOPAC and HVA.

Histology

After the completion of the microdialysis procedure, each animal was given an overdose of chloral hydrate and perfused intracardially with saline and 4% formalin. The animals' brains were removed and stored in formalin, until they were frozen and cut into coronal sections (50μm) for verification of probe placements. Only those probe placements that were within the nucleus accumbens (either core or shell) were used in further analysis.

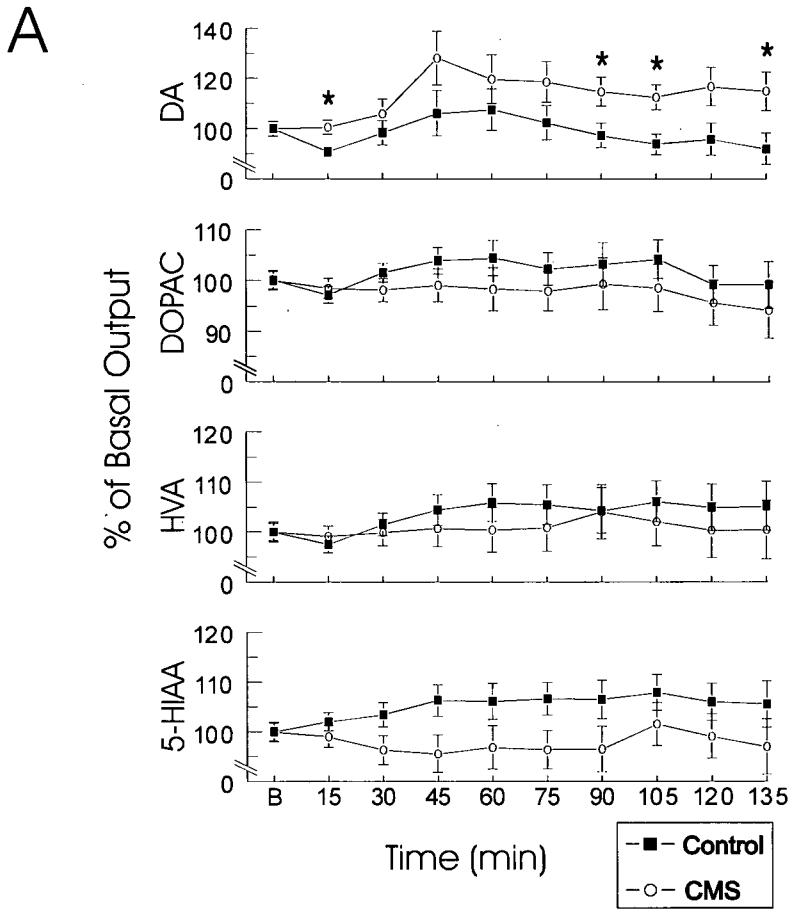
Results:

The concentration of analyte dopamine, its metabolites DOPAC and HVA, and the 5-HT metabolite 5-HIAA were determined by measuring the peak heights on chromatographs and interpolating them on a standardised calibration curve. The mean values (± SEM) for basal levels of dopamine in the nucleus accumbens in CMS-exposed and control animals were 2.71 ± 0.40 vs 1.66 ± 0.40 nM, with no significant difference between the two groups [$t(1,15) = 1.311$, NS]. Similarly, the basal concentrations of both metabolites were not significantly different between the groups, with a mean value for DOPAC of 0.729 ± 0.075 μM for CMS-exposed rats vs 0.594 ± 0.075 μM for controls [$t(1,15) = 0.881$, NS], and for HVA the values were 0.221 ± 0.018 μM (CMS) vs 0.177 ± 0.018 μM (controls) [$t(1,15) = 1.217$, NS]. The basal levels of the

serotonin metabolite 5-HIAA in the two groups were 0.219 ± 0.011 μM (CMS) vs 0.183 ± 0.011 μM (controls), which were not significantly different from each other [t(1,15) = 1.644, NS].

As basal levels of dopamine and the three metabolites were not significantly different between the groups, the levels of these compounds after presentation of the sucrose solution were expressed as percentages of the average basal values for each individual animal. These data were analysed by a one-factor repeated-measures ANOVA, with stress condition as the factor, measured across time [Figure 3.1].

The results of the ANOVA indicated that there was a significant group effect on levels of dopamine after presentation of the 1% sucrose solution [$F(1,15) = 5.92, p < 0.05$], as well as a significant effect of time [$F(8,120) = 3.07, p < 0.05$]. Post-hoc analysis using tests of simple main effects revealed that CMS-exposed animals exhibited significantly higher levels of dopamine release at different time points for up to 2½ hours after the presentation of the 1% sucrose solution [Figure 3.1A]. Analysis of the levels of the dopamine metabolite DOPAC with ANOVA failed to observe a significant group effect [$F(1,15) = 0.771$, NS] [Figure 3.1B]. Similarly, there was no significant group effect upon levels of the dopamine metabolite HVA either [$F(1,15) = 0.331$, NS] [Figure 3.1C]. The serotonin metabolite 5-HIAA was lower in CMS-exposed animals than in controls after presentation of the 1% sucrose solution, an effect that was marginally significant [$F(1,15) = 3.18$, $0.05 < p < 0.10$) [Figure 3.1D].



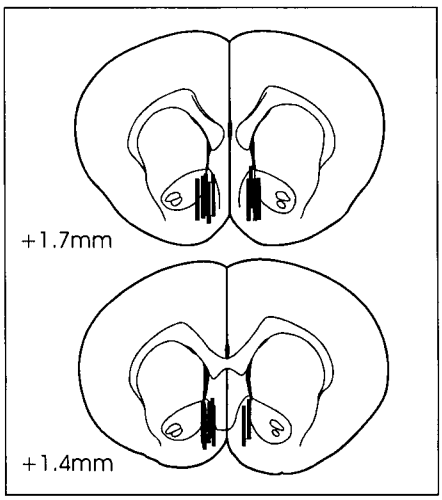


Fig. 3.1. (A) Effects of exposure to chronic mild stress for 6 weeks on levels of dopamine and metabolites after consumption of a small fixed volume (5mL) of a 1% sucrose solution. (B) Microdialysis probe placements.

Discussion:

The main findings from the present study are that rats that have been exposed to chronic mild stress for a period of 6 weeks do not exhibit differences from controls in the basal levels of nucleus accumbens dopamine, its metabolites HVA and DOPAC, or the serotonin metabolite 5-HIAA. In contrast, there was a greater relative increase in the release of dopamine by CMS-exposed rats compared to control animals after they were allowed to consume a small fixed volume (5ml) of a 1% sucrose solution.

The findings from the current study would appear to stand in contrast to the present body of data derived from the CMS model. It is a basic tenet of the CMS model of depression that several of the behavioural sequelae of the model, including the primary symptom of anhedonia, are reflections of a deficit in the underlying physiology of the mesoaccumbens dopamine pathway (Papp et al. 1994). The evidence for this includes deceased D2 and D3 receptor binding in the nucleus accumbens (Willner 1997a), subsensitivity to the drug apomorphine at doses that stimulate the dopamine autoreceptor (Muscat et al. 1988), and the capacity of direct dopamine agonists, such as quinpirole, to reverse the hedonic deficits of the model (Papp et al. 1993). However, it should be noted that a number of these changes (such as reduced D2/D3 receptor binding and DA autoreceptor subsensitivity) are more consistent with chronically elevated levels of dopamine than reduced dopamine levels (Pierce et al. 1995; Flores et al. 1996). Indeed, a study that used *in vivo* voltammetry to measure dopamine levels in the nucleus accumbens of chloral hydrate anaesthetised rats found that direct stimulation of the medial forebrain bundle led to greater increases in dopamine in CMS-exposed animals than in control animals (Stamford et al. 1991).

More recently, Di Chiara et al. (1999) have used microdialysis within both the nucleus accumbens and prefrontal cortex to examine the effects of CMS on dopamine release in response to rewarding and aversive stimuli. Consistent with the results of our study, there was no observed difference in basal levels of dopamine in CMS and control animals. In contrast, animals that had been exposed to CMS for 4 weeks exhibited a blunted dopamine response in the nucleus accumbens after consumption a highly palatable food ("Fonzies") compared to control subjects. This effect was the opposite of our findings, as we observed a greater dopamine response in CMS-exposed animals. The nature of these different findings are not immediately clear. There are, however, several important methodological differences between the study of Di Chiara et al. and ours. Firstly, the animals in our experiment were surgically implanted with guide cannulae for the microdialysis procedure before exposure to CMS (i.e. seven weeks before microdialysis), unlike the study of Di Chiara et al. in which rats were subjected to anaesthesia and intracranial surgery and then tested within 24 hrs, representing only a brief recovery time for these animals. Secondly, the study of Di Chiara et al. allowed animals to consume a "highly palatable" food for 20 minutes, which has a high caloric and fat content (Di Chiara et al. 1999), whereas we used the standard mildly rewarding stimulus of a 1% sucrose solution, available for 1 hour. Finally, the animals in the study by Di Chiara et al. were exposed to the highly palatable food for the first time on the day of the microdialysis procedure. Under our protocol, and consistent with most prior studies that have used the CMS model, all animals were given repeated, prior exposure to the rewarding stimulus before microdialysis was performed. The differences in findings between our lab and those of Di Chiara et al. (1999) may be related to the amount of pre-exposure that stressed and non-stressed animals had to the rewarding stimulus, as the mesoaccumbens dopamine system has been shown to habituate in response to repeated

presentations of the same food stimulus (Bassareo and Di Chiara 1997). It is possible that stressed animals exhibited higher levels of dopamine release as a result of a failure of the dopamine system to adapt to this stimulus, a hypothesis that is consistent with prior studies on the effects of dopamine release and its adaptation to stress (Imperato et al. 1993).

The elevated levels of dopamine release in CMS-exposed animals after consumption of a 1% sucrose solution are incompatible with a hypodopaminergic model of depression, as proposed by Willner et al. (1987; Willner 1997a). The original basis for the role of dopamine in this model was founded on the dopamine hypothesis of reward, which postulated that reduced levels of dopamine result in anhedonia (Wise 1978). Current theories of the role of mesoaccumbens dopamine have elaborated dopamine's function in motivated behaviour to include are more general role, including incentive motivation (Berridge and Robinson 1998) and attention to salient stimuli (Horvizt JC 2000). Nevertheless, reduced levels of dopamine have been associated with anhedonia in humans (Belmaker and Wald 1977; Heinz et al. 1994) and also in major depression (Lambert et al. 2000). These results suggest that the CMS model does not accurately mimic the neurochemical milieu that is associated with MDD, although further study is required to examine specifically the effect of depression on mesoaccumbens dopamine release in response to rewarding stimuli, possibly with the use of neuroimaging techniques (Elliot et al. 2000; Drevets et al. 2001).

Chronic Mild Stress – Synthesis

The results of the experiments described in Experiments 1 - 3 may be evaluated within the traditional boundaries of pharmacological, face and construct validity (Willner 1991). With respect to the validation criterion of pharmacological validity, the experiments that are described in this dissertation do not contribute to the large body of evidence which suggests that CMS exhibits high pharmacological isomorphism. The specificity that the CMS model displays for appropriately therapeutic compounds is perhaps the greatest strength of this model (Willner 1997b), and therefore no studies were undertaken to expand the range of drugs that are known to be effective in this model.

With respect to face and construct validity, the results of the present studies do not support the contention that the CMS paradigm is a valid model of depression. Although the first experiment, described in Experiment I, indicated that an appetitively-mediated task could used to provide a more sensitive indicator of anhedonia in the CMS model than the traditional consummatory task (Willner et al. 1987), these initial findings were overshadowed by the findings from subsequent studies. Most importantly, animals that were exposed to CMS did not demonstrate the expected decrease in motivation to obtain a natural reward, as assessed by a progressive ratio task, under a variety of different motivational conditions. The use of a progressive ratio task provides a way of assessing motivation for rewarding stimuli that is independent of the body weight of the animals, and thus the findings of this experiment lend greater support to those who hypothesise that CMS–induced decreases in sucrose solution consumption represent factors more related to weight loss of the stressed animals (Matthews et al. 1995; Forbes et al. 1996). The data that were obtained in the microdialysis experiment, in

Experiment 3, also challenge the face and construct validity of the CMS model. Unlike the decreased dopaminergic tone that is observed in numerous studies of depressed patients (Fibiger 1995), we observed augmented mesoaccumbens dopamine release in response to a rewarding stimulus in CMS-exposed rats. These findings are not readily interpreted as providing support to the hypothesis that CMS represents a model of anhedonia that arises from hypodopaminergic activity.

In addition to these major criticisms of face and construct validity, the CMS model has also received widespread censure for its lack of reproducibility and reliability. While several laboratories have been able to reproduce the CMS model with some degree of reliability (Moreau 1997), most have not. Even Willner and his colleagues have had problems replicating their results (personal communication from P. Willner). Although the data are not presented in this dissertation, we conducted 6 separate experiments (excluding the microdialysis experiment) over a period of 2½ years, using the CMS model. During this time, we were able to observe decreased fluid consumption in only 2 of 6 studies (i.e. Experiments 1 and 2). Of the remaining experiments, all had to be terminated preliminarily, as we observed no changes in fluid consumption in 3 of these studies after exposure of rats to up to 8 weeks of CMS. In one experiment we actually observed an increased consumption of 1% sucrose solution after chronic exposure to CMS.

Given the nature of these combined results, it was decided to conclude experiments with the CMS model and focus further studies on an alternate model of depression.

Amphetamine Withdrawal

The use of monoamine-depleting drugs represents one of the earliest forms of animal models of depression (Hendley and Welch 1975). After the discovery that administration of the antihypertensive drug reserpine (Serpasil) induced the symptoms of clinical depression in approximately 15% of human patients (Goodwin and Bunney 1971), a large number of experiments were conducted to establish the nature of this phenomenon. As a consequence, the resperine animal model of depression was established, in which behavioural alterations such as sedation and ptosis, as well as physiological changes such as hypothermia and hyperalgesia, could be induced by injecting rodents with reserpine (Willner 1984). Several of the behavioural sequelae of reserpine treatment could be ameliorated with co-administration of tricyclic antidepressants (Sing and Gautam 1977).

However, the reserpine animal model of depression, while useful in establishing the importance of monoamine-based models of depression, exhibited some important limitations. Primary amongst these was the low face validity of the model: the behavioural effects of treatment with reserpine, such as ptosis and hypothermia, bear little relation to the diagnostic symptoms of depression (Willner et al. 1984). The construct validity of the model was also weakened by the use of much higher relative doses of the drug in rats compared to humans. The use of very high doses of reserpine in rodents implied that the behavioural effects of the model were of questionable relevance when being compared to the "depressive" effects that were noted in humans (Bein 1978).

The current list of drugs that can induce depressive-like symptoms in humans is extensive, and ranges from calcium channel blockers to antihyperlipidemic agents (Patten and

Love 1997). In the case of the majority of these drugs, the side effect of depressive symptoms is simply one of many other side effects, and psychological effects are rarely exclusive. However, one class of compounds is particularly prominent in their capacity to engender depressive-like effects in humans, with few additional somatic side-effects. This class of compounds consists of the psychostimulant drugs, that include the amphetamines (e.g. *d*-amphetamine, methamphetamine and 3,4-methylene dioxy methamphetamine) and cocaine. In contrast to the acute effects of self-administration of these drugs, which include increased vigilance, arousal and euphoria, the withdrawal of these drugs leads to anhedonia, depressed mood, fatigue and hypersomnia (Gawin and Kleber 1986; Gillin et al. 1994). The psychological effects of psychostimulant withdrawal bear a strong similarity to endogenous depression, and require a separate diagnostic category to differentiate them from endogenous depression (American Psychiatric Association 1994) in the clinic.

As a result of the selective yet potent psychological effects of psychostimulant withdrawal in humans, animal models have been developed to simulate these symptoms. In particular, the anhedonia that is associated with psychostimulant withdrawal has been successfully modeled, with the use of reward-related tasks such as responding for reinforcing electrical brain stimulation (Leith and Barrett 1976; Markou and Koob 1991). In these studies, it has been shown that animals that have received medium to high doses of psychostimulant drugs followed by their withdrawal exhibit a striking subsensitivity of the neural systems that are hypothesised to mediate "pleasure" in normal subjects. Due to the importance of anhedonia as a core symptom of depression, it has therefore been suggested that psychostimulant withdrawal may provide a valid animal of depression (Leith and Barrett 1980; Kokkinidis et al. 1986; Baumann and Rothman 1998). In support of this hypothesis, several preclinical studies have

observed that the anhedonia associated with psychostimulant withdrawal can be ameliorated by treatment with drugs that have antidepressant properties (Seltzer and Tonge 1975; Markou et al. 1992). To date, the psychostimulant model of depression remains a model with high potential that requires further development and assessment of its different types of validity. The purpose of the following series of experiments was thus to determine the pharmacological, face and construct validity of this model, using in part the techniques (and lessons learned) during earlier research with the CMS animal model of depression.

EXPERIMENT 4:

WITHDRAWAL FOLLOWING REPEATED EXPOSURE TO *D*-AMPHETAMINE DECREASES RESPONDING FOR A SUCROSE SOLUTION AS MEASURED BY A PROGRESSIVE RATIO SCHEDULE OF REINFORCEMENT

***Synopsis*:**

Numerous studies have shown that withdrawal from sustained high doses of psychostimulant drugs such as cocaine or *d*-amphetamine produces depressive-like symptoms in both rats and humans. The majority of experiments with rodents have assessed the effects of amphetamine withdrawal on reinforcing electrical self-stimulation in different brain regions, but relatively few have examined effects on responding for natural reinforcers. In the present study two groups of mildly food and water deprived male rats were trained to respond on a lever for a 4% sucrose solution under a Progressive Ratio schedule of reinforcement. One group was subsequently administered a four day regimen of injections of increasing doses of *d*-amphetamine based on a schedule shown previously to reduce self-stimulation behaviour. Break points were significantly reduced for up to four days after the termination of drug administration, suggesting a decreased motivation to obtain the natural reward. A further experiment demonstrated that the identical drug regimen produced no effect upon consumption of the 4% sucrose solution when it was freely available. These results demonstrate that the Progressive Ratio procedure may be a useful technique for evaluating changes in motivation for natural reinforcing stimuli following withdrawal from psychostimulant drugs.

Introduction:

For more than twenty years, the psychological effects of drug withdrawal have been explained within the theoretical framework of an opponent-process theory of motivation (Solomon 1977; Koob et al. 1997). According to this theory, during withdrawal the previously pleasurable effects of a variety of different drugs of abuse are inevitably followed by emotional states opposite in affect, and of a longer duration, as the body seeks to restore its "hedonic equilibrium" (Solomon and Corbit 1974). Thus, drugs such as the psychostimulants cocaine and *d*-amphetamine which produce the acute effects of euphoria, increased energy and self-confidence, generated a withdrawal syndrome characterized by dysphoria, lethargy and anxiety (Gawin and Kleber 1986). This psychostimulant-induced withdrawal syndrome bears a remarkable similarity to human endogenous depression, and indeed depression is one of the most commonly described side-effects of cocaine and amphetamine withdrawal in humans (Pathiraja et al. 1995). The resemblance of psychostimulant withdrawal to endogenous depression has therefore prompted its development as an isomorphic animal model of depression (Seltzer and Tonge 1975; Leith and Barrett 1980; Kokkinidis et al. 1986; Geyer and Markou 1995).

One major symptom common to both psychostimulant withdrawal and depression is anhedonia, which represents a decreased interest in and pleasure from normally rewarding activities (Willner 1991). A variety of behavioural measures have been used to evaluate drug withdrawal and anhedonia in rodents, including a range of operant schedules (Denoble and Begleiter 1976, Carroll and Lac 1987), but the most frequently applied technique is the assessment of drug withdrawal on intracranial self-stimulation (ICSS) response thresholds from electrodes placed in either the lateral hypothalamus (Simpson and Annau 1977, Markou and

Koob 1991), substantia nigra (SN) (Borowski and Kokkinidis 1992) or ventral tegmental area (VTA) (Frank et al. 1992). Typically, rats or mice administered (by self-administration or the experimenter) medium to high doses of either cocaine or amphetamines show an increase in the frequency or current intensity required to support ICSS, which is interpreted as a reduced responsiveness of the brain's reward systems (Phillips and Fibiger 1989, Wise 1996). These effects generally last from between four days to two weeks, and in agreement with psychostimulant withdrawal as a model of depression, are shown to be alleviated by tricyclic antidepressants (Kokkinidis et al. 1980, Markou et al. 1992).

As anhedonia in humans is assessed by its effects on natural rewards, it is of obvious interest to ascertain how psychostimulant withdrawal, as a model of anhedonia in animals, affects their motivation to respond for natural rewarding stimuli. Furthermore, while drug-induced modulations of ICSS responding return to normal within two weeks (Wise and Munn 1995), there are numerous reports of human cocaine addicts experiencing long term periods of anhedonia during drug withdrawal (Gawin and Kleber 1986, 1988), which can reduce their interest in natural rewards for many months. If similar results were found in animal models of psychostimulant withdrawal, this would significantly increase the utility of psychostimulant withdrawal as an animal model of depression (Willner 1995).

The purpose of the present experiment was therefore to determine if a drug regimen of repeated administration of *d*-amphetamine, which had been shown previously to produce anhedonic effects on ICSS responding (Leith and Barrett 1976, Cassens et al. 1981), could induce a state of withdrawal which would also reduce an animal's responding for a natural reward (4% sucrose solution), as measured by a Progressive Ratio (PR) schedule. The PR schedule of reinforcement has seen widespread use as a sensitive technique to measure

motivation to respond for a variety of different reinforcers, including sweet solutions (Hodos 1961), electrical brain stimulation (Hodos 1965) and drugs (Roberts et al. 1989, Markou et al. 1993, Mendrek et al. 1998). Under this schedule, subjects are required to increase their operant responding for a fixed reward until they reach a "break point" which determines the maximal amount of effort animals will expend to procure the desired rewarding stimulus, with the break point providing an objective measure of the subject's motivation (Hodos 1961).

***Methods*:**

Subjects

Thirty male Long-Evans rats, (Charles River, Quebec) weighing 300-350g at the beginning of the experiment, were housed individually in a temperature regulated colony (21±1°C) under a 12-hr light-dark cycle (lights on at 07:00 hrs); all training and testing took place during the light phase. Three subjects failed to complete the experiment, two due to illness and one failed to meet training criteria, and so their data were not included. All experiments were conducted in accordance with the Canadian Council on Animal Care guidelines for work with laboratory animals.

Apparatus

Experiments were carried out in four Plexiglas test cages (25 × 25 × 25 cm) enclosed within sound and light attenuating chambers. Each test cage was fitted with a removable response lever, which projected 5 cm above the wire grid test cage floor; it was removed

whenever subjects were tested for their free consumption of sucrose (Experiment 2). All test cages were also fitted with a lick activated solenoid valve which provided rats with a drop of sucrose solution each time their tongue contacted the tip of the metal spout. The valve regulated the volume of the drops of sucrose to 0.01 ml, so that for the standard reinforcement of 0.50 ml sucrose solution, the animal was required to lick 50 times. A small light (2.8-W) fitted in the roof of the chamber was turned on to designate the start of each training session, which coincided with activation of both the response lever and lick-activated dispenser. The concentration of the sucrose solution was set at 4% (w/v) because previous experiments (Barr and Phillips 1998) had shown that this concentration was optimal for detecting slight shifts (either increases or decreases) in the reinforcing value of the sucrose reinforcer, when using a PR schedule.

Operant Training

All subjects were given an initial 48-hr exposure to the 4% sucrose solution in their home cages, while water and food were also available *ad libitum*. After 24 hrs, rats were given two 30 min habituation sessions in the test chambers, during which both the response lever and drinking spout were removed. The lick-activated fluid dispensers were returned to the test cages and subjects could freely consume the sucrose solution for 1-hr on each of the next four days. The rats were then placed on an intermittent feeding schedule depriving them of food and water for 20-hr before being placed in the test cages; following testing , subjects were returned to their home cage and given *ad lib* access to both food and water. Throughout the remainder of the experiment rats were trained on alternate days. Response levers were introduced into the test cages and the rats were placed on a 1-hr session Fixed Ratio (FR) schedule of responding as follows: 3 days at FR1/0.05 ml of 4% sucrose solution, 3 days at FR3/0.15 ml sucrose and 3 days

at FR10/0.40 ml sucrose per reinforcement. A minimum of 100 responses per session was required at each level of training, and any rat that did not meet this criterion received additional training sessions until it performed to the required standard.

After completing FR training, subjects were placed on a PR schedule of reinforcement whereby successive reinforcements could be earned according to the following number of bar-presses: 1, 3, 6, 10, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145. The final ratio achieved represented the "break point" value, and the session ended when rats failed to reach the next bar-press criterion within 1-hr. Each reinforcement was set at 0.50 ml of 4% sucrose solution, as this value sustained high levels of operant responding while minimizing any effects of satiety on the PR data. Subjects received eight PR sessions; levels of responding were generally stable (i.e. typically varying by less than ± 1 break point per session) by the fourth session. When training for all animals was completed, subjects were divided into two groups based on their average break points for the last four PR training sessions. One group served as controls (n=12), while the other group (n=15) was placed on a *d*-amphetamine drug administration schedule.

Drug Administration

Escalating doses of the drug *d*-amphetamine sulfate (obtained from SmithKline-Beecham, Oakville, Ontario) were administered to one group (n=15) of rats based on a schedule modified from one shown previously to affect thresholds of ICSS responding (Leith and Barrett 1976). In a pilot study, animals subjected to the level of food and water deprivation used in training were more vulnerable to the toxic effects of high doses of *d*-amphetamine, and thus the final day of drug administration was modified. In this modified schedule, rats were injected IP three times per day (9 am, 5pm, 12 pm), starting with a dose of 1 mg/kg and escalating by 1

mg/kg on each subsequent dose, for the first three days for 9 doses. On the fourth day, subjects received one final dose (10 mg/kg) at 9 am; animals therefore received a total of ten injections over the four day period. 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 behaviour, 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 9 am injection so that any decreases in body weight would be compensated for by adjusting the dose. Control subjects were injected with isotonic saline under the same schedule as rats in the *d*-amphetamine group.

Operant Testing (Post treatment)

After the final dose of *d*-amphetamine, subjects in both groups were again deprived of food and water for 20-hr before being tested on the PR schedule for the 4% sucrose solution reward, as described above. Thereafter, subjects were tested every subsequent 48 hrs for an additional five sessions.

Experiment 4B: Free sucrose consumption

A separate group of six male Long Evans rats (300-400g) were given access to the 4% sucrose solution in their home cages, for 48 hrs. Following the familiarization period, subjects were given two 30 min sessions in the test cages with no access to sucrose, and then placed on the same 20-hr food and water deprivation schedule as the operant-trained rats. Subjects received four 1-hr free consumption sessions in order to obtain a baseline measure, and were

then subjected to the same ten-dose drug or vehicle injection regimen. Animals were subsequently tested for their free consumption of 4% sucrose solution at both 1 and 3 days after the tenth injection.

Data Analyses

Baseline operant response rates were averaged for subjects over their last four training sessions. The break point values obtained in the PR test sessions were subjected to a two-factor ANOVA. Body Weights and Inter-Lick-Intervals were also subjected to two-factor ANOVAs, while latency measures were compared using the Students t-test. For Experiment 2 (free sucrose consumption), data were analyzed by a within-subjects repeated measures ANOVA. Post-hoc comparisons, when appropriate, were made using a test of simple main effects.

Results:

Experiment 1

Figure 4.1 shows the break point scores (mean ± SEM) for vehicle and *d*-amphetamine administered groups, from both pre-drug test sessions and during drug withdrawal. Administration of an escalating series of ten *d*-amphetamine injections over four days produced a significant decrease in the break point value of responding for a 4% sucrose solution, as measured by a PR schedule, during the period of initial withdrawal on post-drug test sessions 1 and 2 (i.e. 1-3 days). The break point values for the *d*-amphetamine group were a mean of 5.3 ± 0.5 and 4.9 ± 0.7 for the 1 and 3 days sessions respectively. In contrast, the break point values

for the vehicle control group were a mean of 8.7 ± 0.6 and 8.8 ± 0.8 respectively. Analysis of the data by a two-factor ANOVA indicated a significant group effect [$F(1,25) = 4.54$, $p < 0.05$], as well as a significant effect of time of withdrawal [$F(6,150) = 4.55$, $p < 0.001$] and an interaction of group × time [$F(6,150) = 5.45$, $p < 0.001$]. A simple main effects post-hoc comparison between groups revealed a significant difference between the two groups on the first two test post-drug sessions (1 and 3 days withdrawal). By the third post-drug test session (5 days withdrawal) the break point scores were no longer statistically significant.

Table 4.1 contains data on the average inter-lick interval (computed from 50 licks per reinforcement) when consuming the sucrose solution during the PR test sessions. Values were averaged across all reinforcements per test session for each rat, and analysis of the data by a two-factor ANOVA showed a trend towards a significant group effect [$F(1,25) = 2.95$, $p < 0.10$] but no effect of time of withdrawal or interaction.

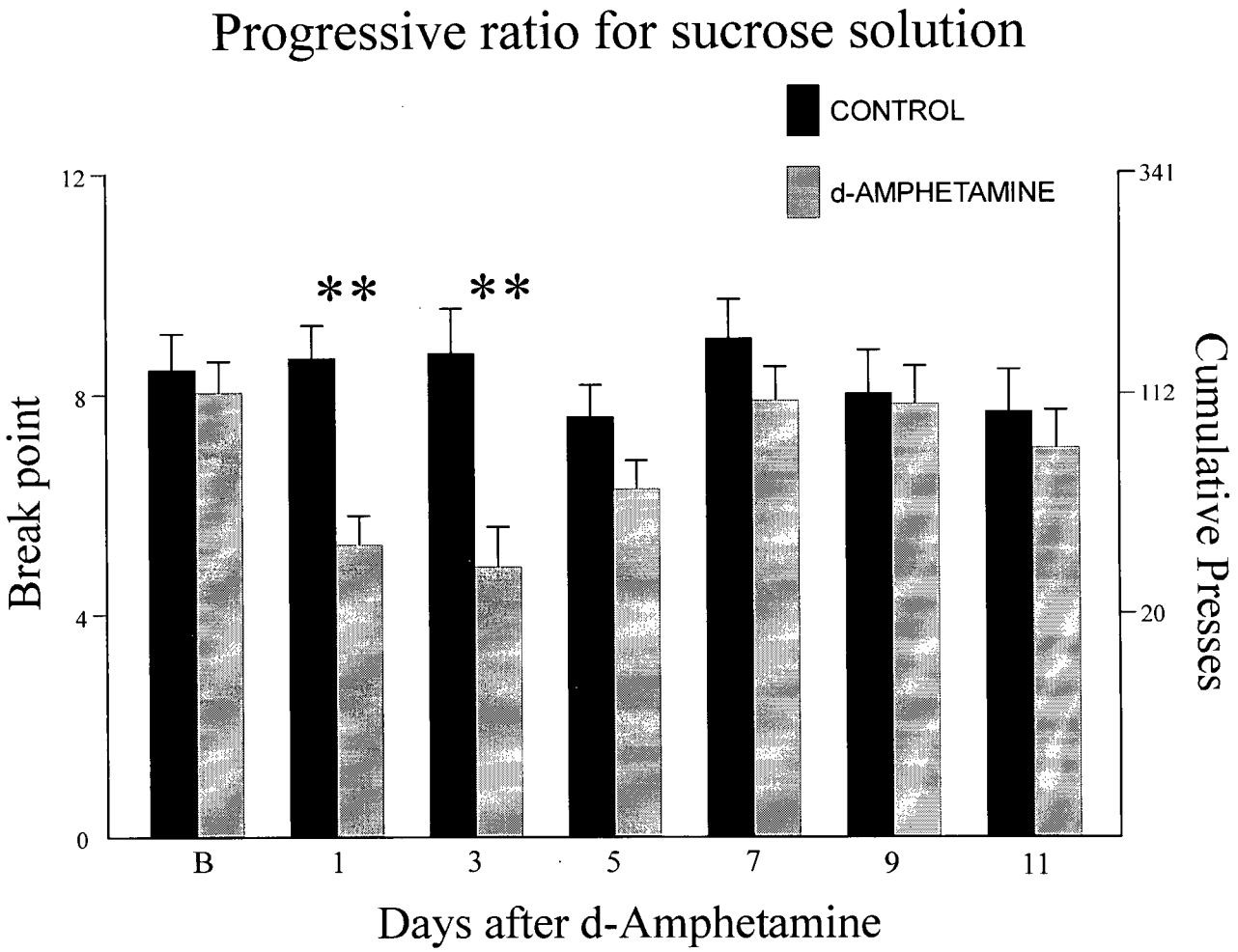


Fig. 4.1 The effect of *d*-amphetamine withdrawal on responding for a 4% sucrose solution under a progressive ratio schedule of reinforcement, across different test sessions. Values represent the break points (±S.E.M.) and cumulative total responses of both groups (n=15, drug [grey bars]; n=12, control [black bars]), during baseline condition (B) and after drug administration (1-11 days). The stars indicate a significant difference between groups ($p < 0.001$=**).

Table 4.1 The effect of *d*-amphetamine withdrawal upon the Inter-Lick-Intervals taken by rats when consuming a 0.50ml sucrose solution reinforcer (50 licks per reinforcement). Intervals (±SEM), measured in seconds (s), are averaged with each rat for all reinforcements per session, and for all rats per group (n=15, drug; n=12, control). The stars denote a significant difference ($p < 0.10$).

Days after *d*-Amphetamine	Drug (s)	Vehicle (s)
Pre-drug baseline	0.34 (0.04)	0.27 (0.05)
1	1.60 (0.92)*	0.28 (1.03)
3	1.74 (0.77)*	0.37 (0.86)
5	0.31 (0.04)	0.21 (0.05)
7	0.33 (0.07)	0.30 (0.08)
9	0.33 (0.08)	0.27 (0.07)
11	0.27 (0.04)	0.30 (0.04)

Further analysis of these data revealed that the differences in inter-lick intervals between groups on the two test sessions following drug administration were due to pauses in consumption of the solution by animals treated previously with *d*-amphetamine rather than a consistent increase in the inter-lick interval.

Latencies to initiate operant responding following commencement of the test on the first two post-drug sessions differed significantly [$t(14) = 6.99$, $p < 0.01$] between the *d*-amphetamine and vehicle-treated groups. The latency values, in seconds, were a mean of 148.1 ± 174 and a mean of 14.6 ± 9.6 for the *d*-amphetamine and vehicle groups, respectively. Latencies to begin responding for the next five reinforcements on post-drug test days 1 and 3, referred to as Post-Reinforcement Pauses [Table 4.2], were not significantly different [$F(4,88) < 1$, NS]. Table 4.2 also includes the durations taken by each group to attain each of the first five reinforcements (representing the averaged break point value for the *d*-amphetamine treated group) on days 1 and 3 of withdrawal, and these data demonstrate that while rats treated with *d*-amphetamine achieved lower break points, they attained each reinforcement at approximately the same rate as vehicle treated rats, [$F(4,88) = 1.7$, NS].

The weights of animals during and after drug administration are shown in Figure 4.2. These data were analyzed by a two-factor ANOVA which showed a significant between-group difference [$F(1,25) = 8.22$, $p < 0.01$] as well as significant effects of day [$F(6,150) = 94.75$, $p < 0.001$] and group × day interaction [$F(6,150) = 21.43$, $p < 0.001$]. Administration of *d*-amphetamine therefore caused long lasting decreases in the weights of subjects, which were still evident when animals were weighed three weeks later, [$t(14) = 4.40$, $p < 0.01$].

Table 4.2 The effects of drug treatment on both the Post-Reinforcement Pause and the Time to attain each reinforcement, for the first five reinforcements. Values represent the means (± SEM) of the combined average scores from post-drug test days 1 and 3. No significant effects were observed.

Response / Reinforcement Ratio	Post Reinforcement Pause (s)		Time to attain each reinforcement (s)	
	DRUG	VEHICLE	DRUG	VEHICLE
1	24.1 (5.5)	14.9 (6.0)	0.01	0.01
3	79.7 (45.9)	22.3 (49.5)	15.2 (5.5)	6.6 (6.0)
6	15.2 (4.2)	18.2 (4.4)	91.1 (34.1)	22.9 (36.9)
10	32.1 (5.6)	17.7 (5.6)	73.3 (24.3)	84.8 (25.3)
15	48.3 (11.7)	20.6 (10.6)	328.7 (95.1)	135.9 (95.0)

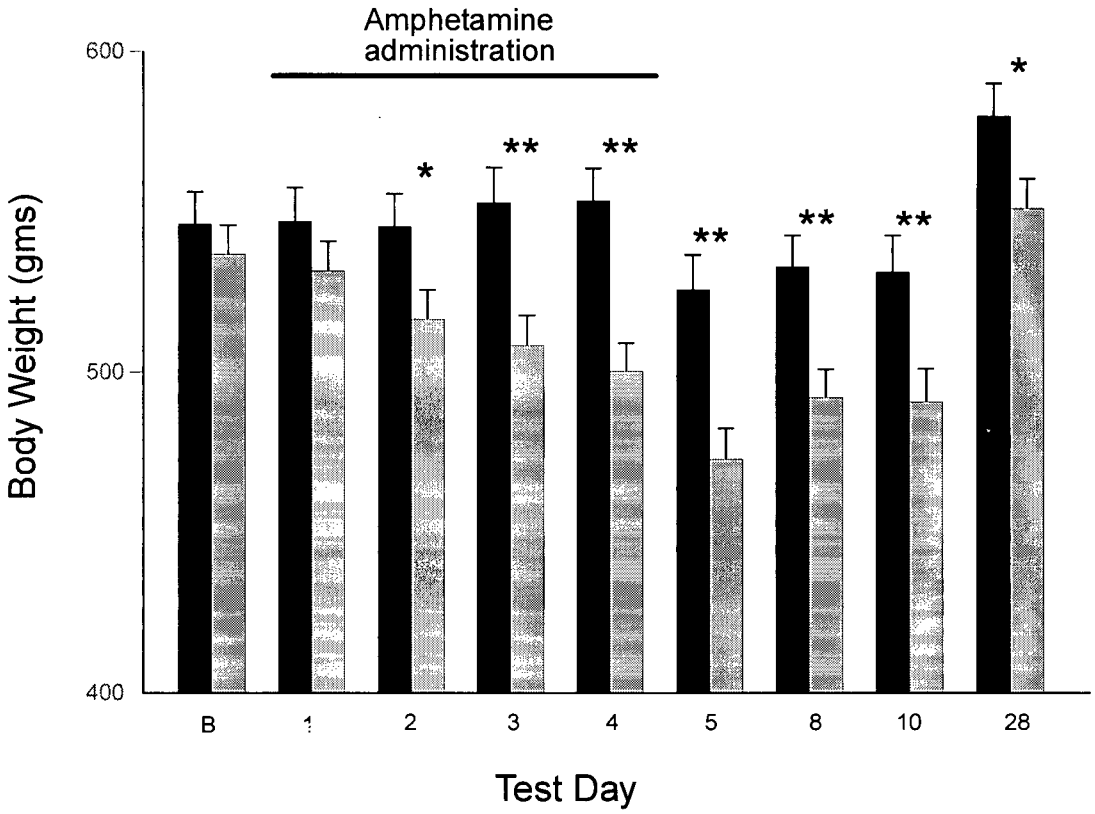


Fig. 4.2 Body weights of subjects in the drug (n=15) [grey bars] and vehicle control (n=12) [black bars] groups prior to *d*-amphetamine administration (B), during *d*-amphetamine administration (test days 1-4), and for up to three weeks after *d*-amphetamine administration (test days 5-28). Data are the body weights (±S.E.M.) measured at 9 a.m. each morning. Stars indicate a significant difference ($p < 0.01$=*, $p < 0.001$=**).

Experiment 2

Figure 4.3 illustrates the mean volumes of 4% sucrose solution consumed by six rats in four pre-drug free consumption baseline tests, and in tests at 24 and 72 hrs following *d*-amphetamine administration. Analysis of these data by a repeated measures ANOVA indicated no treatment effect [$F(1,30) = 0.81$, NS], confirming withdrawal following repeated injections of *d*-amphetamine had no effect on consumption of 4% sucrose when it was freely accessible.

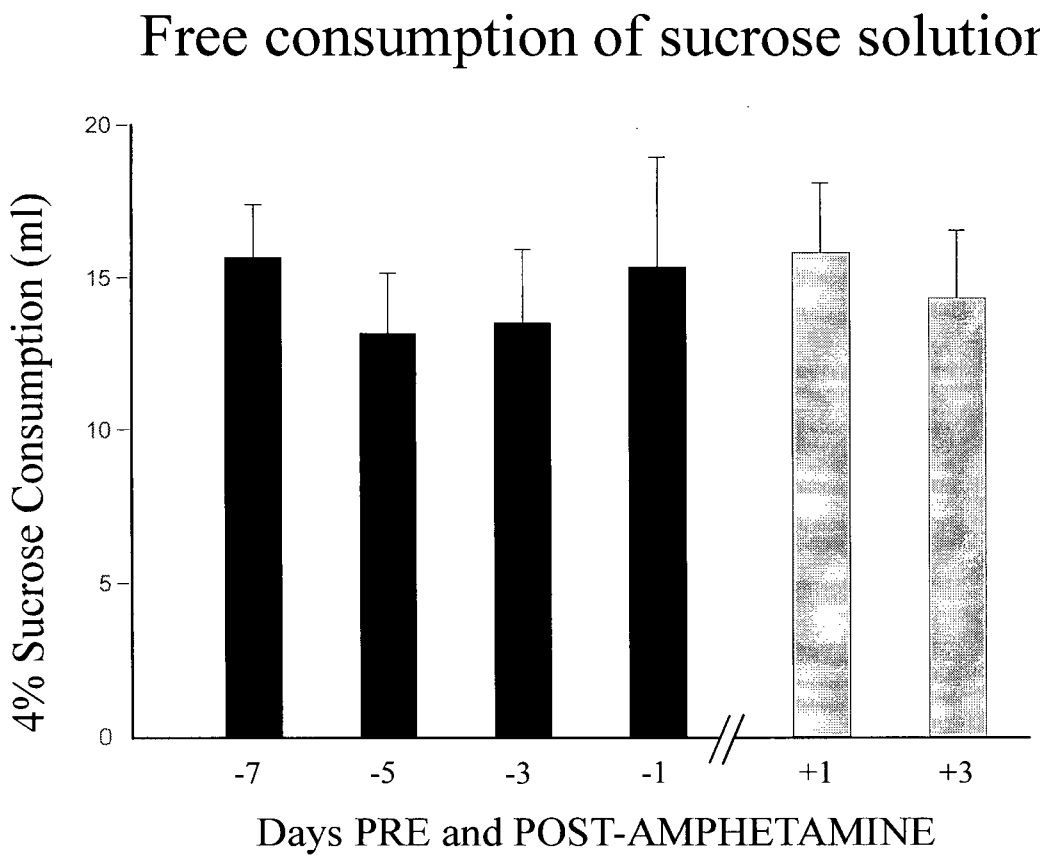


Fig. 4.3 The effect of *d*-amphetamine on free consumption of a 4% sucrose solution from a lickometer, including the four baseline sessions prior to drug administration. Each session lasted for 1-hr (n=6). No significant differences were observed.

Discussion:

The present study investigated the effects of an escalating dose regimen of *d*-amphetamine administration (Leith and Barrett 1976) on rats' motivation to work for a natural reward in a post-drug withdrawal period that extended from 1-11 days. The main finding was that subjects pre-treated with the drug exhibited decreased break points on the first and third day post-drug tests when responding for a 4% sucrose solution on a PR schedule of reinforcement. These results indicate that the escalating dose drug regimen used in the current study which was chosen to represent a "binge-like" pattern (Segal and Kuczenski 1997), may subsequently produce a period of anhedonia which may reflect either a decrease the reinforcing properties of naturally rewarding stimuli or a reduction in motivation to obtain such rewards.

While the induction of a state of anhedonia represents the most likely explanation for the current findings, a number of alternative hypotheses need to be refuted. Firstly, it is unlikely that the reduced motivation shown by rats is due to the anorectic effects of the drug (Caul et al. 1988) causing a decrease in the motivational value of the sucrose, because Experiment 2 failed to observe any effect of the same drug regimen on free consumption of a 4% sucrose solution. Secondly, it is also improbable that the reduction in break points of animals in the post-*d*-amphetamine condition represents a motoric deficit; although decreased levels of locomotor activity are commonly seen during psychostimulant withdrawal (Tonge 1974; Schreiber et al. 1976; Hitzemann et al. 1977; Paulson et al. 1991; Pulvirenti and Koob 1993; Schindler et al. 1994; but see Kokkinidis et al. 1986), numerous studies using ICSS protocols have elegantly demonstrated, through the use of rate-independent techniques, that operant responding for reinforcement during psychostimulant withdrawal is dependent on changes in reward value,

rather than performance factors (Cassens et al. 1981, Markou and Koob 1992). Furthermore, the increase in inter-lick intervals observed by animals during the first 3 days of drug withdrawal was characterised by short duration intervals interspersed with clear pauses (> 3 secs) in consumption of the sucrose solution: these results do not resemble the typical pattern of a uniform increase in inter-lick intervals, without pauses, seen in rats that are administered doses of neuroleptics sufficient to generate motor impairments (Fowler and Mortell 1992).

The decrease in break points observed in *d*-amphetamine-treated subjects in withdrawal may represent a reduction in energy allocation and operant response maintenance (anergia) as is observed following decreased function in the mesoaccumbens and mesostriatal dopamine systems (Salamone 1992). This explanation is unlikely for several reasons. Previous studies which have investigated the impact of different variables in PR paradigms have found that break points, in untreated rats, are far more sensitive to alterations in the reinforcing value of the food reward (such as reward size) than changes in effort requirements (such as the height of the response lever) (Skjoldager et al. 1993). Additionally, a recent study by Sokolowski and Salamone (1998) has demonstrated that dopamine depletions in the nucleus accumbens only induce deficits in operant behaviour on schedules which generate high levels of responding, such as an FR5 schedule, but not in schedules producing moderate levels of responding, including a VI 30 schedule. Subjects in the present study had 1 hour to attain each reinforcement, and break points in withdrawn rats were relatively low, suggesting that the current operant schedule placed only a low energy demand on subjects. Evidence from the present study also implies that rats were not anergic, because the *d*-amphetamine-treated rats did not show an increase in the post-reinforcement pause or a decrease in their rate of responding, as measured by latency to attain each successive reinforcement. Therefore these data indicate that the *d*-amphetamine-treated and

control subjects responded with equal vigor. Other recent data from our laboratory have also shown that *d*-amphetamine withdrawal impaired certain motivational components of male rat sexual behaviour, whereas these rats displayed high levels of physically demanding copulatory activity for a 25 min period, consistent with an absence of either motoric or anergic deficits (Barr et al. 1998).

The PR procedure has been used previously in our laboratory to show that reductions of the concentration of the sucrose solution, or a decrease in the level of food and water deprivation, both produced corresponding decreases in break points (Barr and Phillips 1998). This procedure therefore provides a reliable technique for assessing changes in motivation to respond for a natural reward, and hence the present data strongly imply that following *d*-amphetamine withdrawal rats experience significant reductions in their motivation to obtain a previously preferred reward. This reduced motivation may correspond to what Berridge and his colleagues (Berridge and Valenstein 1991, Robinson and Berridge 1993, Berridge 1996) refer to as "wanting", as distinct from "liking", which is more closely related to alternative hedonic processes. Indeed, in a recent study (Potts et al. 1997) depressed patients with anhedonic symptoms showed the same ability to discriminate the sensory qualities of sweet solutions as non-depressed control subjects. In the present study, high levels of motivation were not required to maintain the reflexive lick response necessary to consume the freely available sucrose solution, whereas in the PR paradigm the motivation to attain the next reinforcement had to be maintained for up to one hour. We interpret the lack of effect of drug withdrawal on the free consumption of sucrose as evidence for normal hedonic processes and attribute a motivational deficit to the finding that these rats were unable to maintain the level of responding required to maintain higher ratio reinforcements. Two other behavioural measures recorded during the

experiment provide additional support for this hypothesis. The increase in latencies taken by subjects to begin responding once the test sessions had begun, as well as the pauses between bouts of licking, are consistent with a decrease in motivation to obtain the sucrose reward.

Previous experiments have examined the effects of a similar regimen of drug administration on ICSS responding and observed results consistent with those from this study. In an early study, Leith and Barrett (1976) demonstrated that amphetamine withdrawal depressed the facilitation of ICSS responding normally seen by low doses of *d*-amphetamine, and that this effect lasted for approximately four days. Similarly, Cassens et al (1981) observed an increase in the current intensities required to sustain ICSS responding, with a return to baseline within 120 hrs after the final drug injection. Thus, there appears to be little difference between the effects of amphetamine withdrawal on ICSS responding and operant responding for naturally rewarding stimuli such as a sucrose solution. The present findings therefore provide additional support for the use of ICSS paradigms to investigate changes in the responsiveness of neural reward systems in models of drug withdrawal and depression (Geyer and Markou 1995, Koob 1995).

In a related study, Markou and Koob (1991) employed a drug self-administration procedure to establish post-cocaine anhedonia measured by a significant increase in ICSS current threshold. Current thresholds were elevated for 5 days post-drug treatment. Pretreatment with the tricyclic antidepressant desmethylimipramine returned ICSS thresholds to pre-drug values 12 hours after cessation of cocaine self-administration.

The post-drug depression of ICSS responding associated with *d*-amphetamine withdrawal has also been shown to be responsive to the tricyclic antidepressants imipramine and amitriptyline. Kokkinidis et al. (1980) demonstrated a mitigation of the effects of a ten day amphetamine regimen by these tricyclics, when ICSS responding at sites in the substantia nigra

was measured. It is not known how effective other classes of antidepressant drugs, such as the selective serotonin reuptake inhibitors, or other antidepressant therapies (such as electro-convulsive therapy (White and Barrett 1981) or REM sleep deprivation) might be in animal models of psychostimulant withdrawal-induced anhedonia. It would therefore be of interest to examine the effects of antidepressant treatment on the suppressed responding for a sucrose solution, on a PR schedule, following the escalating dose of *d*-amphetamine protocol used in the present experiment.

One problem facing such research is the short duration of the anhedonic effects which are typically observed in animals. With most drug regimens producing observable effects for only a few days, and at most a couple of weeks, this may not allow for the development of a theoretically viable model with which to examine the effects of many antidepressant treatments, which require a minimum of two to three weeks before changes in mood are seen in humans (Post et al. 1987) and certain animal models of depression (Willner et al. 1987).

Much important research has focused on individual differences in susceptibility to drugs of abuse (Piazza and LeMoal 1996), however to-date little has been done to identify factors which might predispose certain subjects to display more prolonged and severe psychostimulant withdrawal symptoms. Identification of such factors might lead to the development of an animal model of anhedonia in which the observable effects last for notably longer than in current models. Alternatively, repeated bingeing schedules such as those engaged in by human cocaine addicts (Gawin and Kleber 1986; 1988), or dosing with other types of psychostimulants (such as with MDMA, which has produced long-lasting deficits in monkeys on PR performance for natural rewards (Frederick et al. 1995)) may also provide opportunities for the generation of a longer duration animal model of anhedonia.

In conclusion, the present study has provided additional support for animal models of psychostimulant withdrawal-induced anhedonia by observing reductions in motivation for a natural reward by rats, as assessed by performance on a PR schedule of reinforcement. The duration of the effects, including measures of latency and inter-lick intervals, lasted approximately the same duration as previously reported alterations of ICSS responding, and so provide further support for ICSS techniques as a tool for the measurement of reinforcement. Further developments in the use of the PR paradigm as an instrument for assessing anhedonia in psychostimulant withdrawal may be related to its capacity to measure reversals of drug induced anhedonia by antidepressant treatments.

EXPERIMENT 5:

EFFECTS OF WITHDRAWAL FROM AN ESCALATING DOSE SCHEDULE OF D-AMPHETAMINE ON SEXUAL BEHAVIOR IN THE MALE RAT

Synposis:

The present study sought to determine the effect of withdrawal from an escalating dose schedule of *d*-amphetamine on sexual behavior in male rats. Rats were introduced into testing chambers and, after a 5-min period during which locomotor activity was monitored, receptive females were presented. Copulation was observed for 25 min. Tests were conducted every 5 days until stable levels of sexual behavior were obtained. Upon repeated testing, male rats displayed an increase in the amount of activity associated with the anticipation of an estrous female. Half of the male rats were then subjected to a four day, escalating dose schedule of *d*-amphetamine administration, while half received vehicle. 12 hours after the final drug injection, subjects were tested for sexual behavior. Withdrawal from the drug was associated with decrements in several motivational components of sexual behavior, including decreased anticipatory locomotor activity and increased post-ejaculatory intervals, while consummatory measured remained largely unaffected. This pattern of sexual deficits resembles those seen in human depressive disorders, and therefore provides additional support for the use of psychostimulant withdrawal as a rodent model of depression.

Introduction:

Withdrawal from binge-like doses of various psychostimulant drugs, including cocaine and *d*-amphetamine, reliably induces a state of dysphoria in humans (Satel et al. 1991; Volkow et al. 1991; Pathiraja et al. 1995). This state is characterized by symptoms which include anhedonia, anergia and anxiety, and may last from weeks to months (Gawin and Kleber 1986; 1988). The symptoms associated with psychostimulant withdrawal bear a strong resemblance to those of major depressive disorder (MDD), to such an extent that the DSM-IV contains a specific category for the diagnosis of substance-induced mood disorders to differentiate them from isomorphic endogenous depression (American Psychiatric Association 1994).

Administration of binge-like doses of psychostimulants to animals, followed by a period of drug withdrawal, produces many of the same symptoms that are observed in humans who experience drug withdrawal (Markou and Koob 1991). Extensive research has demonstrated that withdrawal from either cocaine or *d*-amphetamine in rodents generates a period of anhedonia, which is most commonly assessed by alterations in responding for reinforcing electrical brain self-stimulation (Leith and Barrett 1980; Wise and Munn 1995). Decreased locomotor activity is another commonly observed effect of withdrawal from psychostimulants in rats (Hitzemann et al. 1977), and may model anergia, while withdrawal-induced anxiety has also been demonstrated through the use of sophisticated behavioral paradigms (Mutschler and Miczek 1998). This combination of withdrawal-associated behavioral sequelae and their resemblance to the symptoms of MDD in humans has led to the utilization of psychostimulant withdrawal as a rodent model of depression.

Most animal paradigms of depression are developed with the hope that they will accurately model many of the major symptoms of MDD. One widely reported human symptom of MDD is a decrease in sexual behavior, noted as either a loss of libido or problems in sexual functioning (Casper et al. 1985; American Psychiatric Association 1994; Baldwin 1996). Despite the high incidence with which this symptom occurs in human depressives, there have been relatively few studies in which animal models of depression have been used to examine alterations in the sexual behavior of sexually experienced subjects. Several deficits in both the appetitive and consummatory components of sexual behavior have been reported in rats with limited or no copulatory experience using other models of depression (Edwards et al. 1990; Neill et al. 1990; D'Aquila et al. 1994; Vogel et al. 1996).

In the present study, the psychostimulant-withdrawal model of depression was evaluated by using rats that were well-trained and exhibited high levels of sexual activity before the drug treatment conditions were implemented. Although the use of sexually naive rats is valid, the study of sexual behavior in experienced rats in this context has several advantages. First, it increases the face validity of a drug induced model of depression, because many depressed patients have had sexual experience prior to the time of their period of sexual dysfunction - emphasis should be placed on studying the disturbance of established sexual behavior, rather than its acquisition. Second, the activity associated with the anticipation of a receptive female in well-trained rats can provide a valuable measure of sexual motivation. A number of reports have demonstrated the utility of this approach by using bilevel chambers for the study of male sexual behavior (Mendelson and Pfaus 1989). An increase or decrease in the number of level changes made prior to the presentation of a receptive mate in these chambers is hypothesized to reflect an increase or decrease in motivation, respectively.

The present study assessed the psychostimulant-withdrawal model of depression by examining the effect of *d*-amphetamine withdrawal on sexual behavior in experienced male rats. A modified unilevel testing apparatus, analogous to the bilevel chamber, was employed in order to provide a more detailed evaluation of male rat sexual motivation.

Methods:

Male Long-Evans rats (225-250g; Charles River, QU) were group housed in wire cages (18 X 25 X 65 cm) and entrained on a reverse light-dark cycle (light off 7 a.m. - 7 p.m.). One week after arrival, male rats were housed individually in plastic cages with bedding (Carefresh, Absorption Corp., Bellingham, WA) for the remainder of the experiment. The colony room temperature was approximately 20°C and rats had unlimited access to food (Purina Rat Chow) and water. Training and testing occurred during the middle third of the dark phase.

Female rats, housed in a separate colony, were anesthetized with ketamine hydrochloride (80 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and bilaterally ovariectomised at least four weeks prior to testing. Sexual receptivity in the stimulus females was induced by subcutaneous injections of estradiol benzoate (10 μg) and progesterone (500 μg), 48 and 4 h, respectively, before each test session.

Throughout the experiment, male rats were tested every five days for sexual behavior in Plexiglas unilevel testing chambers. The chambers (24 x 32 x 48 cm) were fitted with a central Plexiglas partition (32 cm) creating a perimeter "racetrack" area around which the female could pace sexual behavior. Like the bilevel chambers, these chambers encourage the exposure of the

rats' flanks to the experimenter during copulation thereby facilitating scoring of sexual behavior (Mendelson and Pfaus 1989). Another advantage to this simple design is the relative ease of introducing and removing rats from the chamber. The chambers were equipped with infrared photobeam emitter/detector pairs (one beam across its length on each side of the partition; 3 beams across its width; 12 cm apart) to monitor activity. The number of side changes (SCs), counted as the complete movement from one side of the chamber to other side of the partition, were recorded automatically by computer (2 Hz scan rate).

At the beginning of each test session, male rats were introduced into the chambers and activity was recorded for 5 min. Following the introduction of a sexually receptive female, copulatory behavior was monitored for 25 minutes. Each session was videotaped and subsequently scored for standard measures of sexual behavior using a computer and appropriate software (courtesy of Sonoko Ogawa). Testing chambers were washed thoroughly between tests to remove residual odors/pheromones.

Once consistent measures of anticipatory and copulatory behavior were obtained (i.e. anticipatory SC (< 10% variation), intromission latencies (IL) < 5 min, and ejaculation latencies (EL) < 10 min for 3 consecutive days), male rats were assigned to either the *d*-amphetamine (AMP; n=9) or saline vehicle (SAL; n=9) control groups. Assignment was based upon the average of their EL over the last four test sessions, such that there was an alternating appointment of rats into each group down a rank-ordered list of ELs. Analyses of variance (ANOVAs) were conducted on the SC, IL, and EL from the last test session to ensure there were no statistical differences between groups.

On the morning following the last baseline test session, male rats were placed on a 12-injection regimen over 4 days. Rats received three injections (1 mL/kg) per day: 9 a.m., 4-5

p.m., and 11 p.m.-1 a.m. (i.e. 7-8 h apart) of either *d*-amphetamine (SmithKline-Beecham) dissolved in saline or saline vehicle (Baxter Corp., Toronto, ON).

The AMP group received escalating doses of drug starting at 1 mg/kg and incrementing by 1 mg/kg every injection to a final dose of 12 mg/kg. This regimen has been shown to induce behavioral withdrawal in rats (Leith and Barrett 1976).

Sexual behavior testing continued at 5 day intervals so that the first post-regimen test occurred approximately 12 h after the last injection. After two more tests of sexual behavior, the identical injection regimen was carried out a second time. Subsequently, rats were tested three more times for sexual behavior.

A separate group of rats (n=9) were used as anticipatory activity controls and tested every five days for a total of 7 sessions. These rats were introduced into the chambers and SCs were monitored for the first 5 min of each test.. Rats were left alone in the chambers for an additional 25 min and then returned to their home cages. As earlier studies have demonstrated that the increased locomotor activity that occurs before the presentation of the estrous female provides a reflection of previous sexual experience, it was hypothesized that this group would not demonstrate a conditioned increase in SCs across the 7 training sessions.

Results:

Precopulatory Anticipatory Activity

Figure 5.1A shows the mean (± SEM) anticipatory side changes for the control, vehicle and drug groups during the 5 min precopulatory period for the seven training sessions. An ANOVA of the data revealed a significant group effect [$F(2,24) = 5.19, p < 0.05$], as well as a significant effect of training session [$F(6,144) = 12.48, p < 0.001$] and an interactive effect of group × training session [$F(12,144) = 4.12, p < 0.001$]. Post-hoc analysis of the data demonstrated that the control group, which was never exposed to the estrous females, maintained a constant number of anticipatory side changes throughout the seven training sessions. In contrast, the drug and vehicle groups, which were given the opportunity to copulate for 25 min following the 5 min appetitive period, exhibited a gradual increase in anticipatory side changes which gained significance from both the control group and their own first training session by the fourth session. These results support the use of precopulatory side changes in the unilevel chambers as an index of appetitive sexual behavior in the male rat.

The number of anticipatory side changes during the final training session and the six testing sessions (in which the *d*-amphetamine drug regimen was administered twice) for both the drug and vehicle groups is shown in Figure 5.1B. Analysis of the data by an ANOVA revealed a significant effect of test session [$F(6,96) = 9.81, p < 0.001$] as well as an interactive effect of group × test session [$F(6,96) = 10.58, p < 0.001$]. Further analysis of the data with the appropriate post-hoc test confirmed a significant reduction in the number of anticipatory side changes by rats on sessions following both periods of *d*-amphetamine administration. The effect of drug withdrawal was limited to the single test session immediately following each of the two series of administration of *d*-amphetamine, with a return to baseline activity by the next test session.

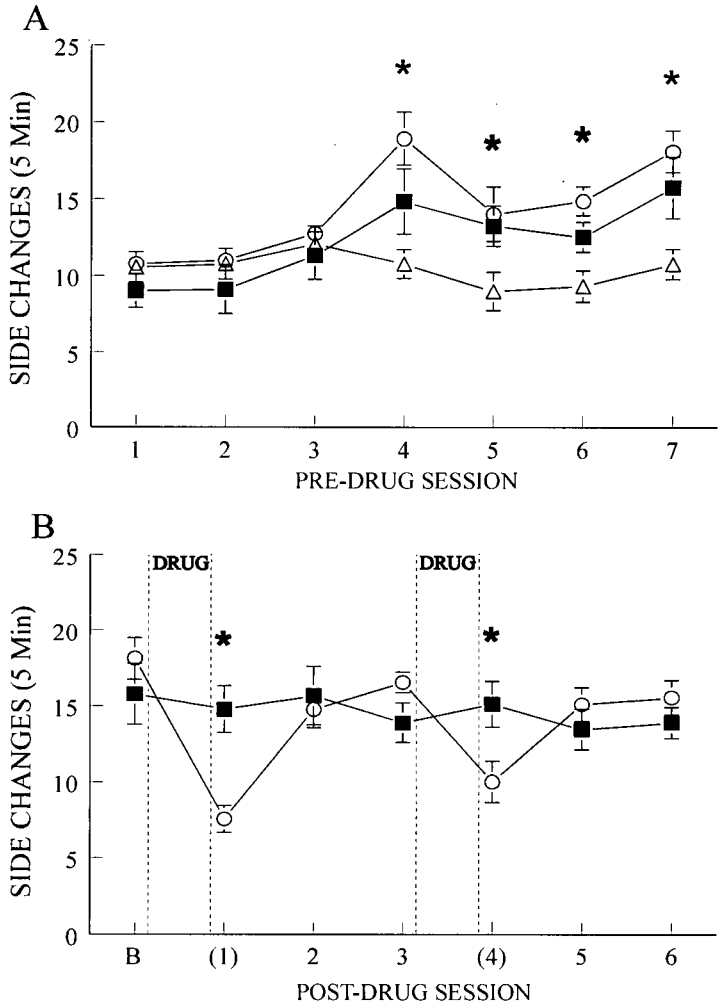


Fig 5.1. The effect of withdrawal from an escalating dose schedule of *d*-amphetamine on anticipatory locomotor activity. **A**: Values represent anticipatory side changes (±S.E.M.) after repeated training sessions in which both drug (O) and vehicle (■) groups are allowed to copulate for 25 min after the 5 min anticipatory period, while the control (Δ) group is not provided with access to estrous females. Stars indicate significantly different from control group, ($p < 0.05$=*, $p < 0.01$=**). **B**: effect of withdrawal from *d*-amphetamine on anticipatory side changes (parentheses indicate test 12 hr after withdrawal from drug regimen). Tests occur every 5 days. Stars indicate a significant difference between groups, ($p < 0.05$=*).

Sexual Behavior

Withdrawal from *d*-amphetamine produced no significant effect on the mount latencies in the rats, [Fig. 5.2A] which were defined as the latency until the animal's first mount or intromission. Intromission latencies in both drug and vehicle groups were short (averaging 5-30 secs), reflecting the sexual experience of the subjects. An ANOVA on post-ejaculatory interval (PEI) data, another appetitive measure of sexual behavior, revealed a significant group effect [$F(1,13) = 4.39, p < 0.05$], an effect of test-session [$F(6,78) = 3.79, p < 0.005$] and also an interactive effect of group × test session [$F(6,78) = 3.06, p < 0.01$]; post-hoc analysis of these results showed that there was a significant increase in the PEIs on each test session following withdrawal from *d*-amphetamine, and that latencies returned to normal by the next test session [Fig. 5.2D].

No significant effect of drug withdrawal was found on either the ejaculation latencies [Fig. 5.2C] or the number of ejaculations, although a significant effect of test-session was observed on the number of ejaculations, [$F(6,96) = 3.51, p < 0.005$] [Fig. 5.3C]. In contrast with an absence of any effect of withdrawal from *d*-amphetamine on ejaculation frequencies, a significant group effect was found for intromission frequencies (defined as the total number of intromissions during the 25 min) throughout the entire session [Fig. 5.3B], [$F(1,16) = 6.86, p < 0.05$] and also an effect of test session, [$F(6,96) = 2.92, p < 0.05$], although the interactive effect was marginally non-significant, [$F(6,96) = 1.67, p = 0.14$]. Similarly, a large group effect was noted for mount frequencies (which were defined as the total number of mounts in the 25 min), [$F(1,16) = 10.35, p < 0.005$], as well as an effect of test session, [$F(6,96) = 1.85, p < 0.10$], but with a non-significant interaction [Fig. 5.3A]. Withdrawal from *d*-amphetamine was associated

with fewer intromissions throughout the test session, while the ejaculation frequency remained constant, indicating a possible facilitation of this copulatory measure.

Other measures of copulatory ability remained unaffected by drug withdrawal. No effect was seen on either the inter-intromission interval, [$F(1,16) < 1$, NS] [Fig. 5.2B] or the intromission ratio (i.e. "hit rate") [$F(1,15) = 1.58$, NS] [Fig. 5.3D].

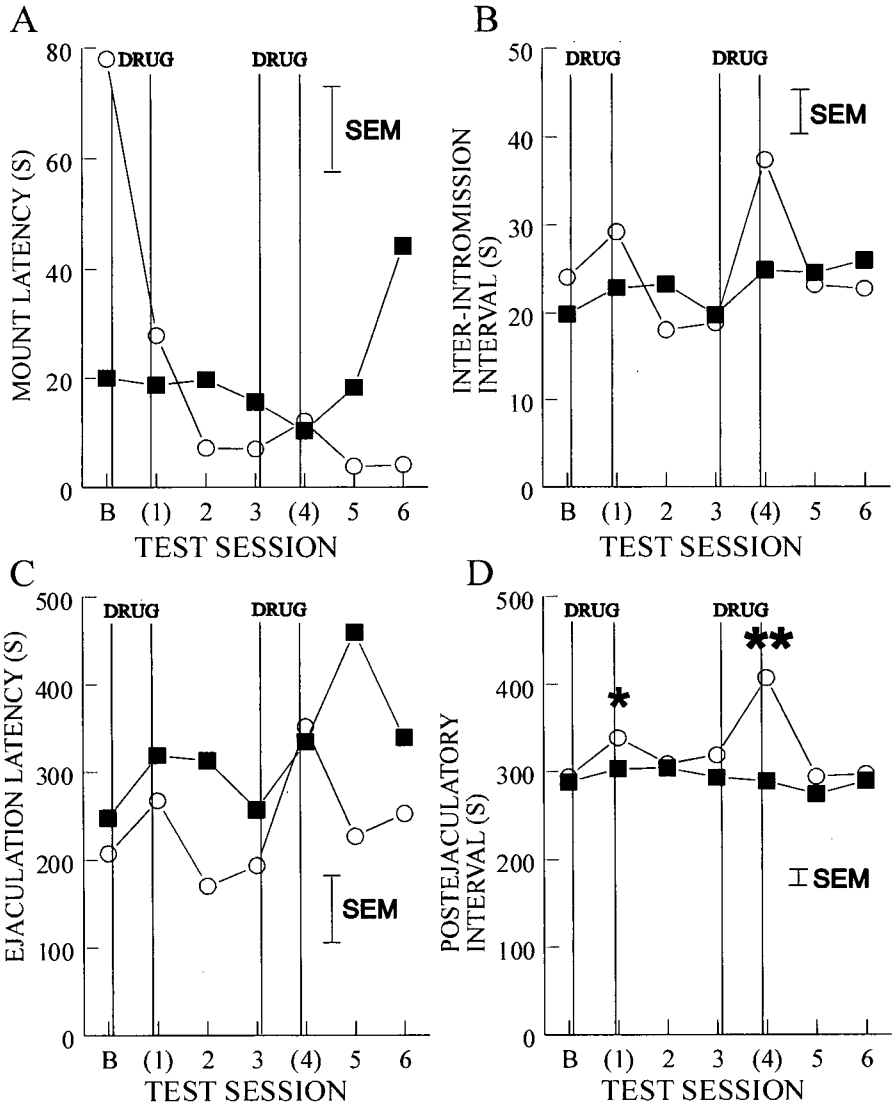


Fig 5.2. The effects of withdrawal from an escalating dose schedule of *d*-amphetamine on different temporal measures of sexual behavior in male rats. (B) represents pre-drug values corresponding to final training session values. Test sessions in parentheses indicate tests occurring 12 hr after withdrawal from drug regimen. Tests occur every 5 days. Stars indicate a significant difference between drug (O) and vehicle (■) groups, ($p<0.05$=*, $p<0.01$=**).

A: effect of withdrawal from drug on latency to first mount or intromission. **B**: effect of withdrawal from drug on inter-intromission interval. **C**: effect of withdrawal from drug on ejaculation latency. **D**: effect of withdrawal from drug on post-ejaculatory interval.

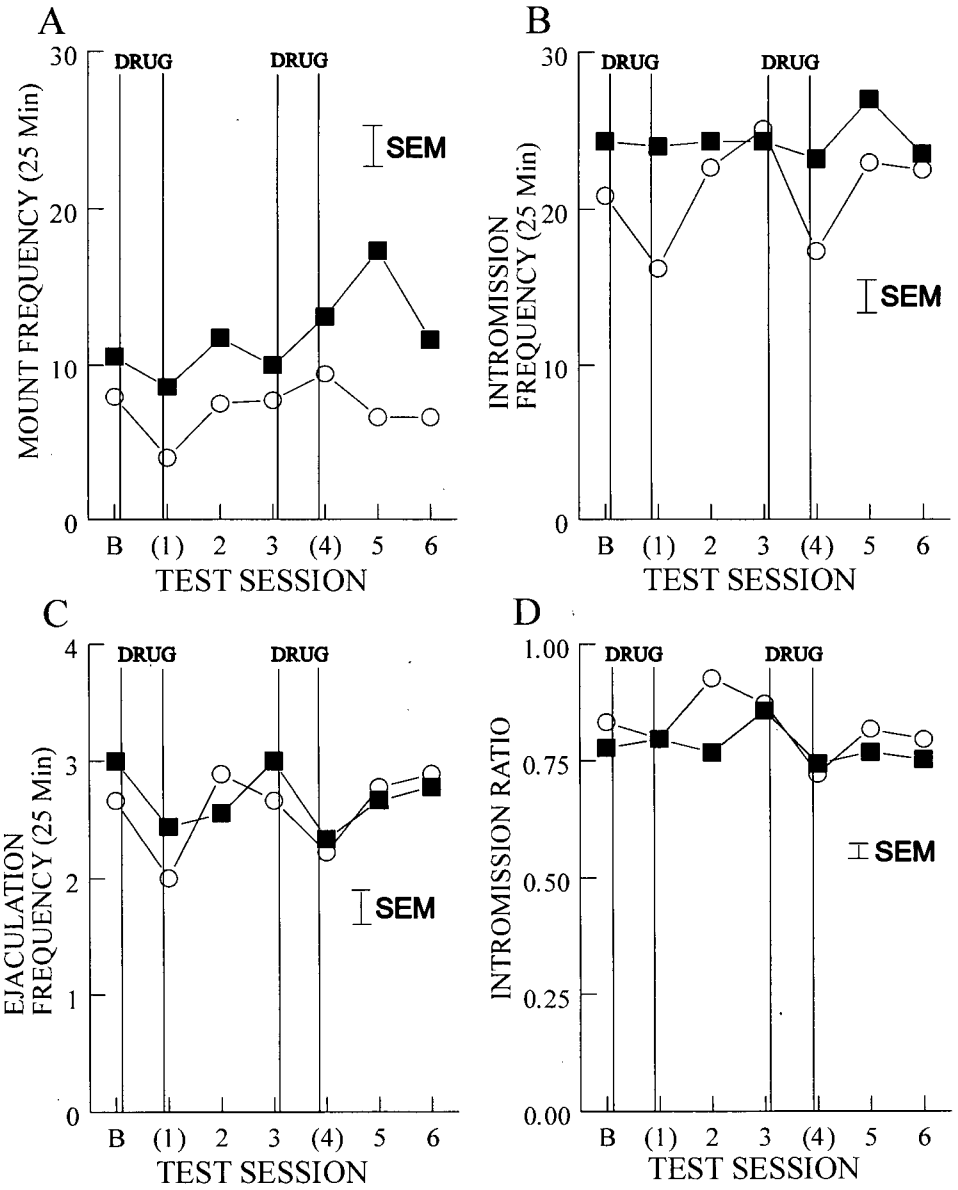


Fig 5.3. The effects of withdrawal from an escalating dose schedule of *d*-amphetamine on different copulatory measures in male rats. (B) represents pre-drug latencies corresponding to final training session values. Test sessions in parentheses indicate tests occurring 12 hr after withdrawal from drug regimen. Tests occur every 5 days. No significant differences were observed between drug (O) and vehicle (■) groups.

A: effect of withdrawal from drug on total number of mounts. **B**: effect of withdrawal from drug on total number of intromissions. **C**: effect of withdrawal from drug on total number of ejaculations. **D**: effect of withdrawal from drug on "hit rate", i.e. total number of intromissions/ total number of mounts + total number of intromissions.

***Discussion*:**

The results of the present study indicate that withdrawal from an escalating dose schedule of *d*-amphetamine decreases certain appetitive components of sexual behavior in sexually experienced male rats, but leaves their copulatory behaviors fundamentally unaltered. Twelve hours after the administration of a 4-day escalating-dose schedule of *d*-amphetamine, anticipatory locomotor activity (measured as the side changes in a modified bilevel chamber) in the 5 minute period prior to the presentation of an estrous female was reduced, reflecting a decrease in appetitive search behaviors. Furthermore, post ejaculatory intervals were significantly longer in *d*-amphetamine-treated rats, demonstrating a reduction in an additional component of motivated sexual behavior. These effects were replicated within the same experiment after the animals were exposed to the same drug schedule a second time, and similar results were obtained. There was also a strong trend towards fewer mounts and intromissions in *d*-amphetamine-treated rats during the period of drug withdrawal, although these effects were not statistically significant.

In the present experiment, there was an absence of augmented "anticipatory" activity by control rats that were not presented with a female, in agreement with a previous study which reported that male rats that were presented with non-estrous females failed to show an increase in level changes after repeated testing in bilevel chambers (Mendelson and Pfaus 1989). These results are consistent with the concept of precopulatory activity as a sensitive measure of appetitive sexual behavior in male rats; post ejaculatory intervals represent an additional appetitive behavior which may provide a further index of sexual motivation. Substantial data exist that support the dissociation of rodent sexual behavior into both appetitive and copulatory

components (Beach 1956), which depend in turn on the normal functioning of different neural substrates (Pfaus and Phillips 1989; Everitt 1990). Appetitive behaviors are more closely linked to activity within the ventral striatum, and may reflect a subject's sexual motivation (Pfaus 1996), while copulatory behaviors are more closely associated with the activity of the medial pre-optic area of the hypothalamus (Everitt 1990). The nature of the sexual deficits observed in the present study suggests that withdrawal from an escalating dose schedule of *d*-amphetamine preferentially disrupts appetitive behaviors over consummatory behaviors, and hence generates a reduction in subjects' sexual motivation rather than in their physical capacity to engage in copulatory activity.

The escalating 12-dose schedule of *d*-amphetamine utilized in the current experiment has been used previously to demonstrate that withdrawal from a psychostimulant can generate anhedonic effects in rats. Two previous studies have shown that withdrawal from a drug schedule identical to the present one reduces intracranial self stimulation (ICSS) of the lateral hypothalamus in rats (Leith and Barrett 1976; 1980), which indicates that there is a subsensitivity of the neural systems involved in behavioral reinforcement. Additionally, recent research in our laboratory has found that withdrawal from a similar drug schedule of *d*-amphetamine decreases rats' motivation to obtain a naturally rewarding sucrose solution, as measured by decreased breakpoints on a progressive ratio schedule of reinforcement (Experiment 4). The results of the present study are therefore consistent with those of previous studies and imply in turn that the reductions in sexual behavior in the current experiment reflect a drug withdrawal-induced state of anhedonia and a consequent decrease in motivation. To our knowledge, this is the only experiment which has specifically examined the effects of psychostimulant withdrawal and its associated anhedonia on sexual behavior, in either rats or humans.

The similarity of the effect that cocaine and amphetamine withdrawal has to the features of human depression has led to psychostimulant withdrawal in rats being forwarded as a rodent model of depression (Leith and Barrett 1980; Kokkinidis et al. 1986; Baumann and Rothman 1998). Anhedonia, one of the two core symptoms of depression, is widely noted in rodent paradigms of drug withdrawal, and many of the deficits observed in ICSS responding during early psychostimulant withdrawal can be alleviated by administration of a tricyclic antidepressant (Markou et al. 1992). Symptoms other than anhedonia are also frequently modeled in animal paradigms of depression, and the high incidence of sexual dysfunction associated with MDD has led to the testing of sexual behavior of rodents with alternate animal models of depression, as decreased sexual behavior in humans may represent a valid behavior which can be modeled in animal models of MDD. Exposure of rats to continuous low-intensity stress in the Chronic Mild Stress model of depression was found to reduce mounting activity to almost nil (D'Aquila et al. 1994), while the removal of the olfactory bulbs in rats reduced mounting activity similarly and also disrupted ejaculatory capacity (Edwards et al. 1990). Pharmacologically-induced models of depression, such as through the administration of clomipramine to rat neonates, generate comprehensive deficits in nearly all aspects of sexual activity, including both motivational and copulatory components of sexual behavior (Vogel et al. 1996).

The results from the present study thus appear milder in effect than most of those from other animal models of depression. Much of this difference may be ascribed to the side-effects of alternate techniques which are used to induce a "depressive"-like state in subjects but which do not necessarily contribute to the effectiveness of that animal model; for example, the olfactory bulbectomy model requires the removal of olfactory sensory systems critical for rodent stimulus-

bound sexual behavior (Wysocki 1979), while the administration of REM-suppressing serotonergic drugs, such as clomipramine, to rat neonates may have unknown effects upon the sexual development of the maturing brain (Lauder 1990). Administration of the current escalating-dose schedule of *d*-amphetamine and its subsequent withdrawal does not create general sensorimotor deficits, and the behavioral effects are reversible with time (potentially modeling the natural remission to normal activity which is commonly seen in most patients with MDD (American Psychiatric Association 1994)).

While it is generally perceived that MDD is associated with a range of deficits in sexual functioning in humans (Baldwin 1996), few studies have actually measured these deficits prospectively and in an objective manner. Two such contemporary studies have shown that depressed men, who maintained daily diaries of their sexual activity, reported decreases in both sexual interest and satisfaction (i.e. reduced libido) yet showed deficiencies in neither sexual capacity nor activity (Howell et al. 1987; Nofzinger et al. 1993). These studies indicate that the sexual problems facing male depressives are primarily motivational and emotional, rather than of a physical nature. Therefore, the absence of an effect of withdrawal from *d*-amphetamine on any of the copulatory behaviors in rats in the present study therefore provides additional support for the use of psychostimulant withdrawal as a valid model of depression, by its clear discrimination between alterations in sexual activity of a motivational and of a motoric origin.

In conclusion, the present study found that withdrawal from an escalating-dose schedule of *d*-amphetamine in male rats was associated with significant decreases in several motivational components of sexual behavior, although most copulatory components were resistant to the effects of the drug withdrawal. The pattern of behavioral changes resembles the types of sexual deficits seen in humans diagnosed with MDD, and therefore supports the development of

psychostimulant withdrawal in rats as a rodent model of depression. Further studies, using more subtle techniques to assay specifically the motivational components of sexual behavior, are required to elucidate the nature of psychostimulant withdrawal-associated deficits in sexual behavior.

EXPERIMENT 6:

INCREASED SUCCESSIVE NEGATIVE CONTRAST IN RATS WITHDRAWN FROM AN ESCALATING-DOSE SCHEDULE OF *D*-AMPHETAMINE

Synopsis:

The exposure of humans and animals to high doses of psychostimulant drugs, followed by their withdrawal, leads to a number of aversive psychological symptoms. These symptoms include increased anxiety and anhedonia, and may be manifested behaviorally as a decreased interested in normally rewarding stimuli. In the present study, we determine the effects of withdrawal from an escalating dose schedule of *d*-amphetamine on the consumption of a 4% sucrose solution under normal conditions, and after an incentive downshift. The downshift was induced by subjecting animals to a consumatory negative contrast paradigm, by switching them from a familiar 32% sucrose solution to a novel 4% solution. In unshifted animals, there was no effect of *d*-amphetamine withdrawal on consumption of the 4% solution. In contrast, drug withdrawn animals displayed an exaggerated negative contrast effect, primarily reflected as a delayed recovery from the downshift lasting for at least 60 hrs. This effect is interpreted as a consequence of the increased emotionality of withdrawn animals, and may be related to disruption of normal search behaviours.

Introduction:

The psychological effects of withdrawal from psychostimulant drugs in animals have been cogently described (Koob et al. 1997). Animals, like humans, are subject to the aversive affective states that arise from the discontinuation of high doses of psychostimulant drugs such as cocaine and *d*-amphetamine. In humans, post-"binge" drug abusers report dysphoric symptoms that include depression, psychomotor retardation and anxiety (Gawin and Kleber 1986; American Psychiatric Association 1994; Gillin et al. 1994; Pathiraja et al. 1995; Coffey et al. 2000). Animal paradigms have been developed that allow the objective measurement of these dysphoric states. For example, rodents that have been allowed to self-administer binge-like doses of cocaine have been shown to exhibit high levels of post-drug anxiety, as measured by increased acoustic startle and distressful ultrasonic vocalizations (Barros and Miczek 1996; Mutschler and Miczek 1998). Similarly, animals that have been experimenter-administered high doses of psychostimulants display increased anxiety during the post-drug withdrawal, when tested in tasks such as the elevated-plus maze and defensive burying (Sarnyai et al.1995; Basso et al. 1999).

Evidence for a state of anhedonia in drug-withdrawn animals has been determined with the refined use of rodent models of reinforcement. The decreased hedonic capacity of rodents that are in post-drug withdrawal has been well characterized by reductions in their responding for rewarding electrical brain stimulation (Leith and Barrett 1980; Cassens et al. 1981; Kokkinidis and McCarter 1990; Markou and Koob 1991; Wise and Munn 1995; Lin et al. 1999). In our laboratory, we have recently shown that rats will exhibit reduced motivation to obtain natural reinforcers, including a sucrose solution and access to a sexually receptive conspecific, for up to

5 days after the termination of a binge-like regimen of *d*-amphetamine administration (Experiments 4 and 5). These results indicate that post-drug withdrawal may be typified by a reduction in the motivation to respond for normally rewarding stimuli. What remains unknown, however, is how animals that are in a state of anhedonia will respond for a rewarding stimulus when its incentive properties have been unexpectedly devalued.

In the successive negative contrast paradigm, animals are trained reliably to expect to receive a reward of a consistent value. If this reward is unexpectedly replaced by one of lesser value, animals will normally consume lower levels of the reward than subjects that have only ever been exposed to the lesser reward will. This phenomenon, referred to as a "successive negative contrast", has been widely demonstrated across different species, including rodents, primates and humans (Schnorr and Myers 1967; Peters and McHose 1975; Flaherty 1982; Grigson et al. 1994; Flaherty 1996; Mustaca et al. 2000). Numerous explanations have been provided to account for the expression of successive negative contrast, many of which are based on the induction of negative affective states in the animals. Prominent amongst these affect-based theories are the development of emotional constructs such as disappointment and frustration (Crespi 1942; Amsel 1951; Flaherty 1982), as the animal fails to find the reward that it had predicted to receive and instead finds one of a lesser value. These data are also consistent with a decrease in the incentive salience of the lesser reward compared to its salience in unshifted animals (Berridge and Robinson 1998), as "downshifted" animals not only consume less of the reward but also decrease their approach speed towards it (Flaherty 1996).

Given that rodents exhibit a state of anxiety and anhedonia after withdrawal from psychostimulant drugs, we were interested in determining the effect of a further downshift in the incentive value of a stimulus by subjecting *d*-amphetamine withdrawn rats to a successive

negative contrast effect. Hypothetically, the adverse affective state that accompanies psychostimulant withdrawal should render animals especially sensitive to the emotionally disruptive effects of successive negative contrast, in which one stressful situation is "superimposed" atop another. The purpose of the present experiment was therefore to determine the effects of withdrawal from a binge-like regimen of *d*-amphetamine on the consumption of a 4% sucrose solution in rats that had been downshifted from prior experience with a 32% sucrose solution.

***Methods*:**

Subjects

Thirty-two male Long-Evans rats, (Charles River, Quebec) weighing 250-275g at the beginning of the experiment, were housed individually in a temperature regulated colony (21 ± 1°C) under a 12-hr light-dark cycle (lights on at 07:00 hrs); training and testing occurred during the light phase. All procedures were conducted in accordance with the Canadian Council on Animal Care guidelines for work with laboratory animals. Water was always available *ad libitum* in the home cage.

Apparatus

Subjects were trained and tested in four Plexiglas test cages (25×25×25cm) that were enclosed within sound and light attenuating chambers. Each test cage was fitted with a lick activated solenoid valve which provided rats with a drop of sucrose solution each time their

tongue contacted the tip of a metal drinking spout, located 6cm above the chamber floor. The solenoid valve regulated the volume of the drops of sucrose to 0.01ml. A small light (2.8-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 then 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 10msec (100Hz).

Training and Testing

All animals were placed on a deprived feeding schedule, which reduced body weights of the rats to approximately 85% of their free-feeding weights. After subjects had attained the desired body weights, they were randomly assigned into 2 different groups (n=16 per group). One group of animals was subsequently exposed to and trained with a 32% sucrose solution, while the remaining group was exposed to and trained with a 4% sucrose solution. Initially, subjects were given two 1 hr habituation sessions to the sucrose solutions in their home cages, on alternate days. Animals were then given access to their respective sucrose solutions for a 5 min period once per day in the testing apparatus. Daily training sessions continued for 10 days, by which time most of the rats had reached an asymptotic level of consumption of the sucrose solutions. At the conclusion of the tenth day of training, each of the 2 groups of animals was sub-divided into 2 further groups (n=8 per group), based upon a rank-ordered division of animals with respect to the number of licks that they exhibited in the final 5 min training session. One group from each of the 4% and 32% sucrose solution exposed-animals was then subjected to a 4 day regimen of *d*-amphetamine injections, while the remaining groups received injections with the vehicle solution. Following the conclusion of the drug regimen, all groups were tested

for their consumption (measured as the number of licks) of a 4% sucrose solution during an additional 8 daily test sessions. For the 2 groups of animals that had had been trained with the 32% sucrose solution, the presentation of the 4% solution represented an unexpected decrement in the rewarding value of the stimulus.

Drug Administration

Escalating doses of the drug *d*-amphetamine sulfate (SmithKline-Beecham, Oakville, Ontario) were administered to two groups (n=8 per group) of rats based on a schedule modified from one that we have shown previously to affect motivated responding for rewarding stimuli (Barr and Phillips 1999; Barr et al. 1999). In this schedule, rats were injected IP three times per day (9 am, 5pm, 12 PM), starting with a dose of 1 mg/kg and escalating by 1 mg/kg on each subsequent dose, for the first three days for 9 doses. On the fourth day, subjects received 3 doses of 10 mg/kg; animals therefore received a total of twelve injections over the 4-day period. 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 9 am injection so that any decreases in body weight would be compensated for by adjusting the dose. As *d*-amphetamine-treated animals typically display a loss of bodyweight during the 4 day drug regimen, each drug-treated animal was "yoked" to a vehicle-treated animal, matched by bodyweight. The amount of food that each drug-treated animal consumed over 24 hr was measured and the yoked animal was limited to consume this amount.

Control subjects were injected with isotonic saline under the same schedule as rats in the *d*-amphetamine group.

Data Analysis

The data were analyzed by repeated-measures analysis of variance (ANOVA) in a two factor design, with drug treatment (*d*-amphetamine vs. vehicle) and pre-shift sucrose solution (32% vs. 4%) as the two factors, measured across time. When the ANOVA indicated the presence of a significant effect, further analysis was conducted with Tukey's post-hoc tests. Data from only the first 4 test sessions after the drug regimen were analyzed, as the effect of reward downshift was no longer evident after this point.

Results:

Prior to drug administration, all animals exhibited high rates of licking for either the 32% or the 4% sucrose solution. Analysis of the data during the 84 hr period following drug termination with the repeated-measures ANOVA indicated a significant main effect of drug treatment, [$F(1,28) = 7.31, p < 0.05$], as animals that were exposed to the escalating dose regimen of *d*-amphetamine exhibited reduced consumption of the 4% sucrose solution, compared to vehicle-treated subjects. There was also a significant main effect of pre-shift sucrose solution [$F(1,28) = 4.38, p < 0.05$], whereby both groups of rats that were allowed to consume the 32% sucrose solution displayed dramatically reduced consumption of the 4% solution after the animals were downshifted to this new reward, confirming a negative contrast effect. The ANOVA also

indicated a significant interaction of drug treatment × pre-shift solution × time [$F(4,112) = 6.43$, $p < 0.001$].

The significant interaction was analyzed further with the use of post-hoc tests [Figure 6.1]. These tests revealed that both of the downshifted groups (32%→4%) displayed reduced consumption of the novel 4% sucrose solution compared to the unshifted (4%%→4%) groups during their first two exposures to the 4% solution. On the third exposure to the 4% solution, at 60 hr after drug termination, the vehicle-treated group displayed an unexpected increase in consumption of the 4% solution, compared to both of the unshifted groups. This effect had diminished by the fourth test session, when there was no longer any difference between these groups. In comparison, the downshifted group that had been exposed to *d*-amphetamine displayed reduced levels of consumption of the 4% solution for 3, as opposed to 2, test sessions, and returned to control levels of consumption by the fourth test session. When the two downshifted groups were compared to each other, the *d*-amphetamine-treated group exhibited lower levels of consumption across the first three test sessions, although this effect was only marginally significant on the first test day.

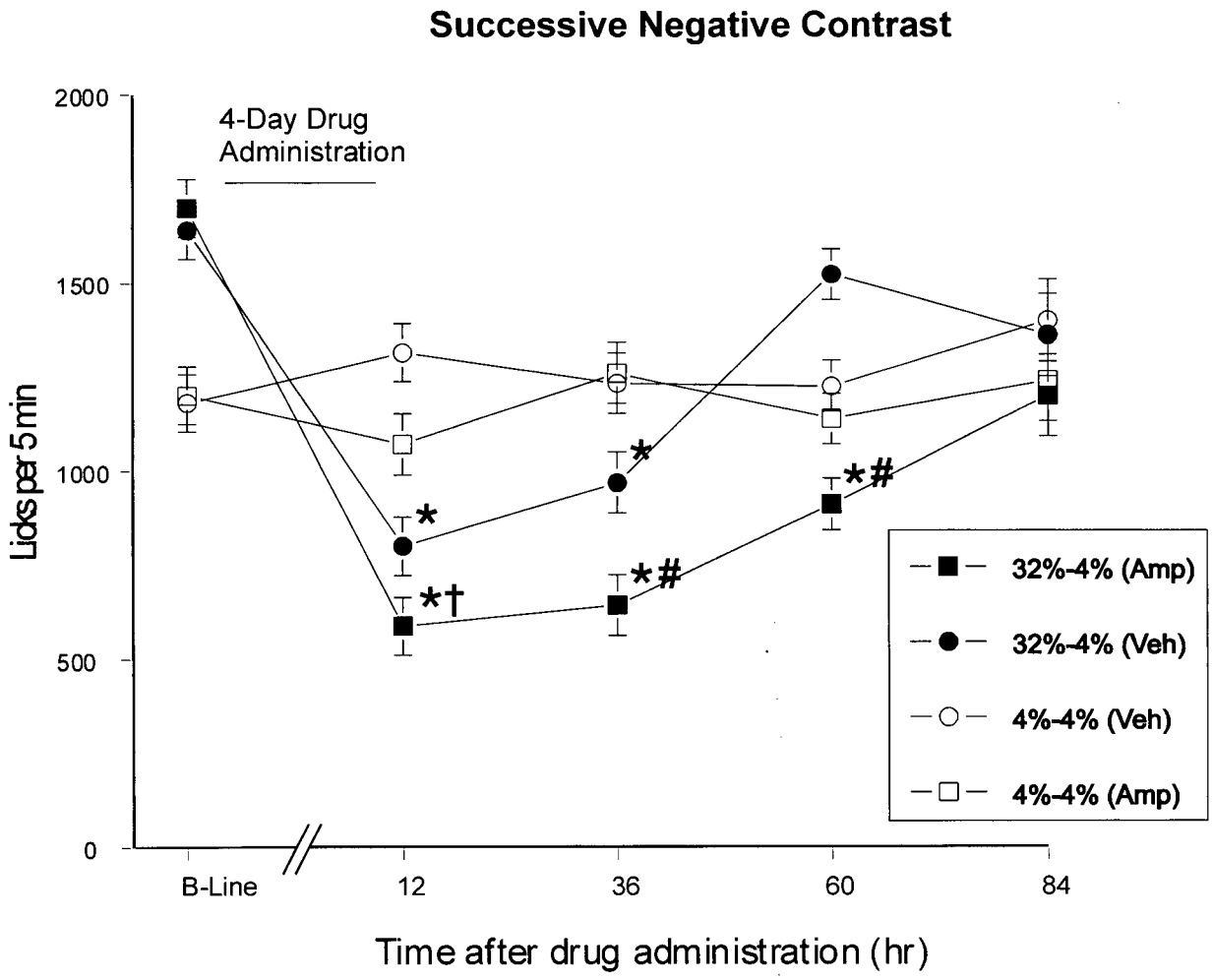


Fig 6.1

Effects of withdrawal from a 4-day regimen of *d*-amphetamine, or vehicle, on number of licks for a 4% sucrose solution. Animals were given 5 min fluid consumption tests at different time points before (B-Line) and after withdrawal from drug administration.

* = significantly different from 4%-4% (VEH) group, $p < 0.05$
= = significantly different from 32%-4% (VEH) group, $p < 0.10$
= significantly different from 32%-4% (VEH) group, $p < 0.05$

Discussion:

In the present experiment, we have demonstrated that rats exhibit a greater consumatory negative contrast compared to control subjects when they are tested after withdrawal from a binge-like regimen of *d*-amphetamine. This effect was manifested as a marginally significant increase in the size of the contrast effect on the first day of exposure to the devalued sucrose solution. By the second day of exposure to the devalued stimulus, the magnitude of the contrast effect was substantially greater between the downshifted groups, as vehicle treated animals showed a more rapid recovery from the exposure to the devalued stimulus. On the third day of exposure to the devalued sucrose solution, only the *d*-amphetamine withdrawn animals continued to exhibit a contrast effect, indicating that withdrawal from a psychostimulant drug can perpetuate negative contrast effects in rodents.

A number of different hypotheses have been proposed to account for the phenomenon of negative contrast effects. One of the most influential of such theories postulates that contrast effects arise from associative generalization decrements (Spear and Spitzer 1966; Capaldi 1972), whereby changes in either the rewarding environment or the rewarding stimulus lead to a reduced association between the two, with a commensurate decrease in consumption of the reward. Although generalization decrements fail to account for several important aspects of contrast effects, such as the existence of positive contrast effects (Flaherty 1996), it may be argued than in this case animals in the novel state of drug withdrawal would exhibit potent generalization decrements when exposed to the devalued stimulus for the first time. This explanation is unlikely, however, as the animals in the group that received *d*-amphetamine that was not downshifted did not reduce their consumption of the 4% sucrose solution. It is also

unlikely that the increased negative contrast observed in drug withdrawn animals, measured by a decreased fluid consumption, is a reflection of psychomotor deficits that arise from the withdrawal of high doses of psychostimulant drugs. Although several studies have reported reduced locomotor activity by drug withdrawn animals (Paulson et al. 1991; Pulvirenti and Koob 1993), we have shown in previous experiments, using a similar regimen of drug administration, that animals are capable of vigorous physical activity during the withdrawal state when they respond for naturally rewarding stimuli such as a sexually receptive conspecific or a sucrose solution (Barr and Phillips 1999; Barr et al. 1999). In addition, psychomotor deficits should have affected the unshifted, *d*-amphetamine treated group equally, for which there was no evidence.

Alternate theories for the basis of negative contrast effects have focused on the role of psychological constructs such as "emotionality" and anxiety (Weinstein 1972; Becker 1984; Flaherty 1996). The withdrawal from high doses of psychostimulant drugs in rodents has been shown reliably to provoke aversive affective states (Koob et al. 1997). In particular, two of the more commonly described sequelae of drug withdrawal are increased anxiety (Mutschler and Miczek 1998; Basso et al. 1999) and anhedonia (Markou and Koob 1991; Wise and Munn 1995). With respect to anxiety, there is a substantial body of evidence which suggests that negative contrast effects may be mediated, in part, by increased levels of anxiety in subjects. The capacity of a wide range of anxiolytic drugs to ameliorate the effects of successive negative contrast (Flaherty 1990; Morales et al. 1992), as well as the anti-contrast effects of selective amygdaloid lesions (Becker et al. 1984; Salinas et al.1996), indicate that anxiety-like processes may be involved in the expression of negative contrast effects. In addition, it was reported that Syracuse Low Avoidance rats, which exhibit greater levels of anxiety (Brush et al. 1988), displayed increased levels of consumatory negative contrast when compared to the less

emotionally reactive Syracuse High Avoidance strain (Flaherty and Rowan 1989). It is theoretically possible that the withdrawal from a binge-like dose of *d*-amphetamine could perpetuate the effects of negative contrast by sustaining high levels of anxiety in rats, and thus potentiate the weaker anxiogenic effects of the contrast paradigm in later exposures to the devalued stimulus. However, a recent study of consumatory negative contrast in humans failed to detect increases in anxiety when subjects were presented with a devalued sweet solution, despite perceptions of reduced absolute sweetness (Specht and Twining 1999). Furthermore, rats that were selectively bred for high levels of consumatory negative contrast did not display an expected increase in anxiety-related behaviors when compared to the control animals (Flaherty et al. 1994). These contrary findings suggest that, while anxiety may contribute some measure towards the expression of negative contrast effects, there are clearly other psychological factors that are involved.

Flaherty has postulated that the expression of successive negative contrast involves a multistage process of distinct yet interacting cognitive and affective processes (Flaherty 1996; Mitchell and Flaherty 1998). One of the more important of these stages involves a pattern of search activity by the downshifted animal, after it detects and avoids the devalued stimulus and instead searches for the familiar high-reward stimulus. The existence of this component of Flaherty's multistage model is supported by the recent findings of Pecoraro et al. (1999), who observed that downshifted animals engaged in systematic investigative activity after detection of the devalued stimulus, presumably seeking the more rewarding stimulus. The effects of *d*-amphetamine withdrawal may be particularly disruptive at this point in the multistage process, for several reasons. Firstly, it has been shown that animals in a state of psychostimulant withdrawal exhibit a suppressed response towards novel stimuli (Persico et al. 1995). Secondly,

and in a similar manner, psychostimulant withdrawal is associated with reduced investigative activity in rodents (Hitzemann 1977; Meert 1992; Barr et al. 1999). A suppressed response to the novel, downshifted reward, followed by inhibited investigation for the familiar high-reward stimulus, could in theory delay the sequence of recovery that is predicted by the multistage hypothesis of successive negative contrast, and account for the prolonged contrast effects that were observed in the present study. This hypothesis would also be consistent with the large body of evidence which has demonstrated that drug withdrawn animals experience anhedonia (prior refs). Anhedonic animals would be less interested in both the previous high-reward and the novel, downshifted reward, leading to a delayed return to consumption of the 4% sucrose solution. However, this hypothesis remains to be tested empirically, and future studies should measure search behaviors when animals are downshifted.

In conclusion, the results of the present study indicate that withdrawal from a psychostimulant drug is associated with increased consumatory negative contrast effects, primarily reflected in a delayed recovery by these animals. The exact nature of the extended negative contrast effect remains unknown, but may be related to increased emotionality in withdrawn animals, and deficits in their exploratory activity.

EXPERIMENT 7:

REPEATED ELECTROCONVULSIVE SHOCK ATTENUATES THE DEPRESSIVE EFFECTS OF *D*-AMPHETAMINE WITHDRAWAL ON BRAIN REWARD FUNCTION IN RATS

Synopsis:

Rationale: The withdrawal of humans from high doses of psychostimulant drugs can result in a transient syndrome which appears isomorphic to endogenous depression. One of the more prominent symptoms is a loss of hedonic capacity; in animals, the anhedonia associated with amphetamine withdrawal has been measured objectively by decrements in responding for intracranial self-stimulation (ICSS). *Objective*: To date, the effects of amphetamine withdrawal on ICSS responding have been reversed by different antidepressant drugs. In the present study, we sought to reverse withdrawal-induced anhedonia by administration of repeated electroconvulsive shocks (ECS). *Methods*: Rats with electrodes in the lateral hypothalamus were trained on an ascending-series current intensity ICSS paradigm until stable levels of responding were attained. Half of the animals were then administered a 4-day escalating dose schedule of *d*-amphetamine, and tests for ICSS responding started 12 hr after the final injection. During withdrawal, all animals received daily treatment with either ECS or sham-ECS. *Results*: Amphetamine withdrawal was associated with reduced ICSS responding; animals treated with ECS exhibited a facilitated recovery compared to sham-ECS treated animals, and returned to control levels of ICSS responding 24 hr earlier. *Conclusions*: ECS was able to mitigate the anhedonic effects of *d*-amphetamine withdrawal, and provides additional support for the use of psychostimulant withdrawal as a model of depression.

Introduction:

The class of drugs referred to as the "psychostimulants" has the common property of inducing highly pleasurable mood alterations in humans. This class of drugs, which includes the amphetamines, substituted amphetamines and cocaine, produces increased arousal and hyperactivity, as well as euphoria when taken at higher doses (Breiter et al.1997; Drevets et al. 2001). With continuous access, tolerance typically develops quickly to the reinforcing effects of these drugs (Brauer et al. 1996; Schenk and Partridge 1997; Mendelson et al. 1998), and ever-increasing amounts of the drug must be taken to maintain the mood of the individual (Ward et al.1997). Eventually, drug intake ceases and opponent-processes prevail (Koob and Le Moal 1997), and the individual is subject to the effects of psychostimulant withdrawal (sometimes referred to as the "crash"). During withdrawal, the previously pleasurable effects of the drug are countered in an unopposed manner by the homeostatic mechanisms of the body (Koob et al. 1997). Psychostimulants are characterized by a withdrawal syndrome that is predominantly psychological in nature, and includes anxiety, lethargy and anhedonia (Gawin and Kleber 1986; Coffey et al. 2000).

The effects of psychostimulant withdrawal in humans bear a remarkable similarity to endogenous depression. The dysphoria of drug withdrawal may appear indistinguishable from depression (American Psychiatric Association 1994; Pathiraja et al. 1995). Animal models of psychostimulant withdrawal have been developed which allow for the accurate and objective measurement of several of the more prominent psychological sequelae of drug withdrawal. These include increased levels of anxiety (Mutshcler and Miczek 1998; Basso et al. 1999) and psychomotor retardation (Fung and Richard 1994; Persico et al. 1995; Baldo et al. 1999b).

Commensurate with the depressogenic effects of drug withdrawal that are observed in humans, there is also a substantial body of evidence which describes the reduced hedonic capacity, or anhedonia, of animals in the early stages of drug withdrawal. This phenomenon is most commonly measured by a reduction in rodents' responding for rewarding electrical brain stimulation (Leith and Barrett 1980; Kokkinidis et al. 1986; Markou and Koob 1991; Wise and Munn 1995; Lin et al. 1999). In our laboratory, we have recently demonstrated that rats which have been withdrawn from an escalating-dose schedule of *d*-amphetamine exhibit a reduced motivation to obtain natural reinforcers, such as a sucrose solution or access to a sexually receptive conspecific (Experiments 4 and 5).

Given the strong similarities between psychostimulant withdrawal and endogenous depression, it is therefore not surprising that there have been a number of preclinical studies that have sought to reverse the anhedonia of drug withdrawal with the use of established antidepressant therapies. The tricyclic antidepressants imipramine and amitriptyline were shown to mitigate the effects of amphetamine withdrawal on intracranial self-stimulation (ICSS) (Kokkinidis et al. 1980), while cocaine withdrawal was alleviated by the administration of the tricyclic drug desmethylimipramine (Markou et al. 1992). More recently, Harrison et al. (2001) have demonstrated that the effects of amphetamine withdrawal on ICSS can be diminished by antidepressants from a different chemical class, namely the selective serotonin reuptake inhibitors (SSRIs). The elevated ICSS reward thresholds in *d*-amphetamine withdrawn rats, which are indicative of anhedonia, were attenuated by a higher dose of fluoxetine (Harrison et al. 2001); furthermore, the therapeutic effects of this drug were facilitated by coadministration of the selective serotonin-1A receptor antagonist p-MPPI.

Consistent with the capacity of antidepressant drugs to alleviate the effects of psychostimulant withdrawal, it may be predicted that non-drug antidepressant therapies also would be effective in this paradigm. The most frequently applied of such treatments in humans is electroconvulsive therapy (ECT). The use of ECT is well established due to its relatively fast onset of therapeutic efficacy, as well as its capacity to alleviate the symptoms of depression in drug-refractory patients (Lam et al. 1999; Sackeim et al. 2000). The purpose of the present study was therefore to determine if the reductions in ICSS responding in rats that result from withdrawal of an escalating-dose schedule of *d*-amphetamine could be alleviated by the repeated administration of electroconvulsive shock (ECS).

Methods:

Subjects

Forty male Long-Evans rats, (Charles River, Quebec) weighing 250-275g at the beginning of the experiment, were housed individually in a temperature regulated colony (21±1°C) under a 12-hr light-dark cycle (lights on at 07:00 hrs); training and testing occurred during the light phase. All procedures were conducted in accordance with the Canadian Council on Animal Care guidelines for work with laboratory animals.

Surgery

Rats were anesthetized with xylazine (7 mg/kg, i.p.) and ketamine hydrochloride (100mg/kg, i.p.), and placed in a standard stereotaxic apparatus. The dorsal surface of the skull was exposed

and a single hole was drilled to allow implantation with a stainless steel bipolar electrode. Electrodes were directed at a site in the medial forebrain bundle corresponding to the level of the posterior lateral hypothalamus [anteroposterior, -0.5mm from bregma; mediolateral, +1.7mm; dorsoventral, -8.3mm from dura; tooth bar, 5.0mm above the interaural line. The electrodes were secured to the skull with surgical stainless steel screws and dental acrylic cement. All animals were allowed to recover from surgery for at least 1 weak before starting ICSS training.

ICSS Training

Training and testing were conducted in 4 Plexiglas boxes (30 × 30 × 24cm), housed within sound attenuating chambers. Depression of a lever delivered sine wave current (60Hz) of a fixed duration (200msec), via a flexible lead connected to the chronically implanted intracranial electrode assembly. During the initial training period, the current was set at 16μA, and only those animals that maintained consistent lever pressing were used for the second stage of training. This stage consisted of training subjects on an ascending-series rate-intensity protocol, whereby current intensities were preset by a computer (Nova-3; Manx software) and incremented in 2μA steps, from an initial value of 8μA to 28μA. Five priming pulses of stimulation were delivered to each animal at the beginning of the first minute of testing at a given current level. The number of bar presses was recorded for the subsequent 4-min period, after which the current intensity was set at the next level. Data collection was controlled by the computer and individual rate-intensity curves were plotted daily for each subject, from which three measures were calculated; the current at which responding was half maximal (M_{50}), the minimum current required to maintain a threshold level of responding of 30 presses/min, and the asymptotic level of responding. The half-maximal and threshold currents provide an accurate measurement of the

individual's sensitivity to reward, while the asymptotic level of responding provides a measure of the animal's capacity to perform motor tasks, hence allowing the influence of performance-altering effects of drugs to be determined (Liebman 1983; Markou and Koob 1992).

After stable levels of ICSS responding had been achieved by subjects ($M_{50} < \pm 10\%$, for 3 consecutive days), animals were rank ordered based upon their level of responding over the previous 3 baseline sessions. The rats were then assigned sequentially to 4 groups, from the highest to the lowest M_{50} values. Two groups (n=10 per group) were assigned to receive administration of *d*-amphetamine based on a schedule that we have used previously to induce withdrawal symptoms (Barr and Phillips 1999; Barr et al. 1999), while two groups (n=10 per group) received vehicle. One of each of the drug and vehicle groups was then assigned to receive electroconvulsive shock at the conclusion of drug or vehicle administration, while the remaining two groups received sham electroconvulsive shock.

Drug Administration

Escalating doses of the drug *d*-amphetamine sulfate (obtained from SmithKline-Beecham, Oakville, Ontario) were administered to two groups of rats based on a schedule modified from one shown previously to affect thresholds of ICSS responding (Leith and Barrett 1976). In this modified schedule, rats were injected IP three times per day (9 am, 5pm, 12 pm), starting with a dose of 1 mg/kg and escalating by 1 mg/kg on each subsequent dose, for the first three days for 9 doses. On the fourth day, subjects received three doses at 10 mg/kg; animals therefore received a total of twelve injections over the four day period. 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 9 am injection so that any decreases in body weight would be compensated for by adjusting the dose. As *d*-amphetamine-treated animals typically display a loss of bodyweight during the 4 day drug regimen, each drug-treated animal was "yoked" to a vehicle-treated animal, matched by bodyweight. The amount of food that each drug-treated animal consumed over 24 hr was measured and the yoked animal was limited to consume this amount. Control subjects were injected with isotonic saline under the same schedule as rats in the *d*-amphetamine group.

Electroconvulsive Shock

Treatment with ECS commenced the morning after the final injection of drug or vehicle. All animals were induced with a mixture of 2% isoflurane and oxygen. As soon as animals exhibited the effects of anaesthesia, they were given a brief electroconvulsive shock (ECS). Treatments were delivered using a constant current UGO Basile (Varese, Italy) apparatus for small mammals; this device delivers a unidirectional brief pulse stimulus. Treatment parameters were set at 90 mA, 70Hz for 8 sec, or sham ECS (no current applied). ECS was applied bilaterally, with earclips that were coated in electroconductive gel. Animals in the ECS group typically exhibited tonicoclonic seizures for approximately 25-30 sec. ECS was administered every day at 07:30, i.e. at least 3 hrs before behavioral testing occurred.

Histology

At the conclusion of the experiment, all subjects were given an overdose of chloral hydrate and perfused intracardially with saline and formalin (4%). Frozen brains were sliced, and coronal sections were stained with cresyl violet to determine placement of electrodes.

Data Analysis

The data were analyzed by repeated-measures analysis of variance (ANOVA) in a two factor design, with drug treatment (*d*-amphetamine vs. vehicle) and electroconvulsive shock treatment (ECS vs sham) as the two factors, measured across test sessions. When the ANOVA indicated the presence of a significant effect, further analysis was conducted with Fisher's LSD post-hoc tests.

Results:

The data from 3 animals had to be excluded, due to complications that arose from repeated stimulation with ECS. Two of these rats were from the vehicle-treated ECS group, while one animal was from the amphetamine-treated ECS group.

The analysis of the M_{50} ICSS data with ANOVA indicated a significant main effect of treatment with *d*-amphetamine [$F(1,33) = 6.89, p < 0.05$], as animals in withdrawal from the drug required significantly greater current to maintain half maximal responding, consistent with a decrease in hedonic capacity. The ANOVA also revealed a significant effect of test session [$F(3,99) = 5.00, p < 0.005$)] and a significant interaction of drug treatment × test session [$F(3,99) = 6.99, p < 0.001$)]. These effects were followed up with post-hoc tests, which revealed that in

the first ICSS session (12 hr) after the final injection of *d*-amphetamine, both of the groups (ECS and sham-ECS) experiencing withdrawal from the drug had significantly greater M_{50} values (16.49μA ± 1.19 and 16.65 ± 1.07μA) than both of the vehicle-treated groups (12.93μA ± 1.12 and 12.74μA ± 1.07) [Figure 7.1]. By 36 hr after drug termination, the M_{50} values of the *d*-amphetamine-treated group that had received ECS (14.16μA ± 1.01)were no longer significantly different from either of the vehicle-treated groups (12.63μA ± 0.96 and 12.92μA ± 0.91). In contrast, the *d*-amphetamine group given only sham-ECS had a significantly higher M_{50} value (16.68μA ± 0.91) than both of the vehicle-treated groups, and a marginally higher value than the *d*-amphetamine ECS group ($p = 0.073$). At 60 hr after drug termination, there were no longer any significant differences between any of the groups in the M_{50} current values.

ANOVA analyses of the current required to maintain threshold levels of responding (≥ 30 presses per min) indicated a significant main effect of drug treatment [$F(1,33) = 4.58$, $p < 0.05$], a significant effect of test session [$F(3,99) = 10.17$, $p < 0.001$], and a significant interaction of drug treatment × test session [$F(3,99) = 12.18$, $p < 0.001$]. Further analysis of these results with post-hoc tests revealed that 12 hr after drug termination, both the *d*-amphetamine ECS and sham-ECS groups displayed significantly higher threshold current values than either of the vehicle-treated groups [Figure 7.1]. By 36 hr after drug termination, threshold currents for the *d*-amphetamine sham-ECS group displayed threshold currents that were significantly greater than currents for both of the vehicle-treated groups, and marginally greater than the *d*-amphetamine ECS group ($p = 0.081$). At this time point, mean threshold currents for the *d*-amphetamine ECS group did not differ from the vehicle-treated groups. On the final test session, at 60 hr after drug termination, threshold currents were significantly greater for the *d*-amphetamine sham-ECS group than the other groups.

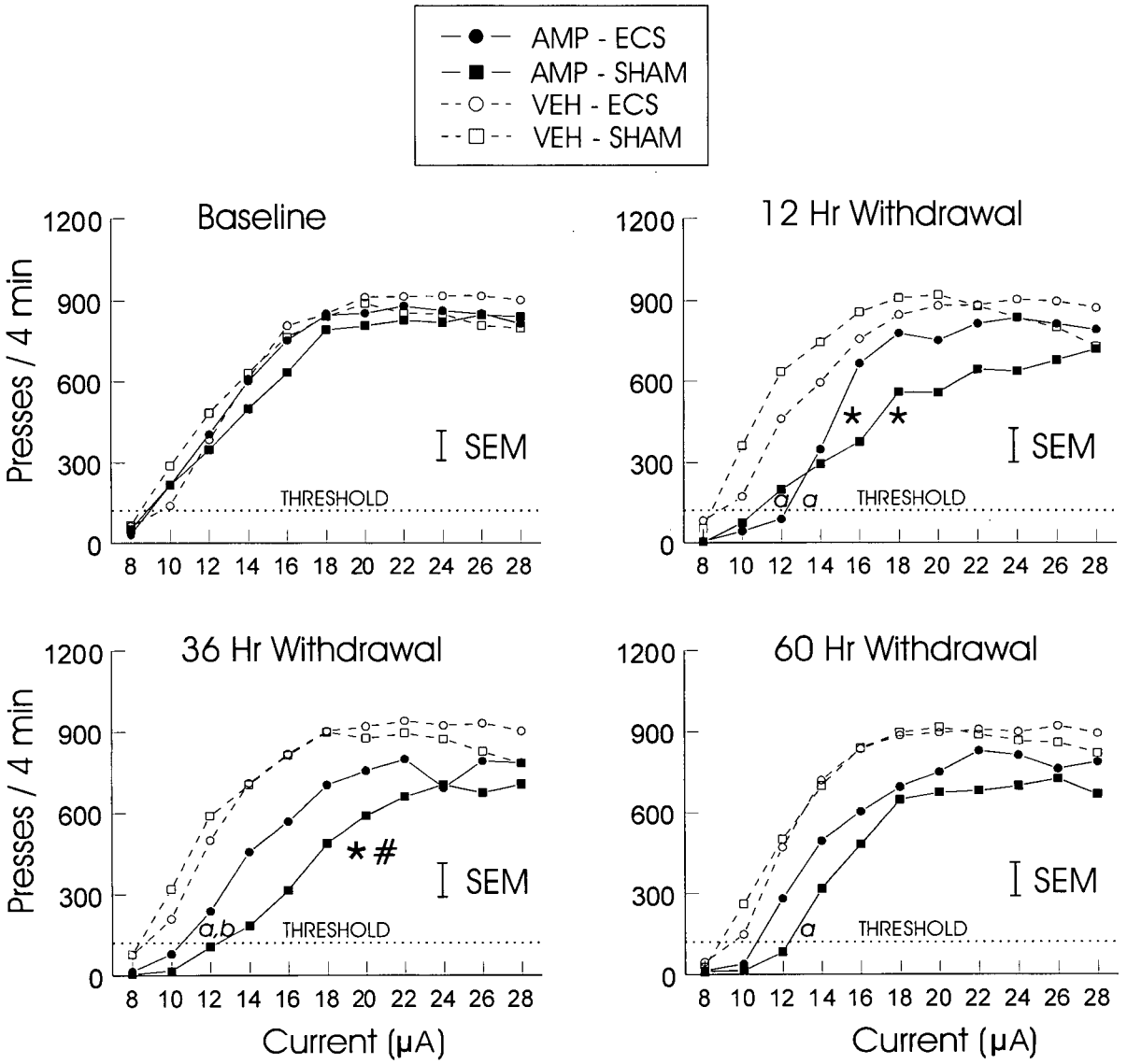


Fig 7.1 Effects of withdrawal from a 4-day escalating dose schedule of *d*-amphetamine (AMP) or vehicle (VEH) on ICSS responding. Values represent mean group levels of responding under an ascending-series current intensity paradigm. Data are displayed prior to drug administration (Baseline) and at 12, 36 and 60 hrs following cessation of drug. AMP and VEH groups received daily treatment with either ECS (ECS) or sham-ECS (SHAM).

* = M_{50} values significantly different from control group (VEH-SHAM), $p < 0.05$
# = M_{50} value marginally different from AMP-ECS group, $0.05 < p < 0.10$
a = threshold current significantly different from control group (VEH-SHAM), $p < 0.05$
b = threshold current marginally different from AMP-ECS group, $0.05 < p < 0.10$

The ANOVA indicated no significant main effect of drug treatment [$F(1,33) = 1.66$, NS] on the asymptotic levels of responding. Similarly, there was also no significant main effect of test session [$F(3,99) = 1.57$, NS] nor a test session × drug treatment interactive effect [$F(3,99) = 2.46$, NS] on asymptotic levels of responding. Although maximal levels of responding appear to be lower in the *d*-amphetamine treated animals in Figure 7.1, this can be attributed to individual variability in the maximal levels of responding.

Histology placements indicated that electrode tips were located entirely within the boundaries of the lateral hypothalamus, in an anterior/posterior range extending from –1.8mm to –2.3mm from bregma [Figure 7.2].

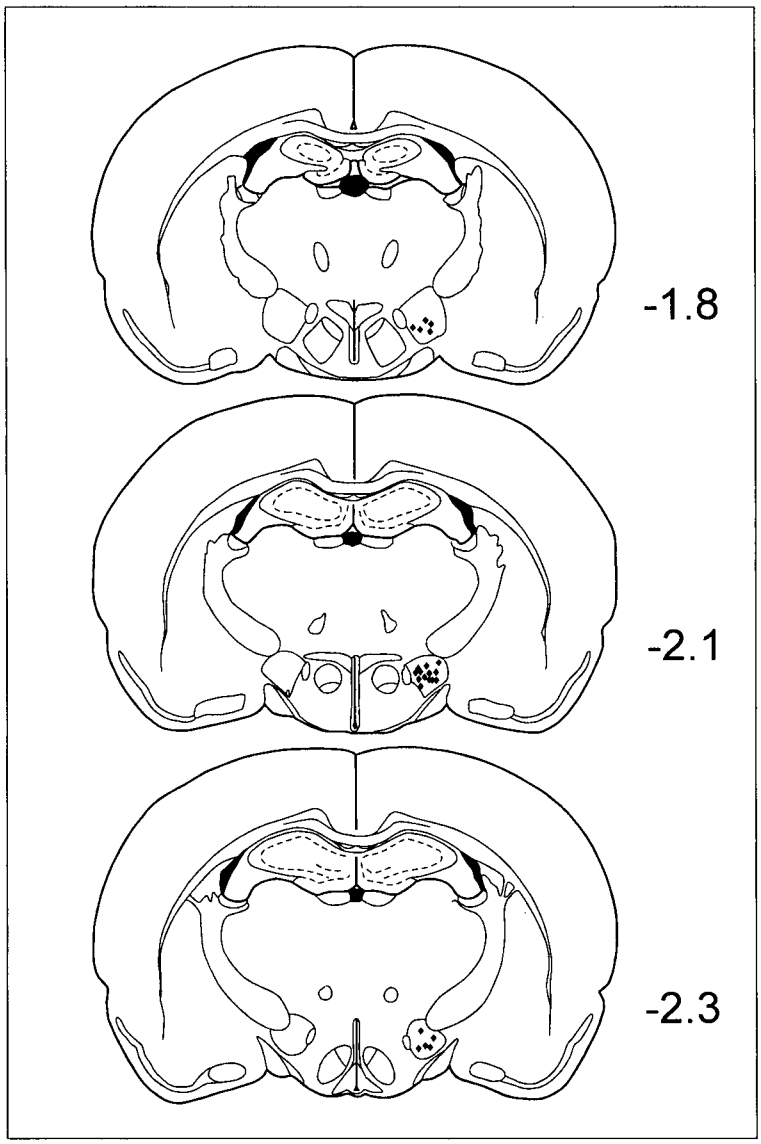


Fig 7.2

Histological placements of stimulating electrodes located within the lateral hypothalamus. Black diamonds (♦) indicate tips of electrodes. Placements shown are representative of electrode tips across entire anteroposterior range (-1.8 to –2.3 mm compared to bregma).

Discussion:

In the present study, rats exhibited reduced levels of ICSS responding at sites in the lateral hypothalamus for up to 60 hr following the termination of a 4-day escalating-dose schedule of *d*-amphetamine. We were able to demonstrate, for the first time, that repeated administration of ECS, which commenced after the termination of the drug schedule, facilitated the recovery of subjects from the anhedonia of *d*-amphetamine withdrawal. The ameliorative effect of ECS was manifested by an earlier recovery, relative to the sham-ECS condition, to control values of the currents that were required to maintain half-maximal (M_{50}) and threshold levels of responding for ICSS. Rats that had received ECS displayed no obvious signs of altered motor activity, as determined by their maximal levels of ICSS responding.

The effects of *d*-amphetamine withdrawal on M_{50} values in the sham-ECS group were evident for greater than 36 hrs in the present ICSS paradigm, while threshold currents were elevated for over 60 hrs. These effects represent a duration of hedonic disequilibrium that is comparable to our observations of attenuated responding for natural reinforcers, such as a sucrose solution or a sexually receptive conspecific (Experiments 4 and 5), in animals exposed to a similar regimen of *d*-amphetamine. Decrements in ICSS responding have been observed for longer periods when extended schedules of drug administration or different ICSS protocols were used (Kokkinidis et al. 1986; Wise and Munn 1995; Harrison et al. 2001). For example, Markou and colleagues reliably observe increases in reward thresholds, which are indicative of anhedonia, for up to 5 days following termination of a 6-day treatment with *d*-amphetamine (Lin et al. 1999; Harrison et al. 2001). Their use of a discrete-trials current procedure (Kornetsky and Esposito 1979) may be optimal for detecting the more subtle anhedonic effects of

psychostimulant withdrawal in its latter stages. Although the ascending-series rate/intensity paradigm used in the present study is widely employed to measure hedonic changes in rodents (e.g. Fibiger and Phillips 1981; Zacharko et al. 1997; Barr et al. 2000), this protocol may induce positive contrast effects (Phillips and LePiane 1986), which could feasibly mask minor deficits in ICSS responding. Our recent observation that rats withdrawn from *d*-amphetamine display protracted negative contrast effects (Barr and Phillips 2001) suggests a note of caution in the use of ICSS protocols that depend upon the presentation of currents in either an ascending or descending order.

Nevertheless, the duration of the effects of *d*-amphetamine withdrawal on ICSS responding was sufficient to reveal a restorative effect of repeated administration of ECS. These findings are in general agreement with previous studies that have examined the influence of repeated ECS in other animal models of anhedonia or depression. Electroconvulsive shock that is administered to rats has been shown reliably to exert antidepressant effects when rats or mice are subjected to a behavioral despair paradigm (Porsolt et al. 1978; Danysz et al. 1989; Lisanby and Belmaker 2000), by decreasing the time that animals spend immobile in this task. In a learned helplessness paradigm, rats displayed a potent antidepressant response to treatment with repeated ECS, which was not observed in subconvulsive control animals (Sherman et al. 1982). More recently, it was shown that rats that exhibited decrements in responding for ICSS after exposure to chronic mild stress demonstrated a rapid and complete reversal of the stress-induced anhedonia when treated with ECS (Moreau et al. 1995). 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 (Seltzer and Tonge 1975; Leith and Barrett 1980; Kokkinidis et al. 1986; Swerdlow et al. 1991; Baumann and Rothman 1998; Harrison et al. 2001).

In addition to these ameliorative effects of ECS, the anhedonic sequelae of psychostimulant withdrawal in rodents have also been reversed by a broad range of antidepressant drugs. Deficits in ICSS responding that result from the withdrawal of high doses of either cocaine or amphetamines have been reversed or mitigated by the administration of several different tricyclic antidepressants. Both imipramine and amitriptyline were shown to reverse the anhedonic effects of *d*-amphetamine withdrawal (Kokkinidis et al. 1980), while the effects of cocaine withdrawal were attenuated by treatment with desmethylimipramine (Markou et al. 1992). The treatment of animals with lithium, which also possesses therapeutic qualities in unipolar depression (Bauer et al. 2000), exhibited prophylactic effects and prevented the post-amphetamine depression of ICSS responding (Predy and Kokkinidis 1981). Additionally, other compounds that have been shown to exhibit antidepressant properties in preclinical studies were able to attenuate the effects of drug withdrawal, and included the indirect dopamine agonist bromocriptine (Markou and Koob 1992) and adenosine A2 antagonist DMPX (Baldo et al. 1999a).

The capacity of antidepressant drugs, such as the tricyclics, to reverse the hedonic deficits of psychostimulant withdrawal in a rapid manner may pose a theoretical challenge to the validity of this paradigm as a model of depression (Geyer and Markou 1995). The therapeutic onset of these drugs in humans typically requires a period of 2-4 weeks before a significant effect is observed (Gelenberg and Chesen 2000), whereas studies with drug withdrawal and ICSS in rodents observe mitigative effects within several days. The reasons for this discrepancy are unclear, but similar issues have been faced in alternate models of depression that maintain a high

level of pharmacological validity (Willner 1984). For instance, animals that are subjected to the forced swim test display a rapid and selective response to acute antidepressant drug treatment (Borsini and Meli 1988; Lucki 1997; Galea et al. 2001). It has been hypothesized that the timely response of rodents in this task may reflect differences in drug metabolism or receptor modulation (Porsolt et al. 1978; Duncan et al. 1985). It therefore remains possible that the rapid response of rodents to antidepressant drug treatment during psychostimulant withdrawal simply reflects differences in the metabolism of neuroactive metabolites, or the use or proportionally higher doses of drugs than are used in humans. In addition, the pharmacological validity of the current model is supported by the recent findings of Harrison et al. (2001), who noted that the SSRI fluoxetine was able to attenuate the effects of *d*-amphetamine withdrawal on ICSS responding after the fifth day of administration. Furthermore, the addition of the selective 5-HT1A antagonist p-MPPI in combination with fluoxetine significantly shortened the onset of therapeutic efficacy. Currently, there is a strong interest in the 5-HT1A receptor as a substrate for the development of rapidly-acting antidepressants (Briner and Dodel 1998; Andree et al. 1999; Beique et al. 2000), and the present data and those of Harrison et al. (2001) suggest that the psychostimulant withdrawal model may be able to differentiate between fast-acting and conventional antidepressants.

At present, the physiological mechanisms by which repeated ECS is able to alleviate the effects of psychostimulant withdrawal remain obscure. Electroconvulsive shock produces a wide range of molecular changes in the brain (Fochtmann 1994), although many of these may be unrelated to the therapeutic effects of treatment (Sackeim and Devanand 1990). The current schedule of *d*-amphetamine administration results in reduced levels of extracellular dopamine within the nucleus accumbens for at least 48 hrs after the final injection (Taepavarapruk et al.

1998), and may affect other monoamines as well (Segal and Kuczenski 1997). ECS has been shown to exert a number of effects on pre- and postsynaptic dopamine function, including large, transient increases in interstitial dopamine within the striatum (Zis et al. 1991). Additionally, there is evidence for reduced dopamine autoreceptor sensitivity after ECS (Tepper et al. 1982), while the consistent increases in behavioral activation that occur in response to direct or indirect dopamine agonists following repeated ECS (Green 1984; Fochtmann 1994) are indicative of postsynaptic changes in dopamine function. Future studies should determine the role of neurochemical changes in monoamine systems in the mitigation of the depressant effects of psychostimulant withdrawal, as well as those observed in other animal models of depression (Moreau et al. 1995; Phillips and Barr 1997).

General Discussion

The experiments described in this dissertation have charted the development and demise of one animal model of depression, followed by the development and successful maturation of another. In the first part of the dissertation, the Chronic Mild Stress model of depression was adopted for study, due to its academic credentials, which included seemingly high levels of pharmacological, face and construct validity. The model appeared to utilize realistic "inducing conditions" and provided a less severe, but logical, extension of models of learned helplessness. However, over an approximately 3-5 year period (1994-1998), the CMS model dropped out of popularity, and new studies that utilize this model have almost disappeared from the literature.

There are several reasons for the demise of this model, most of which are manifest in the first three experiments of this dissertation. First, and most importantly, the central premise that rats that are exposed to CMS exhibit anhedonia has been called into question. The results of Experiment 2, which indicate that stressed animals exhibit no motivational deficits for a rewarding stimulus, are not consistent with a model of anhedonia. A more plausible explanation for the reduced levels of sucrose solution consumption in CMS-exposed rats is that the large decreases in body weight occasioned by CMS may lead to reduced overall consumption. Secondly, the model displays a poor degree of reproducibility and reliability, reflected in our inconsistent results when attempting to generate the basic phenomenon of reduced consumption of a sucrose solution in CMS-exposed rats. Finally, the neurochemical results obtained in Experiment 3 are not readily interpretable within the current theories of the role of hypodopaminergic activity in depression (Willner 1995), indicating that the CMS model displays poor construct validity.

The subsequent shift in research and focus upon an alternate rodent model of depression was guided by the lessons learned with CMS. The basis for the development of an animal model of depression was more likely to prove fruitful if it could be constructed upon observations taken from the human literature, in which self-reports of depression could be confirmed. Many of the animal models of depression that are currently in use (cf. Introduction, pp 25-31) rely upon experimental manipulations to induce depressogenic symptomatology, that have an unknown relevance to the human condition. Clearly, a manipulation that leads to depressive-like symptoms in humans acts as a better starting point for a model than one which has no obvious correlate. For instance, there is little evidence that humans who are forced to swim for a relatively brief period, as in the forced swim test, ultimately develop depressive symptoms. Similarly, the exposure of neonatal infants to high doses of REM-suppressing drugs or lesions of the olfactory bulbs in humans may induce depression, but such bizarre events occur only rarely, and so cannot be easily confirmed with regards to their appropriateness. In contrast, a number of different pharmacological treatments can be shown to produce transient depression in humans (Patten and Love 1997).

Others have already suggested that the withdrawal from medium-to–high doses of psychostimulant drugs, which increases self-reports of depression in humans, may provide the basis for an animal model of depression (Seltzer and Tonge 1975; Leith and Barrett 1980; Kokkinidis 1986). Feelings of depression are described by humans during the withdrawal from many different classes of psychoactive drugs, including psychostimulants, benzodiazepines, opiates, alcohol and nicotine (West and Gossop 1994). However, all of these drugs, with the exception of psychostimulants, produce a host of other withdrawal symptoms, as evinced by various somatic symptoms. The relatively "clean" and selective psychological effects of

psychostimulant withdrawal therefore make it a better candidate for a model, because behavioral (and possibly physiological) effects will not be interfered with, or obfuscated by, irrelevant phenomena. However, there has never been a comprehensive review of the effects of psychostimulant withdrawal in humans and how they relate to MDD as a whole, including their response to antidepressant treatment; the first part of the General Discussion therefore fills this void. Subsequently, this comparative approach is then extended to the body of preclinical and animal research, wherein the effects of withdrawal from psychostimulant drugs in animals is compared to the symptomatology of MDD. These reviews provide a necessary evaluation of the face validity and overall level of empirical support for the psychostimulant withdrawal model of depression.

The relevance of the findings of Experiments 4-7 to this model of depression are individually described in detail in the Discussion section at the conclusion of each Experiment. In review, the results of Experiments 4 and 5 were able to add significantly to the face validity of the model in two main ways. Firstly, they indicated that psychostimulant withdrawal was associated with reduced interest or pleasure in "natural" rewards; perhaps surprisingly, very few studies have examined the effects of psychostimulant withdrawal in rodents on responding for natural reinforcers (most have used ICSS protocols). Second, the behavioral paradigms that we utilized were sophisticated and allowed a careful dissection of the motivational constructs that were selectively modulated in these tasks, with little room for future ambiguity. The findings of Experiment 6, in which rats displayed a protracted recovery from the effects of a negative contrast paradigm, emphasized the capacity of this model to disrupt more complex psychological experiences. In the final Experiment 7, we were able to add substantially to the pharmacological

validity of the paradigm, by demonstrating that one of the most widely-used treatments for MDD was also effective in this model.

The remainder of the General Discussion is committed to providing a general analysis of the validity of the psychostimulant withdrawal model of depression. By first providing an overview of the effects of psychostimulant withdrawal and its treatment in humans and animals, an evaluation of the face and pharmacological validity of this model may be determined. An ensuing section then deals with the more complex task of assessing the construct validity of this model. The dissertation concludes by pointing out the limitations of the model (as well as approaches to minimizing these limitations), and suggests areas of immediate future application.

Psychostimulants and Psychostimulant Withdrawal in Humans

The class of drugs known as the "psychostimulants" includes a broad range of psychoactive compounds, which have the common property of causing elevated behavioral and cognitive activity. Frequently-used psychostimulants, which are legally available in most countries, include caffeine and nicotine, while narcotic psychostimulants include cocaine, amphetamine, and the substituted amphetamines, such as para-methoxyamphetamine (PMA) and 3,4-methylenedioxymethamphetamine (MDMA) (Withers et al. 1995; Milroy et al. 1996; Christophersen 2000). Although abstinence symptoms have been widely reported after withdrawal from caffeine (Mitchell et al. 1995; Evans and Griffith 1999; Kendler et al. 1999) these symptoms are rarely of a magnitude sufficient to require hospitalization. The psychological effects of nicotine withdrawal are more severe (Hughes et al. 1994; Shiffman et al. 2000; Watkins et al. 2000a) and have been effectively modeled in animal paradigms (Helton et al. 1993; Bozarth et al. 1998; Epping-Jordan et al. 1998). However, nicotine abstinence differs from cocaine and amphetamine withdrawal in that is also associated with numerous somatic symptoms (Hughes and Hatsukami 1986; Carboni et al. 2000; Watkins et al. 2000b) that are not observed in MDD and will therefore not be part of the focus of the present review.

The mechanism of action of cocaine and the amphetamines is not entirely understood, but most likely involves their ability to bind to plasmalemmal monoamine transporters (Fleckenstein et al. 2000) and vesicular monoamine transporters (Brown et al. 2001). Cocaine increases interstitial levels of synaptically–released monoamines, primarily by preventing their reuptake via plasmalemmal transporters (Pifl et al. 1995), whereas amphetamines facilitate neurotransmitter release by depleting vesicular stores of monoamines (Sulzer et al. 1995; Jones

et al. 1998; Leviel 2001). Both types of drug result in voluminous increases in synaptic levels of dopamine, norepinephrine and serotonin (Parsons and Justice 1993; Reith et al. 1997; Rothman et al. 2001). It is generally assumed that these increases in monoamines are essential for the euphorigenic effects of the drugs (Dackis and Gold 1985; Fibiger et al. 1992; Koob and Nestler 1997), although the relationship between the levels of these neurotransmitters and subjective mood is likely to be quite complex (Volkow et al. 1999).

The psychological effects of psychostimulant drugs vary somewhat, depending on the drug and the individual (Mattay et al. 2000), as well as his/her prior experience with the drug. In general, both cocaine and the amphetamines increase alertness, concentration and energy when taken in lower doses (Stillman et al. 1993; Johnson et al. 2000; Wachtel et al. 2001), while higher doses produce additional euphorigenic effects that are often described as causing a "high" or "rush" (Sherer et al. 1989; Darke et al. 1994; Jones et al. 1999; Hart et al. 2000). These pleasurable effects decline rapidly, within minutes in the case of cocaine (Breiter et al. 1997), or over a period of several hours for *d*-amphetamine (Justice and De Wit 2000), depending in part upon the route of self-administration. As the rewarding properties of the drug diminish, negative affective states begin to emerge, which addicts will often seek to reverse by further administration of the psychostimulant drug. However, if none is available, or if the individual chooses to terminate drug self-administration, the affective sequelae of withdrawal from the drug become entirely apparent.

The psychological effects of drug withdrawal have often been explained within the theoretical framework of the opponent-process theory of motivation (Solomon 1977; Koob et al. 1997; Ettenberg et al. 1999). According to this theory, during withdrawal the previously pleasurable effects of drugs of abuse are inevitably followed by emotional states opposite in

affect, and of a longer duration, as the body seeks to restore its "hedonic equilibrium" (Solomon and Corbitt 1974). Thus, the acutely rewarding properties of psychostimulant drugs, which include euphoria, increased energy and self-confidence (Watson et al. 1972), generate a withdrawal syndrome characterized by anhedonia, lethargy and anxiety. In a seminal paper, Gawin and Kleber (1986) described in detail the abstinence symptomatology of chronic cocaine users, and determined that the course of the post-binge withdrawal syndrome could be categorized into three distinct phases. The first of these, referred to as the "crash", was characterized by "extreme dysphoria...full anhedonia...irritability, anxiety [and] a subjective sense of confusion," as well as "temporary suicidal ideation" in 43% of the sample patient group. The crash phase lasted for up to 4 days, after which subjects entered the "withdrawal" phase; this stage of abstinence was represented by milder dysphoria, substantial anhedonia and anergia, with elevated levels of anxiety and irritability. The withdrawal phase continued for up to 10 weeks as patients gradually entered the "extinction" phase, which reflected a return to near-normal functioning. More recent studies of the effects of cocaine withdrawal have reported a similar constellation of abstinence symptoms, although there has been relatively little support for the 3-phase sequence that Gawin and Kleber (1986) originally described. Instead, most studies have reported a gradual, linear decrease in the severity of withdrawal symptoms over time (Satel et al. 1991; Lago and Kosten 1994; Kampman et al. 1998). Although substantially fewer in number, studies of withdrawal from binge-like doses of amphetamines in humans reported similar effects to those observed in cocaine-withdrawn patients (Kramer et al. 1967; Watson et al. 1972; Gawin and Ellinwood 1988; Gillin et al. 1994; Jittiwutikan et al. 1997).

The cognitive and emotional effects of psychostimulant withdrawal bear a remarkable similarity to the symptoms of unipolar depression. Indeed, the semblance can be so great that the

clinician may have to differentiate between the two based solely upon the suspected etiology of the condition (APA 1994). A comparison of the effects of psychostimulant abstinence with the DSM-IV diagnostic criteria for MDD, independent of symptom duration, in Table 1 indicates that almost all of the presenting symptoms of MDD are widely observed during psychostimulant abstinence, with the possible exception of "feelings of worthlessness or excessive or inappropriate guilt" (Criterion A7). Both of the core symptoms of MDD, (i.e. depressed mood and anhedonia, Criteria A1 and A2), either one of which must be present for a diagnosis of MDD, are reliably induced by psychostimulant withdrawal (Schildkraut et al. 1971; Weddington et al. 1990; Volkow et al. 1991; Pathiraja et al. 1995; Malison et al. 1998). Changes in appetite, another defining symptom of MDD (Criterion A3), are also commonly reported during post-binge abstinence (Dackis et al. 1987; Brower et al. 1988; Srisurapanont et al. 1999), most commonly reflected as a pronounced hyperphagia. Hypersomnia (Criterion A4) is a cardinal symptom of psychostimulant withdrawal (Gawin and Kleber 1986; Gillin et al. 1994; Thompson et al 1995; Volkow et al. 1998) and is also a distinguishing sign of atypical depression (Silberman and Sullivan 1984; Nierenberg et al. 1998). Feelings of fatigue (Criterion A6), often combined with psychomotor retardation (Criterion A5), represent symptoms common to both MDD and psychostimulant withdrawal (Tuma 1993; Uslaner et al. 1999), while the cognitive sequelae of psychostimulant abstinence include impaired concentration and confusion (Satel et al. 1991; Roberts and Bauer 1993) (Criterion A8). Finally, suicidal thoughts and ideations (Criterion A9) have been reported extensively during psychostimulant withdrawal (Kaplan and Saddock 1983; Lowenstein et al. 1987; Kaminer 1992; Kampman 1998).

In addition to the DSM-IV diagnostic criteria for MDD, there are also numerous other shared behavioral and affective symptoms common to both depression and psychostimulant

withdrawal. For instance, both syndromes are characterized by an increased craving for carbohydrates (Wurtman 1990; Moller 1992; Kampman et al. 1998). Depressed individuals and patients in the later stages of psychostimulant withdrawal also exhibit an increased craving for drugs of abuse, which has been hypothesized to reflect a desire to self-medicate the dysphoria common to both conditions (see Markou et al. 1998 for review). Irritability is prominent in MDD and during psychostimulant abstinence (Brower et al. 1988; Fava 1998), as are feelings of restlessness (Gawin and Kleber 1986; Schatzberg and DeBattista 1999). The similarities between the two conditions has led to the widespread measurement of psychostimulant withdrawal symptoms with diagnostic tools that are more commonly used to gauge depression, such as with the Beck Depression Inventory, the Hamilton Rating Scale for Depression and the ICD-10.

Given the similarity of the phenomenology between MDD and psychostimulant withdrawal, it would be expected that the two disorders might share a common underlying physiology. To confirm this hypothesis, however, would require that the physiology of each condition be understood *a priori*, which does not reflect the current state of affairs. Nevertheless, major depression has been consistently associated with a number of physiological markers, including hormonal, electrophysiological, and metabolic indices, many of which are present during psychostimulant withdrawal. Disruptions of the normal functioning of the hypothalamic-pituitary-adrenal (HPA) axis are frequently observed in MDD, including elevated levels of cortisol, decreased dexamethasone-mediated negative feedback, and increased cerebrospinal levels of corticotropin-releasing factor (CRF) (Plotsky et al. 1998; Holsboer 2000). In a study by Vescovi et al. (1992), levels of the neuropeptides ACTH and β-endorphin, as well as the glucocorticoid hormone cortisol, were monitored at different times throughout the diurnal

period in recently abstinent cocaine addicts. The authors of the study recorded elevated diurnal levels of all three hormones during early withdrawal, a pattern that resembles the neurohormonal profile of severely depressed patients reported in the majority of clinical studies (Goodwin et al. 1993; Deuschle et al. 1997). Elevated levels of prolactin are also frequently observed during psychostimulant abstinence (Mendelson et al. 1988; Satel et al. 1991) which are consistent with the results of studies indicating that elevated levels of prolactin are associated with depressive symptomatology (Kellner et al. 1984).

Neuroimaging and EEG techniques have provided the opportunity to compare the patterns of regional cerebral activity during psychostimulant abstinence and MDD. Although widespread and diffuse effects have generally been reported, certain similarities between the two disorders have been noted. For example, EEG changes during cocaine abstinence have been reflected by altered power in the beta (18-26Hz) band (Herning et al. 1997), resembling the changes noted in some melancholic depressives (Kano et al. 1992). More convincingly, there is a striking similarity between the EEG activity of depressives and abstinent psychostimulant abusers during sleep. One of the more reliable physiological markers of MDD has been a characteristic disruption of the normal architecture of the nocturnal EEG, reflected in a reduced REM latency and increased REM density throughout the night (Kupfer 1976; Thase et al. 1998). Abstinence from both amphetamine (Watson et al. 1972) and cocaine (Thompson et al. 1995) mimics the effects of MDD on these parameters.

Measures of regional cerebral activity can be provided by neuroimaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI). Neuroimaging studies of MDD have observed diffuse changes in cerebral metabolism, although certain limbic regions appear to show more consistent changes in activity than do others (Drevets

2000). In particular, regions of the dorsolateral prefrontal cortex, subgenual anterior cingulate cortex and the striatum display reduced cerebral blood flow (CBF) (Drevets et al. 1992; Mayberg et al. 1999), while the orbitofrontal cortex and amygdaloid complex exhibit increased CBF (Drevets 2000). A PET study of cocaine addicts in the early phase of withdrawal observed elevated metabolic activity within the orbitofrontal cortex, similar to that found in MDD, and also in the basal ganglia (Volkow et al. 1991), which is not characteristic of MDD. More recently, Breiter et al. (1997) used PET to correlate regional brain activity with mood during and after an infusion of cocaine. There were no significant associations of regional brain metabolism with the "low" that subjects experienced during cocaine withdrawal. The reasons for the discrepancy in the findings of Volkow et al. (1991) and Breiter et al. (1997) are not immediately clear, but may be related to the amount of drug that was consumed, as subjects in the Volkow et al. (1991) study had self-administered binge-like doses of cocaine, in contrast to the single, lower-dose infusion of drug in the Breiter et al. (1997) study. Neuroimaging techniques have also enabled the measurement of monoamine transporter densities in MDD and during psychostimulant abstinence. In both syndromes, increases in dopamine transporter (DAT) densities were observed in the striatum (Malison et al. 1998; Laasonen-Balk et al. 1999). In contrast, serotonin transporter (SERT) binding was increased in the brainstem of recently abstinent cocaine addicts (Jacobsen et al. 2000), whereas SERT binding was decreased in the brainstem of patients with MDD (Malison et al. 1998) – although this effect was reversed in adolescent MDD (Dahlstrom et al. 2000). These diverse findings are of interest, and may represent an important difference between the two disorders.

Overall, it can be concluded that MDD and psychostimulant withdrawal display many of the same behavioral and physiological symptoms. The similarity is greater for the psychological

and phenomenological effects, wherein the two conditions are nearly identical. Physiological similarities are extensive, and include hormonal, EEG and some neuromolecular parallels. Differences between the two syndromes have been demonstrated using PET, and may be related to regions of metabolic activity as well as differences in brainstem SERT density. It should be noted, however, that the human subjects in most studies represent a heterogeneous population, with diverse backgrounds, and substantial variability occurs when comparing measures across studies. Drug addicts, who are the most common type of subjects used in the investigation of psychostimulant withdrawal, frequently exhibit polydrug abuse (Liu et al. 1998), and display higher levels of psychiatric conditions than the general population (Gawin and Kleber 1986). Furthermore, inpatient psychostimulant abusers rarely experience abstinence symptoms as severe as outpatient addicts who require hospitalization (Dudish-Poulsen and Hatsukami 2000), suggesting that the additional stresses of life "on the street" may be a contributing factor to the intensity of withdrawal symptoms. Similarly, depression is a heterogeneous disorder, with numerous different subtypes, and many depressives have a long history of substance abuse (Markou et al. 1998). These large sources of potential variability, and the limitations that they impose, must be considered when attempting to compare the behavioral and physiological sequelae of psychostimulant withdrawal and MDD.

Treatment of Psychostimulant Withdrawal in Humans

At present, there is no effective pharmacological treatment available for the complete reversal of the aversive effects of psychostimulant withdrawal. Numerous therapies have been evaluated,

but only a few have reliably been shown to exhibit efficacy significantly superior to that of a placebo. As a consequence of the similarity of the symptoms of psychostimulant withdrawal to those of MDD, the majority of treatments for psychostimulant abstinence have consisted of drugs that display antidepressant properties. The results of many studies, however, are complicated by the high rates of comorbid MDD with substance abuse; often, it is not clear which is being treated – the effects of early psychostimulant withdrawal or an underlying depression. Additionally, many clinical studies have treated abstinent drug addicts with antidepressant drugs, with the intent to reduce drug craving and prevent relapse, without measuring the effects of these drugs on mood during withdrawal. Evidence for reduced craving during early abstinence in patients given antidepressant drugs suggests that such drugs may alleviate withdrawal symptoms, but more evidence is needed.

A smaller number of studies have specifically sought to treat the symptoms of psychostimulant withdrawal in drug addicts. Due in large part to the crucial role of dopamine in the rewarding properties of psychostimulant drugs, treatment therapies have often focused on pharmacological manipulation of the mesolimbic dopamine system. One of the more promising drugs used in earlier studies was the D2 receptor agonist bromocriptine. Several reports indicated that bromocriptine could provide rapid relief to the dysphoria of cocaine withdrawal (Tennant and Sagherian 1987; Giannini et al. 1987). More recent studies have generally failed to reproduce these findings (Eiler et al. 1995; Handelsman et al. 1997), although the reason for the discrepancy in results is unclear. When bromocriptine was used in combination with the tricyclic antidepressant desipramine, it acted synergistically to attenuate the symptoms of cocaine withdrawal (Giannini and Billett 1987), indicating that bromocriptine may be more useful as an adjunct therapy. Lisuride, an ergot derivative with D2 agonist properties similar to

bromocriptine, was also examined for its putative therapeutic properties in the treatment of psychostimulant withdrawal (Gillin et al. 1994). The benefits of lisuride were limited, and predominantly involved its capacity to alleviate sleep-related deficits. Similarly, the D1/D2 agonist pergolide was shown to be most effective in treating sleep deficits during cocaine abstinence (Malcolm et al. 1991). The D2 agonist apomorphine, which has both pre-and postsynaptic affinities, produced a rapid reversal of depressive symptomatology during cocaine withdrawal (Hollander et al. 1990). When compared with the results of studies that have used direct agonists at the postsynaptic D2 receptor, such as bromocriptine and lisuride, the observations of Hollander et al. (1990) suggest that further study with D2 autoreceptor binding drugs may be warranted. 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), particularly when it is used by patients with more severe withdrawal symptoms (Kampman et al. 2000). Treatment of cocaine addicts with dopamine precursors, such as tyrosine (Galloway et al. 1996) and l-dopa/carbidopa (Wolfsohn et al. 1993) has generally not provided a successful approach to the alleviation of withdrawal symptoms.

Aside from dopamine-focused treatment strategies, more conventional antidepressant drugs have also proven to be of use in the treatment in psychostimulant abstinence. Desipramine is frequently administered to psychostimulant addicts during drug withdrawal (Halikas et al. 1993), and shows some potential in reducing cocaine cravings (Gawin et al. 1989), as well as hypersomnia (Baxter 1983). It was also shown to reduce depressive symptoms during abstinence, although this effect was not apparent until after 20-40 days of treatment (Giannini et al .1986). In a case study, amitriptyline was successful in reversing the dysphoria of

amphetamine withdrawal (Tuma 1993), while the antidepressant amineptine has been shown repeatedly to provide a rapid attenuation of the symptoms of amphetamine abstinence (Jittiwutikan et al. 1997; Srisurapanont et al. 1999). The combined serotonin/norepinephrine reuptake inhibitor venlafaxine reduced depressed symptomatology during cocaine withdrawal in patients with comorbid MDD (McDowell et al. 2000). A role for reduced levels of serotonin in the aversive effects of cocaine withdrawal was given further support by the recent findings of Kampman et al. (2000), who demonstrated that cocaine abstinence could be reversed with a combination of the serotonin-releasing compounds phentermine and fenfluramine. This group has also indicated that withdrawal-induced depressive symptomatology can be alleviated rapidly by treatment with propanolol (Kampman et al. 2001), presumably due to its beta-blocking properties.

The broad range of pharmacological compounds that is available for the treatment of psychostimulant abstinence indicates that there are likely a number of different neurochemical substrates involved in the symptomatology of this condition. Almost all successful treatments have used drugs that act upon the monoamine systems, either directly of indirectly. In this manner, the mechanisms of action of drugs used to treat psychostimulant withdrawal strongly resemble the pharmacological profile of most antidepressants, which also act on central monoamine systems (Berman and Charney 1999; Feighner 1999a), with a few exceptions (Kramer et al. 1998; Altar 1999). Furthermore, many of the drugs that can alleviate the symptoms of psychostimulant withdrawal also display antidepressant properties in humans, including bromocriptine (Bouras and Bridges 1982), amantadine (Huber et al. 1999) and fenfluramine (O'Rourke et al. 1989), in addition to the conventional antidepressants desipramine, amitriptyline and venlafaxine. These similarities suggest that psychostimulant withdrawal and

MDD share some degree of pharmacological isomorphism, as they respond to similar psychoactive compounds. However, direct comparisons of pharmacological responsivity between the two conditions are often hindered by the relatively short duration of the effects of psychostimulant withdrawal and the delayed onset of action of most conventional antidepressant treatments. Clearly, it will be of interest to determine how future rapidly-acting antidepressant treatments perform in alleviation of the symptoms of psychostimulant withdrawal.

Psychostimulant Withdrawal as a Model of Depression in Rodents

Face Validity

The similarity of psychostimulant withdrawal to MDD in humans may provide the theoretical foundation for the development of an animal model of depression, if valid comparisons may be made between the behavioral, physiological and pharmacological aspects of psychostimulant withdrawal in animals and humans. A large body of evidence suggests that rodents willingly self-administer similar types of drugs of abuse as humans do (Markou et al. 1993; Gardner 2000), indicating that they are selectively responsive to the reinforcing properties of these drugs. With the use of sophisticated behavioral paradigms, it has been shown that rodents are also subject to the aversive psychological states that are associated with drug withdrawal. The degree of similarity between the effects of psychostimulant withdrawal in rodents and the symptoms of MDD in humans reflects the "face validity" of this model of depression (Mosier 1947; McKinney and Bunney 1969; Willner 1991). An animal model of a psychiatric disorder must exhibit a high degree of face validity, measured by strong behavioral and psychological correlates between the animal paradigm and the human condition, before it can be considered to be a legitimate model (Geyer and Markou 1995).

The effects of withdrawal from high doses of psychostimulant drugs in rodents have been measured on a number of different parameters, including both behavioral and physiological indices. The former range from changes in simple tasks such as measures of locomotor activity to complex instrumental behaviors that can provide a substantial amount of information about the affective state and motivation of the animal. These alterations may be compared to

behavioral or self-reported affective changes in depressed humans, and, given a certain amount of license, the similarities between the two conditions may be evaluated. The physiological changes that arise from psychostimulant withdrawal in rodents may also be compared to known physiological changes that are associated with MDD, although the more invasive nature of preclinical research often entails that comparable studies do not exist in the human literature.

One of the more frequently described psychological effects of withdrawal from high doses of cocaine in humans and rodents is an increased level of anxiety. In animals, this has been demonstrated using behavioral tasks such as the suppression of punished responding (Fontana and Commissaris 1989), conditioned avoidance (Fung and Richard 1994), performance on the elevated plus maze (Sarnyai et al. 1995), fear potentiated startle (Gordon and Rosen 1999), defensive burying (Basso et al 1999) and ultrasonic vocalization (Mutschler and Miczek 1998). Increased anxiety during amphetamine withdrawal is less well established, despite being noted in human studies (Srisurapanont et al. 1999), and further research in this field is required. The presence of elevated levels of anxiety during psychostimulant abstinence represents an important potential confound when utilizing the behavioral sequelae of psychostimulant withdrawal as markers of depressive symptomatology. For instance, decreases in locomotor activity that occur during psychostimulant withdrawal when a rat is exposed to a novel open field environment (Lynch and Leonard 1978) may reflect psychomotor retardation (a defining symptom of MDD), but could equally as well indicate increased levels of anxiety (Meert 1992). The different roles of these two factors may be determined by selective responding to a specific class of drugs, such as antidepressants, but as many compounds in this class also exhibit alternate therapeutic properties (Feighner 1999b; Baker et al. 2000), firm conclusions cannot always be drawn. Increased levels of anxiety frequently occur comorbidly with MDD (Sullivan and

Kendler 1998; Kaufman and Charney 2000), and hence increase the face validity of psychostimulant withdrawal as a model of depression. However, they also introduce a note of caution into interpreting the results of certain behavioral tasks that may be affected by elevated levels of anxiety.

In general, animal models of depression seek to induce quantifiable behavioral alterations that parallel a specific symptom of MDD (Willner 1984; Geyer and Markou 1995; Redei et al. 2001). Of the nine diagnostic symptoms that the DSM-IV describes for MDD (APA 1994), it has been suggested that a majority of these can be modeled in rodent paradigms (Willner 1991). While it is clear that certain symptoms which rely on self-report cannot be modeled in rats, such as "depressed mood" (Criterion A1), "feelings of worthlessness" (Criterion A7) and "suicidal ideation" (Criterion A9), these symptoms are commonly described in humans experiencing psychostimulant withdrawal, and hence reflect a limitation of the species used for modeling, rather than an inherent weakness in the model itself. The remaining six diagnostic criteria can all be modeled to a greater or lesser degree in rodents.

Changes in simple homeostatic behaviors, such as sleeping and eating, are readily measured in rodents after withdrawal from psychostimulant drugs. Alterations in weight or food consumption are commonly exhibited in MDD (Criterion A3) and may be manifest as either an increase or decrease in appetite. The administration of medium-to-high doses of psychostimulants to rats typically produces a biphasic effect upon food consumption, whereby animals initially reduce eating during the earlier anorectic phase, and display hyperphagia during the later "rebound" phase (Jones and Caul 1989). In this manner, psychostimulant withdrawal is more closely associated with the hyperphagic phase, and it is of interest that rats exhibit a preference for carbohydrate and fat over protein during this period (Bane et al. 1993), similar to

the preference that many depressives exhibit for foods richer in fat and carbohydrates, such as chocolate (Moller 1992; Willner et al. 1998). Furthermore, we have recently analyzed the microstructural licking behavior of rats when freely consuming a 4% sucrose solution for a 10 minute period during withdrawal from an escalating-dose schedule of *d*-amphetamine (Barr et al., in preparation). Results from this study indicated that in the earlier stages of fluid consumption, the pattern of rats' licking resembled that observed with a more concentrated sucrose solution in non-withdrawn animals, suggesting that rats may exhibit a transient initial increase in liking for a sucrose solution.

Another important diagnostic symptom of MDD is a change in sleep patterns (Criterion A4), reflected as either insomnia or hypersomnia. The biphasic effects of psychostimulants on this parameter resemble those observed with food consumption, as cocaine and amphetamines produce an initial decrease in sleep with a subsequent "rebound" effect which is characterized by increased time spent sleeping (Touret et al. 1995). Consistent with the depressive-like changes in sleep architecture that humans in psychostimulant withdrawal manifest, rats also display a post-drug increase in REM sleep density (Dugovic et al. 1992) after withdrawal from chronic cocaine. The withdrawal from cocaine is also associated with disruptions of rats' circadian rhythm, with respect to their regular pattern of feeding activity (Giorgetti and Zhdanova 2000).

Two closely related diagnostic criteria of MDD are "psychomotor retardation" (Criterion A5) and "fatigue/ loss of energy" (Criterion A6). In animals, decreases in locomotor activity are often interpreted as representing psychomotor retardation, although the concept of "fatigue" implies a phenomenological component that may be less amenable to measurement. Numerous studies have reported that during psychostimulant withdrawal, animals exhibit decreased locomotion (Herman et al. 1971; Seltzer and Tonge 1975; Fung and Richard 1994; Gauvin et al.

1997), often with accompanying mild catalepsy (Tonge 1974; Pulvirenti and Koob 1993; Malin et al. 2000). However, a number of other studies have failed to observe decreased locomotor activity in psychostimulant withdrawn animals (Schreiber et al. 1976; Kokkinidis et al. 1986). While this may reflect discrepancies in methodology, it is also evident that differences in locomotion between withdrawn and non-withdrawn animals are greater during periods of higher activity, such as upon exposure to a novel environment (Schindler et al. 1994) or during the active/ night phase (Paulson et al. 1991; Paulson and Robinson 1996; personal observations), indicating that "floor effects" during periods of hypoactivity may mask putative differences in locomotion. This appears to be a reasonable explanation, as evidence for psychomotor retardation in depressives would be less obvious if they were compared to normals who were in a state of rest. Whether or not differences in locomotor activity in rodents are due to an internal state of "fatigue" is more difficult to determine, although it should be noted that fatigue is frequently reported during abstinence in human psychostimulant abusers (Gawin and Kleber 1986). Salamone et al. (1994) have developed a novel behavioral task, based on the ratio of energy requirements to reward benefits, which purportedly is able to measure "anergia" in rodents, and it will be of interest to determine how psychostimulant withdrawn animals perform in this task.

Cognitive deficits in MDD represent a cardinal set of symptoms (Criterion A8) that are receiving increased attention, as more is learned about the specific types of deficits that are prevalent with this disorder. With the use of sophisticated neuropsychological testing batteries, there is emerging evidence that different sub-types of depression are associated with distinct cognitive profiles (Elliott et al. 1997; Austin et al. 2001) which may be extrapolated to the dysregulation of specific brain regions. In humans, psychostimulant withdrawal is characterized

by a range of different cognitive aberrations, including deficits in attention and memory (Roberts and Bauer 1993; Bolla et al. 2000; Coffey et al. 2000). Many of these cognitive constructs can be measured in rodents (D'Mello and Steckler 1996), and will represent an important area of future focus for animal models of depression. The author is not aware of any study to date that has assessed cognitive function in rats during the withdrawal of high doses of psychostimulant drugs, although one study found perseverative deficits in mice during amphetamine withdrawal (Kokkinidis 1983), but given the neurocognitive deficits that are observed in humans during psychostimulant withdrawal, there is good reason to believe that such deficits may exist.

The DSM-IV (as well as previous editions of the manual) places particular emphasis on two core symptoms for the diagnosis of depression, either one of which must be present for confirmation of the disorder. The first of these, i.e. "depressed mood" (Criterion A1), relies upon the self-report of subjective experience and is therefore impossible to determine in rodents. However, the results of certain studies have indicated that psychostimulant withdrawal may be associated with a dysphoric state; for instance, rats will develop a conditioned place aversion towards an environment that is associated with the withdrawal from cocaine (Ettenberg et al. 1999). In a three choice drug discrimination task, Stadler et al. (1999) recently demonstrated that rats in amphetamine withdrawal pressed on a lever which was normally associated with a low dose of haloperidol, suggesting that the interoceptive cue of drug abstinence generalized to the dysphoria caused by a low dose of haloperidol (King et al. 1995). Two studies have examined the effects of amphetamine withdrawal on animals' behavior in the forced swim test: this task is a common screen for antidepressant drugs (Borsini and Meli 1988; Lucki 1997) but has seen increased use recently as a paradigm for measuring "depressive" symptomatology in rodents (Hansen et al. 1997; Galea et al. 2001). In one of the two studies, it was noted that mice

exhibited increased immobility during amphetamine withdrawal (Kokkinidis et al. 1986), which is suggestive of depressogenic factors. In the other study (Schindler et al. 1994), however, the authors found opposite results, as amphetamine withdrawn rats displayed reduced immobility during the second exposure to the testing apparatus, which would theoretically indicate an antidepressant effect of drug withdrawal. It is unclear why the two studies found such discrepant results, and species differences are probably insufficient to explain the differences. The effects described by Schindler et al. clearly need to be replicated, and reconciled with current interpretations of behavioral changes displayed in the forced swim test.

Anhedonia, the second core symptom of depression, which is evinced by a "markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day" (Criterion A2), has proven to be much more easily measured in rodent models of reward and depression. Due to the relative ease of training rats to respond for rewarding stimuli, manipulations which decrease the salience or reinforcing properties of these stimuli can be readily quantified in an objective manner. Thus, the reduced value of rewarding stimuli during psychostimulant withdrawal can be reliably demonstrated.

The vast majority of studies that have examined the anhedonic effects of psychostimulant withdrawal have done so by assessing animals' responding for rewarding brain stimulation. The use of intracranial self-stimulation (ICSS) is a well-validated technique that allows experimenters to determine the amount or frequency of current that is required to maintain different patterns of responding by animals, depending on the paradigm, and hence provides a measure of the reactivity of the reward system (Olds and Fobes 1981; Liebman 1983; Phillips 1984; Wise 1996). Typically, drugs of abuse such as amphetamines and cocaine reduce the amount/frequency of current necessary to maintain threshold or half-maximal levels of

responding (Ivanona and Greenshaw 1997), indicating that the reward system is being stimulated. In contrast, during withdrawal from psychostimulant drugs animals require greater electrical brain stimulation to maintain responding, verifying a loss of reward function. Studies have demonstrated this effect using electrodes placed in the lateral hypothalamus (Simpson and Annau 1977; Leith and Barrett 1980; Cassens et al. 1981; Lin et al. 1999), substantia nigra (Kokkinidis et al. 1980) and ventral tegmental area (Borowski and Kokkinidis 1992; Frank et al. 1992). Similar types of effect are found with both amphetamines and cocaine, and the duration and magnitude of withdrawal effects are in proportion to the amount of drug that the animal consumed (Markou and Koob 1991). A similar degree of anhedonia is evident in rats that self-administer (Markou and Koob 1992), receive passive injections (Leith and Barrett 1976), or subcutaneously absorb drug from implanted minipumps (Paterson et al. 2000). The duration of the effects of psychostimulant withdrawal typically range from between 48hr (Barr et al. 2001) up to six days (Harrison et al. 2001), although some indices may be present for up to three weeks (Wise and Munn 1995), and in the one study that examined the effect of amphetamine withdrawal on ICSS from electrodes placed in the substantia nigra (Kokkinidis et al. 1980) longer term effects were observed.

Natural rewards are also reduced in value during psychostimulant withdrawal. We have recently shown that that when rats are trained to respond instrumentally for a sucrose solution under a progressive ratio (PR) schedule of reinforcement, animals will exhibit high rates of lever pressing for this reward (Experiment 4). According to the requirements of this schedule, rats must increase the number of lever presses for each subsequent reward, until a point is reached at which they fail to obtain the next reward within the time available (Hodos 1961; Roberts et al. 1989; Mendrek et al. 1998), an effect known as the "breaking point". When rats were tested

after withdrawal from a 4 day escalating dose schedule of *d*-amphetamine, they exhibited lower breaking points than control animals for up to three days when responding for a 4% sucrose solution (Barr and Phillips 1999), an effect recently replicated by Orsini et al. (2001), indicating a reduced motivation to obtain the rewarding stimulus. A more detailed analysis of the data revealed that at lower ratios of responding, both groups took approximately equal times to complete response requirements, but at higher ratios, when the amount of effort necessary to obtain a fixed reward was substantially greater, the withdrawn animals took significantly longer than controls. These data suggest that the effects of psychostimulant withdrawal become more pronounced either as the task becomes more demanding, or as the ratio of the reward/cost decreases. In support of this hypothesis, it was found that withdrawn animals did not differ in their consumption of the sucrose solution when it was freely available. The use of the PR paradigm may therefore be an effective tool for measuring anhedonia in rodent models of depression, and it is of interest that depressed humans also exhibit lower breaking points compared to normals in a PR task when responding for a monetary reinforcer (Hughes et al. 1985). Withdrawal from an escalating dose schedule of *d*-amphetamine in rodents also decreases motivation for access to a sexually receptive conspecific (Barr et al. 1999). Using a similar regimen of *d*-amphetamine administration to the experiment described above, we observed that withdrawn rats engaged in normal copulatory behavior, but their interest in obtaining the conspecific, both before and after ejaculation, was significantly diminished. These results provide strong support for the face validity of this model of depression, as human depressives often exhibit decreased interest in sexual activity (Phillips and Slaughter 2000) but typically maintain normal copulatory abilities (Nofzinger et al. 1993; Karp et al. 1994).

Anhedonia has also been appraised in psychostimulant withdrawal using a number of alternate techniques. For instance, Carroll and Lac (1987) reported that rats decreased responding under a fixed-ratio schedule for a sweetened drinking solution during cocaine withdrawal, and this effect was reversed by reinstatement of the drug. Several studies have observed that rats display reduced exploratory activity and a decreased interest in a novel object during psychostimulant withdrawal (Schreiber et al. 1976; Hitzemann et al. 1977; Persico et al. 1995) providing another measure of anhedonia. Perhaps in order to "self-medicate" their own anhedonia, human depressives engage in much higher rates of substance abuse than the general population (reviewed in Kosten et al. 1998; Markou et al. 1998), and this effect is mimicked in animal studies, as rodents demonstrate more intense drug-seeking behavior during withdrawal than prior to drug self-administration (Tran-Nguyen et al. 1998).

In addition to the behavioral similarities between psychostimulant withdrawal in rodents and MDD in humans, there is also a range of physiological parallels between the two conditions. Numerous studies have examined the neurochemical alterations associated with cocaine withdrawal (reviewed in Kuhar and Pilotte 1996; Pilotte 1997). The most frequently described effects of psychostimulant withdrawal are changes in the function of monoamine systems, with particular emphasis on mesolimbic dopaminergic and serotonergic pathways. Through the use of *in vivo* techniques including microdialysis and electrochemistry, it has been shown that extracellular levels of dopamine are reduced in limbic nuclei, such as the nucleus accumbens, during cocaine withdrawal (Parsons et al. 1991; Weiss et al. 1992). Equivocal findings in this region have been reported during amphetamine withdrawal, with some groups finding evidence for reduced levels of dopamine (Rossetti et al. 1992; Weiss et al. 1997; Taepavarapruk et al. 1998) while others do not (Crippens and Robinson 1994; Paulson and Robinson 1996). A

hypofunctioning of the dopaminergic system during cocaine withdrawal is supported by behavioral data from studies which show that rodents are more sensitive to the locomotor depressing effects of D_2 antagonists during this period (Baldo et al. 1999). As there is a substantial body of evidence which indicates that unipolar depression is related to reduced functioning of the mesolimbic dopamine system (Willner 1995; Lambert et al. 2000; Martinot et al. 2001), these findings suggest a degree of face validity between the physiology of this model of depression and the human disorder. Similarly, deficits in serotonergic function have been reported in both MDD and during psychostimulant withdrawal. The list of serotonergic deficits in MDD is exhaustive (Graeff et al. 1996; Deakin 1998; Mann 1999), and includes both pre- and postsynaptic modifications, as well as regional decreases in neurotransmission. Corresponding changes in pre- (Baumann et al. 1995; Darmani et al. 1997) and postsynaptic (Baumann and Rothman 1998) serotonergic activity have been observed during cocaine withdrawal in rodents, with parallel decreases in serotonergic neurotransmission in the nucleus accumbens (Parsons et al. 1995). To date, relatively few studies have examined the role of norepinephrine function during psychostimulant withdrawal (Herman et al. 1971; Paulson et al. 1991), but based upon the large amount of evidence that indicates an important role for this catecholamine in MDD (Leonard 1997; Anand and Charney 2000), further research is warranted.

Neuroendocrine modifications are another common physiological marker of MDD. In particular, disruption of the HPA axis are frequently observed, including elevated levels of cortisol, decreased dexamethasone-mediated negative feedback, and increased cerebrospinal levels of corticotropin-releasing factor (CRF) (Plotsky et al. 1998; Holsboer 2000). Several studies have examined levels of corticosterone during psychostimulant withdrawal, and elevated levels of the hormone were observed 24 hours after termination of a "binge"-like dose of cocaine

(Sarnyai et al. 1998). In apparent contrast, there was no effect of amphetamine withdrawal upon levels of corticosterone or ACTH when measured at one, five or ten days after termination of drug treatment (Swerdlow et al. 1991). However, before conclusions can be drawn about the similarity of HPA axis function in amphetamine or cocaine withdrawal and MDD, a number of further studies are required. Most importantly, studies should seek to determine the presence of putative alterations in dexamethasone-mediated feedback, as these are perhaps the cardinal indicators of HPA axis dysfunction in depressives. In agreement with a potential role for CRF in depression (Arborelius et al. 1999), it has been demonstrated that withdrawal from cocaine is coincident with increased levels of CRF in regions of the brain such as the hypothalamus and the amygdala (Sarnyai et al. 1995; Richter and Weiss 1999). While it is likely that increased levels of this neuropeptide contribute significantly to the anxiety associated with cocaine withdrawal (Basso et al. 1999), it has been shown recently that intracerebral infusion of CRF decreases ICSS responding (Macey et al. 2000), indicating a possible role for elevated levels of CRF in anhedonia.

Pharmacological Validity:

The primary purpose of many animal models of depression is to identify compounds with putative antidepressant properties. In order for a model to perform this task successfully, it must be shown to respond selectively to drugs that also exhibit antidepressant effects in humans, and hence provide a strong measure of "pharmacological validity". The model should respond to all classes of compounds (such as tricyclics, MAOIs, SSRIs etc.), and a failure to detect appropriate

drugs represents an important challenge to the validity of the model. Furthermore, the model should provide "discriminant validity" by not responding to drugs of a different class, such as anxiolytics or antipsychotics (Geyer and Markou 1995). As a number of studies have reported effects of treatment with different psychoactive compounds on the behavioral symptoms of psychostimulant withdrawal in rodents, these findings provide the basis for an evaluation of the pharmacological validity of this model of depression.

The decrease in locomotor activity that is frequently associated with psychostimulant withdrawal has been treated with several different antidepressant compounds. In an earlier study, Lynch and Leonard (1978) demonstrated that subchronic treatment with the antidepressants pargyline and mianserin diminished the effects of amphetamine withdrawal on locomotor deficits in an open field task, although the tricyclic antidepressant amitriptyline was ineffective. However, Seltzer and Tonge (1975) were able to show that treatment with the tricyclic drug imipramine was able to reverse the behavioral depression associated with methamphetamine withdrawal, indicating that certain drugs from the tricyclic class are effective in this model. The direct dopamine agonist lisuride was able to reverse completely the decreased locomotor activity of rats during amphetamine withdrawal (Pulvirenti and Koob 1993). Although the antidepressant efficacy of lisuride in MDD remains undetermined, alternate preclinical models have indicated antidepressant properties of this drug (Golda et al. 1986; Nakamura et al. 1989), and it was shown to decrease depression in patients who were recovering from a stroke (Hougaku et al. 1994).

The anhedonia of psychostimulant withdrawal, which is measured by ICSS paradigms, has also been modulated by treatment with various antidepressant drugs. The tricyclic antidepressant desmethylimipramine attenuated the effects of cocaine withdrawal on ICSS

responding (Markou et al. 1992), while both imipramine and amitriptyline were able to mitigate the effects of amphetamine withdrawal (Kokkinidis et al. 1980). Animals that were pretreated with lithium, which possesses therapeutic qualities in MDD (Bauer et al. 2000), exhibited prophylactic effects and prevented the post-amphetamine depression of ICSS responding (Predy and Kokkinidis 1981). Furthermore, other compounds that have been shown to exhibit antidepressant properties in preclinical studies were able to attenuate the effects of drug withdrawal, and included the indirect dopamine agonist bromocriptine (Markou and Koob 1992) and adenosine A2 antagonist DMPX (Baldo et al. 1999). In a recent study, Harrison et al. (2001) determined the capacity of the SSRI fluoxetine to attenuate the effects of amphetamine withdrawal on ICSS responding. The authors observed that on the fifth day of drug withdrawal, fluoxetine-treated animals exhibited a return to normal levels of ICSS responding, unlike vehicle-treated animals, indicating a positive antidepressant response. More interesting, though, the addition of the selective 5-HT1A antagonist p-MPPI significantly shortened the onset of therapeutic efficacy, implying that the psychostimulant withdrawal model of depression may be able to differentiate fast-acting antidepressant treatments from standard ones. There has been a substantial interest in the 5-HT1A receptor as a substrate for the development of rapidly acting antidepressants (Briner and Dodel 1998; Andree et al. 1999; Beique et al. 2000), and the findings of Harrison et al. indicate that this model of depression may offer unique potential in detecting putative antidepressants in this class of drugs. The findings of the present dissertation, whereby we were able to mitigate the effects of *d*-amphetamine withdrawal after two daily administrations of ECS (Barr et al. 2001), provide additional support for the hypothesis that this model is responsive to rapidly-acting antidepressant treatments.

As a caveat, it should be noted that some of the behavioral effects of cocaine withdrawal have also been ameliorated with anxiolytic drugs, posing a potential threat to the discriminant validity of the psychostimulant withdrawal model of depression. However, as has been discussed previously, elevated levels of anxiety appear to occur in a comorbid manner with the depressive symptomatology during psychostimulant withdrawal. Thus, drugs which exhibit anxiolytic properties, such as propanolol (Harris and Aston-Jones 1993;), ritanserin (Meert 1992) and CRF antagonists (Sarnyai et al. 1995; Basso et al. 1999) all mitigate the effects of cocaine withdrawal, but only in tasks which measure anxiety, such as defensive burying and the elevated plus maze. It is unlikely that these drugs would alleviate the anhedonic deficits measured in paradigms such as ICSS responding, although this remains to be determined empirically.

Construct Validity:

Even if an animal model of a psychiatric disorder exhibits a high degree of face and pharmacological validity, it should also meet a high standard of "construct validity" before it may be considered a robust model (Willner 1991). Construct validity, which is often difficult to determine (Geyer and Markou 1995), reflects the overall accuracy with which the model measures what it purports to measure, as well as the logical consistency behind the design of the model. The latter issue may be assessed by explicating and validating the inherent assumptions of the model. In the case of the psychostimulant withdrawal model of depression, these premises may be expressed in a syllogistic manner as follows:

1/ Depressed humans exhibit a number of distinct symptoms, which are an essential expression of the underlying disorder.

2/ Non-depressed humans who experience psychostimulant withdrawal exhibit a similar constellation of symptoms to depressed humans.

3/ As a consequence of the similarity between the two conditions, is may be assumed that psychostimulant withdrawal in humans provides a valid model of depression.

And to extend this argument:

4/ Humans who experience psychostimulant withdrawal provide a model of depression (point 3 above).

5/ Rodents that are withdrawn from high doses of psychostimulant drugs can be shown to exhibit similar behavioral deficits to humans who experience psychostimulant withdrawal.

6/ Therefore, it may be concluded that psychostimulant withdrawal in rodents also provides a valid animal model of depression.

Of course, these arguments are only as valid as the scientific accuracy of their predicates, which also contain other implicit assumptions. However, they cogently express the basic logic behind the design of this model, and when compared to the basic assumptions underlying a number of other animal models of depression, they offer a superior degree of construct validity. From the overview of the strong similarities between MDD and psychostimulant withdrawal in humans that was provided at the beginning of the Discussion of this dissertation, it can be concluded that the first argument (points 1-3) is largely true. Similarly, the review of the data in the Discussion which demonstrate that rodents that are withdrawn from large doses of psychostimulant drugs exhibit similar behavioral "symptoms" both to humans experiencing

psychostimulant withdrawal as well as human depressives, indicates that the conclusion of the second argument is also valid. The major assumption of the second argument, which is contained within point # 5, is that rodents exhibit similar behavioral deficits to depressed humans and humans in drug withdrawal; clearly, this point rests upon the degree to which the paradigms that are used to assess behavior in rodents actually measure what they are hypothesized to measure. In general, the tasks that have been used to assess behavior during psychostimulant withdrawal are amongst the most sophisticated that are available, with paradigms such as ICCS, progressive ratio responding and sexual behavior providing specific measures of distinct psychological constructs. The establishment of construct validity is an ongoing process, depending on the continuing refinement of paradigms in the literature, but it may be concluded that the current status of the psychostimulant withdrawal model of depression is that of a model which exhibits a high degree of construct validity.

Reproducibility and Reliability:

For an animal model to be of use to the appropriate field of study, it must exhibit certain qualities that pertain to practical considerations, aside from the theoretical criteria that it must satisfy. The most frequently desired qualities include that the model be "reproducible," meaning that it should be easily established in different laboratories, as well as "reliable", meaning that it should generate consistent results over time (Geyer and Markou 1995). The reproducibility of the psychostimulant withdrawal model is attested to by the large number of different groups that have used this model, and its reliability is indicated by the converging data that these groups

have reported, such as consistent deficits in ICSS responding, often despite the use of different strains of animals and other complicating factors.

Limitations:

All animal models have limitations, which determine their ultimate boundary of usefulness. In the present paradigm a number of important limitations are apparent, which do not seriously inhibit the use of this model, but indicate that further development of the model may be required to optimize its maximal utility.

Perhaps the greatest limitation of the current model of depression is the duration of the effects of psychostimulant withdrawal. Excluding a few disparate results, the majority of studies have observed behavioral sequelae that extend for up to, but infrequently beyond, one week. As most antidepressants require a period of 2-3 weeks before therapeutic effects are observed (Leon 2001), the short duration of the effects of psychostimulant withdrawal represent both a theoretical and practical challenge to the validity of this model. However, similar issues have been faced in alternate models of depression that exhibit a high level of pharmacological validity (Willner 1986). For example, in the forced swim test, rodents display a rapid and selective response to acute treatment with antidepressant drugs (Borsini and Meli 1988; Lucki 1997; Galea et al. 2001). It has been hypothesized that this rapid response may reflect species-specific differences in drug metabolism and production of neuroactive metabolites or receptor modulation (Porsolt et al. 1978; Duncan et al. 1985). Furthermore, the doses of antidepressant drugs that are normally administered to rodents in animal models of depression, such as the

psychostimulant withdrawal model, are disproportionally higher than those given to depressed humans. A number of studies have reliably demonstrated that when patients with MDD receive high doses of antidepressants administered intravenously, such as clomipramine (Pollock et al. 1989; Sallee et al. 1997) and amitriptyline (Deisenhammer et al. 2000), patients exhibit significant reductions in depressive symptoms within five to seven days, consistent with the effects observed in the animal paradigms.

Another potential concern with the current animal model is that the pharmacological validity of the model requires more extensive evaluation. There clearly needs to be a comprehensive investigation of all the psychoactive compounds that are responsive in this model, as well as a discrimination of those compounds that are not. This is an intimidating task, though, as consistent procedures must be established first, such as which behavioral testing procedure is to be used; even after a task is chosen, such as ICSS, other consistencies need to be set, such as choice of electrode location, type of psychostimulant drug and method of delivery, etc.. In this manner, the current model lags significantly in practicality behind other models which have more uniform operating procedures, such as the forced swim test and olfactory bulbectomy models. Nevertheless, these are obstacles that can be readily overcome, given the potential benefits of the psychostimulant withdrawal model.

Other limitations of the psychostimulant withdrawal model of depression include issues that are also of concern to most other animal models of depression. Given the overwhelming evidence that females are 1½ – 3 times more vulnerable to developing MDD than males (Kendler 1998), an animal model should be able to replicate this phenomenon, if it may be assumed that the gender difference in the epidemiology of MDD are due to biological factors. There has been no systematic study of the effects of psychostimulant withdrawal in female rats, as a comparison

with males, and this represents an important area of future study. Similarly, individual vulnerability factors to the development of MDD are receiving increased interest; these predispositions are often described within the theoretical framework of a "stress-diathesis" model (Mann et al. 1999), whereby genetic or early developmental factors interact with environmental insults later in life to induce episodes of MDD. In the present animal model of psychostimulant withdrawal, there is no attempt to identify individual differences in vulnerability to the effects of psychostimulant abstinence, despite sometimes obvious differences in the magnitude or duration of the effects of drug withdrawal. As it has been shown that certain traits in rats predict their individual differences in reactivity to the reinforcing effects of psychostimulant drugs (DeSousa et al. 2000), the converse identification of markers that can predict greater vulnerability to the aversive effects of psychostimulant withdrawal may increase the utility of the model by segregating a subset of particularly vulnerable animals, and hence ultimately increase the statistical power of the model.

Future Directions:

The development and validation of an animal model represents only its beginning. Based upon the promise that the psychostimulant withdrawal model exhibits, reflected in its unparalleled degree of face validity, high construct validity and limited but consistent pharmacological validity, there are a number of important future directions for this model. Firstly, the model should be assessed in its capacity to detect selectively rapidly-acting antidepressant treatments. Within the human literature, several putatively fast-onset treatments

have been developed, including SSRI/ 5-HT1A antagonist cocktails (Briner and Dodel 1998; Beique et al. 2000), ECT and pindolol (Shiah et al. 2000), transcranial magnetic stimulation (Klein et al. 1999), REM-sleep deprivation (Wirz-Justice and Van den Hoofdakker 1999) and other drugs such as venlafaxine (Amsterdam et al. 1998); these treatments should all be assessed in the model.

The current model may also be used to help in determining the neurobiological systems that are involved in psychostimulant withdrawal, and hence depression. *In vivo* techniques have already been used to quantify regional changes in neurotransmission, which mimic some of those observed in MDD. The identification of distinct circuits which mediate the aversive effects of psychostimulant withdrawal, through the measurement of immediate-early gene expression (Mutschler et al. 2000) and the use of other techniques, will help to provide insight into the neuroanatomical substrates of depression in humans.

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