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Effects of quetiapine on anhedonia induced by withdrawal from chronic amphetamine administration

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Résumé

Contexte L'anhédonie, un état caractérisé par une capacité réduite d'éprouver du plaisir. Des études cliniques récentes montrent qu'un médicament antipsychotique atypique, la quétiapine, est bénéfique pour le traitement de la toxicomanie qui est supposé d'atténuer les symptômes de sevrage associés à l'usage abusif des drogues psychotropes. Le but de la présente étude était d'étudier les effets de l'administration aiguë de quétiapine sur la récompense chez des animaux en état de sevrage après un traitement chronique avec l'amphétamine. Notre hypothese est que la quetiapine va diminuer l'anhedonie causer par le sevrage.

Méthodes Les expériences ont été effectuées avec des rats mâles de la souche Sprague-Dawley entraînés à produire une réponse opérante pour obtenir une courte stimulation électrique au niveau de l'hypothalamus latéral. Des mesures du seuil de récompense ont été déterminées chez différents groupes de rats avant et pendant quatre jours après le traitement avec des doses croissantes (1 à 10 mg/kg, ip toutes les 8 heures) de d-amphétamine sulfate, ou de son véhicule, au moyen de la méthode du déplacement de la courbe. L'effet de deux doses de quétiapine a été testé 24 h après le sevrage chez des animaux traités avec l'amphétamine ou le véhicule.

Résultats Les animaux traités avec l'amphétamine ont montré une augmentation de 25% du seuil de récompense 24 h après la dernière injection, un effet qui a diminué progressivement entre le jour 1 et le jour 4, mais qui est resté significativement plus élevé en comparaison de celui du groupe contrôle. La quétiapine administrée à 2 et 10 mg/kg pendant la phase de sevrage (à 24 h) a produit une augmentation respective de 10 % et 25 % du seuil de recompense; le meme augmentation du seuil a été observe chez les animaux traitées avec le véhicule. Un augmentation de 25 % du seuil de recompense a aussi été observés chez les animaux en état de sevrage à l'amphétamine. Un test avec une faible dose d'amphétamine (1 mg/kg) avant et après le sevrage a

révélé une légère tolérance à l'effet amplificateur de cette drogue sur la récompense, un phénomène qui pourrait expliquer l'effet différent de la quétiapine chez les animaux traités avec le véhicule et ceux traités avec l'amphétamine.

Conclusions Ces résultats reproduisent ceux des études précédentes montrant que la quétiapine produit une légère atténuation de la récompense. Ils montrent également que le sevrage à l'amphétamine engendre un léger état d'anhédonie et que dans cet état, une dose élevée de quetiapine et non pas une dose faible accentue l'état émotionnel négatif. Ils suggèrent qu'un traitement à faibles doses de quétiapine des symptômes de sevrage chez le toxicomane devrait ni aggraver ni améliorer son état émotionnel.

Mots clefs: Anhédonie, Amphétamine, Dopamine, Dysphorie, Quétiapine, Récompense, Tolérance

Résumé en anglais

Background Anhedonia, a condition in which the capacity of experiencing pleasure is reduced, is observed in patients that are under withdrawal from drugs of abuse. Recent clinical studies show that quetiapine may be beneficial in the treatment of substance abuse by alleviating the withdrawal-negative affect stage of addiction. This study investigated the effects of acute quetiapine on reward in animals under withdrawal from d-amphetamine.

Methods Experiments were performed on male Sprague-Dawley rats trained for intracranial self-stimulation. Measures of reward threshold were determined with the curve-shift method in different groups of rats before, and during four days after treatment with escalating doses (1 to 10 mg/kg, i.p) of d-amphetamine sulphate or its vehicle. At 24h after withdrawal, the effects of two doses of quetiapine (2 and 10 mg/kg ip) were tested in all the animals.

Results Animals treated with d-amphetamine showed 25% reward attenuation at 24h of withdrawal, an effect that decreased over the next three days. Quetiapine administered acutely at 2mg/kg and 10mg/kg on the first day of withdrawal produced 10% and 25% reward attenuation, respectively, in the vehicle-control animals, an effect also observed in the animals under withdrawal from d-amphetamine but only at the high dose.

Conclusions These results show that quetiapine produced a mild attenuation of reward in normohedonic and in anhedonic animals. They suggest that quetiapine should be used at low doses for the treatment of substance abusers under withdrawal from psychostimulant drugs to avoid enhancement of the anhedonic state.

Key words: Anhedonia, Amphetamine, Dopamine, Quetiapine, Reward, Tolerance

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Liste des abreviations

5-HT: serotonin
6-OHDA: 6-Hydroxydopamine
AADC: aromatic amino acid decarboxylase
ACTH: adrenocorticotropic hormone
BDNF: brain-derived neurotrophic factor
BSR: brain stimulation reward
COMT: catechol-O-methyltransferase
CPu: caudate-putamen
CRF: corticotrophin-releasing factor
CMS: conditioned mild stress
CS: conditioned stimulus
DA: Dopamine
DAT: dopamine transporter
DPDPE: [D-Pen2,D-Pen5]-Enkephalin
DOPAC: 3,4-dihydroxy-phenylacetic acid
EPS: extrapyramidal symptoms
GABA: gamma-aminobutyric acid
HVA: homovanillic acid
ICV: intracerebroventricular
L-DOPA: L-3,4-dihydroxyphenylalanine
MAO: monoamine oxidase
MFB: medial forebrain bundle
mRNA: messenger ribonucleic acid
MPP+: N-methyl-4-phenylprindium
NAS: nucleus accumbens
NE: norepinephrine/noradrenalin
PCP: phencyclidine
PFC: prefrontal cortex
p-MPPI: [4-(2-methoxy-phenyl)-1-[2-(n-(2-pyridinyl)-p-iodobenzamido]-ethyl-piperazine]
PR: progressive ratio
SNc: substantia nigra
SNC: successive negative contrast
SSRI: selective-serotonin reuptake inhibitor
TCA: tricyclic antidepressant
TH: tyrosine hydroxylase
US: unconditioned stimulus
VMAT: vesicular monoamine transporter
VTA: ventral tegmental area

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1. Introduction

1.1 Drug addiction: a brief history

Although alcohol and drug addiction have no doubt been with us for millennia, the modern medical conceptualization of addiction can be traced back to Benjamin Rush, an American physician who lived during the 19th century in the period known as the enlightenment, a time when disease began to be conceptualized as an imbalance in the nervous system (Meyer, 1996). Rush postulated that since distilled spirits were considered to be strong nervous system stimulants and could cause an imbalance in the nervous system, then alcoholism could be considered as a disease, the cure to which could only come from total abstinence. As a result of this rationale, public education campaigns were mounted and succeeded in reducing public drunkenness between 1810 and 1830 (Meyer, 1996).

The next step in the evolution of the concept of addiction came at the end of the 19th century when notions of disease became increasingly rooted in findings from pathology and biology (Meyer, 1996). It was during this period that Emile Kraepelin, considered as the founder of contemporary scientific psychiatry, published the first psychometric data on the influence of common recreational (tea, coffee, alcohol) and medical drugs (amyl nitrite, chloral hydrate, chloroform, ethyl ether, morphine, paraldehyde) (Muller et al. 2006; Crocq, 2007). Using himself as the first (and sometimes only) research subject, Kraepelin concluded that alcohol, ether, chloroform, chloral hydrate, paraldehyde and morphine produce "initial excitation of sensory and intellectual domains accompanied by simultaneous central motor impairment…This is a common trait of all toxins that induce a personality change after chronic abuse…[These drugs] if in different strength, [induce] this persistent weakness of will in addition to acute central motor paralysis" (as quoted in Muller et al. 2006). Moreover, he was one of the first to scientifically demonstrate the dangers of alcoholism. He noted that chronic alcoholism provoked cortical brain

lesions that led to a permanent cognitive decline and became a fervent proponent of abstinence as a result (Crocq, 2007). Although Kraepelin championed the psychopharmacological approach to addiction, it was Sigmund Freud who laid the foundation for the psychological approach to addiction whereby addiction would come to be viewed as a disease of the mind, in addition to being a neuropharmacological phenomenon. In a letter to his friend and confidant Wilhelm Fleiss (1897), Freud wrote: "…it has dawned on me that masturbation is the one major habit, the "primal" addiction and that it is only as a substitute and replacement for it that the other addictions—for alcohol, morphine, tobacco, etc—come into existence" (as quoted in Crocq, 2007). It was a result of the psychological approach that addiction became to be defined not only by dependence on drugs of abuse, but also by any persistent self-destructive behaviors such as gambling (Crocq, 2007).

In the latter half of the 20th century as science became increasingly systematic, researchers built upon the initial observations of pioneers such as Kraepelin and Freud to come up with more objective conceptualizations of addiction. One such conceptualization was the opponent process theory, originally proposed by Leo Hurvich and Dorothea Jameson (1955) as a model of color vision and expanded by Richard Solomon (1974) through his research on motivation and addiction. Solomon posited that the central nervous system automatically modulated hedonic states, once they were initiated, by way of counteradaptive mechanisms that reduced their intensity and brought the system back to hedonic neutrality (Solomon & Corbit, 1974). Solomon termed this process the standard pattern of affective dynamics, a five stage pattern that was said to underlie all addiction and indeed all hedonic or pleasure-seeking behavior. The stages of this pattern are as follows: (a) the peak of the primary hedonic state, brought on by onset of the stimulus; (b) a period of adaptation during which the intensity of the

hedonic state decreases, despite the intensity of the stimulus remaining the same; (c) a stable level of the now reduced hedonic state which continues as long as stimulus intensity is maintained; (d) a peak of affective after-reaction or withdrawal state, which quickly follows discontinuation of the stimulus; and finally, (e) the decay and disappearance of the withdrawal state. These stages were then further grouped into two states: State "A" and State "B", whereby State A is the primary reaction to the stimulus and State B is the after-reaction (Solomon & Corbit, 1974).

A more concrete example that was given by the authors is that of opiate addiction. Initially the opiate user experiences an intense "rush" directly after the injection of the drug followed by a period of less intense euphoria. Next, as the drug wears off, anhedonia (a term coined in 1897 by the French psychologist Théodule-Armand Ribot to denote a diminished ability to experience pleasure), irritability, physical discomfort and intense craving set in (Solomon & Corbit, 1974; Willner, 1995). This baseline -> State A -> State B -> baseline sequence shifts as a result of prolonged opiate use. With repeated doses over several weeks, the strength of State A declines, while the strength of State B increases and takes longer to return to baseline. Furthermore, as both states are pushed lower and lower on the hedonic scale and modify the 'hedonic set point', State A become "normal" and State B ushers in extremely unpleasant and enduring agony that may last for days on end.

The opponent process theory of motivation has provided one of the most influential explanations of addiction to drugs of abuse to date. The model not only gave testable hypotheses concerning addiction, but also testable hypotheses on any process of acquired motivation such as love, social attachment and risk-taking behavior. The difference between drug addiction and other forms of acquired motivation, however, is that in the case of the latter, the B process fades

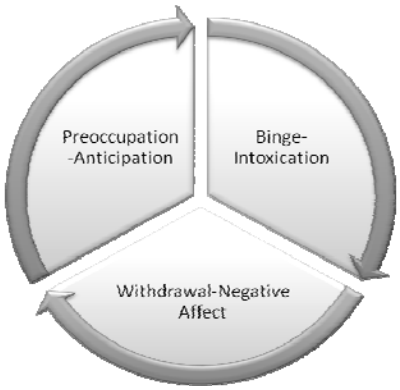
to baseline very quickly and consequently, the downward spiral of repeated lowering of the hedonic state does not occur (Solomon & Corbit, 1974).

1.2 Drug addiction as a cycle of spiraling distress

More recently, Koob & Le Moal (1997) have expanded the opponent process theory to encompass the neurobiology and neurocircuitry of addiction. In this model, drug addiction is conceptualized as a cycle of spiraling distress and dysregulation of brain reward/anti-reward systems, whereby drugs of abuse progressively alter the normal homeostatic state thereby causing the reward-relevant system to become imbalanced and form an allostatic state (Koob & Le Moal, 2008). The normally homeostatic counteradaptive opponent processes that fall into disequilibrium can be subdivided into two categories: within-system neuroadaptations and between-system neuroadaptations. In a within-systems opposing process, the first-order elements on which the drug exerts its effects adapt to neutralize these effects and produce tolerance to the reinforcing effects of further administration of the drug. Within system adaptations include decreases in function of the same neurotransmitter systems that are responsible for the reinforcing effects of drugs of abuse as well as molecular changes such as cell down-regulation and cell desensitization. In this case, the affective after-reaction or withdrawal state would be caused by persistence of the opposing effects after the drug begins to leave the body (Koob & Bloom, 1988). Indeed, compensatory changes in reward-relevant circuits leading to reward deficits in the form of depression and anhedonia have been evidenced in withdrawal from all of the major drugs of abuse including cocaine, nicotine, amphetamines, phencyclidine (PCP), opiates, and alcohol (Melinchar et al. 2001; Spielewoy & Markou, 2003; Volkow et al. 2009). In a between-systems opposing process, a drug's effect on primary drug response neurons leads to overactivation of neurons of a different circuit involved in the restoration of homeostasis of

bodily and brain function (Koob & Bloom, 1988). Between-system opposing processes may include long lasting increases in craving, anxiety, irritability and stress during withdrawal, and protracted abstinence. This form of adaptations has been witnessed in alcohol, nicotine, cannabis, cocaine, amphetamine and opiate dependence and is an important factor in relapse to drug taking (Bruijnzeel & Gold, 2005; Koob, 2009). One criticism of the opponent-process model, however, is that it does not provide a satisfactory explanation of drug craving by addicts and does not deal with the earliest stages of drug taking behavior (Meyer & Quenzer, 2005).

Figure A: The cycle of drug addiction



In an effort to bring the various theoretical interpretations of drug addiction under the same roof, the model of spiraling distress proposes characteristics of the addiction cycle on which different theoretical perspectives such as that of psychiatry, behavioral psychology and neuroscience can be superimposed. Here, drug addiction is characterized as a disorder that progresses from impulsion to compulsion and consists of three stages: preoccupation-anticipation (craving), binge-intoxication, and withdrawal-negative affect (Figure A). Individuals with impulse control disorders such as kleptomania feel a sense of tension and arousal before carrying out an impulsive action as well as pleasure and gratification at the time of the action. On the other hand, individuals with compulsive disorders such as obsessive compulsive disorder feel

stress and anxiety before carrying out a compulsive action and relief from the stress after performing the compulsive behavior (Koob & Le Moal, 2008). As an individual's addiction progresses from being motivated by impulsions to being motivated by compulsions, there is a shift from pleasure and gratification driving their behavior to relief from anxiety and dysphoria. Thus, using behavioral psychology as a framework, Koob & Le Moal (1997) have proposed that different types of reinforcement correspond to different components of the addiction cycle. Specifically, the binge-intoxication phase is said to be associated with positive reinforcement (i.e. an increase in the future frequency of a behavior due to the addition of a stimulus immediately following a response), whereas the withdrawal-negative affect phase is said to be associated with negative reinforcement (i.e. an increase in the future frequency of a behavior when the consequence is the removal of an aversive stimulus). The preoccupation-anticipation phase is said to be associated with conditioned positive and negative reinforcement (i.e. a stimulus that has acquired its function as a reinforcer after pairing with a stimulus which functions as a primary reinforcer).

From a neurochemical perspective, the binge-intoxication stage is reflected by activation of dopaminergic and opioid peptide systems. All major drugs of abuse including cocaine, amphetamine, alcohol, PCP, opiates, and nicotine increase extracellular dopamine (DA) levels (as measured by microdialysis), a neurotransmitter that is associated with reward and motivation (Carboni et al. 1988; Di Chiara & Imperato, 1988). Alcohol, opiates, nicotine, and cannabis also bind to the body's endogenous mu- and delta-opioid receptors, which play a crucial role in the body's ability to relieve pain and whose stimulation reverses the tonic inhibition that these receptors have on DA neurons, thereby leading to DA release (Lingford-Hughes & Nutt, 2003). The withdrawal-negative affect stage is characterized by decreases in dopamine and mu- and

delta-opioid function, as well as hyperactivity of brain anxiety and stress systems. Anxiety is thought to be mediated by a number of neurotransmitters including norepinephrine (noradrenalin, NE), serotonin (5-HT), and corticotrophin-releasing factor (CRF). Noradrenergic neurons originate in the locus coeruleus (LC) and serve to generate arousal, orienting, and response to fear-evoking stimuli, also known as the 'fight or flight' response. LC neurons are known to contain receptor sites for CRF as well as 5-HT, which serve an excitatory and inhibitory function on NE release, respectively (Meyer & Quenzer, 2005). Additionally, NE and CRF mediate the neuroendocrine response to stress. In response to stress, multiple neurotransmitters (including NE) regulate the secretion of CRF from the cells of the hypothalamus. CRF, in turn, acts to release adrenocorticotropic hormone (ACTH) form the pituitary gland into the blood. ACTH then acts on the adrenal gland to increase the secretion of cortisol and other glucocorticoids. This hypothalamic-pituitary-adrenal (HPA) axis activation would normally be shut down by feedback from cortisol, resulting in brief and transient increases in cortisol involved in the mobilization of energy to deal with a particular stressful event (Offermanns & Rosenthal, 2008). During drug withdrawal, however, anxiety/stress systems become dysregulated and normally effective feedback mechanisms become overwhelmed and dysfunctional. Indeed, abnormally high and persistent increases in levels of CRF, ACTH, cortisol and NE are characteristic of withdrawal from all major drugs of abuse (Koob & Le Moal, 2001; Sinha, 2008). Depletion of DA as well as dysregulation of the anxiety/stress have also been implicated in the phenomenon of craving (the preoccupation-anticipation) stage of drug addiction (Adinoff, 2004; Weiss, 2005).

The cycle of spiraling distress, of course, is not the same for all drugs of abuse; different drugs emphasize different aspects of the addiction cycle (Koob & Le Moal, 2008). Opioid addiction is primarily driven by a profoundly pleasurable intoxication as well as relief from

dramatically dysphoric withdrawal symptoms and intense craving associated with desire to obtain the drug. The intoxication from cigarette smoking, on the other hand, is weak or absent and thus, nicotine addiction is primarily driven by the preoccupation-anticipation and withdrawal-negative affect aspects of the cycle. Finally, abuse of psychostimulants such as cocaine and amphetamine is focused on the binge-intoxication stage in which binges may last for days but also features significant withdrawal symptoms that are characterized by extreme anxiety, anhedonia, and lethargy (Uslaner et al. 1999; Koob, 2009).

Different aspects of drug addiction have been modeled in animals. The pre-occupation-anticipation stage of addiction has been studied using animal models of relapse such as drug-, cue-, and stress-induced reinstatement. The binge-intoxication stage has been studied using paradigms of rewarding behavior such as drug self-administration, conditioned place preference, responding for a sucrose solution, and brain stimulation reward (BSR). Animal models of the withdrawal-negative affect stage include measures of stress and anxiety such as elevated plus-maze, light/dark test, and open field as well as measures of decreased reward function (anhedonia) such as conditioned place aversion, reduced responding for a sucrose solution and increased reward thresholds (Koob & Le Moal, 2008). With the goal of mimicking the complexity of human drug withdrawal syndromes, the aforementioned models of negative affect are usually administered in the context of withdrawal from chronic administration of drugs of abuse.

The present dissertation will focus on this last point: drug-induced anhedonia. Anhedonia has classically been defined as a reduced capacity to experience pleasure. We will begin by investigating the nature of the reward-relevant system and its organization. We will consider which animal models are most suited to study anhedonia. We will describe the neurobiology of

withdrawal-induced anhedonia and how it may be treated. We will present our own research findings and discuss their relevance to the overarching phenomenon of drug addiction.

1.3 Reward and the anhedonia hypothesis

In order to study withdrawal-induced anhedonia in animals, we must first understand the nature of the hedonic system. The mammalian reward-relevant circuitry was uncovered in the early 1950s by James Olds and Peter Milner of McGill University. While investigating the effects of electrical stimulation of various brain regions on cognitive function, the researchers made the serendipitous discovery that animals evidenced approach behaviors characterized by increased locomotor activity when pulses of electricity were administered to a brain region called the hypothalamus. Olds and Milner subsequently modified the experiment so that the rats could stimulate themselves by pressing a lever located inside the cage (Milner, 1989). Further research demonstrated that a variety of limbic, forebrain, and even brain stem sites supported self-stimulation; some of which were considered to be sensory, others associational, and yet others motor (Porter et al. 1959, Wise, 1996). The burning question in everyone's mind at the time was what precise mechanism linked these regions as mediators of self-stimulation?

Although self-stimulation was powerful evidence for the existence of a central reward-relevant neurotransmitter system, its exact identity was unknown. From the outset there was a fierce debate as to whether it was the neurotransmitter dopamine, norepinephrine (NE), or both that mediated these rewarding properties of BSR (Crow, 1972). Wise and Stein (1969) made the case for NE by demonstrating that disulfiram, a dopamine-beta-hydroxylase inhibitor (the enzyme responsible for the final step of NE biosynthesis) suppressed medial forebrain bundle (MFB; a complex bundle of DA-rich axons which run between the ventral tegmental area and the lateral hypothalamus) stimulation; an effect that was reversed by l-norepinephrine. This idea,

however, was countered by another study which showed that disulfiram induced sedation, hence making the case that the decreases in self-stimulation were due to a reduced ability to respond and not reward deficits (Roll, 1970). Other researchers made the case for the facilitative effect of DA on self-stimulation (Lippa et al. 1973) and this issue remained unresolved until Yokel and Wise (1975) decided to test the effects of DA and NE blockers using a different paradigm. The researchers hypothesized that although the BSR paradigm could not dissociate nonspecific deficits from reward deficits, amphetamine self-administration could. Indeed, rats were known to lever press for intravenous injections of d-amphetamine; an effect that was also thought to be mediated by catecholamines. Moreover, because the duration of effectiveness of each infusion determines the lever pressing rate for self-administration of amphetamine and varies inversely with the dose (i.e. the lower the dose the higher the lever-rate since more is needed for satiation), a decrease in responding signals the presence of nonspecific rather than reward deficits. Thus, whereas noradrenergic blocking agents decreased responding, pimozide, a dopaminergic antagonist, increased lever pressing for intravenous infusions of amphetamine, an effect indicative of a decrease in the rewarding property of amphetamine (Yokel & Wise, 1975). Wise et al. (1978) further demonstrated that pimozide attenuated lever-pressing and running for food in hungry rats at doses that did not cause motor side-effects. The decline in responding under DA blockade was progressive and paralleled that of extinction (non-reinforcement), suggesting that due to the memory of devalued reinforcement from previous days, the expectancy of the animal became progressively weaker (Wise, 2004). These findings prompted for a universal theory of reward that encompassed both natural stimuli and drugs of abuse. Consequently, the ‘anhedonia hypothesis’ proposed a central role for DA based on evidence that dopaminergic antagonists “appear to take the pleasure out of normally rewarding brain stimulation, take the euphoria out of

normally rewarding amphetamine, and take the "goodness" out of normally rewarding food" (Wise et al. 1978).

Even though the original anhedonia hypothesis was a major breakthrough, it has since proven to be too simplistic to encompass all facets of the phenomenon of reward. In particular, certain effects of neuroleptics have been difficult to explain by a simple devaluation of the hedonic properties of rewards (Di Chiara & Bassareo, 2007). For example, pimozide pre-treatment impaired lever pressing for food in rats trained on a variable interval schedule even during the first few minutes of the test session before they had received any reinforcement (Gray & Wise, 1980). Indeed, there is much support for the notion that when the DA system is blocked in animals that have already learned a task, reward predictors that were once established become associated with devalued rewards and are no longer effective motivators (Wise, 2004). There is also evidence that pimozide pre-treatment produced a dose-dependent attenuation of learning to lever press for food in hungry rats (Wise & Schwartz, 1981) and a growing number of studies have confirmed the finding that when the DA system is blocked in animals that are about to learn a task, rewards that would normally confer motivational value on predictive stimuli fail to do so (Wise, 2004). Taken together, this evidence suggests that in addition to devaluating the rewarding properties of primary reinforcers, neuroleptics also impair the creation and maintenance of normal stimulus-reward associations and the motivational arousal that accompanies such associations. Consequently, we may say that anhedonia is not merely a reduction or absence of pleasure but also a deterioration of the normal incentive-motivational processes that are inexorably linked to pleasure (see Wise, 2004 for detailed review). Although, these distinctions lead one to conclude that the term reward could be an umbrella term for both reinforcement (motivation) and pleasure, this paper will use reward and reinforcement

interchangeably as this is what is commonly done in the literature.

1.4 Characteristics of the reward-relevant system

The catecolaminergic system was first mapped in the 1960s by Swedish researchers who developed a classification system in which the catecholamine cell groups were designated with the letter A; noradrenergic cell groups were designated A1 to A7 and dopaminergic cell groups (Figure B) were designated A8 to A16. The A9 cell group is associated with a structure called the substantia nigra, from which dopaminergic axons ascend to a forebrain structure termed the striatum and form the mesostriatal tract (Dahlstrom & Fuxe, 1964). This pathway plays a crucial role in the control of movement and is damaged in Parkinson's disease, a disorder that is characterized by deficits in motor function such as tremors, postural disturbances, and difficulty in initiating voluntary movement (Winograd-Gurvich et al., 2006). More important to our discussion on anhedonia is the A10 cell group which originates in the VTA and projects dopaminergic axons to various structures of the limbic system such as the nucleus accumbens (NAS), olfactory tubercle, amygdala, septum, and hippocampus. This group of axons forms the mesolimbic dopamine pathway. Other DA-containing fibers travel from the VTA to the prefrontal (PFC), cingulate, and perirhinal cortex and constitute the mesocortical dopamine pathway. There is considerable overlap between the VTA cells that project to these various regions, and therefore, the two systems are often collectively referred to as the mesocorticolimbic system (Chinta & Andersen, 2005). These systems have been implicated in the rewarding effects of drugs of abuse as well as in affective dysfunction in drug withdrawal, schizophrenia, bipolar disorder, and Parkinson's disease (Winograd-Gurvich et al. 2006; Kato, 2008).

Figure B: Mesocorticolimbic dopamine system

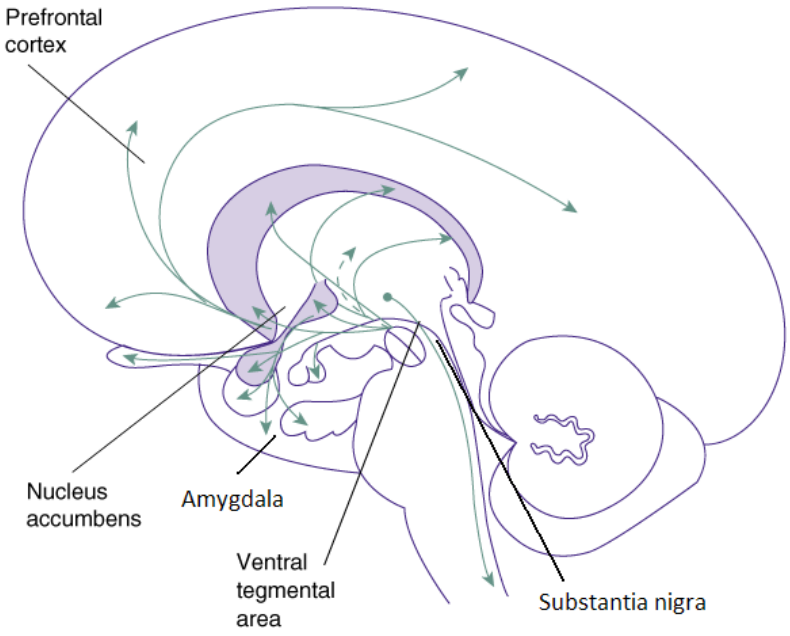


Image modified from (http://pubs.niaaa.nih.gov/publications/arh26-2/26_2images/brain.gif)

Dopamine synthesis is a two step process whereby the enzyme tyrosine hydroxylase (TH) turns the amino acid tyrosine into L-DOPA (L-3,4-dihydroxyphenylalanine), which is subsequently turned into DA by the enzyme aromatic amino acid decarboxylase (AADC). Dopamine, in turn, serves as the precursor to NE which is synthesized from DA by the enzyme dopamine beta-hydroxylase. TH is the rate-limiting enzyme in this biochemical pathway due to the fact that the conversion of tyrosine occurs at a slower rate than the ensuing reactions in the pathway (Offermanns & Rosenthal, 2008). Drugs which induce the release of catecholamines also tend to affect TH because high catecholamine levels serve as a negative feedback mechanism to inhibit the enzyme and this may contribute to anhedonic symptoms. Following synthesis DA is transported into synaptic vesicles by the vesicular monoamine transporter (VMAT) for later release into the synaptic cleft. The importance of VMAT can be deduced from actions of the drug reserpine which blocks uptake of catecholamines into vesicles. As a result of

this blockade, DA and NE are left vulnerable to breakdown within the nerve terminal and their levels drop significantly. The behavioral consequence of this catecholamine depletion is anergia and anhedonia (Meyer & Quenzer, 2005).

Five main DA receptor subtypes, designated D1 to D5, have been identified thus far. They are all metabotropic receptors meaning that they interact with G-coupled proteins and function through second messengers. The subtypes can be further grouped into two families: D1-like (D1 and D5) and D2-like (D2, D3, and D4) (Offermanns & Rosenthal, 2008). The most common subtypes are D1 and D2, both of which are found in large quantities in the striatum and the NAS (the major termination sites of the mesostriatal and mesolimbic DA pathways, respectively). D2 receptors, in some cases, also function as pre-synaptic autoreceptors which inhibit cell firing and decrease DA release when the cell is firing rapidly and the synaptic cleft is overloaded with high amounts of DA (Meyer & Quenzer, 2005). Drugs that stimulate D2 autoreceptors inhibit DA release whereas drugs that antagonize D2 autoreceptors increase dopamine release by blocking the inhibitory action of autoreceptors (Lee & Ellinwood, 1989). Neuroleptics such as pimozide and haloperidol, which are the basis for the anhedonia hypothesis, are D2 receptor antagonists, presumed to be preferential for post-synaptic receptors because their net effect is a reduction in the activity of DA and alleviate positive symptoms (psychosis) in schizophrenia (Wise et al. 1978).

Like to pre-synaptic autoreceptors, the DA transporter (DAT) is an important mechanism that acts to extinguish the synaptic signal induced by each release of the neurotransmitter in order for the postsynaptic cell be may activated again (Offermanns & Rosenthal, 2008). In each case that the pre-synaptic cell releases dopamine into the synaptic cleft, DAT recycles the neurotransmitter by reuptaking it back into the cell. Cocaine is a drug that derives its rewarding

effects from blockade of the DAT thereby increasing and prolonging the presence of DA in the synaptic cleft and enhancing its effects on the postsynaptic cell (Karoum et al. 1994). Amphetamines (e.g. d-amphetamine, methamphetamine) on the other hand, are thought not only to reverse the DAT but also to act on VMAT to provoke a massive release of DA from synaptic vesicles into the synaptic cleft (Fleckenstein et al. 2007).

In addition to reuptake, another way in which dopamine's actions are terminated is through metabolic breakdown by enzymes that function to prevent excessive neurotransmitter accumulation. These enzymes, termed catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO), are responsible for the breakdown of catecholamines. The actions of COMT and MAO on dopamine give rise to the DA metabolites 3,4-dihydroxy-phenylacetic acid (DOPAC) and homovanillic acid (HVA), the concentrations of which can be an indice of dopaminergic activity (Offermanns & Rosenthal, 2008). Drugs which inhibit these enzymes increase catecholamine concentrations (and 5-HT in the case of MAO) by preventing their breakdown. COMT and MAO inhibitors can be beneficial in the treatment of anhedonia, depression, and Parkinson's (Meyer & Quenzer, 2005).

1.5 Animal models of anhedonia

1.5.1 Conditioned place-preference/aversion

Conditioned place-preference involves the explicit pairing of a distinctive environment with drugs that act as positive reinforcers such as cocaine, opiates and amphetamines (Bardo et al. 1986; Tzschentke, 1998). These environments may differ in size, shape, color, pattern, and smell. Conditioning involves an animal receiving repeated access to the reinforcing stimulus (unconditioned stimulus or US) in a particular context (conditioned stimulus or CS) and receiving no US in another context. When animals are later presented with equal opportunity to

spend time in the compartment paired with the appetitive US or in a compartment not paired with the US, they show significantly more preference for the former (Bardo et al. 1986). By contrast, animals avoid environments paired with aversive drugs and aversive drug states. For example, administration of naloxone (an opiate receptor antagonist used to counter opiate overdose) in opiate dependent rats precipitated withdrawal and produced a place-aversion (Schulteis et al. 1994); an effect that was reversed by administration of clonidine (an alpha 2-adrenoreceptor agonist that is used to alleviate withdrawal severity during detoxification from chronic opiate use) (Kosten, 1994). Likewise, administration of the nicotine receptor antagonist mecamylamine produced a place aversion in nicotine-dependent rats (Suzuki et al. 1996).

Although the place-conditioning/place-aversion paradigm may be used to study drug withdrawal-induced anhedonia, it has a number of important drawbacks. First, it is subject to a number of confounds associated with the conditioning itself that can produce inconsistent results when used to measure motivation. There are reports of both conditioned place preferences and aversions to some drugs such as amphetamine, methamphetamine and apomorphine. Ethanol has been mainly found to have aversive effects but has also produced place-preference (Tzschentke, 1998) and this may be dependent on whether the ethanol is administered just before or just after exposure to the conditioned stimulus CS (Cunningham et al. 2002). These discrepancies may also be due to differences in a preference towards one of two distinctive environments before conditioning occurs. Furthermore, the nature of the conditioned stimuli (visual, tactile, or olfactory) can influence the motivational properties of the drug that it is paired with (Tzschentke, 1998). Another limitation of the paradigm is that while it may shed light on whether a drug state is pleasant or aversive it is not particularly useful for defining the qualitative nature of the state in question such as increased or decreased anxiety, anhedonia, somatic symptoms etc. Finally,

conditioned place preference cannot be used to map a dose-response curve because the same group of animals cannot be tested more than once (Bardo & Bevins, 2000). Consequently, it seems that the place-conditioning/place-aversion paradigm is better suited to study the effects of drugs on stimulus conditioning or on drug-induced positive/negative affect in general rather than the anhedonic withdrawal state *per se*.

1.5.2 Sucrose preference

There is an abundance of research that makes a strong case for the ability of sugar, like drugs of abuse, to activate the endogenous reward-relevant system. For instance, when rats are maintained on a daily diet of intermittent access to sucrose they show behavioral changes similar to rats that are dependent on drugs of abuse (Colantuoni et al., 2002). Moreover, they demonstrate a pattern of binging in the first hour of daily access and progressively augment their daily sugar intake. If they are then food deprived for 24 hours or administered naloxone, they demonstrate anxiety-like behavior and somatic signs of withdrawal such as teeth chattering, forepaw tremor and head shakes (Colantuoni et al., 2002). Sugar binging has also been shown to cause neurochemical changes such as increased DA turnover and up-regulation of D1 and mu-opioid receptors in the NAS that parallel adaptations to drugs of abuse (Hajnal & Norgen, 2002; Rada et al. 2005). Additionally, withdrawal from repeated daily sugar intake was shown to induce a DA/acetylcholine imbalance similar to that which is seen in nicotine and morphine withdrawal (Colantuoni et al., 2001).

Due to the striking similarities between sucrose intake and administration of addictive drugs, preference for sucrose has been used as a dependant variable when measuring hedonic shifts in rats. There is evidence that sucrose increases DA metabolism in the hypothalamus in sham fed rats (rats that have a gastric cannula which, when opened, does not allow the sucrose

solution to accumulate in the stomach thereby suggesting that this was due to hedonic and not postingestive effects), while D2 receptor antagonists sulpiride and raclopride, and the selective D1 receptor antagonist, SCH 23390 decrease sucrose intake in a manner that is functionally equivalent to that of decreasing sucrose concentration (Smith & Schneider, 1988). Other studies have found that intra-VTA application of 6-Hydroxydopamine (6-OHDA; a neurotoxin used by neurobiologists to selectively kill dopaminergic neurons), as well as treatment with pimozide and morphine withdrawal decreased free sucrose consumption without decreasing water intake and without decreasing rats' discrimination of sucrose from water (Towel et al. 1987; Lieblich et al. 1991; Shimura et al. 2002).

Free sucrose consumption, however, may not be the most reliable or valid way to measure anhedonia (Barr & Phillips, 1998). There is evidence that doses of the neuroleptic flupenthixol, which inhibited place-preference to sucrose, did not alter free sucrose consumption, whereas naloxone, at doses which had no effect on place-preference, had deleterious effects on free sucrose consumption (Agmo et al. 1995). This phenomenon may underlie the distinction between sucrose reinforcement and sucrose reward. Hence the reinforcing (motivational) properties of sucrose may be more sensitive to DA antagonism than its rewarding (palatable) properties and vice-versa for naloxone (Agmo et al. 1995). Indeed, naloxone has been found to be more effective at decreasing intake of a sucrose-rich than cornstarch-rich diet and to have a greater effect on drinking palatable solutions than on drinking water suggesting that endogenous opioids are involved in mediating the enhancement of feeding through food palatability (Yeomans & Gray, 2002). On the other hand, pimozide and 6-OHDA lesions of the nucleus accumbens and neostriatum that caused 99% DA depletion did not suppress unconditioned hedonic reactions (rhythmic tongue protrusions and paw licks) to sucrose in the taste reactivity

paradigm (Pecina et al. 1997; Berridge & Robinson, 1998). Likewise, DA antagonists reduced appetitive behaviors (investigatory and exploratory behaviors) for sucrose and food, while leaving consummatory behaviors (eating and drinking) intact (Blackburn et al. 1987; Ikemoto & Panksepp, 1996). Finally, genetically engineered mice lacking the ability to synthesize DA were found to preferentially choose sucrose over water, and also preferred saccharin, a non-caloric sweetener (Cannon & Palmitter, 2003). As a whole, these studies suggest that free sucrose consumption may be an indice of unconditioned hedonic reactions, which is not mediated by DA.

It is unclear why some studies have found reductions of free consumption of sucrose as a result of DA lesions and DA antagonists while others have not. Nevertheless, there is good reason be believe that, at least in the case of palatable food such as sucrose, DA plays a greater role in reinforcement than in reward (see Di Chiara, 2002 for detailed review). One paradigm that has proven to be a reliable indice of motivation to obtain sucrose is the progressive ratio (PR) schedule, which requires that the animal emit an increasing number of responses in order to obtain each reward. The breakpoint (i.e. the point at which an animal ceases to respond for further reward) may reflect the relative motivation to put in work to obtain the reward (Hodos, 1961). Studies have demonstrated that manipulations which are thought to induce anhedonia decrease breakpoint for sucrose reinforcement on a PR schedule whereas manipulations that are thought to reduce anhedonia increase it. For example, conventional antipsychotics (neuroleptics) haloperidol and chlorpromazine decreased break point for sucrose reinforcement on a PR schedule (Reilly, 1999; Mobini et al. 2000). Conversely, acute amphetamine and atypical antipsychotics clozapine, quetiapine, olanzapine, and ziprasidone (characterized by low affinity for D2 but strong affinity for 5-HT, histaminergic and adrenergic receptors) increased breakpoint

(Mobini et al. 2000; Zhang et al. 2005). Moreover, morphine, nicotine, and amphetamine withdrawal have also been shown to decrease break point for sucrose responding (Barr & Phillips, 1999; Lesage et al. 2006; Zhang et al. 2007). In the Barr and Phillips (1999) study, the same treatment that decreased breakpoint for sucrose responding (amphetamine withdrawal) produced no effect upon consumption of sucrose when it was freely available. Similarly, clinical studies with patients presenting depression and anhedonia have consistently failed to find alterations of sucrose taste perception (Potts et al. 1997; Scinska et al. 2004). Taken together, these evidences suggest that, in contrast to the motivation to work for sucrose reward, the hedonic reaction to the taste of sucrose may not be mediated by the dopaminergic system.

In addition to free sucrose consumption and PR schedule responding, sucrose has been used to measure changes in the reward-relevant system using the consummatory negative contrast paradigm. In this procedure, rats are exposed to one reward level (e.g. 32% sucrose) for some period of time and unexpectedly shifted to a reward with a lesser hedonic value (e.g. 4% sucrose). The shifted animals normally consume less of the reward and run slower on a runway with a sucrose 'goal' at the end (a measure of reinforcement rather than reward) than a control group which had been exposed to only the lower level of reward (Flaherty et al. 1996). The explanation for these effects seems to be that of frustration and disappointment as the animal finds a reward of a lesser value than one which was expected based on previous experience (Barr & Phillips, 2002). Some studies have supported a link between consummatory negative contrast and manipulation of the reward-relevant system. For example, rats withdrawn from chronic amphetamine administration displayed an exaggerated negative contrast effect, which was evident due to their delayed recovery from the downshift in reward as compared with controls (Barr & Phillips, 2002). Amisulpride, an atypical antipsychotic with affinity for D2 and D3

receptors (Schoemaker et al. 1997), has been shown to be effective in the treatment of anxiety and depression as well as anhedonia in schizophrenia and to reduce consummatory negative contrast in rats (Pani & Gessa, 2002; Genn et al. 2002). Finally, whereas control rats consuming a 4% sucrose solution displayed increased DA efflux in the NAS, rats downshifted form 32% to 4% sucrose displayed no such increase (Genn et al. 2004) suggesting that the negative contrast effect may be DA dependant.

The relationship between consummatory negative contrast and reward has not been unequivocal, however. Lesions of the nucleus accumbens did not attenuate the negative contrast effect or reward magnitude discrimination but they did reduce running speed toward the sucrose goal (Leszczuk & Flaherty, 2000). Furthermore, classical antipsychotics chlorpromazine and haloperidol had no effect on the negative contrast effect, whereas sedative/hypnotics that bind to the benzodiazepine site on inhibitory GABAA neurons such as chlordiazepoxide, flurazepam, and zolpidem have consistently and significantly reduced the contrast effect (Flaherty et al. 1992; Flaherty et al. 1996; Mitchell et al. 2004). In addition, consummatory negative contrast was attenuated by morphine and the opioid agonist DPDPE ([D-Pen2,D-Pen5]-Enkephalin) but potentiated by naloxone (Pellegrini et al. 2005; Wood et al. 2005). This may not be surprising since both benzodiazepines and opioids have been shown to increase sucrose intake in a free choice paradigm and are hypothesized to mediate taste 'liking' (Berridge & Robinson, 1998; Kelley et al. 2002; Yamamoto, 2003). Taken together, these results suggest that the mechanisms responsible for consummatory negative contrast and free consumption of sucrose may have more substrates in common with each other than with reinforcement for sucrose under a progressive ratio schedule. That is, dopamine is necessary for reinforcement but is only loosely related to taste hedonia. This conclusion does not negate the role of DA in drug reward and drug-induced

anhedonia because, as some have argued, euphoria and not palatability (being a form of 'stimulus-bound hedonia') is an appropriate test of the anhedonia hypothesis (Di Chiara, 2002).

1.5.3 Brain stimulation reward

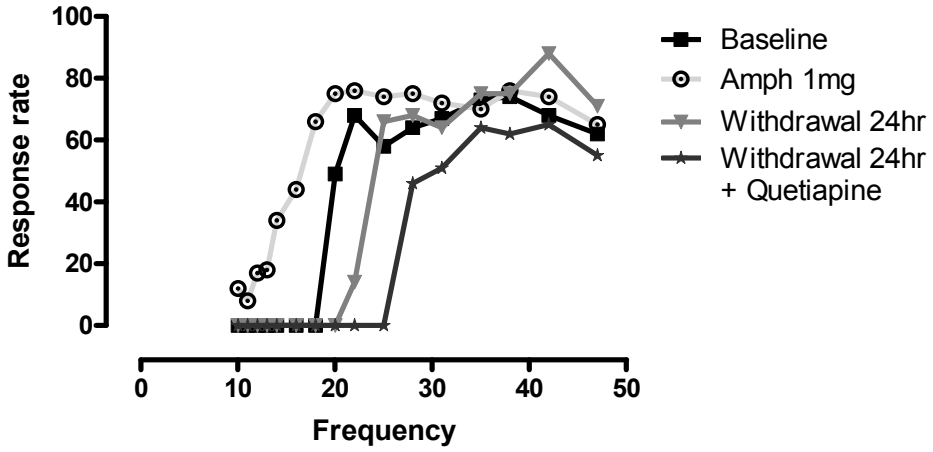
Electrical stimulation of the brain can be used to activate cells at the tip of the electrode while recording the behavior that occurs as a result of that activation. This is possible because very tiny amounts of electric current alter the membrane potential of proximate cells and generate action potentials which cause the release of neurotransmitters into synaptic terminals, thereby imitating normal synaptic transmission. As a result, BSR can mimic the neurobiological and behavioral effects that are produced by natural and drug rewards. There are a number of advantages to using BSR over natural and drug reinforcement, however. Whereas drugs and natural rewards such as food are not anatomically specific and can depend on a host of pharmacokinetic factors such as absorption and metabolism, electrical stimulation activates discreet brain regions and the degree of activation can be controlled with extreme precision (Meyer & Quenzer, 2005). The degree of activation of a specific brain area can be varied according to the duration of individual pulses, the number of pulses per stimulation train as well as the intensity and frequency of stimulation. Pulse duration affects the size of the stimulation field. Longer pulse durations tend to be more rewarding but this value is typically kept at 100 microseconds so that pulses depolarize axons only once thereby simplifying theoretical interpretation. Longer train durations also tend to be more rewarding but can cause animals to respond for additional stimulation before the last train ceases. Hence train duration is typically fixed at 200 to 500 milliseconds. Intensity is most frequently varied when anatomical localization is the goal. Like pulse duration, the higher the stimulation the larger the size of the stimulation field, and consequently, manipulation of intensity is useful in attempts to localize the

boundaries of reward sites (Wise, 1996). Stimulation frequency determines the number of times per stimulation that action potentials are triggered in a set quantity of neurons (Wise et al. 1992). Manipulation of frequency is most useful in pharmacological studies where the area of stimulation should be held constant so that the effect of drugs on sensitivity of reward sites can be measured (Wise, 1996). While increasing any of the aforementioned stimulation parameters has been established to be more rewarding (higher stimulation parameters are consistently chosen over lower ones in double-lever tests), this motivational variation cannot be seen in single-lever tests because responding is constrained by motor capacity. Thus, a higher asymptotic response rate in single lever tests cannot be interpreted as being more rewarding since different stimulation sites produce different response rates depending on their proximity to motor fibers (Wise & Rompre, 1989).

Unfortunately, in the early history of BSR there was no method to separate response rate from reward (and consequently, these studies should be interpreted with caution). Researchers were largely concerned with identifying the brain regions which mediated reward. For these studies, response rate was the dependant variable since it signified that the animal was willing to work for stimulation, and thus, it must have been rewarding (Wise, 1996). In the case of pharmacological studies with BSR, however, it became obvious that simple response rate was often a contaminated variable. We may recall that in the Wise and Stein (1969) study disulfiram-induced sedation reduced response rate and made theoretical interpretation about the role of norepinephrine in reward extremely difficult. Such confusion between motor disturbance and reward were remedied by the curve-shift paradigm, originally proposed by Edmonds and Gallistel (1974). This method involves the creation of dose-response curves by manipulation of a single parameter in BSR (Figure C). In the case of pharmacological studies, this means varying

frequency while keeping pulse duration, train duration, and intensity constant. Then by taking an arbitrary point on the resulting rate-frequency curves (e.g. 50% of maximal response rate) parallel shifts can be quantified to represent changes in reward threshold. If a drug treatment induces a rightward shift of the rate-frequency curve then the treatment increased reward threshold (decreased reward). Conversely, if a drug treatment induces a leftward shift then the treatment decreased reward threshold (increased reward). If, however, administration of a drug leads to a downward shift in the dose-response curve (decrease in maximal response rate), then this indicates that the treatment interfered with the animal's capability to perform the task and not the rewarding properties of the stimulation (Miliaressis et al. 1986). The ability of the curve-shift paradigm to distinguish between reward alterations and motor impairment has been validated using different response criteria such as a minimal absolute rate of responding (e.g., 10 or 20 responses per minute) or minimal relative rate (e.g., 10% or 50% of asymptotic rate). In each case, it was shown that the effectiveness of reward altering drugs was the same regardless of what response criterion was used (Wise et al. 1992).

Figure C: Rate-frequency curve for one rat



There is considerable evidence that BSR shares the ability of drugs of abuse to activate the mesocorticolimbic DA system. For instance, electrical stimulation of the VTA produced significant increases in primary DA metabolites, namely, DOPAC and HVA in NAS, striatum and olfactory tubercle whereas 6-OHDA lesions that depleted 95% of DA in these regions produced large increases in reward thresholds. The ratios of DOPAC and HVA to DA are considered to be indices of DA utilization (Fibiger et al. 1987). Moreover, MFB self-stimulation at different stimulation frequencies and pulse widths increased levels of dopamine, HVA and DOPAC in the NAS and VTA and local perfusion with the dopamine uptake inhibitor nomifensine (a drug that works in a similar fashion to cocaine) increased DA levels in the NAS and potentiated the increase of DA induced by self-stimulation (You et al. 2001).

Recently, it has been suggested that the ability of BSR to induce DA release is true only when the electrical stimulus is unpredictable, and hence, that DA is a mediator of the novelty of reward rather than the hedonistic properties of reward. Garris et al. (1999) used in vivo voltammetric microsensors to measure DA release in the NAS in animals that received electrical stimulation for the first time and in those that had received stimulation continuously. They found that extracellular DA levels were augmented during the first experimenter-delivered electrical stimulation but decreased to unmeasurable levels once the animal learned to self-stimulate – so that although responding was vigorous, increases in DA were not observed (Garris et al. 1999). The problem with the hypothesis that BSR is capable of inducing only transient rather than continuous DA release is the fact that dopaminergic antagonists attenuate self-stimulation even after the animal has learned the task and has been at it for long periods of time (Benaliouad et al. 2007). Indeed, a number of subsequent studies have seriously put in doubt the role of predictability in BSR-induced DA release (Hernandez et al. 2006; Hernandez et al. 2008). These

studies have found that delivery of MFB stimulation at a low rate produced sustained DA elevation regardless of whether the stimulation was predictable. On the other hand, when the rate of stimulation was high, DA levels increased initially and then decreased toward the baseline range. Such a decrease was shown not to be contingent upon predictability but was hypothesized to reflect attenuated release capability of DA neurons as a result of a bombardment of stimulation (Hernandez et al. 2006; Hernandez et al. 2008). Consequently, it can be said that BSR-induced DA release, and more specifically DA itself, is not merely a mediator of novelty.

Amphetamine derives its rewarding effects from preferentially blocking DA reuptake in the NAS (preventing removal of DA from its sites of action and prolonging neurotransmission) and facilitating DA release in the NAS (Di Chiara & Imperato, 1988; Karoum et al. 1994). Amphetamine injections into the NAS are readily self-administered (Wise & Hoffman, 1992) and systemic and intra-accumbens injections of amphetamine have been shown to cause shifts to the left of the rate-frequency function, thereby reducing the amount of stimulation required to sustain responding (Broekkamp et al. 1975; Colle & Wise, 1988). While there is evidence that cocaine also elevates extracellular DA levels in the NAS (Di Chiara & Imperato, 1988) and D1 and D2 antagonists attenuate cocaine self-administration (Robeldo et al. 1992), cocaine is not readily self-administered directly into the NAS (Goeders & Smith, 1983; Wise & Hoffman, 1992). Rather, the cocaine seems to preferentially affect the mesocortical dopamine system as it blocks DA reuptake in the PFC (Karoum et al. 1994), rats will work for cocaine injections into the PFC (Goeders & Smith, 1983) and systemic cocaine injections reduce PFC as well as MFB reward thresholds (McGregor et al. 1992; Bauco & Wise, 1997). These and a myriad of other evidence had made strong case that drugs which activate the mesocorticolimbic DA pathway also cause leftward shifts in the self-stimulation dose-effect curve (decreased reward thresholds)

(Wise & Rompre, 1989).

Opiates such as morphine and heroin also activate the mesolimbic dopamine system but by a different mechanism. They bind to endogenous opioid receptors which inhibit GABA-inhibitory cells found in the VTA, thereby facilitating DA release (Kelley et al. 1980). Studies using microdialysis, a technique that allows the measurement of extracellular fluid from deep within the brain in freely moving animals, have consistently demonstrated that both systemic and intra-VTA administration of opiates or opioid receptor agonists increases DA cell firing, which subsequently increases the release of DA and its metabolites in the NAS (Spanagel et al. 1990; Leone et al 1991). In addition, opiates and opioid receptor agonists are self-administered into the VTA (Devine & Wise, 1994; Wise & Hoffman, 1992) and reduce thresholds for BSR when administered both systemically and directly into the NAS (Duvauchelle et al. 1996; Jha et al. 2004). By contrast, there is evidence that 6-OHDA lesions of the VTA blocked morphine-induced reductions in reward thresholds (Hand & Franklin, 1985) and the opioid receptor antagonist naloxone increased reward thresholds when administered alone as well as blocked intra-VTA morphine and opioid agonist-induced facilitation of BSR (Jenck et al. 1987). As is the case with amphetamine, cocaine, and opiates, the mesolimbic DA pathway from the VTA to the nucleus accumbens plays at least a key role in the reinforcing effects of additional drugs of abuse such as nicotine, alcohol and cannabis. All three of the latter drugs increase the firing rate of DA neurons in the VTA, enhance DA release in the NAS (Di Chiara & Imperato, 1988; Wise, 1998; Meyer and Quenzer, 2005) and induce leftward shifts in the self-stimulation dose-effect curve (Wise & Rompre, 1989; Lepore et al. 1996).

As with any other operant task, the principle limitation of BSR is that it cannot differentiate reward from reinforcement However, despite this the aforementioned research

suggests is that BSR is a unique, anatomically specific paradigm that is sensitive to pharmacologically-induced changes in reward and whose effects have been extensively validated using other paradigms (i.e. good external validity). In addition, unlike other paradigms used to measure reward deficits (e.g. sucrose intake), the number of reward-relevant neurons that are activated during stimulation can be precisely controlled and easily quantified. These properties make BSR probably the best behavioral paradigm to study withdrawal-induced anhedonia.

1.6 Neurobiology of anhedonia induced by psychostimulant withdrawal

Microdialysis and BSR studies have repeatedly demonstrated that withdrawal from virtually all drugs of abuse including cocaine, amphetamine, alcohol, nicotine, and opiates decreases mesolimbic dopamine levels and increases reward thresholds (Rossetti et al. 1992; Paterson & Markou, 2007). Apart from psychostimulants, however, abstinence from other classes of drugs is associated with a considerable amount of somatic symptoms that may act as a confounding factor (Barr & Markou, 2005). Consequently, the forthcoming discussion will focus on withdrawal from psychostimulants since these drugs induce an anhedonic state that is minimally obfuscated by physical symptoms.

Numerous studies have shown that withdrawal from cocaine and amphetamines produce significant 'within system' adaptations that lead to hypofunctioning of the mesocorticolimbic dopamine pathway. Some of these adaptations are characterized by a decrease in TH, the rate-limiting enzyme in dopamine synthesis, as well as a reduction in extracellular levels of DA and its metabolites. One study using the method of immunohistochemistry (the process of localizing proteins in cells of a tissue section by introducing antibodies that bind to specific proteins such as neuropeptides, receptors, or enzymes, that researchers want to locate in the brain), has delineated that abstinence from chronic cocaine self-administration reduced TH immunoreactivity in the

NAS by 17% and 71% by day one and day seven of withdrawal, respectively (Schmidt et al. 2001; Meyer & Quenzer, 2005). Another study using a different method to ascertain levels of TH, termed in situ hybridization, has revealed that mRNA (messenger ribonucleic acid) levels of the enzyme were reduced by 25% in the substantia nigra (SNc) after 5 hours of abstinence from intraperitoneal methamphetamine injections (Zhang & Angulo, 1996). Since the rate of synthesis of the specific protein is signified by the amount of mRNA, an increase in TH mRNA means that there is more of that protein being synthesized (Meyer & Quenzer, 2005). Indeed, methamphetamine-induced deficits of mesostriatal TH are well established and have been observed in a number of different studies (Hotchkiss & Gibb, 1980; Schmidt et al. 1985). Analogously, there is evidence for decreased TH activity in the caudate-putamen (CPu) following withdrawal from chronic amphetamine administration (Ellison et al. 1978). The CPu is a region within the dorsal striatum which is highly innervated by dopaminergic neurons that originate from the VTA and SNc (Chinta & Andersen, 2005). Since high catecholamine levels act as a negative feedback mechanism to inhibit TH, which in turn, inhibits further DA synthesis, low levels of TH during withdrawal may be an opponent process adaptation that counters the acute rewarding effects of psychostimulants.

Microdialysis studies have revealed that withdrawal from chronic cocaine administration leads to reductions in basal extracellular DA levels in the NAS (Parsons et al. 1991; Weiss et al. 1992) and the basolateral amygdala (Tran-Nguyen et al. 1998). Furthermore, regional cerebral glucose (energy) metabolism was reduced in DA rich areas including the NAS, olfactory tubercle, basolateral and central amygdaloid nuclei and the lateral hypothalamus following abstinence from cocaine self-administration (Hammer et al. 1993). For their part, amphetamines have also been shown to induce a marked reduction in extracellular DA concentration in the

NAS, CPu, PFC, and amygdala during abstinence (Rossetti et al. 1992; Persico et al. 1995; Paulson & Robinson, 1996; Weiss et al. 1997; Broom & Yamamoto, 2005; Peleg-Raibstein et al. 2006).

Another adaptation that has been observed during psychostimulant withdrawal is a decrease in dopaminergic metabolites. Abstinence from chronic cocaine administration decreased DA turnover as indicated by decreased DOPAC concentration in the NAS, septum, striatum, hypothalamus and frontal cortex and attenuated HVA concentrations in the frontal cortex (Karoum et al. 1990). Analogously, there is evidence that methamphetamine withdrawal reduced DOPAC and HVA concentrations in the striatum (Schmidt et al. 1985) and abstinence from chronic amphetamine treatment reduced DOPAC concentration in the CPu of rats (Swerdlow et al. 1991).

In addition to decreased DA neurotransmission, abstinence from cocaine and amphetamines has been found to produce molecular adaptations within dopaminergic neurons themselves. For example, three days after discontinuation of repeated cocaine injections, NAS neurons recorded in brain slices evidenced decreased excitability by being less responsive to depolarizing current injections and having higher action potential thresholds and lower spike amplitudes. These changes seem to indicate that cocaine-induced augmentation of DA levels lead to compensatory changes in the neuronal signaling mechanisms that moderate the excitability of NAS neurons, which in turn, caused the NAS to be significantly less receptive to excitatory input (Zhang et al. 1998). Psychostimulant withdrawal has not only been demonstrated to render NAS neurons less responsive to current injections but to drug challenge as well. In particular, whereas challenge with amphetamine in saline-treated rats produced a 10-fold increase in DA levels in the NAS, the same injections in rats undergoing 7 days of amphetamine

withdrawal only produced a 50% increase in DA (Imperato et al. 1996; Di Ciano et al. 2002). This result suggests not only a tolerance to amphetamine itself but perhaps a tolerance to the ability of natural reinforcers to increase DA efflux as well, since they too have been hypothesized to be primarily DA dependant (Wise et al. 1978). On the other hand, there is evidence that repeated drug exposure induces sensitization of drug-induced stimulation of DA neurotransmission (Di Chiara, 2002); however, this process is more likely to predominate during the period of protracted rather than acute withdrawal.

D2 autoreceptor supersensitivity and upregulation is another adaptation that has been witnessed in rats withdrawn from psychostimulants. This effect may be an opponent process that serves to normalize DA neurotransmission following psychostimulant-induced DA release and could be related to anhedonia that is often observed following high-dose, long-term psychostimulant addiction (Davidson et al. 2000). For instance, it was demonstrated that quinpirole, a D2-like agonist that preferentially activates D2 autoreceptors at low doses, was significantly more effective at inhibiting electrically evoked DA release in slice preparations of the NAS and SNc of rats abstinent from cocaine self-administration as compared to controls (Gao et al. 1998; Davidson et al. 2000). Similarly, slice preparations of SNc cells of rats undergoing withdrawal from chronic amphetamine treatment evidenced supersensitivity to apomorphine, another D2-like receptor agonist with autoreceptor affinity at low doses (Lee & Ellinwood, 1989).

The last major within system adaptation that may be associated with psychostimulant withdrawal-induced anhedonia is a hyperfunctioning of the DA transporter. DAT-mediated DA uptake induced by nomifensine was augmented by as much as 31% in the CPu and 86% NAS in rats withdrawn from cocaine self-administration as compared with saline treated controls

(Samuvel et al. 2008). Moreover, increased levels of the DAT protein were also found in the PFC of cocaine abstinent rats (Grimm et al. 2002). Because cocaine blocks the DAT during intoxication which, in turn, floods synapses with dopamine, a hyperfunctioning of the DAT during withdrawal lowers the amount of DA in the synaptic cleft and may contribute to post-cocaine anhedonia (Meyer & Quenzer, 2005). There is also evidence that amphetamines – which function by reversing and/or blocking the DAT, increase – DAT levels in various regions of the mesocorticolimbic DA pathway. Specifically, DAT mRNA was significantly elevated in the SNc and VTA of rats abstinent from repeated amphetamine treatment (Lu & Wolf, 1997; Shilling et al. 1997) and repeated intraperitoneal injections of methamphetamine increased DAT immunoreactivity by over 50% in the NAS of rats during abstinence (Broom & Yamamoto, 2005). Taken together, the aforementioned research suggests that a number of within system changes occur in the dopaminergic pathways in response to psychostimulants which may result in anhedonic symptoms. These include reduced levels of TH and DA, subsensitivity of DA neurons and supersensitivity of D2 autoreceptors and DA transporters.

1.7 Treatment of anhedonia

1.7.1 Full and partial dopamine receptor agonists

There is no doubt that any drug which induces pleasure can also reverse anhedonia and ease the withdrawal process. This is why dopamine receptor agonists, drugs which are rewarding but not always to the same degree as psychostimulants, have been proposed as potential treatments of drug addiction (Markou & Koob, 1992; Markou et al. 1992). The mechanism of action of these drugs differs from cocaine and amphetamines because they produce their rewarding effects by directly stimulating DA receptors instead of inducing DA release indirectly through action at transporters. Studies have shown that while direct DA agonists such as

bromocriptine, apomorphine, pergolide and quinpirole were able to be partially substituted for cocaine and amphetamine in a two lever choice procedure whereby animals were trained to discriminate psychostimulant from vehicle infusions, the maximally effective doses of these agents produced markedly lower response rates on the psychostimulant associated lever (Kamien & Woolverton, 1989; Spealman et al. 1991; Witkin et al. 1991).

Further adding to their potential for the treatment of drug addiction DA agonists were found to relieve withdrawal-induced anhedonia. For instance, bromocriptine was demonstrated to reverse post-cocaine reward thresholds in a dose dependant manner (Markou & Koob, 1992). In the progressive-ratio schedule responding for sucrose paradigm, the DA agonist ropinirole ameliorated post-methamphetamine anhedonia by increasing breakpoints to near control levels (Hoefer et al. 2006). Unfortunately, however, the method of substituting indirect DA agonists with direct DA agonists in order to treat drug addiction has had limited success in clinical trials. This may be because these compounds induce similar side-effects to the drugs that they are supposed to substitute, including increased autonomic activation and tendency to commit impulsive behaviors such as gambling and further drug taking. They have also failed to reduce the acute subjective effects of cocaine and have shown to increase dangerous side-effects such as increased heart rate and blood pressure when used in combination with cocaine (Preston et al. 1992; Bergman, 2008).

There is some promising preliminary data on the utility of partial DA agonists to treat drug addiction. Partial DA agonists bind to the dopamine receptor with high affinity but produce only a low level of activation. The activity of these compounds is believed to be dependent on the dopaminergic tone of the brain. In normal animals and animals under the influence of acute psychostimulant treatment these agents act as functional antagonists because they compete for

binding sites with endogenous dopamine. Conversely, during conditions of low dopaminergic activity such as after 6-OHDA lesions or reserpine administration, partial agonists activate DA neurons that would otherwise not be activated (Carlsson, 1983; Clark et al. 1991). These drugs may be particularly useful in the treatment of drug addiction because they do not have abuse potential themselves (e.g. they are not self-administered in rats) and have shown some preliminary positive results in the treatment of anhedonia induced by withdrawal from amphetamines. In particular, treatment with the partial D2 agonist terguride after chronic amphetamine and methamphetamine administration increased breakpoint for sucrose responding on a progressive-ratio schedule (Orsini et al. 2001; Hoefer et al. 2006). Further research needs to be done to corroborate these evidences using other paradigms of withdrawal-induced anhedonia before firm conclusions can be made regarding the utility of partial DA agonists in the treatment of drug addiction.

1.7.2 Antidepressants

Dopaminergic activation is not the only modus operandi whereby drugs can achieve anti-anhedonic effects. Some evidence exists for the ability of serotonergic and noradrenergic manipulations to treat anhedonia, potentially by indirectly augmenting DA concentration. Administration of tricyclic antidepressants (TCAs), which inhibit the reuptake of NE and 5-HT, and selective serotonin reuptake inhibitors (SSRIs), which selectively inhibit the reuptake of 5-HT, are some of the more conventional treatments that have evidenced anti-anhedonic properties in the self-stimulation paradigm in cocaine and amphetamine abstinent rats. When administered during two days prior to the cessation of chronic amphetamine treatment, the TCAs imipramine and amitriptyline reversed depressed response rates that were a result of withdrawal (Kokkinidis et al. 1980). In addition, chronic administration of imipramine shortened the duration of deficits

in reward thresholds elicited by cocaine withdrawal (Markou et al. 1992). Analogously, the SSRIs paroxetine and fluoxetine when combined with p-MPPI (4-(Methoxyphenyl)-1-[2′-(n-2″-pyridinyl)-p-iodobenzamido]-ethyl-piperazine ([^{125}I), a 5-HT1A antagonist, shortened the number of days that rats exhibited elevations in reward thresholds following amphetamine abstinence (Harrison et al. 2001; Markou et al. 2005). The 5-HT1A receptor is the autoreceptor for serotonin and counterbalances the increased synaptic 5-HT during the early stages of reuptake blockade by antidepressants; an effect that is believed to be responsible for the delayed onset of action of many antidepressant drugs. Thus, the administration of 5-HT1A antagonists in the aforementioned experiments served to block autoreceptors that would otherwise take weeks to downregulate thereby allowing the antidepressant drugs to increase levels of 5-HT (Meyer & Quenzer, 2005).

The effects of serotonergic and noradrenergic manipulations on reward thresholds can be partly explained by the fact that 5-HT and NE receptors are thought to localize on DA cells as well as on glutamatergic and GABAergic neurons which excite and inhibit DA neurons, respectively (Adell & Artigas, 2004; Roth et al. 2004). Serotonergic neurons, most of which originate from the median and dorsal raphe nuclei found along the midline of the brainstem send projections to nearly all forebrain areas including the neocortex, striatum, VTA, NAS, amygdala and hypothalamus (Mylecharane, 1996; Meyer & Quenzer, 2005) and local application of 5-HT to VTA brain slices has been shown to augment DA efflux in that area (Beart & Mcdonald, 1982). Similarly, the NE-containing neurons within the brain are located in an area within the brainstem called the locus coeruleus, and likewise, project to virtually all areas of the forebrain (Meyer & Quenzer, 2005). Moreover, intra-VTA injection of fluoxetine and the NE reuptake blocker nisoxetine was evidenced to increase DA levels in that region (Chen & Reith, 1994).

Behavioral studies have suggested that the role of 5-HT and NE on brain reward function may be more modulatory than facilitatory, however. Both acute paroxetine and p-MPPI and acute fluoxetine and p-MPPI significantly elevated reward thresholds in control animals. Some researchers have hypothesized that the putative anti-anhedonic consequences of enhanced serotonergic and noradrenergic neurotransmission may depend on the original "hedonic" state whereby antidepressants elevate mood in anhedonic subjects but have no effect or depress mood in individuals free of mental illness (Harrison et al. 2001; Markou et al. 2005).

1.7.3 Atypical antipsychotics

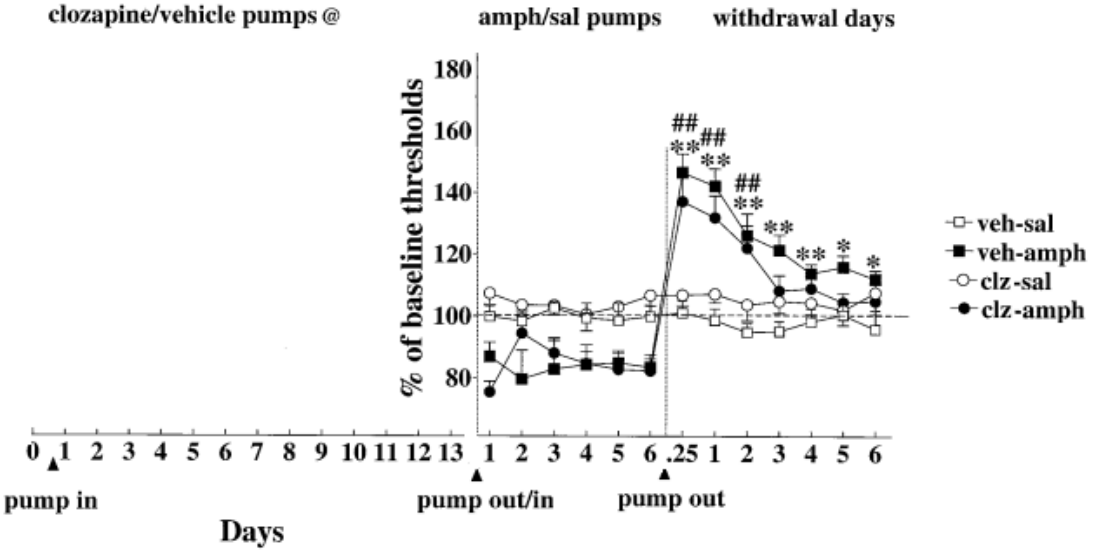
By far the most research that has been done thus far on treatment of anhedonia in animals has been with atypical antipsychotics. The first atypical or second-generation antipsychotic medication, clozapine, was discovered in 1958 and subsequently introduced into clinical practice for the treatment of schizophrenia. The drug was hailed for its decreased propensity to induce extrapyramidal side effects (motor impairment) and sustained prolactin elevation; both dangerous side effects of typical or first-generation antipsychotic drugs (also known as neuroleptics; Healy, 2002). Aside from a lower side-effect profile, clozapine was found to be more effective than any other antipsychotic drug in treating negative symptoms of schizophrenia such anhedonia, apathy, avolition and affective flattening (Sirota et al. 2006; Roth et al. 2004). Unfortunately, clozapine itself was found to cause agranulocytosis, an acute condition involving a severe and dangerous decrease in white blood cell count. This has led pharmaceutical companies to develop a number of analogues such as olanzapine, risperidone, and quetiapine; drugs that share crucial pharmacological properties with clozapine but do not induce the deadly disease. Recent research has made a strong case that these agents are not only useful in the treatment of schizophrenia, but also in the treatment of drug addiction (Green et al. 2008). One

of the possibilities that has been explored in animal models is that atypical antipsychotics may treat substance dependence by alleviating anhedonia associated with drug withdrawal. One model that has been used to demonstrate the anti-anhedonic effect of atypical antipsychotics is the chronic mild stress (CMS) protocol. This model involves presenting a series of unpredictable innocuous stressors such as tilting of the cage, crowding up to 8 animals in a single cage, food deprivation for up to 24 hours, and intermittent overnight lighting. Animals exposed to this protocol for 2-3 weeks begin to exhibit depressed voluntary sucrose intake and this effect is reliably reversed by antidepressants (Willner et al. 1987; Ferretti et al. 1995). Studies with quetiapine and olanzapine have demonstrated that chronic administration of these agents alleviated CMS-induced anhedonia; an effect not witnessed with chronic haloperidol administration (Orsetti et al. 2006, 2007). These results have to be interpreted with caution, however, because CMS failed to induce anhedonia in the progressive-ratio schedule for sucrose reinforcement. That is, animals that underwent CMS treatment did not evidence lower breakpoints for responding for a sucrose solution (Phillips & Barr, 1997; Barr & Phillips, 1998). Judging from these results it seems that the CMS protocol may depress hedonic reactions to sucrose but does not produce the motivational deficits that are the hallmark of psychostimulant withdrawal, and thus, may not be a valid model of drug addiction.

In addition to reversing CMS depressions in sucrose responding, there is also evidence that atypical antipsychotics may help alleviate anhedonia induced by amphetamine withdrawal. Specifically, a study by Semenova & Markou (2003) demonstrated that two week clozapine pretreatment reduced the number of days that animals exhibited reward deficits in the BSR paradigm whereas acute clozapine treatment elevated reward thresholds in control animals and had no effect on reward thresholds in anhedonic animals. These results suggest that a long-term

adaptation may take place when clozapine is administered chronically that serves to modulate dopaminergic activity according to the prevailing dopaminergic tone. This sort of adaptation reminds one of the mitigation of anhedonia that is induced by chronic administration of 5-HT and NE reuptake inhibitors. Indeed, clozapine has moderate affinity for serotonergic receptors and high affinity for adrenergic receptors and these mechanisms may very well underlie its putative benefits in the treatment of anhedonia (Roth et al. 2004).

Figure D: The effect of chronic clozapine administration on anhedonia induced by amphetamine withdrawal



The effects of 14-day clozapine pretreatment (6mg/kg/day through osmotic minipumps) on reward thresholds in rats under amphetamine withdrawal. The @ sign denotes statistically significant elevations in thresholds during clozapine pretreatment ($p < 0.05$; data not shown). Asterisks denote statistically significant differences between thresholds of amphetamine- and saline-exposed rats pretreated with vehicle. Pound signs denote statistically significant differences between thresholds of amphetamine- and saline-exposed rats pretreated with clozapine. Modified from: Semenova and A. Markou, Clozapine treatment attenuated somatic and affective signs of nicotine and amphetamine withdrawal in subsets of rats exhibiting hyposensitivity to the initial effects of clozapine, *Biol. Psychiatry* **54** (2003), pp. 1249–1264.

The present study sought to extend the research on atypical antipsychotic medications

and d-amphetamine withdrawal-induced anhedonia by investigating the effects of acute quetiapine on brain stimulation reward (BSR) in rats under withdrawal from d-amphetamine. Quetiapine was chosen because it possesses one of the best side-effect profiles of its class (Masand & Narasimhan, 2006) and has been effective in treatment abuse of drugs such as alcohol, cocaine, amphetamine, methamphetamine, and opioids (Brown et al. 2003; Potvin et al. 2006; Kennedy et al. 2008; Martinotti et al. 2008).

1.8 Hypotheses

1. Acute injection of amphetamine (1mg/kg) will reduce reward thresholds.
2. The reduction in reward thresholds hypothesized above will be inferior in amphetamine-treated rats when they are challenged with amphetamine (1mg/kg) at the end of the experiment, compared to vehicle-treated controls.
3. Rats withdrawn from systemic escalating amphetamine treatment will display significantly elevated reward thresholds compared with controls.
4. Acute quetiapine injection will dose-dependently elevate reward thresholds in control rats.
5. Acute quetiapine injection will dose-dependently reverse elevated reward thresholds observed in amphetamine withdrawn rats.

2. Article soumis à European Neuropsychopharmacology

Contribution a l'article

I contributed to the article contained herein by completing the entirety of the experimentation as well as by writing the first draft.

Acute quetiapine dose-dependently enhances anhedonia induced by withdrawal from escalating doses of d-amphetamine

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Abstract

Background Anhedonia, a condition in which the capacity of experiencing pleasure is reduced, is observed in patients that are under withdrawal from drugs of abuse. Recent clinical studies show that quetiapine may be beneficial in the treatment of substance abuse by alleviating the withdrawal-negative affect stage of addiction. This study investigated the effects of acute quetiapine on reward in animals under withdrawal from d-amphetamine.

Methods Experiments were performed on male Sprague-Dawley rats trained for intracranial self-stimulation. Measures of reward threshold were determined with the curve-shift method in different groups of rats before, and during four days after treatment with escalating doses (1 to 10 mg/kg, i.p) of d-amphetamine sulphate or its vehicle. At 24h after withdrawal, the effects of two doses of quetiapine (2 and 10 mg/kg ip) were tested in all the animals.

Results Animals treated with d-amphetamine showed 25% reward attenuation at 24h of withdrawal, an effect that decreased over the next three days. Quetiapine administered acutely at 2mg/kg and 10mg/kg on the first day of withdrawal produced 10% and 25% reward attenuation, respectively, in the vehicle-control animals, an effect also observed in the animals under withdrawal from d-amphetamine but only at the high dose.

Conclusions These results show that quetiapine produced a mild attenuation of reward in normohedonic and in anhedonic animals. They suggest that quetiapine should be used at low doses for the treatment of substance abusers under withdrawal from psychostimulant drugs to avoid enhancement of the anhedonic state.

Key words: Anhedonia, Amphetamine, Dopamine, Quetiapine, Reward, Tolerance

1. Introduction

Anhedonia, a condition in which the capacity of experiencing pleasure is reduced, is frequently observed in patients that are under withdrawal from chronic, alcohol, opioid or psychostimulant abuse (Newton et al. 2004; Janiri et al. 2005). Anhedonia induced by drugs of abuse is believed to occur due to decreased reward system function as a result of depletion of mesolimbic dopamine (DA) (Rossetti et al. 1992). This reward deficit, in turn, is hypothesized to act as an 'opponent process' to perpetuate the cycle of drug addiction (Solomon & Corbit, 1974). As abuse of a drug progresses, it is hypothesized to shift from being motivated by the strong positive reinforcing property of the drug, to being motivated by a strong negative reinforcing property of withdrawal (i.e. the desire to alleviate withdrawal symptoms such as anhedonia, anxiety, irritability and dysphoria) and a weaker positive reinforcing property (Koob & Le Moal, 2001).

Recent clinical studies show that the atypical antipsychotic medication, quetiapine, may be beneficial in the treatment of drug abuse, alleviating the negative affective symptoms that are thought to predominate and drive the cycle of addiction (Koob & Le Moal, 2001; Potvin et al. 2006; Martinotti et al. 2008). Unlike typical antipsychotic medications, which bind to DA receptors with a high affinity and interfere strongly with DA neurotransmission, atypical antipsychotic medications, like quetiapine, are characterized by a higher ratio of serotonergic (5-HT), and noradrenergic (NE), to DA blockade; this pharmacodynamic profile has been proposed to account for their presumed better effectiveness in reducing negative symptoms of schizophrenia such as anhedonia, alogia, affective flattening and avolition (Meltzer et al. 1989).

A number of studies employing different animal models of hedonic behavior have testified to the ability of atypical antipsychotic medications to mitigate deficits in reward system function. For instance, there is evidence that quetiapine reversed anhedonia-like depression in

free consumption of sucrose induced by chronic mild stress (CMS) (Orsetti et al. 2007). Moreover, treatment with amisulpride reduced decreases in sucrose consumption in rats that were shifted from 32% to 4% sucrose solution, a procedure known as the successive negative contrast (SNC) (Genn et al. 2002). Finally, administration of clozapine decreased the number of days that rats evidenced elevated thresholds for brain stimulation reward (BSR) as a result of withdrawal from chronic infusion with d-amphetamine (Semenova & Markou, 2003).

Withdrawal from d-amphetamine, which is associated with a significant reward deficit, is a validated model of the anhedonia – a component of the opponent process model of motivation (Solomon & Corbit, 1974; Koob & Le Moal, 2001; Barr & Markou, 2005). The rewarding action of d-amphetamine, like several other drugs that are initially abuse for their positive reinforcing property, is highly DA-dependant. Animal studies have shown that blockade of central DA receptors, or selective neurotoxic lesions of mesolimbic DA pathways, reduce d-amphetamine reward (Wise and Rompré, 1989). During d-amphetamine withdrawal extracellular DA concentrations have been evidenced to decrease by as much as 50, 35 and 25% on days 1, 3 and 5 of abstinence, respectively (Rossetti et al. 1992), reductions that are correlated with decreases in break-points for sucrose responding on a progressive-ratio (PR) schedule and attenuation of reward induced by medial forebrain bundle electrical stimulation (Barr & Phillips, 1999; Cryan et al. 2003). In addition, whereas withdrawal from many other drugs of abuse is associated with a considerable amount of somatic symptoms that may act as confounding factors, amphetamine withdrawal is believed to be minimally obfuscated by physical symptoms (Barr & Markou, 2005).

The present study sought to extend the research on atypical antipsychotic medications and d-amphetamine withdrawal-induced anhedonia by investigating the effects of acute

quetiapine on brain stimulation reward (BSR) in rats under withdrawal from d-amphetamine. Brain stimulation reward is a behavioral paradigm that is highly sensitive to changes in central DA neurotransmission (Wise & Rompré, 1989). Administration of drugs of abuse such as opiates, ethanol, nicotine, amphetamine, and cocaine increases extracellular DA release in the mesolimbic dopamine system and decreases BSR threshold (Di Chiara & Imperato, 1988; Wise & Rompré, 1989). By contrast, withdrawal from chronic treatment with ethanol, morphine, cocaine and amphetamine have been reported to decrease extracellular DA concentrations in the mesolimbic dopamine system and to elevate BSR threshold (Wise & Rompré, 1989; Rossetti et al. 1992). The use of the curve-shift method with BSR, unlike other paradigms, (e.g. sucrose intake), allows one to dissociate reward from performance deficit, hence to obtain a specific measure of a change in reward system function (Edmonds and Gallistel, 1974; Miliaressis et al, 1986). Quetiapine was chosen because it possesses one of the best side-effect profiles of its class (Masand & Narasimhan, 2006) and has been effective in treatment abuse of drugs such as alcohol, cocaine, amphetamine, methamphetamine, and opioids (Brown et al. 2003; Pinkofsky et al. 2005; Potvin et al. 2006; Kennedy et al. 2008; Martinotti et al. 2008).

2. Materials and Methods

2.1 Animals and surgery

Male Sprague–Dawley rats (Charles River, St-Constant, Quebec) weighing between 300 and 350 g at the surgery time were used. They were initially housed two per cage, and one per cage after the surgery, in a temperature and humidity-controlled room (21 ± 1 °C; 53 ± 2%) with a 12 h light-dark cycle (lights on at 06:30 am). They were allowed to habituate to the new housing environment for 7 days before the surgery and had access to food and water *ad libitum*. Experiments were performed during the light cycle and were carried out in accordance with

guidelines of the Canadian Council on Animal Care; all efforts were made to minimize suffering and number of animals used. Rats were anaesthetized with isoflurane (2.5–3.5%, O_2 0.6 L/min) and mounted on a stereotaxic apparatus. A 0.2 ml solution of the local anaesthetic marcaine (0.25%) was injected subcutaneously at the site of incision. Two stainless steel wires of 0.27 mm in diameter insulated with Epoxy, except for the round tip, were implanted within each hemisphere into the lateral hypothalamus using the following flat skull coordinates: 2.8 mm posterior to bregma, 1.7 mm lateral to the saggital suture and 8.6 mm below the surface of the skull (Paxinos and Watson, 1986). An uninsulated wire serving as the inactive electrode (anode) was wrapped around four stainless steel screws threaded into the cranium and the whole assembly was fixed with dental acrylic. A 0.1 ml (im) solution of Duplocillin LA containing 300,000 I.U. of penicillin was administered to prevent infections.

2.2 Behavioral Test

2.2.1 Apparatus and training procedure

One week after surgery, rats were placed in a test cage (25 × 25 cm) made from three opaque polymer walls and one front Plexiglas wall that allowed observation. Each cage was equipped with an infrared photocell located inside a hole (3 cm diameter × 3 cm deep) 2 cm above a wire-mesh floor. To minimize disturbance due to external noise test cages were encased in ventilated wooden boxes insulated with Styrofoam. Rats were trained to produce a nose-poke response to trigger a constant-current pulse generator (Mundl, 1980) that delivered a single 500 ms train of 0.1 ms cathodal rectangular pulses. Each stimulation train was followed by an inter-train interval (500 ms) during which the pulse generator could not be triggered; the animal could not self-administer more than one train per sec (see Boye and Rompré, 1996). The effects of the stimulation on the behaviour were initially evaluated on each of the electrodes at different

current intensities (200–500 μA); the site at which the stimulation induced exploratory behaviour and forward locomotion with no, or minimal, motor effects was selected for the training test. Once operant responding was established (see Rompré, 1995 for details), the animals were trained to respond during discrete 55-s trials, each trial being followed by an interval of 30-s during which stimulation was not available. The beginning of each trial was signaled by five trains of non-contingent priming stimulation delivered at a rate of one per second. With the current intensity held constant, the frequency was varied from 94 to 20 Hz in approximately 0.05 log unit steps to obtain a function relating the total number of nose pokes per trial to the stimulation frequency (rate/frequency or R/F curve). An index of reward was derived from each R/F curve and was defined as the pulse frequency sustaining a half-maximal rate of responding (M50, see data analysis). The current intensity was set for each rat to generate a M50 value between 30 and 40 Hz. Four R/F curves were determined during daily test session and this until the lower and the higher M50 values determined within the session varied by less than 0.1 log unit for three consecutive days.

2.2.2 Drug testing

Different groups of rats were administered escalating doses of d-amphetamine (1-10 mg/kg, i.p), or its vehicle, every 8 h (9am, 5pm, 12pm), for three days; this regimen of d-amphetamine treatment was reported to induce a decrease sensitivity to reward following withdrawal (anhedonia; Barr & Phillips, 1999). The magnitude of anhedonia was measured by determining R/F curves on each day for 4 consecutive days, beginning twenty-four hours after the last d-amphetamine injection. The effect of an acute low and moderate dose of quetiapine (2 and 10 mg/kg) on sensitivity to reward was tested on the first day of withdrawal in all the animals. On this day, we first determined three R/F curves, and then we injected quetiapine, or its vehicle,

and determined three new R/F curves starting 15 min after the injection. In order to determine whether tolerance developed to the enhancing effects of d-amphetamine following exposure to escalating doses, we determined R/F curves before and after the first injection of d-amphetamine (1 mg/kg, only in those animals that received an escalating doses of d-amphetamine), and we repeated this test in the same animals at 96 h of withdrawal.

2.3 Drugs

Dextro-amphetamine sulphate (Sigma-Aldrich, United Kingdom) was dissolved in 0.9% isotonic saline and quetiapine (a generous gift of Astra Zeneca) was dissolved in 0.9% saline solution that contained 2 % glacial acetic acid; pH of quetiapine solution was adjusted to 5.4 with sodium hydroxide. Both drugs were injected intraperitonealy in a volume of 1 ml/kg and the doses are expressed as salt.

2.4 Data analysis

Measure of reward (M50 index, referred to as reward threshold later on) was derived from each R/F curve obtained before (baseline) and after withdrawal from d-amphetamine or the vehicle; M50 values were expressed as the percentage of baseline and group means were calculated. Maximum response rate was determined from each R/F curve using a procedure previously described (Benaliouad et al., 2007); data were expressed as percentage of baseline and group means were calculated. M50 values and maximum rates obtained following injection of quetiapine, or its vehicle, on the first day of withdrawal, and following the first and the second test acute d-amphetamine (1 mg/kg) were expressed as percentage pre-injection. Mean changes of both reward threshold and maximum rate were analyzed with a two-way (drug and vehicle × time) analysis of variance (ANOVA) for repeated measures on time. Homogeneity of variances was tested with Bartlett Chi-Square test and square root or log data transformation was

performed when necessary. Comparisons among means when justified were made with Duncan's test with the level of significance set at 0.05 (Statistica V6.1, Statsoft Inc., Tulsa, OK, USA).

2.5 Histology

At the end of the experiment, animals were anaesthetized with urethane (1.4 g/kg i.p.) and the stimulation site was lesioned by passing through the electrode a direct anodal current (0.15 mA during 15 sec). They were then perfused with a 10% of formalin solution containing 3% potassium ferrocyanide, 3% potassium ferricyanide and 0.5% trichloroacetic acid (Prussian blue technique). Brains were removed, stored in a 30% of sucrose solution until they sank, then frozen with 2-methylbutane (99.2%) and kept at − 80 °C. Brains were subsequently sliced in 40 μm sections that were mounted on gelatine-coated glass slides. Slices were stained using Nissl's technique and stimulation sites were localized under light microscopic examination.

3. Results

3.1 Histology

Histological analysis revealed that the stimulation sites were located within the MFB in the anterior and posterior part of lateral hypothalamus between 2.30 and 3.14 mm posterior to bregma (Fig. 1).

3.2 Effects of d-amphetamine withdrawal on reward threshold and maximum rate.

Figure 2 illustrates the R/F curves obtained from four rats 24h after the last d-amphetamine (bottom panels) and vehicle (top panels) injections. Withdrawal from d-amphetamine resulted in a rightward shift in R/F curves reflecting a reduction in reward sensitivity or anhedonia; such an effect was not seen in the animals that were injected repeatedly with the vehicle. It is noteworthy that maximum rate of responding was not altered in animals treated with the vehicle nor in those treated with d-amphetamine. Group mean changes in reward threshold and maximum rate

measured on each of the four days after withdrawal are shown in Figure 3. An increase in reward threshold ranging between 25% (24h) and 15% (96h) was observed on each day in the d-amphetamine treated rats; on the other hand, reward threshold remained near baseline level on each tested day in the vehicle treated rats (top panel). A two-way ANOVA performed on reward threshold data yielded a significant treatment by time interaction ($F(3, 123)=7.3$, $p<0.001$). Post-hoc test showed that reward threshold was significantly elevated in the d-amphetamine withdrawal group compared to vehicle on each testing day. A two-way ANOVA performed on maximum rate data (bottom panel) yielded no effect of treatment ($F(1, 41)=2.3$, $p=0.14$), time ($F(3, 123)=2.55$, $p=0.06$), and no treatment by time interaction by time interaction ($F(3, 123)=2.0$, $p=0.112$).

3.3 Effects of acute 2 mg/kg and 10 mg/kg of quetiapine on reward on the first day of withdrawal.

Figure 4 illustrates changes in reward threshold (top panel) and maximum rate (bottom panel) observed in different groups of rats on the first day of withdrawal before (black bars) and after (gray bars) injections of different doses of quetiapine or its vehicle. A two-way ANOVA performed on reward threshold data yielded a significant treatment by time interaction ($F(5, 37) = 11.6$, $p<0.001$). Post-hoc tests confirmed that reward threshold was significantly elevated (different than vehicle) in all groups that were under withdrawal from d-amphetamine. In the animals that were under withdrawal from vehicle (V-V, VQ2 and VQ10 groups), quetiapine produced a dose-orderly attenuation of reward that translates into a 15 and 25% increase in threshold at the low and moderate dose respectively. An attenuation effect of the same magnitude was observed at the moderate but not at the low dose of quetiapine the anhedonic animals. These changes in reward threshold cannot be attributed to an alteration in performance

because maximum rates did not change significantly (Figure 4, bottom panel). The ANOVA yield no effect of treatment ($F(5,37) = 0.60$ $p=0.69$), no effect of time ($F1,37=1.45$ $p=0.25$) and no treatment by time interaction ($F5,37=2.13$ $p=0.08$).

3.3 Effect of acute low dose of d-amphetamine on reward: evidence for a weak tolerance following repeated injections.

Changes in reward threshold and maximal rate were measured following injection of the first d-amphetamine injection (1 mg/kg); this test was repeated in all the d-amphetamine-treated animals at 96h of withdrawal. Results were then grouped according to the treatment administered at 24h of withdrawal (vehicle, 2 mg/kg or 10 mg/kg of quetiapine) and analyzed with a two-way ANOVA. The analysis performed on reward threshold data yielded no significant effect of group ($F2,18 = 1.7$, $p=0.21$), no group by time interaction ($F2,18 = 1.47$, $p=0.26$) but a significant effect of time ($F1,18 = 5.46$, $p <0.05$), showing that the enhancing effect of d-amphetamine on BSR differs between the first and the second test independent of the groups. Consequently, reward threshold data were averaged across groups and means were compared with Student's T-test for dependent sample (Fig. 5). As can be seen, d-amphetamine produced a 40% decrease in reward threshold on the first test, an effect that was slightly but significantly smaller (near 33%) on second test performed at 96h of withdrawal, suggesting that tolerance developed with repeated d-amphetamine injections ($t = 2.28$, $df = 20$, $p<0.05$). A two-way ANOVA performed on maximum rate data yield no group effect ($F2,18 = 0.30$, $p=0.75$), no time effect ($F1,18 = 0.003$, $p=0.95$) and no group by time interaction ($F1,18 = 1.28$, $p=0.30$), showing that tolerance to the reward enhancing effect cannot be attributed to an alteration in performance (Fig. 5).

Discussion

Animals that were under withdrawal from d-amphetamine showed a reward deficit (anhedonia) that is reflected by the increase in the amount of electrical stimulation necessary to sustain operant responding at threshold level compared to vehicle-control animals. This reward deficit was maximal at 24h after withdrawal and remained present for at least 4 days. The fact that on each day of withdrawal the increase in reward threshold was not accompanied by a change in maximum response rate confirms that it was not due to a performance deficit (Miliaressis et al., 1986). This finding is consistent with those reported in previous studies where changes in reward sensitivity following withdrawal from d-amphetamine were measured with BSR, and with other paradigms such as the breakpoint of a progressive ratio schedule for sucrose reinforcement and delayed recovery from SNC (Barr & Phillips, 1999; Barr & Phillips, 2002; Barr & Markou, 2005). It is well established that d-amphetamine produces a dose-dependent increase in ventral striatal DA release, an effect that is hypothesized to account for its reward enhancing effect (Wise, 1996). However, during withdrawal from repeated high doses of d-amphetamine (such as in the present study), the opposite effect on DA release occurs. Rossetti et al (1992) have reported a decrease in ventral striatal extracellular DA ranging from 50 to 25% between day 1 and day 5 of d-amphetamine withdrawal. Moreover, several studies have shown that BSR is highly sensitive to changes in central DA neurotransmission (Wise and Rompré, 1989; Wise, 1996), and a reduction in ventral striatal DA most likely explains the increase in reward threshold that we measured. This hypothesis is further supported by results that we obtained following injection of the first dose (1 mg/kg) of d-amphetamine and at 96h after withdrawal. On the first test, this dose of d-amphetamine produced near 40% decrease in reward threshold, a magnitude consistent with that reported in previous studies (Colle and Wise, 1988; Wise and Munn, 1993) and with an increase in ventral striatal DA release (Di Chiara &

Imperato, 1988; Karoum et al. 1994). But on the second test, performed at a time period when animals still expressed a reward deficit, the same dose of d-amphetamine produced a slightly, but significantly, smaller decrease in reward threshold. This weak tolerance effect can be interpreted as reflecting a decrease in the ability of d-amphetamine to increase ventral striatal DA release; it may result from within-system adaptations such as down-regulation and/or desensitization of postsynaptic DA receptors (see White & Kalivas, 1998). Another hypothesis to account for the reward deficit, and for the tolerance to the reward enhancing effect of d-amphetamine, is dopaminergic neurotoxicity. Although the bulk of research has focused on methamphetamine-induced neurotoxicity, there is evidence that repeated treatment with high doses of d-amphetamine induces a decrease in DA tissue level and in tyrosine hydroxylase immunoreactivity in the striatum (Ryan et al. 1990; Segal & Kuczenski, 1997; He et al. 2005, 2006). Semenova and Markou (2003) also observed that rats under withdrawal from 6-day infusion of d-amphetamine evidenced significant reward threshold elevations that lasted at least 144h.

The main objective of this study was to determine the effect of an acute quetiapine injection on reward during d-amphetamine withdrawal-induced anhedonia. Quetiapine was tested at two doses on the first day of withdrawal, a time period at which reward deficit was maximal. It produced a dose-orderly attenuation of reward in the vehicle-treated control animals that were normohedonic (no change in reward sensitivity). These findings replicate previous results that showed an attenuation of reward following acute injection of similar doses of quetiapine (Lapointe et al. 2006). On par with this finding is evidence that clozapine, which displays a similar receptor binding profile to quetiapine, dose-dependently elevated reward thresholds when given acutely in vehicle-treated animals (Semenova & Markou, 2003). Quetiapine displays a low

affinity for DA D2 receptors and yet competes with endogenous DA to produce a significant striatal D2 receptor occupation which likely accounts for its reward attenuation (Kapur et al., 2003). When tested in animals that were under withdrawal from d-amphetamine, quetiapine produced significant reward attenuation at the moderate dose, an effect that was similar in amplitude to that seen in vehicle-control animals. This result predicts that quetiapine administered acutely at a clinically-effective dose for schizophrenia (the dose that produces near 50% striatal D2 receptor occupancy) is likely to accentuate rather than reverse anhedonia (Kapur et al., 2003). Most interestingly, the reward threshold elevation elicited by the low dose (2 mg/kg) of quetiapine was not observed in the anhedonic animals. One explanation for this finding is that the neural adaptations such as down-regulation of DA receptors in response to systemic escalating d-amphetamine treatment may have blunted the ability of a low dose of quetiapine to act effectively at post-synaptic DA receptors (see White & Kalivas, 1998). This interpretation is problematic, however, because the effect of the moderate dose of quetiapine should have been blunted in these animals as well and this was not what was found. One of the primary metabolites of quetiapine, *N*-desalkylquetiapine, displays strong partial agonist activity at the 5-HT1A receptors. *N*-desalkylquetiapine is 10 times more potent at activating human 5-HT1A receptor in binding assays than quetiapine (45 vs. 450nM) and the action of this metabolite has been proposed to account for the efficacy of quetiapine monotherapy in the treatment of major and bipolar depression (McIntyre et al. 2007; Jensen et al. 2008). The presence of significant 5-HT1A action of quetiapine is underlined by findings that quetiapine treatment altered 5-HT1A and 5-HT2A but not DA receptor labelling in rat forebrain regions *in vivo* (Tarazi et al. 2001, 2002). In BSR studies, the selective 5-HT1A agonist 8-OH-DPAT exhibited a biphasic effect, lowering reward threshold at low doses and elevating it at high doses

– effects which are attributed to action at pre- and post-synaptic 5-HT1A receptors, respectively (Fletcher et al. 1995; Harrison & Markou, 2001). Furthermore, although d-amphetamine withdrawal has been most commonly associated with depleted DA levels, there is also evidence for a decrease in 5-HT levels in post-mortem brain tissue of rats treated systemically with the psychostimulant (Kitanaka et al. 2008). Additionally, Bonhomme et al. (1995) reported an increased in dorsal raphé 5-HT1A receptors following repeated d-amphetamine treatment. Consequently, due to the presumed reduction of endogenous 5-HT and the increase in pre-synaptic 5-HT1A receptors, the 5-HT1A partial agonist action of *N*-desalkylquetiapine may come into play at the low dose and act to reduce the reward attenuating effect of quetiapine. At the high dose the action of the primary metabolite at the post-synaptic 5-HT1A receptors may counteract its pre-synaptic action, and the D2 antagonism may drown out any further reward threshold changes related to 5-HT1A agonism.

Clinical studies of quetiapine for the treatment of substance abuse seem to be concordant with our results. That is, studies which have evidenced a benefit of quetiapine on non-psychotic substance abusers have generally treated patients with low doses of the atypical antipsychotic medication (50-300mg/day) (Monnelly et al. 2004; Sattar et al. 2004; Pinkofsky et al. 2005); with higher doses (300-600mg/kg) being used to manage psychosis in schizophrenia (Kapur et al. 2003). The use of high doses is likely to accentuate anhedonia in substance abusers; an effect that is clearly not desirable. The tolerance that we observed to the pro-anhedonic effect of a low dose of quetiapine in the animals under d-amphetamine withdrawal may explain why quetiapine has thus far not been found to worsen substance abuse outcomes in non-psychotic patients. In psychotic patients, however, quetiapine's weak D2 antagonism and fast dissociation from D2 receptors may act to normalize hyperactivity of the mesolimbic DA system, which has been

hypothesized to be responsible for the increased prevalence of substance abuse in this population (Kapur & Seeman, 2001; Chambers & Self, 2002). Typical antipsychotics, on the other hand, dissociate slowly from D2 receptors and the dysphoria which results from this continuous blockade may pull the individual into a negative affective state, requiring alleviation with drugs of abuse (Solomon & Corbit, 1974; Koob & Le Moal, 2001; Kapur & Seeman, 2001). Schizophrenia and bipolar studies show that patients' substance abuse may decrease simply by stopping typical therapy; receiving no antipsychotic medication (Brown et al. 2003; Swanson et al. 2007). Interestingly, the same studies found that substance abuse decreased even further if the patients began atypical therapy.

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Figure legends

Figure 1. Stimulation electrode tip locations for all the rats that were included in the study. Sites stimulated were all located within the medial forebrain bundle. Drawings are adapted from Paxinos and Watson's plates of the rat brain atlas (1998). Numbers on the right of each drawing indicate the distance in mm (posterior) from bregma.

Figure 2. Response-frequency (R/F) curves obtained from four rats before, and 24h after, administration of escalating doses of amphetamine or an equivalent regimen of vehicle treatment. In the animals that were under withdrawal from d-amphetamine higher stimulation frequencies were required to initiate and sustain responding (bottom panels) resulting in a rightward shift of the R/F curves. This effect was not observed in the animals under withdrawal from repeated vehicle treatment (top panels). See text for details.

Figure 3. Group mean changes (±SEM) of reward threshold (top panel) and maximum response rate (bottom panel), expressed as percent of baseline, measured daily for four days after withdrawal from de-amphetamine (n=21) and vehicle (n=22). Stars indicate a statistically significant difference between vehicle and d-amphetamine at the corresponding test day (***$p<0.001$). See text for details

Figure 4. Group mean changes (±SEM) of reward threshold (top panel) and maximum response rate (bottom panel) expressed as percent of baseline measured 24h after withdrawal from vehicle nd d-amphetamine (black bars) and after injection of one of two doses of quetiapine (2 and 10 mg/kg) or its vehicle (grey bars). Groups are divided according to the treatment they received before withdrawal and during this test day respectively: vehicle and vehicle (V-V, n=8), vehicle and 2 mg/kg quetiapine (V-Q2, n=7), vehicle and 10 mg/kg quetiapine (V-Q10, n=7), d-amphetamine and vehicle (A-V, n=7), amphetamine and 2 mg/kg quetiapine (A-Q2, n=7), d-

amphetamine and 10 mg/kg quetiapine (V-Q10, n=7). Stars indicate statistically significant difference between pre- and post-injection (**p<0.01; ***p<0.001); crosses indicate statistically significant difference with vehicle (V-V) pre-injection (+++p<0.001); pound signs indicate a statistically significant difference between V-Q10 and V-Q2, and between A-Q10 and A-Q2 (###p<0.001).

Figure 5. Group mean changes (±SEM) of reward threshold (black bars) and maximum response rate (grey bars) measured following injection a low dose of d-amphetamine (1 mg/kg). Two BSR tests were performed, a first one on following the first injection of d-amphetamine and a second one at the same dose at 96h of withdrawal. Results are expressed as percent of pre-injection values determined on each test day (n=21). Stars indicate a statistically significant difference with test 1. See text for details.

Figure 1

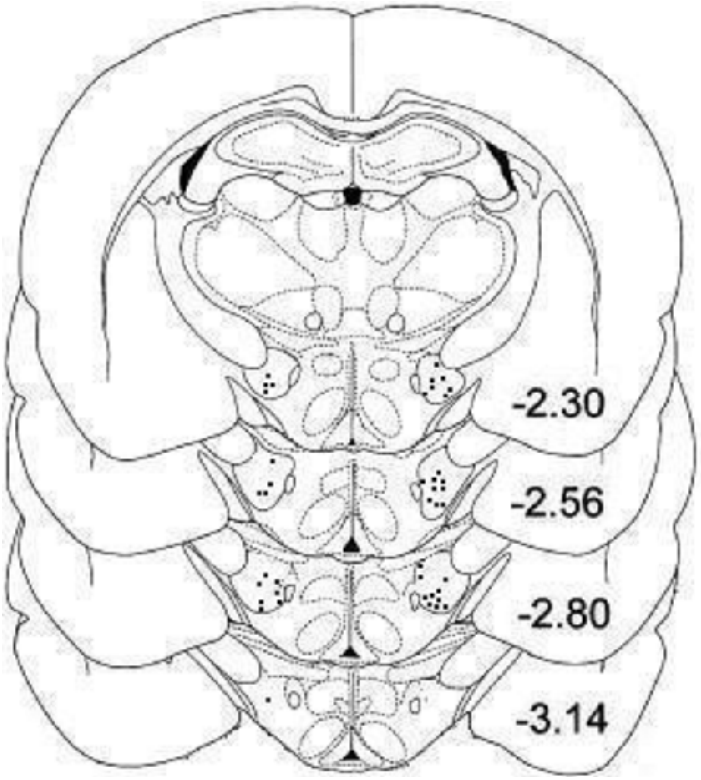


Figure 2

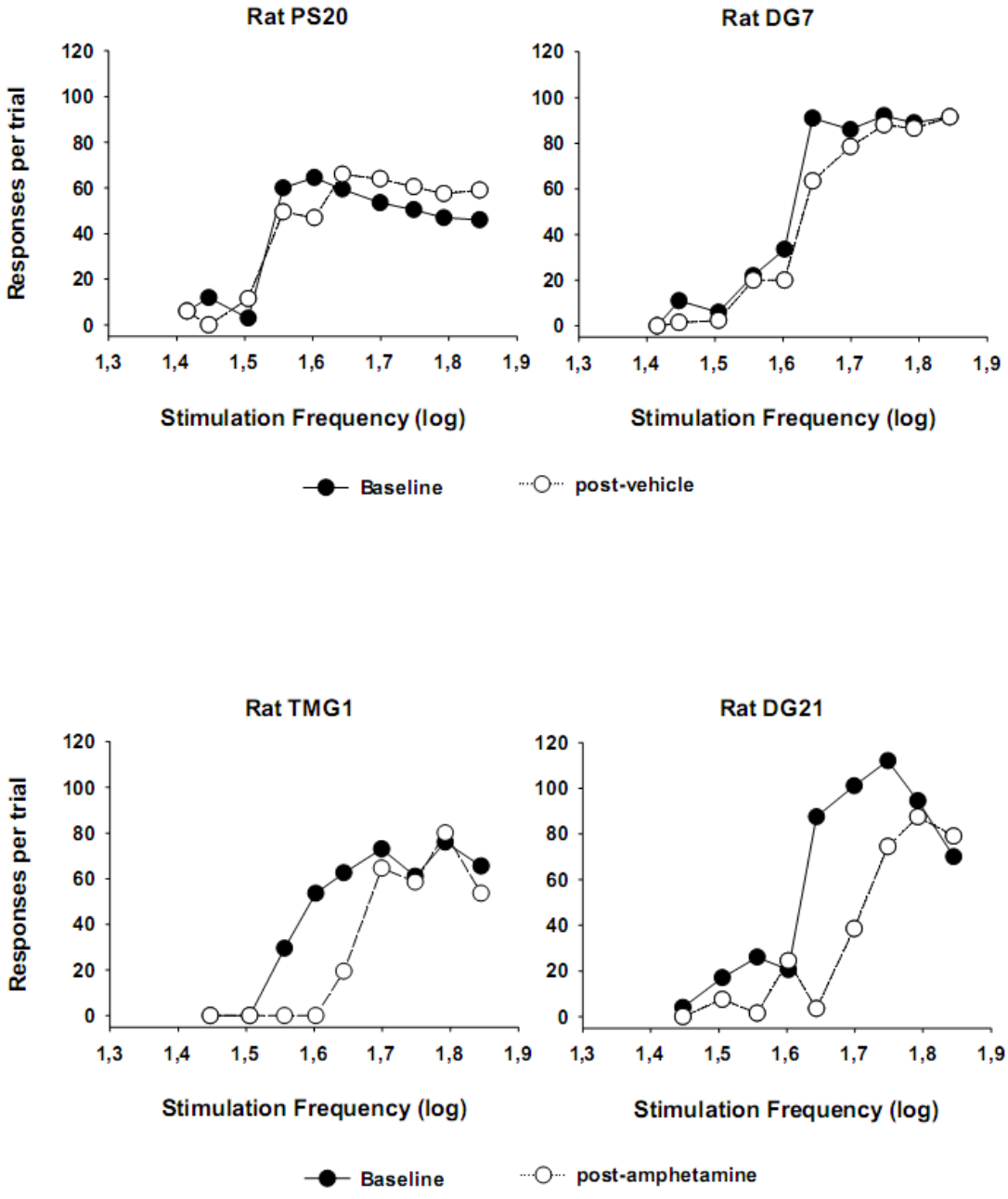

Rat PS20
Rat DG7
Responses per trial
120
100
80
60
40
20
0
1,3
1,4
1,5
1,6
1,7
1,8
1,9
Stimulation Frequency (log)
Baseline
post-vehicle
Rat TMG1
Rat DG21
post-amphetamine

Figure 3

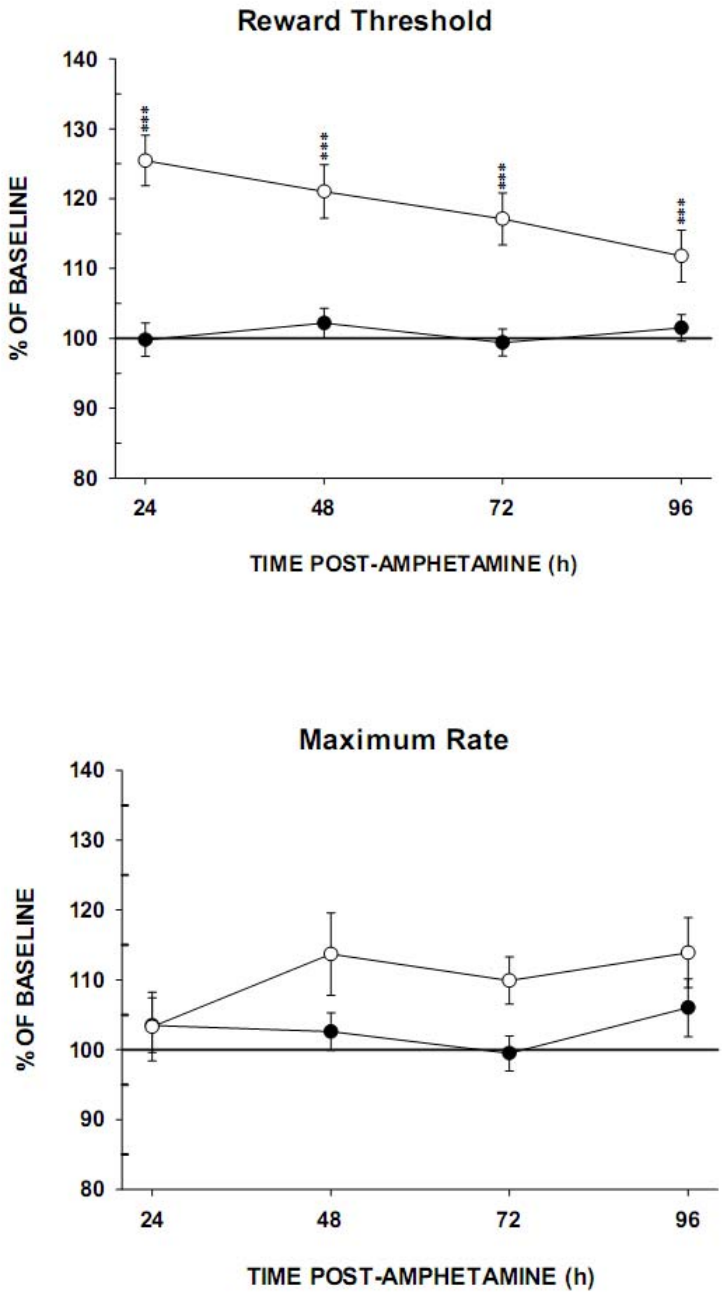

Reward Threshold
% OF BASELINE
140
130
120
110
100
90
80
***
***
***
***
24
48
72
96
TIME POST-AMPHETAMINE (h)
Maximum Rate
% OF BASELINE
140
130
120
110
100
90
80
24
48
72
96
TIME POST-AMPHETAMINE (h)

Figure 4

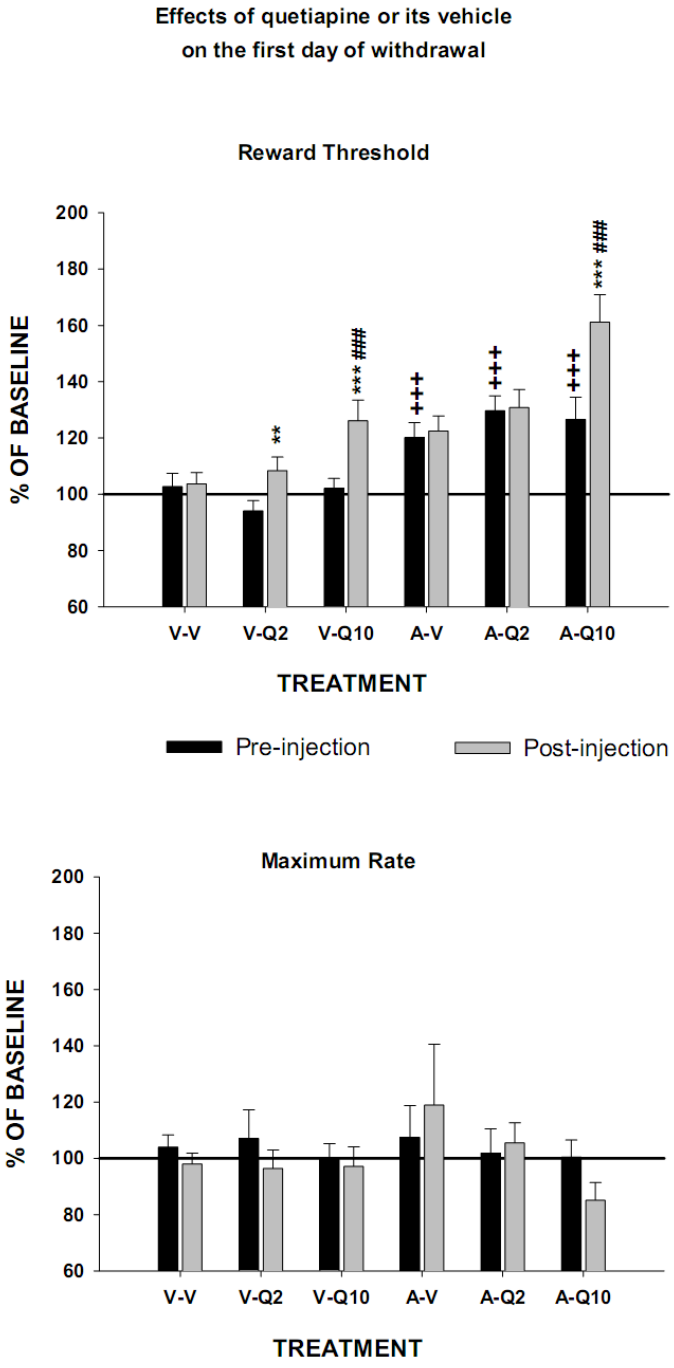
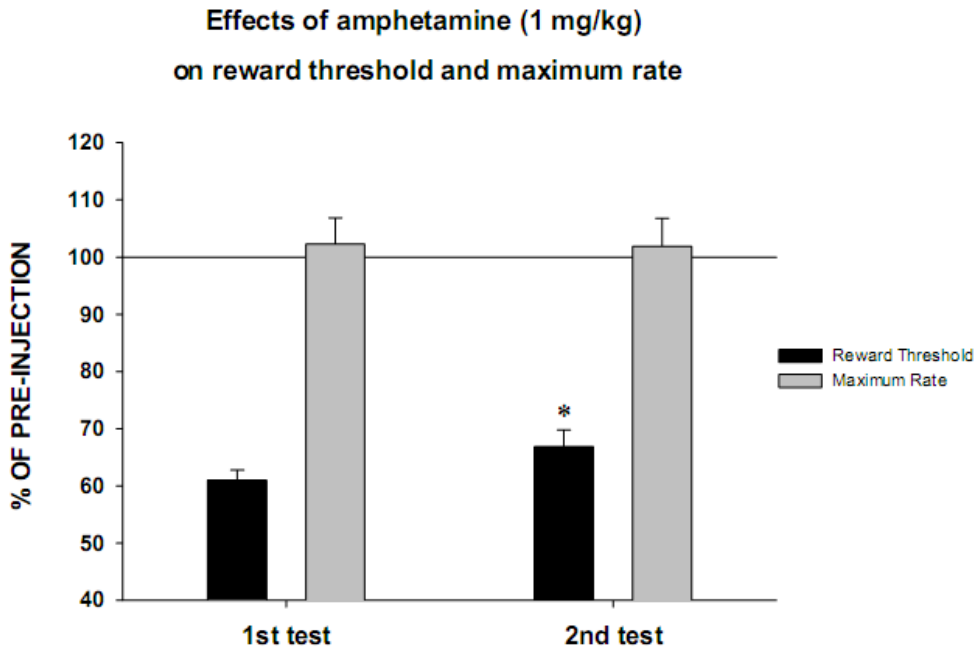

Effects of quetiapine or its vehicle
on the first day of withdrawal
Reward Threshold
% OF BASELINE
200
180
160
140
120
100
80
60
**
*** ###
+++
+++
+++
*** ###
V-V
V-Q2
V-Q10
A-V
A-Q2
A-Q10
TREATMENT
Pre-injection
Post-injection
Maximum Rate
% OF BASELINE
200
180
160
140
120
100
80
60
V-V
V-Q2
V-Q10
A-V
A-Q2
A-Q10
TREATMENT

Figure 5


Effects of amphetamine (1 mg/kg)
on reward threshold and maximum rate
% OF PRE-INJECTION
120
110
100
90
80
70
60
50
40
*
1st test
2nd test
Reward Threshold
Maximum Rate

3. Conclusions

Animals that were under withdrawal from d-amphetamine showed a reward deficit (anhedonia) that is reflected by the increase in the amount of electrical stimulation necessary to sustain operant responding at threshold level compared to vehicle-control animals. This reward deficit was maximal at 24h after withdrawal and remained present for at least 4 days. The fact that on each day of withdrawal the increase in reward threshold was not accompanied by a change in maximum response rate confirms that it was not due to a performance deficit (Miliaressis et al., 1986). This finding is consistent with those reported in previous studies where changes in reward sensitivity following withdrawal from d-amphetamine were measured with BSR, and with other paradigms such as the breakpoint of a progressive ratio schedule for sucrose reinforcement and delayed recovery from SNC (Barr & Phillips, 1999; Barr & Phillips, 2002; Barr & Markou, 2005). It is well established that d-amphetamine produces a dose-dependent increase in ventral striatal DA release, an effect that is hypothesized to account for its reward enhancing effect (Wise, 1996). However, during withdrawal from repeated high doses of d-amphetamine (such as in the present study), the opposite effect on DA release occurs. Rossetti et al (1992) have reported a decrease in ventral striatal extracellular DA ranging from 50 to 25% between day 1 and day 5 of d-amphetamine withdrawal. Moreover, several studies have shown that BSR is highly sensitive to changes in central DA neurotransmission (Wise and Rompré, 1989; Wise, 1996), and a reduction in ventral striatal DA most likely explains the increase in reward threshold that we measured. This hypothesis is further supported by results that we obtained following injection of the first dose (1 mg/kg) of d-amphetamine and at 96h after withdrawal. On the first test, this dose of d-amphetamine produced near 40% decrease in reward threshold, a magnitude consistent with that reported in previous studies (Colle and Wise, 1988; Wise and Munn, 1993) and with an increase in ventral striatal DA release (Di Chiara &

Imperato, 1988; Karoum et al. 1994). But on the second test, performed at a time period when animals still expressed a reward deficit, the same dose of d-amphetamine produced a slightly, but significantly, smaller decrease in reward threshold. This weak tolerance effect can be interpreted as reflecting a decrease in the ability of d-amphetamine to increase ventral striatal DA release; it may result from within-system adaptations such as down-regulation and/or desensitization of postsynaptic DA receptors (see White & Kalivas, 1998). Another hypothesis to account for the reward deficit, and for the tolerance to the reward enhancing effect of d-amphetamine, is dopaminergic neurotoxicity. Although the bulk of research has focused on methamphetamine-induced neurotoxicity, there is evidence that repeated treatment with high doses of d-amphetamine induces a decrease in DA tissue level and in tyrosine hydroxylase immunoreactivity in the striatum (Ryan et al. 1990; Segal & Kuczenski, 1997; He et al. 2005, 2006). Semenova and Markou (2003) also observed that rats under withdrawal from 6-day infusion of d-amphetamine evidenced significant reward threshold elevations that lasted at least 144h.

The main objective of this study was to determine the effect of an acute quetiapine injection on reward during d-amphetamine withdrawal-induced anhedonia. Quetiapine was tested at two doses on the first day of withdrawal, a time period at which reward deficit was maximal. It produced a dose-orderly attenuation of reward in the vehicle-treated control animals that were normohedonic (no change in reward sensitivity). These findings replicate previous results that showed an attenuation of reward following acute injection of similar doses of quetiapine (Lapointe et al. 2006). On par with this finding is evidence that clozapine, which displays a similar receptor binding profile to quetiapine, dose-dependently elevated reward thresholds when given acutely in vehicle-treated animals (Semenova & Markou, 2003). Quetiapine displays a low

affinity for DA D2 receptors and yet competes with endogenous DA to produce a significant striatal D2 receptor occupation which likely accounts for its reward attenuation (Kapur et al., 2003). When tested in animals that were under withdrawal from d-amphetamine, quetiapine produced significant reward attenuation at the moderate dose, an effect that was similar in amplitude to that seen in vehicle-control animals. This result predicts that quetiapine administered acutely at a clinically-effective dose for schizophrenia (the dose that produces near 50% striatal D2 receptor occupancy) is likely to accentuate rather than reverse anhedonia (Kapur et al., 2003). Most interestingly, the reward threshold elevation elicited by the low dose (2 mg/kg) of quetiapine was not observed in the anhedonic animals. One explanation for this finding is that the neural adaptations such as down-regulation of DA receptors in response to systemic escalating d-amphetamine treatment may have blunted the ability of a low dose of quetiapine to act effectively at post-synaptic DA receptors (see White & Kalivas, 1998). This interpretation is problematic, however, because the effect of the moderate dose of quetiapine should have been blunted in these animals as well and this was not what was found. One of the primary metabolites of quetiapine, *N*-desalkylquetiapine, displays strong partial agonist activity at the 5-HT1A receptors. *N*-desalkylquetiapine is 10 times more potent at activating human 5-HT1A receptor in binding assays than quetiapine (45 vs. 450nM) and the action of this metabolite has been proposed to account for the efficacy of quetiapine monotherapy in the treatment of major and bipolar depression (McIntyre et al. 2007; Jensen et al. 2008). The presence of significant 5-HT1A action of quetiapine is underlined by findings that quetiapine treatment altered 5-HT1A and 5-HT2A but not DA receptor labelling in rat forebrain regions *in vivo* (Tarazi et al. 2001, 2002). In BSR studies, the selective 5-HT1A agonist 8-OH-DPAT exhibited a biphasic effect, lowering reward threshold at low doses and elevating it at high doses

– effects which are attributed to action at pre- and post-synaptic 5-HT1A receptors, respectively (Fletcher et al. 1995; Harrison & Markou, 2001). Furthermore, although d-amphetamine withdrawal has been most commonly associated with depleted DA levels, there is also evidence for a decrease in 5-HT levels in post-mortem brain tissue of rats treated systemically with the psychostimulant (Kitanaka et al. 2008). Additionally, Bonhomme et al. (1995) reported an increased in dorsal raphé 5-HT1A receptors following repeated d-amphetamine treatment. Consequently, due to the presumed reduction of endogenous 5-HT and the increase in pre-synaptic 5-HT1A receptors, the 5-HT1A partial agonist action of *N*-desalkylquetiapine may come into play at the low dose and act to reduce the reward attenuating effect of quetiapine. At the high dose the action of the primary metabolite at the post-synaptic 5-HT1A receptors may counteract its pre-synaptic action, and the D2 antagonism may drown out any further reward threshold changes related to 5-HT1A agonism.

Clinical studies of quetiapine for the treatment of substance abuse seem to be concordant with our results. That is, studies which have evidenced a benefit of quetiapine on non-psychotic substance abusers have generally treated patients with low doses of the atypical antipsychotic medication (50-300mg/day) (Monnelly et al. 2004; Sattar et al. 2004; Pinkofsky et al. 2005); with higher doses (300-600mg/kg) being used to manage psychosis in schizophrenia (Kapur et al. 2003). The use of high doses is likely to accentuate anhedonia in substance abusers; an effect that is clearly not desirable. The tolerance that we observed to the pro-anhedonic effect of a low dose of quetiapine in the animals under d-amphetamine withdrawal may explain why quetiapine has thus far not been found to worsen substance abuse outcomes in non-psychotic patients. In psychotic patients, however, quetiapine's weak D2 antagonism and fast dissociation from D2 receptors may act to normalize hyperactivity of the mesolimbic DA system, which has been

hypothesized to be responsible for the increased prevalence of substance abuse in this population (Kapur & Seeman, 2001; Chambers & Self, 2002). Typical antipsychotics, on the other hand, dissociate slowly from D2 receptors and the dysphoria which results from this continuous blockade may pull the individual into a negative affective state, requiring alleviation with drugs of abuse (Solomon & Corbit, 1974; Koob & Le Moal, 2001; Kapur & Seeman, 2001). Indeed, schizophrenia and bipolar studies show that patients' substance abuse may decrease simply by stopping typical therapy; receiving no antipsychotic medication (Brown et al. 2003; Swanson et al. 2007). Interestingly, the same studies found that substance abuse decreased even further if the patients began atypical therapy.

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