Scanning Sex, Stress and Substance Abuse Susceptibility: Neural Correlates of
Vulnerability to Abuse Drugs

by

Tiffany Marie Love

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Doctoral Committee:

       Professor Jon-Kar Zubieta, Chair
       Professor Wayne Aldridge
       Professor Jill Becker
       Professor Margit Burmeister
       Professor Thaddeus Polk

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"Man carries the world in his head, the whole astronomy and chemistry suspended in a thought. Because the history of nature is charactered in his brain, therefore he is the prophet and discoverer of her secrets. Every known fact in natural science was divined by the presentiment of somebody, before it was actually verified."

Ralph Waldo Emerson 1803-1882

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Chapter I

Introduction

"When I first smoked it, I didn't notice the effects too much...As I continued to use, meth gave me energy and alertness... It wasn't long after I started using meth that I could no longer get high like I used to. Instead, I got paranoid, scared and uncomfortable and I started distancing myself from friends and family. I was caught for stealing, possession of marijuana and numerous underage-consumption violations. I found myself in and out of juvenile detention centers and rehab clinics... I just kept thinking I was different in some way because all my friends seemed to be able to handle the drugs just fine."
–Recovering Addict, Partnership for a Drug Free America

The preceding passage highlights a familiar and all too common scenario affecting millions of people each year – the initiation of drug use and the transition to substance dependence. A substantial percentage of US 12th graders reported both being able to 'fairly easily' access alcohol (92.2%) or other illicit drugs (83.9%) and having tried alcohol (71.9%) or illicit drugs (47.4%) within their lifetime (Johnston et al., 2009). However, though large percentages of the population are exposed to drugs of abuse only a fraction (~9%) eventually develop substance abuse or dependence (Johnston et al., 2009; SAMHSA, 2009).

Why is it then, given that drugs are freely available, only a portion of the population seeks out drugs and of these individuals only a small percentage eventually develops substance abuse and dependence problems? The recognition of individual variation to the effects of substances of abuse has been realized for some time. Indeed,

published accounts can be found more than 300 years ago articulating the presence of individual differences in the effects of opiates (Jones, 1700; Bigelow, 1822; Crothers, 1893), alcohol (Crothers, 1893), and cocaine (Brainerd, 1891). Through decades of concentrated research, it is now realized that the vulnerability to use and abuse drugs arises from a complex, interacting set of factors extending across genetic, environmental, psychological and biological realms.

To adequately introduce my dissertation topic, I will describe some of the commonly discussed environmental, trait and genetic factors associated with an increased risk to initiate and maintain drug use. The neurobiological pathway theorized to underlie some of the effects of these factors on drug use risk will then be depicted and outlined as it relates to an animal model of drug use vulnerability. Parallels between animal models and human research will be discussed ending with several proposals for examining substance use vulnerability in humans.

Epidemiological, Developmental, and Genetic Studies on Drug Use Liability

Risk and resilience factors, in this context, describe characteristics present prior to the onset of addiction, which are associated with an increased or decreased probability of drug abuse. These characteristics, henceforth referred to as susceptibility factors, may influence the initiation of drug use, the reinforcing effects of drugs and may ultimately predict substance dependence. A variety of long-term, epidemiological, longitudinal and genetic studies have been conducted with the purpose of uncovering which factors determine future drug use.

Social and Environmental Factors

In the initiation stage of addiction, individuals experiment with drugs either as a result of curiosity or circumstance. Among those drawn to use drugs out of interest, social and environmental factors are most frequently attributed to their initial drug taking behavior.

Familial and peer relationships are regularly cited as important factors in mediating initial drug use. This is particularly true among adolescents and young adults where the risk of illicit drug initiation is the highest. Poor familial bonds, family conflict and associating with antisocial peers are all significant predictors of future drug use (e.g. (Guo et al., 2002)). In addition, having family members who use drugs and affiliating with peers who use drugs are associated with faster initiation of substances use (see (Newcomb et al., 1986; Boyd and Mieczkowski, 1990; Levy and Pierce, 1990; Burton et al., 1996; Hussong and Chassin, 1997; Miller and Miller, 1997; Galea et al., 2004; Dawson, 2006). Social influences appear, from a psychological perspective, to mediate drug use by manipulating opportunity to access drugs, shaping attitudes regarding substance use and acting as a source of conflict where drugs are used as a means to cope. To the latter point, stress has long been recognized as a key factor in the initiation of drugs use. The use of drugs as a coping mechanism is frequently encountered in the literature, particularly among persons dealing with traumatic events such as physical abuse, sexual abuse, and frequent geographic relocation (e.g. (Harrison et al., 1997; DeWit, 1998; Hien et al., 2005)). Indeed, development of positive coping strategies and social problem solving skills serve as protective factors against initiation and increased consumption (e.g. (Hussong and Chassin, 1997; Siqueira et al., 2001; Wills et al., 2001;

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Kaplow et al., 2002)) while disengaging coping strategies (e.g. reacting with anger, hanging out with friends) are associated with escalated substance use (Wills et al., 2001).

Stressful life events can increase drug use and influence escalation from use to abuse (see reviews (Kaplan et al., 1986; Wills et al., 1996; Sinha, 2001; Wills et al., 2001; Butters, 2002; de Wit et al., 2003; Fishbein et al., 2006; Sinha, 2008). Recent stress as well as the total number of stressful experiences reported over a life time is associated with increased risk of drug dependence (see review (Sinha, 2008)). Furthermore, in dependent individuals drug consumption tends to increase following exposure to stressful situations. In periods of abstinence, stress can elicit craving and relapse in heroin addicts, smokers, alcoholics and cocaine users (Miller et al., 1974; Sinha et al., 1999; Sinha et al., 2000; McMahon, 2001; Sinha, 2001). While stress may instigate drug use as a means to cope with current adverse states (e.g. dealing with personal problems, craving, withdrawal) as will be described later stress is also a potent mediator of neurobiological functioning.

Gender Differences

According to the most recent National Survey on Drug Use and Health in persons over the age of 12, men currently show higher rates of illicit drug use and lifetime prevalence of drug use relative to women, though there was an overall significant increase in illicit drug use among females from the previous year (2007, 5.8%; 2008 6.3%; (SAMHSA, 2009). The rates of dependence in persons over the age of 12 are also higher in men relative to women (men, 11.5%; women, 6.4%). Though in adolescents

between the ages of 12 and 17 the rate of dependence is more similar (men, 8.2%;women, 7.0%) (SAMHSA, 2009).

Though women generally show lower rates of substance use and dependence, the escalation from initial use to compulsive use is thought to occur much more rapidly in women (see (Becker et al., 2007)). Women more quickly develop substance abuse problems and enroll into substance abuse treatment sooner relative to men (Haas and Peters, 2000; Hernandez-Avila et al., 2004)). The consequences of drug taking in females are also more severe; for instance in alcoholism, mortality rates for women are higher then men and women develop alcohol related medical issues faster then men (e.g. (Saunders et al., 1981; Bradley et al., 1998)).

The effects of drugs on women and men can also be quite different. At moderate and high alcohol drinking levels, women show greater impairments in short and long term memory and divided attention (Mumenthaler et al., 1999). Following intranasal administration of cocaine, women report more nervousness and take longer to notice its effects compared to men (Kosten et al., 1996; Lukas et al., 1996). Exposure to drug related cues also elicits greater craving in cocaine abusing women (Robbins et al., 1999).

Interestingly, the effects of drug described by women appear to vary by phase of menstrual cycle. Women give higher positive subjective ratings for amphetamine in their follicular phase relative to their luteal phase (Justice and de Wit, 1999; White et al., 2002), lower positive subjective effects for amphetamine when in their luteal phase, and give more similar ratings to men in their follicular phase (White et al., 2002). Similar effects are noted for smoked cocaine where men report more positive drug effects compared with women in the luteal phase but not when compared to women in their

follicular phase (Sofuoglu et al., 1999). In addition, smoking cessation attempts among women are more successful when attempted during the luteal phase (Allen et al., 2008).

How exactly sex plays a role in determining substance abuse liability is not known but as will be explored later differences in stress responsivity may be partially accountable (Becker et al., 2007).

Psychiatric Conditions

Psychological conditions have received some attention in regards to substance use initiation. There is a high degree of co-morbidity between substance abuse and psychiatric disorders. According to the most recent National Survey on Drug Use and Health, persons over the age of 18 with a serious mental illness (e.g. diagnosed mental disorders, other than substance dependence, based on DSM-IV criteria) were much more likely to have used an illicit substance in the past year than those without a psychiatric condition (30.3% vs. 12.9%, (SAMHSA, 2009)). In addition the rate of dependence among those with psychiatric problems is much higher (25.2% vs. 8.3%, (SAMHSA, 2009)).

Attention-deficit hyperactivity disorder, conduct disorder, oppositional defiant disorder, and major depressive disorder all predict early onset of substance use (Sihvola et al., 2008; Munafò et al., 2008b; Elkins et al., 2007; King and Chassin, 2004; Cohen et al., 2007). Early initiation of drug use is also predicted by personality disorders such as paranoid and borderline personality disorders (Cohen et al., 2007). Diagnosis of posttraumatic stress disorder (PTSD) and schizophrenia is also associated with

significantly increased risk of developing substance abuse and dependence (see (Chilcoat and Breslau, 1998b, a; Volkow, 2009)).

The basis for the linkage between substance abuse and mental illness is not entirely clear, however, it has been postulated that these disorders share common underlying biological substrates (Volkow, 2001; Volkow, 2009).

Trait Characteristics

Certain personality characteristics predict earlier initiation to substance use and heightened sensitivity to the effects of drugs. Impulsiveness represents one of the most frequently implicated traits in this regard (e.g. (Dawe and Loxton, 2004)). Though often referred to as a single trait, impulsiveness is a multidimensional concept consisting of characteristics that include sensation seeking, lack of planning, lack of persistence and urgency (Whiteside and Lynam, 2001; Smith et al., 2007). The planning dimension, frequently measured as the devaluation of rewards as a function of time (i.e. delayed discounting), predicts problem behaviors like drinking, problem gambling, and binge eating and the sensation-seeking dimension predicts risk taking in general (Fischer et al., 2004; Smith et al., 2007). Expressing high levels of impulsiveness either in the novelty/sensation seeking or non-planning dimensions are associated with earlier initiation of substance use (Masse and Tremblay, 1997; Kollins, 2003; Ernst et al., 2006; Wills et al., 2006; Audrain-McGovern et al., 2009). The subjective effects of nicotine and amphetamine are also related to measures of sensation seeking in non-users (Hutchison et al., 1999; Perkins et al., 2000; Kelly et al., 2006; Stoops et al., 2007).

Individuals exhibiting more impulsive or sensation-seeking behavior are at greater risk to develop substance use problems and be diagnosed with a substance use disorder (e.g. (Cloninger et al., 1988; Caspi et al., 1996; Zuckerman et al., 2000; Ruiz et al., 2003)). Indeed, severity of dependence is associated with measures of delayed discounting (see review (Reynolds, 2006)).

Personality can also influence relapse and craving. For instance, impulsive smokers have more trouble inhibiting their smoking and show more rapid relapse than their counterparts (Mitchell, 1999; Doran et al., 2004). In addition, smokers exhibiting high sensation-seeking or impulsive characteristics demonstrate greater craving following exposure to smoking cues (Doran et al., 2007; Doran et al., 2009). High impulsivity in adolescence predicts poorer outcomes in individuals attempting to quit smoking (Krishnan-Sarin et al., 2007).

In sum, these data indicate that personality characteristics can significantly influence substance use and abuse susceptibility. The exact mechanisms behind personalities' influence on drug seeking and use are not well understood, though as will be discussed later, much work indicates that differences in neurobiological functioning are responsible.

Genetic Influences

Much work indicates that the heritability of addiction itself is roughly 33-72% (see (Goldman et al., 2005; Li and Burmeister, 2009)). The use of linkage mapping, candidate gene association and genome wide association analyses has enabled the identification of multiple genes associated with an increased risk to develop addiction.

As recently reviewed by several groups, potential gene candidates for addiction susceptibility include gene variants in dopamine, opioid, GABA, and serotonin pathways (Goldman et al., 2005; Kreek et al., 2005; Li and Burmeister, 2009).

How might genetic variability influence susceptibility to addiction? As I will briefly discuss below, genetic variation can directly alter the metabolism of the drug itself, influence the functioning of neurobiological pathways that may underlie addiction processes and in some measure determine traits that place an individual at risk for drug use.

Drug Metabolism

The positive reinforcing effects of drugs, a reasonable predictor of abuse potential (Foltin and Fischman, 1991), can vary quite dramatically from individual to individual. Inherited conditions have been demonstrated to dramatically alter the initial effects of drugs, which can impact future drug use. One striking example of this comes from a mutation in the aldehyde dehydrogenase gene (ALDH2). Normally, when alcohol is consumed it is metabolized within the liver into acetate by two enzymes: alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). ADH first converts alcohol into acetylaldehyde and ALDH converts this into acetate. If ALDH is not functioning properly, toxic levels of acetylaldehyde accumulate and the result is "flushing syndrome" where among other negative reactions, the person experiences facial flushing, dizziness, and nausea (see (McGue, 1999)). Carriers of this inactivated enzyme are relatively protected against alcohol addiction, however, they still initiate drinking at the

same levels (McGue, 1999; Hampton, 2006) and given the right set of conditions will drink despite possessing this resilience factor (see overview (Hampton, 2006)).

Similar findings have been noted for polymorphisms of cytochrome P450 genes. Major enzymes involved in drug metabolism, cytochrome P450 enzymes transform carbon-rich molecules into a form that is more easily excreted from the body. Within our collection of cytochrome P450 enzymes, exist CYP2D6, which converts codeine to its active form morphine, and CYP2A6, which converts nicotine into its inactive form cotinine. Some investigations have observed that individuals who are deficient in CYP2D6 metabolism are protected, albeit incompletely, against opioid dependence, though this assertion is still under discussion (Tyndale et al., 1997; Mikus et al., 1998) and in addition, those with reduced or null CYP2A6 activity tend to smoke less than their wild-type counterparts, possibly due to the higher levels of nicotine afforded by each cigarette, and are more likely to quit smoking (see review (Ray et al., 2009)). Individuals with a genetic predisposition for faster metabolism, on the other hand, may be at increased risk to develop substance dependence (see review (Ray et al., 2009)).

Neurotransmitter Systems

Dopamine acts at several dopamine receptor subtypes (D1-D5) and each show slightly different expression patterns throughout the brain (see (Jaber et al., 1996)). Multiple genetic studies have studied each of these receptor subtypes and have attempted to associate genetic variation with various measures of substance abuse and several interesting observations have been made.

Genetic variations at D2-like receptors, which include subtypes D2-D4, are most frequently associated with addictive behaviors. The dopamine D2 receptor (DRD2) polymorphisms TaqIA (and in ANKK1, a neighboring gene involved in signal transduction) and TaqIB, have been associated with alcoholism and opioid dependence respectively (see throughout review by (Le Foll et al., 2009)). These polymorphisms are also related to the age of initiation and progression of smoking in adolescents and predict higher consumption and craving of opiates following exposure to drug cues (Laucht et al., 2008; Le Foll et al., 2009). Additionally, variation at the dopamine D3 receptor (DRD3) gene and the dopamine D4 receptor (DRD4) variable number tandem repeat (VNTR) polymorphism have been linked to nicotine dependence (Le Foll et al., 2009). Though most work has been carried out on D2-like receptors, some studies have indicated that polymorphisms in D1-like receptors, which include D1 and D5 subtypes, are been associated with alcohol dependence, heavy smoking, and greater consumption of heroin (see review (Le Foll et al., 2009)). There have also been reports linking genes for two enzymes responsible for catecholamine breakdown (which includes dopamine) to addiction risk, catechol-o-methyl transferase (COMT) and monoamine oxidase (MAO). A common polymorphism in COMT results in a valine to methionine change at position 158, which reduces COMT enzymatic activity dramatically. This mutation has been associated with alcoholism and social drinking (see (Kauhanen et al., 2000; Kreek et al., 2005)). A variable number tandem repeat (VNTR) in the MAOA gene has also been linked to alcoholism (see (Kreek et al., 2005)).

Beyond dopamine, genetic variation at opioidergic receptors has also been linked to substance dependence. There are several receptor subtypes to which endogenous

opioids bind to – μ, κ, δ, and orphan. The endogenous opioids, which include endorphins, enkephalins, nociceptins, endomorphins and dynorphins have distinct preferences to the receptor types, which they bind to. Endorphins, endomorphins and enkephalins display preferences to μ- and δ- receptors whereas dynorphin and nociceptin principally bind to κ- and orphan receptors, respectively.

In regards to genetic association with substance dependence, one of the best-studied receptor subtypes is the μ-opioid receptor. Within the μ-opioid receptor gene (OPRM1) a missense single nucleotide polymorphism (SNP) results in an asparagine to aspartic acid substitution at residue 40 (typically referred to as the A118G polymorphism). This alteration results in enhanced binding of β-endorphin at the μ-opioid receptor (Bond et al., 1998). This A118G polymorphism has been associated with opioid addiction and alcoholism (Szeto et al., 2001; Bart et al., 2004; Bart et al., 2005; Drakenberg et al., 2006); however some investigations have failed to observe this (Crowley et al., 2003; Arias et al., 2006) and others have identified associations between nearby SNPs but not A118G with opioid addiction (Levran et al., 2008). A more consistent association has been observed between this polymorphism and the treatment efficacy of naltrexone in alcoholics (e.g. (Oslin et al., 2003; Anton et al., 2008; Kim et al., 2009)).

There are dozens of other examples from genes within serotonergic, cholinergic and GABAergic systems to neurotrophic factors and even signaling molecules within the immune system which are also associated with substance abuse (Goldman et al., 2005; Kreek et al., 2005; Li and Burmeister, 2009). This discussion represents only a short list of genes that have been associated with addiction that will be relevant in later sections.

Personality Characteristics

Estimates on the heritability of personality fall roughly between 30-50% (Cloninger et al., 1988; Bouchard, 1994). Multiple genetic variants have been associated with the expression of particular trait characteristics shown to contribute to both the initiation and maintenance of drug use.

Genes from multiple neurotransmitter systems show linkage with both impulsivity and addiction, including dopamine, serotonin and GABA (Kreek et al., 2005). For instance, a recent meta-analysis by Munafo demonstrated an association between measures of novelty-seeking and impulsivity and the DRD4 C-521T polymorphism (Munafò et al., 2008a). Similarly, within the DRD1 gene, a 5' untranslated region polymorphism called Ddel has been associated with sensation seeking in alcoholic patients (Limosin et al., 2003) and DRD3 variants have been associated with opioid dependence in individuals with high sensation seeking scores (Duaux et al., 1998).

Along these same lines, associations between traits impulsivity and novelty seeking and genes responsible for the degradation of catecholamines like dopamine, COMT and MAOA, have also been described (e.g. (Manuck et al., 2000; Contini et al., 2006; Golimbet et al., 2007)). Beyond dopaminergic genes, a variety of genetic studies have linked serotonin and GABAergic variants to impulsive and novelty-seeking traits including: TPH1, SERT, GABARA1, GABARA6, GABARB1 (see review (Kreek et al., 2005)).

Summary of Genetic Findings

In conclusion, genetic variability appears to significantly contribute to some of the drug taking variability in humans and is related to some measures of trait characteristics.

What should be gleaned from these studies is there is no one gene responsible for an individual's passage into addiction, nor does any one gene confer absolute vulnerability or resilience to use drugs. Though some individuals exhibit relative insensitivity to the positive reinforcing effects and relative sensitivity to the toxic, negative effects of a drug, this does not inevitably lead to cessation of drug use, nor do differences in drug metabolism inexorably govern use and non use, rather an individuals consumption seems determined not only by their genetic sensitivities but owing to other intervening factors as well.

It appears that addiction results from a complex interplay of multiple genetic and environmental factors. These data indicate that variation within multiple neurotransmitter systems may be in part responsible for future drug use and abuse. Identification of other gene targets is therefore of significant interest as it may lead to better understand to the biological mechanisms underlying addiction susceptibility.

Susceptibility Factor Summary

To summarize, there are a number of different factors that can affect initial drug use, subjective effects to the drug itself, and influence the progression to substance dependence. While psychological, social and environmental factors may provide some individuals with greater exposure and opportunity for experimentation, there is data to suggest the impact of these factors go beyond simple differences in availability and social acceptability (see (Dube et al., 2003)) and may involve biological processes. Indeed, the

initiation and maintenance of substance use appears to be heritable (e.g. (Sullivan and Kendler, 1999; Heath and Martin, 1993; Kendler et al., 1999)).

While there has been quite a bit of work done uncovering factors that modify individual vulnerability, understanding the mechanisms that underlie these differences in susceptibility, particularly in humans, are not completely understood. For many of the factors discussed, while they may affect drug use by exposure, opportunity and metabolism of the drug itself, it is possible they may also exert their effect by influencing central biological processes. Indeed, one could hypothesize that variation brought on by susceptibility characteristics within the neural pathways responsible for reward or motivation could certainly impact the drive of an organism towards appealing drug stimuli. This, in fact, appears to be the case.

As I will discuss in the upcoming passages, certain trait and environmental susceptibility factors have been associated with activity within a particular neurobiological pathway responsible for reward and drive, the motivational circuitry. Before describing that research, I will first delve into a description of the motivational circuitry's function and organization to provide a framework for discussing those studies.

Motivational Circuitry

Work done in the 1950s first established the presence of an anatomically distinct 'reward' circuit (Olds and Milner, 1954). In their groundbreaking and serendipitous study, Olds and Milner implanted electrodes into the brains of rats and presented them with the opportunity to self-administer electrical stimulation by way of a lever press. They observed that when the electrodes were placed within certain brain regions, the rats

would press a lever persistently, sometimes thousands of times an hour (Olds and Milner, 1954). From these observations, Olds and Milner surmised they had inadvertently stumbled upon a circuit responsible for reward. Later work would establish the region stimulated was near the ventral striatum (Milner, 1989) and that given the opportunity humans would also self-administer electrical stimulation in equivalent regions (Heath, 1963).

A few years following Olds and Milner's discovery, Falack and Hillarp detected the cell bodies of dopaminergic neurons within the midbrain which projected to the ventral striatum (Falck and Hillarp, 1959) and it was found that dopaminergic antagonists and lesions to the ventral tegmental area (VTA) would result in a substantial reduction in self-stimulation (Rolls et al., 1974; Fibiger et al., 1987). Later studies would expand upon that and demonstrate that in response to natural or artificial rewards (such as food, sex, drugs) neurons within the VTA become activated which results in dopaminergic release within the nucleus accumbens (NAC). Thus, it appeared that dopamine, particularly within this mesolimbic circuitry, was central to reward. Decades later, this circuitry would be elaborated upon and conceptualized more generally as motivational circuitry, broadened to embody the influence of other neurotransmitter systems (e.g. opioids, GABA, glutamate) and expanded to include a number of other regions including: prefrontal cortex, hypothalamus, amygdala, and ventral pallidum.

Motivational circuitry anatomy

Motivated behavior emerges as a result of activity within a series of interconnected regions including the ventral tegmental area, nucleus accumbens, amygdala, and

prefrontal cortex, which interact to signal the significance of stimuli, assign incentive value and select appropriate action ((Kalivas and Volkow, 2005), see Figure 1).
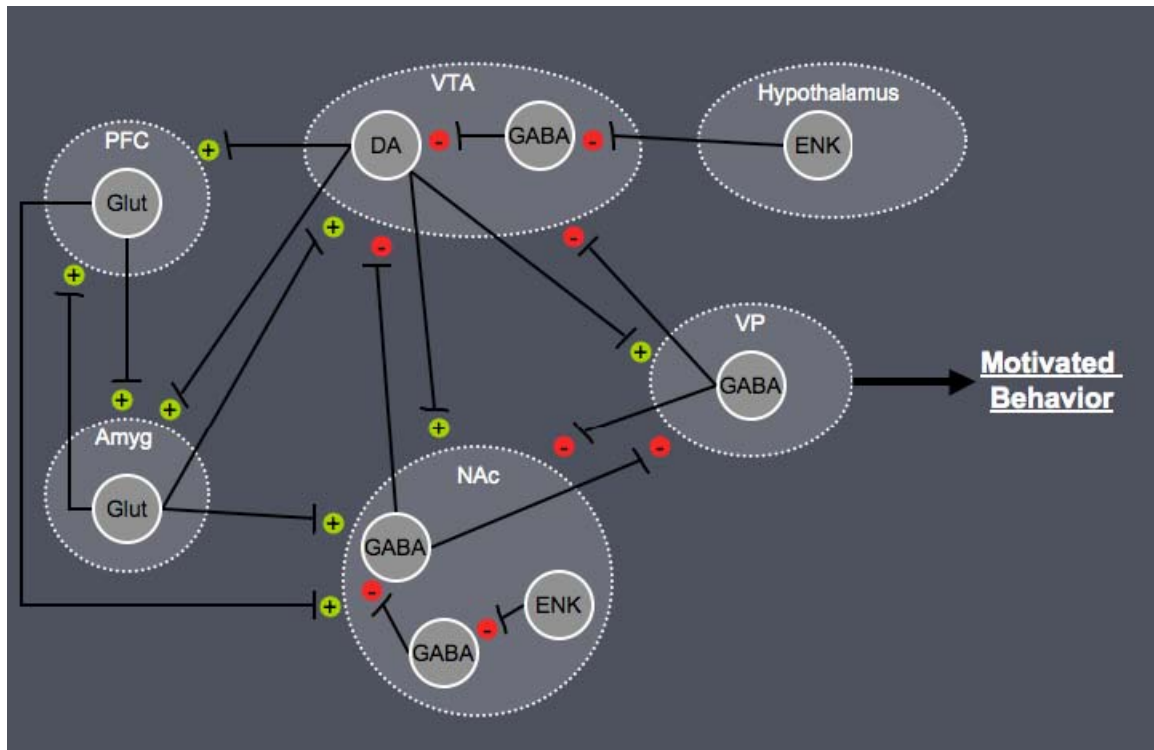
Figure 1. Schematic of critical brain structures involved in motivated behavior and their connections. (adapted from (Kalivas and Nakamura, 1999) and (Blum et al., 2008)). Ventral tegmental area (VTA), nucleus accumbens (NAc), ventral pallidum (VP), amygdala (AmyG) and hypothalamus.

Key to the regulation of motivated behavior is dopaminergic neurotransmission within the nucleus accumbens. As will be described below and is illustrated in the figure above, the nucleus accumbens receives information from sites across the brain. It is hypothesized that dopamine influences the integration of activity arriving at this locus. Indeed, critical to the expression of motivated behavior is the activity from dopaminergic neurons (A10) located within the VTA. These neurons project from the VTA to the nucleus accumbens to form the central pathway of the mesolimbic dopamine system.

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The nucleus accumbens does not act alone but in concert with regions throughout the brain to regulate motivated behavior. In addition to the dopaminergic projection from the VTA to the nucleus accumbens, the VTA also projects to the hippocampus, amygdala, ventral pallidum, and various cortical areas including the prefrontal and cingulate cortex. These regions and their interconnections are collectively referred to as mesocorticolimbic pathways.

To delve into this circuit a bit more deeply, the amygdala, hippocampus and prefrontal cortex all send glutamatergic projections to the nucleus accumbens and VTA which activate dopamine neurons within the VTA and the dopaminoceptive neurons within the nucleus accumbens (see Figure 1). The nucleus accumbens in turn sends GABAergic projections to the VTA, ventral pallidum and substantia nigra (Haber et al., 1990). The ventral pallidum sends GABAergic efferents to the VTA, the pedunculopontine nucleus, which influences locomotor activity and to the medial dorsal nucleus of the thalamus, which ultimately feeds back to the prefrontal cortex.

As illustrated above, the nucleus accumbens receives projections from throughout the brain and is proposed to act in an integrative capacity assimilating goal directed information from the prefrontal cortex, environmental context from the hippocampus, and emotional significance from amygdala (Mogenson and Yang, 1991; Kalivas and Nakamura, 1999). In addition, the nucleus accumbens output to the ventral pallidum[1] and substantia nigra are believed to influence motor planning. As a result of these

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[1] Footnote: The ventral pallidum is being increasingly recognized as a critical reward region. In addition to the connections described above the ventral pallidum also receives inputs from a variety of limbic and midbrain structures (Smith et al., 2009). It appears that together with the nucleus accumbens, the ventral pallidum is key to the manifestation of 'liking' whereas the nucleus accumbens is crucial for 'wanting' (Tindell et al., 2006; Smith and Berridge, 2007; Tindell et al., 2009)).

connections, the nucleus accumbens is believed to be crucial in the translation of will into action (Mogenson and Yang, 1991; Kalivas and Nakamura, 1999).

The mesocorticolimbic dopamine system does not exist in a vacuum, rather it interacts with a number of different neural systems to regulate behavior. Below I will discuss how the opioid system, HPA axis and other neuropeptide systems can influence activity within this pathway with specific attention paid to the VTA and nucleus accumbens given their central roles in motivated behavior.

Opioid System Interactions

Opioidergic activity acts as a critical regulator in the mesocorticolimbic circuitry. As described earlier, there are several main classes of endogenous opioids that display different preferences for the receptor subtype they bind to, but for the purposes of this discussion, the main opioid receptor of interest is the μ-opioid receptor, which has been extensively linked with stress, reward and addiction. Two main classes of endogenous opioid act at the μ-opioid receptor: endorphins and enkephalins, which are a product of the propiomelanocortin (POMC) gene and proenkephalin gene respectively. μ-opioid receptors are localized through the motivational circuitry including within various areas within the limbic system including the hippocampus and amygdala, prefrontal cortex, thalamus, the dorsal and ventral striatum as well as within the VTA (Frost et al., 1985; German et al., 1993).

The μ-opioid receptor belongs to the G-protein-coupled receptor (GPCR) family, which transduces opioid binding signals into an intracellular signal (G-protein activation). Activation of this receptor results in the inhibition of adenlyl cyclase and

voltage-gated Ca2+ channels and the stimulation of inwardly-rectifying K+ channels, the net result being, inhibition of transmitter release.

Endogenous opioids activity can influence the mesocorticolimbic circuitry in several ways, though principally they function via disinhibition. For example, the neuronal population of the VTA is mostly comprised of dopaminergic and GABAergic neurons. These VTA dopaminergic neurons are tonically inhibited by the activity of GABA interneurons (Johnson and North, 1992; Spanagel et al., 1992). μ-opioid receptors are localized on GABAergic interneurons and activation of these receptors releases the VTA dopamine neurons from inhibition and promotes firing (Johnson and North, 1992; Spanagel et al., 1992).

μ-opioid neurotransmission can have effects on dopaminergic activity outside of the VTA; for instance, μ-opioid receptors have been shown to colocalize with D2 receptors in the striatum (Ambrose et al., 2004). In addition, there are also enkephalin projections from the nucleus accumbens to the ventral pallidum and VTA and endorphin projections from the hypothalamus to the nucleus accumbens (Bloom et al., 1978; Finley et al., 1981; Kalivas et al., 1993).

Stress, Dopamine and Neuropeptide Interactions in the Mesocorticolimbic Pathway
Stress

In response to a stressor a myriad of biological reactions occur. Along the hypothalamic-pituitary-adrenal (HPA) axis perception of stress results in release of corticotrophin releasing factor (CRF) from the hypothalamus, this stimulates the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary gland into the

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bloodstream which leads to release of glucocorticoids from the adrenal glands (McEwen et al., 1986). The effects of stress are not limited to the HPA axis and can result in widespread changes throughout the brain.

More than thirty years ago, it was recognized that stress could increase dopamine release within the mesolimbic circuitry (Thierry et al., 1976). These effects appear to be mediated through the actions of glucocorticoids and CRF on a variety of regions. Corticosteroid receptors are widely distributed throughout the brain including within the prefrontal cortex, hippocampus, amygdala and on A10 dopamine neurons within the midbrain (Härfstrand et al., 1986; Seckl et al., 1991). CRF receptors are also found in extrahypophysal regions, in particular within the hippocampus and amygdala (Chalmers et al., 1996; Van Pett et al., 2000).

Stress and glucocorticoid administration can enhance dopamine release within the nucleus accumbens (Kalivas and Duffy, 1995; Piazza et al., 1996). In addition, a reduction in both basal dopamine concentration and stress induced dopamine release within the nucleus accumbens is observed following adrenalectomy (Piazza et al., 1996; Rouge-Pont et al., 1998; Barrot et al., 2001). In addition to glucocorticoid actions at the nucleus accumbens, regions projecting to the nucleus accumbens including the prefrontal cortex, hippocampus and amygdala are sensitive to the changing levels of circulating glucocorticoids. Similarly, stress prompts CRF release from the VTA, which stimulates A10 dopaminergic neurons and enhances dopaminergic release in VTA projection areas (Ungless et al., 2003; Wang et al., 2005; Wanat et al., 2008). Likewise, application of CRF receptor antagonists attenuates cocaine-induced dopaminergic activity in the nucleus accumbens (Lodge and Grace, 2005).

Opioid System

Stress actions within the mesocorticolimbic system can be modulated by other systems. The endogenous opioid system can serve both as a direct regulator of mesocorticolimbic activity and a modulator of HPA axis responsiveness. In general, the endogenous opioid system has an inhibitory effect on stress responsiveness. In response to stress, a large precursor protein called proopiomelanocortin (POMC) is synthesized and cleaved to produce several products including ACTH and the opioid peptide, beta-endorphin (Guillemin et al., 1977). Both ACTH and beta-endorphin exert negative feedback on CRF release within the hypothalamus dampening the stress response. The interaction between μ-opioid system activity and stress responsiveness has been demonstrated through the administration of the opioid antagonist naloxone, which increases ACTH circulation possibly by facilitating CRF (Conaglen et al., 1985; Burnett et al., 1999).

Oxytocin

Another neuropeptide relevant to this discussion is oxytocin which has also been demonstrated to manipulate both stress and mesocorticolimbic activity. Oxytocin is a neuropeptide primarily synthesized within the paraventricular nuclei within the hypothalamus though it has been observed along with its receptors in a variety of extrahypothalamic brain regions including the amygdala, nucleus accumbens and ventral tegmental area (primate: (Fliers et al., 1986; Caffé et al., 1989; Loup et al., 1991), rat: (Brinton et al., 1984; Van Leeuwen et al., 1985; Krémarik et al., 1993).

Oxytocin is released in response to a variety of psychological and physical stressors where it appears to have anxiolytic effects and can influence both central and peripheral stress responsiveness (Lang et al., 1983; Jezova et al., 1995; Robinson et al., 2002; Wigger and Neumann, 2002; Onaka, 2004). For example, chronic infusions of oxytocin can dose dependently lower plasma corticosterone concentrations following stress in ovariectomized female rats (Windle et al., 1997) whereas intracerebral infusion of oxytocin antagonists elevates stress-induced ACTH and corticosterone concentrations in response to a variety of stressors (e.g. (Neumann et al., 2000)). Similar effects have been observed in humans where intranasal administration of oxytocin can reduce cortisol responses to psychological stressors (Heinrichs et al., 2003; Ditzen et al., 2009).

Beyond its effects on HPA activity, oxytocin can influence central nervous system activity as well. As mentioned earlier, oxytocin receptors are expressed throughout the brain. In addition, oxytocin neurons within the hypothalamus project to extrahypothalamic regions including the ventral tegmental area (Sofroniew, 1983). Application of oxytocin into the VTA results in increases in extracellular dopamine concentrations in the nucleus accumbens and hypothalamus (Melis et al., 2007). In addition, application of an oxytocin receptor antagonist intracerbroventricularly can severely diminish apomorphine-stimulated dopaminergic release in the nucleus accumbens (Succu et al., 2007).

Summary of Motivational Circuitry Anatomy

As the information presented thus far indicates, there are numerous systems that contribute to the functioning of the mesocorticolimbic circuitry. Central to this circuitry

lies dopaminergic activity within the nucleus accumbens, facilitated by VTA, which incorporates information from a variety of systems to promote motivated behavior – How does all this relate to substance abuse?

All drugs of abuse increase dopaminergic release within the NAC (see (Nestler, 2004)). Repeated administration of drugs causes sensitization of dopamine release within this region. This dopamine release is hypothesized to result in a progressive increase in the incentive value of the perceived stimuli, in effect increasing desire or "wanting" for the stimuli (Robinson and Berridge, 1993, 2000). Environmental cues that were formerly paired with the drug can also elicit dopamine release and develop conditioned incentive properties as well (Robinson and Berridge, 1993, 2000).

Objects imbued with incentive salience are more attractive and "wanted" and can influence motivated behavior, effectively "drawing" the individual to the drug or conditioned cue (Robinson and Berridge, 1993, 2000; Wise, 2004). In the case of addiction, chronic activation of the mesolimbic system by drugs is hypothesized to result in neural sensitization where dopaminergic systems overreact in response to drugs and environmental cues resulting in compulsive desire to consume drugs (Robinson and Berridge, 1993, 2000). Thus, influencing activity within the nucleus accumbens and within related regions may influence the attribution of incentive salience, motivated responses and ultimately addictive behavior.

A good question at this point in regards to the previous susceptibility conversation - Is there any evidence to suggest that individual differences in the activity of this region exist and are they associated with an increased propensity to use drugs? And if so, do environmental, trait, or genetic characteristics play a role in influencing such activity?

The answer to these questions appears to be yes. As work done by Piazza and colleagues demonstrated more than twenty years ago, certain characteristics are associated with an increased propensity to use drugs and this behavior is related to dopaminergic activity within the nucleus accumbens which can be influenced by multiple factors.

Individual Differences in Motivational Circuitry: Animal Models

Some of the early work establishing individual differences in the propensity to use and abuse drugs observed differences in dopaminergic activity within the nucleus accumbens. When laboratory animals are placed in a novel environment, a mild stressor, Piazza and colleagues observed some animals would exhibit high rates of exploratory locomotion (High Responders, HR) and others would exhibit lower rates (Low Responders, LR) (Piazza et al., 1989). If given the opportunity, HR rats learn to self-administer psychostimulants faster than LR rats (e.g. (Piazza et al., 1989; Piazza et al., 2000; Marinelli and White, 2000; Kabbaj et al., 2000; Kabbaj et al., 2001)). Further study would show that HR rats exhibit increased DA turnover (Piazza et al., 1991b) and enhanced DA in response to cocaine in the nucleus accumbens (Hooks et al., 1991), and lower dopaminergic activity within the prefrontal cortex (Piazza et al., 1991b). Little more than a decade after the original HR/LR observations, Dalley and colleagues observed that animals exhibiting high 'waiting impulsivity' demonstrated higher rates of cocaine self-administration and lower levels of DA D2/3 receptors in the ventral striatum prior to drug use relative to their counterparts (Dalley et al., 2007).

Influence of Stress Responses

Following the observation that one can predict an animals' vulnerability to self-administer psychostimulants by monitoring their locomotor reactivity to a mild stressor, Piazza and colleagues further explored the role of stress in determining addictive behavior by monitoring the animals biological stress responses following exposure to a novel environment (Piazza et al., 1991a). Analyzing corticosterone secretion, the dominant glucocorticoid in rats, in HR and LR animals, it was found that HR rats exhibit greater and longer secretion of corticosterone in response to novelty then their LR counterparts and that the level of secretion in HR rats was related to their propensity to self administer amphetamine (Piazza et al., 1991a; Piazza et al., 1998a; Kabbaj et al., 2000). Additionally, it was observed that the administration of exogenous corticosterone differentially affected the animals, reducing amphetamine intake in HR rats and enhancing it in LR rats (Piazza et al., 1991a). Later it was shown that administration of a glucocorticoid antagonist reduces cocaine self-administration (Deroche-Gamonet et al., 2003). Further, deletion of glucocorticoid receptors on postsynaptic neurons of the dopamine system (existing primarily within the nucleus accumbens, dorsal striatum, and prefrontal cortex) reduces cocaine self-administration (Ambroggi et al., 2009). Together, these data are believed to demonstrate a potentiating role for corticosterone on self-administration behavior (Piazza et al., 1991a).

In addition to innate differences in stress-reactivity, the influence of environmental stress can also play an important role in self-administration behavior. Social stress increases psychostimulant self-administration as does exposure to prenatal

stress, repeated electric shocks and tail pinch (Piazza et al., 1990a; Deminière et al., 1992; Goeders and Guerin, 1994; Haney et al., 1995). How stress modifies self-administration behavior is still a somewhat open question however, modifications have been observed at the level of the mesolimbic circuitry following acute and chronic stress.

Stress and Dopamine Interactions

As discussed earlier, exposure to stress has been observed to increase dopamine release within the mesolimbic circuitry (e.g. (Thierry et al., 1976; Abercrombie et al., 1989)). Repeated exposure to a stressor, like footshock, can result in enhanced activation of dopamine neurons within the nucleus accumbens to further stress encounters (Kalivas and Duffy, 1989); this is similar to what is observed in the sensitization of dopaminergic neurons to psychostimulants (e.g. (Robinson and Becker, 1986; Robinson et al., 1988; Kalivas and Duffy, 1989). The degree to which stress induces changes in dopaminergic activity appears to be somewhat dependent on glucocorticoid transmission. Adrenalectomy, which suppresses the secretion of glucocorticoids, has been demonstrated to dramatically reduce basal dopamine concentrations in stress- or drug-induced dopamine release within the nucleus accumbens (Rouge-Pont et al., 1998; Barrot et al., 2000). Additionally, administration of glucocorticoid antagonists blocks the stress-induced release of dopamine within the nucleus accumbens (Rouge-Pont et al., 1995).

There are individual differences in the degree to which stress and corticosteroids induce dopaminergic release within the mesolimbic system. Returning to the HR/LR rats, intravenously administered corticosteroids induces increases in extracellular dopamine concentrations within the nucleus accumbens in both groups, though the increases tend to

be greater in the HR rats compared to the LR rats (Piazza and Le Moal, 1996). Similar observations have been made in regards to stress-induced dopamine release whereby HR rats exhibit greater dopamine release relative to their counterparts (e.g. (Rouge-Pont et al., 1998)). Further, adrenalectomy eliminated the HR rats' enhanced stress-induced increase of dopamine but the administration of corticosterone reinstated it (Rouge-Pont et al., 1998).

Differences beyond dopamine

Besides dopamine, other neurochemical differences have been noted in these animals. For example, HR animals demonstrate increased pro-enkephalin gene expression in the shell of the nucleus accumbens compared to LR animals (Lucas et al., 1998). The variation of endogenous opioid activity within the HR animals is likely intertwined with the differences in dopaminergic neurotransmission noted earlier. As illustrated previously, there are substantial connections between the μ-opioid system and the dopaminergic system (see Figure 1). μ-opioid receptors are located throughout the motivational circuitry, including on GABAergic neurons within the VTA (Garzón and Pickel, 2001, 2002). As mentioned earlier, the action of μ-opioid receptors results is one of presynaptic inhibition, the result of activating μ-opioid receptors within the VTA is one of disinhibition, potentially resulting in greater dopamine release at the level of the nucleus accumbens (Johnson and North, 1992).

In addition to the behavioral and biological differences observed between HR/LR rats, there may also be some genetic differences. The novelty-seeking characteristic which differentiates HR rats from LR rats can be selectively bred (Stead et al., 2006).

Also, the presentation of novelty seeking behavior seems to be unaffected by prenatal stress and maternal care though early life stress can alter stress-responsivity in a trait dependent manner (Stead et al., 2006; Clinton et al., 2008). There also seem to be differences in gene expression: HR rats exhibit lower D2 receptor mRNA within the striatum (Hooks et al., 1994) which may account for the elevated dopamine levels within the striatum of HR rats (see review (Blanchard et al., 2009); lower CRF mRNA in the amygdala, which may have anxiolytic effects; higher CRF mRNA levels within the hypothalamus and decreased glucocorticoid receptor mRNA in the hippocampus, which may promote enhanced sensation seeking behavior (Kabbaj et al., 2000; Kabbaj, 2004).

Sex also appears to play a role in determining differences in HR/LR behavior. Though there is scarce data in this area, in one report testing for cocaine self-administration behavior, Davis and colleagues found that HR bred females self-administered more cocaine than either HR-bred males or LR females or males (Davis et al., 2008).

Summary

It would appear that the manifestation of individual differences in drug self-administration behavior among the high and low sensation-seeking animals is at least partially related to differences in stress responsiveness. Stress can modulate dopaminergic activity within the mesolimbic system and the degree to which this occurs is related to an individual rat's vulnerability to self-administer drugs. Differences between HR/LR rats were also identified within the endogenous opioid system, which has been shown to interact with both dopaminergic and stress systems. The variation in

behavioral and biological activity noted between the HR and LR rats also appear to be heritable. Thus, it has been observed that some of the same factors which have been shown to predispose/protect an individual to drug use (e.g. trait characteristics, genetic variability, exposure to stress) in humans are associated with variation in some measures of biological activity in animal models. Are there any parallel findings in humans?

Individual Differences in Motivational Circuitry: Human Data

When compared to animal data there is a relative paucity of information available from human studies. However, there have been some similarities noted between some of the animal work described earlier and data collected in humans.

Studies in Current and Former Drug Addicts

As thoroughly discussed earlier and thus won't be repeated here, stress and personality characteristics contribute to initial and future drug use in humans as they do in animals. To illustrate this point further, it has been demonstrated that stress reactivity can predict drug consumption and relapse among current drug users. In particular, a higher cortisol response to stress predicts faster treatment dropout among current drug users (Daughters et al., 2009) and higher cocaine consumption following treatment (Sinha et al., 2006). In regards to trait characteristics, drug addicts tend to discount rewards more (a measure of non-planning impulsivity) than non-addicts though this improves as a result of drug abstinence (see review (Reynolds, 2006).

Some work indicates drug abuse in humans is also associated with neurobiological changes within the dopaminergic and opioidergic systems. Examining

current and former drug addicts compared to healthy controls, numerous differences in dopaminergic activity have been noted particularly within the ventral striatum. D2 receptor availability is lower in the striatum in cocaine, methamphetamine, alcohol and opiate abusing subjects and appears to persist after withdrawal (Volkow et al., 1993; Hietala et al., 1994; Wang et al., 1997; Volkow et al., 1999a). Dopamine transporter (DAT) levels, responsible for the reuptake of dopamine from the synapse, have also been reported to be decreased in the striatum in alcoholics and methamphetamine users (Tupala et al., 2000; Tupala et al., 2001; Chang et al., 2007). In addition, dopamine increases in the ventral striatum in response to methylphenidate are lower in cocaine and alcohol abusers possibly reflecting decreases in dopaminergic cell activity and sensitivity to stimulation by reward (Volkow et al., 2007).

Abstinent alcoholics show an increase in μ-opioid receptor BP with the ventral striatum relative to control subjects, which correlates to alcohol craving (Heinz et al., 2005), though other investigations have failed to demonstrate this (Bencherif et al., 2004). Abstinent cocaine users also show increased μ-opioid receptor availability within a number of regions including the frontal cortex, anterior cingulate, thalamus and caudate relative to healthy controls; in addition, some of these increases are positively related to craving (Zubieta et al., 1996). Furthermore, some of these differences have been found to persist many weeks post abstinence (Gorelick et al., 2005).

Studying individuals at risk

Though the data in current and former drug addicts provide some perspective on functional neurobiological differences between addicts and controls, it isn't possible to

determine whether these differences are a consequence of drug consumption or predisposing factors to their use. This difficulty is present even when studying abstinent drug users as drug use and possible lifestyle differences can result in a number of biological and psychological changes that may persist long after the drug abuse stops (Schuckit, 1987).

In humans, determining whether variation in biological functioning is related to vulnerability to use drugs presents a significant challenge. Unlike animal studies, where environmental factors and exposure to drug can be tightly controlled and individual animals followed from initiation to compulsive use, this is completely unrealistic for human experimentation. The best method available to study vulnerability in humans has been the study of groups at high risk. As Shuckit described for the study of vulnerability for alcoholism, there are numerous ways of obtaining a sample of high risk individuals for study: 1) study children of drug users that were raised by non-drug dependent parents 2) study non-affected relatives of drug-using families 3) study healthy, non-drug using individuals from the population which carry risk factors theorized to place them at higher risk for substance abuse (Schuckit, 1987).

For the purpose of studying specific neurotransmitter activity, typically done using neuroimaging methods that involve the administration of a radioligand, the first method is not acceptable, as one cannot ethically perform these scans in children for the purposes of research, given the necessity for radiation exposure. Other techniques, like functional magnetic resonance imaging can be used however, this only yields a general picture of the neurobiological functioning differences and does not allow for the determination of the specific neurochemical processes involved. The second method

proposed would allow for the use of radioligands however identifying non-affected relatives of drug using families would involve a very complicated recruitment process and would require access to multiple generations of families. The final method proposed, studying populations that carry risk factors, is by far the most common approach used and has yielded some interesting results.

First, in the realm of stress responsiveness, several neuroimaging studies have observed stress induced increases in dopaminergic and opioidergic activity within the striatum in healthy volunteers (e.g. (Adler et al., 2000; Zubieta et al., 2001; Pruessner et al., 2004; Scott et al., 2006)). Increases in dopaminergic activity has been reported to be affected by early life stress, specifically only those displaying low levels of maternal care appear to show significant release within the ventral striatum (Pruessner et al., 2004), however, this finding has failed to be replicated (Montgomery et al., 2006). Other investigations indicate that exposure to high environmental stress blunts dopamine responsiveness, whereas low or moderate life stress blunts dopamine responsiveness in high impulsive individuals to a greater extent (Oswald et al., 2007).

Second, increases in dopamine activity can also be elicited by administration of methylphenidate and amphetamine, which is related to the degree of 'high' reported in healthy volunteers, and associated with cortisol secretion (Volkow et al., 1999c; Leyton et al., 2002; Oswald et al., 2005; Oswald et al., 2007; Wand et al., 2007). Along these same lines, Boileau and colleagues demonstrated long-lasting enhancement of amphetamine-induced dopamine release within the ventral striatum following repeated exposure to amphetamine (Boileau et al., 2006). In addition to associations with dopamine release, akin to the observations made by Dalley, Hooks and coworkers in rats,

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lower D2 receptor levels are associated with greater positive subjective ratings in the effects of methylphenidate in healthy, non-drug using volunteers (Volkow et al., 1999b).

Third, similar to the observations made in rats, trait characteristics of impulsivity, novelty- and sensation-seeking have been shown to be related to amphetamine-induced dopamine release within the ventral striatum and anterior cingulate in healthy human subjects, although the direction of these relationships vary by sex and by study (Leyton et al., 2002; Riccardi et al., 2006a; Oswald et al., 2007). One study also reported an association between reward dependence and opioid receptor availability within the ventral striatum (Schreckenberger et al., 2008). In addition, the degree of sensitization of dopamine release following repeated administration of amphetamine is predicted by measures of novelty seeking and impulsivity (Boileau et al., 2006).

Current Directions

Clearly understanding the neurobiological mechanisms underlying susceptibility factors is of considerable interest. Given the substantial personal and societal costs resulting from substance abuse, it is of great relevance to uncover and understand the factors that convey resistance or resilience to drug abuse. Thus far, it has been established that: 1) behavioral, environmental and genetic factors provide some susceptibility to drug use, 2) that biochemical variations particularly within the motivational circuitry may underlie these differences in vulnerability and 3) the data available in humans to this end is much more sparse and in need of expansion.

The work presented here attempts to further elucidate the association between activity within the motivational circuitry and the personal characteristics and experiences

that have been established to play an important role in determining vulnerability to abuse

drugs in humans. Personal attributes such as sex, personality, and genetic disposition will

be considered along with external influences such as adverse life events.

These experiments utilize positron emission tomography, a neuroimaging technique

employing the use of radiotracers, which allows metabolic processes to be studied in

vivo. In doing so, we are able to track neurochemical activity for the systems of interest

(i.e. dopaminergic and opioidergic). Given the observations made earlier, which indicate

that neural and behavioral activity in response to stress are useful predictors of

vulnerability for self-administration behaviors, we will examine neurochemical activity

changes during performance of a stress challenge that has been previously shown to

result in increases in activity in both endogenous opioid and dopaminergic systems

throughout the motivational circuitry (Zubieta et al., 2001; Scott et al., 2006).

First, we discuss how trait characteristics, specifically measures of non-planning

impulsivity, are associated with μ-opioid receptor activity, expanding upon the former

literature previously describing an association with dopaminergic functioning.

Second, these works will seek to expand upon and confirm some of the associations

observed between susceptibility factors and dopaminergic system activity. The influence

of environmental factors on dopaminergic activity will be examined and determine if sex

plays a significant role.

Finally, we will assess the impact of genetic variation of the oxytocin gene on

stress-induced dopaminergic activity. Though the activity of this gene has not been

previously associated with increased vulnerability to use and abuse drugs, we wanted to

move beyond the typically studied variation at dopaminergic and opioidergic genes and

identify a unique target. The oxytocin gene is an interesting candidate in this regard. As described earlier, oxytocin has effects on both stress and motivational system activity. In addition, oxytocin has been shown to facilitate a variety of social and reproductive behaviors that incorporate motivational systems including maternal behavior, pair bonding, and sexual behaviors (see (Donaldson and Young, 2008; Lee et al., 2009)). For example, oxytocin appears to influence the drive of a lactating dam to approach pups (e.g. (Pedersen et al., 1994) and in a number of compelling studies, oxytocin has been demonstrated to influence pair bonding among monogamous prairie voles, specifically impacting partner preference among females (e.g. Williams et al., 1992, Insel and Hulihan, 1995). The mechanisms underlying oxytocin's ability to facilitate such behaviors are not completely understood, however, it has been postulated that resulting dopaminergic increase within the nucleus accumbens following stimulation by oxytocin may influence the incentive salience attribution to the pups, influencing a mother's drive to their infant (Pedersen et al., 1994; Becker and Taylor, 2008). Oxytocin can also alter central dopaminergic responses associated with non-social behaviors, such as addictive behaviors like self-administration, tolerance, and dependence (see (Sarnyai and Kovács, 1994). In fact, increased oxytocin activity may provide some resilience to addictions (see (Kovács et al., 1998)). Thus, it has been postulated that oxytocin, though it may have evolved as a mechanism to promote maternal, affiliative, and sexual behaviors key to an organisms reproductive success, it also serves to influence other types of motivated behavior, like drug taking (see (Becker and Taylor, 2008)). Given this information, we examined how genetic variation at the oxytocin gene relates to stress-induced dopaminergic activity.

Overall, this research seeks to observe how certain traits, environmental factors and genetic predispositions, associated with an increased risk to develop substance use problems, influence individual differences in dopaminergic and opioidergic neurotransmission within the motivational circuitry.

Chapter II

Positron Emission Tomography Measures of Endogenous Opioid

Neurotransmission and Impulsiveness Traits in Humans[2]

There is substantial interest in the mechanistic understanding of traits that may

predispose individuals to the development of specific behaviors or psychopathologies.

Trait impulsivity has received substantial attention because of its association with risky

behaviors (e.g., experimentation with drugs, sex, problem gambling, reckless driving),

personality disorders, or even the mortality associated with the mood disorders.

Impulsivity, though frequently referred to as a single trait, is better conceptualized as

heterogeneous characteristic consisting of multiple dimensions that include sensation

seeking, lack of planning, lack of persistence and urgency (Whiteside and Lynam, 2001;

Smith et al., 2007). The planning dimension in particular appears to be more strongly

associated with negative risk taking (i.e. binge eating, problem gambling, (Smith et al.,

2007)). The same holds true for the urgency dimension which predicts problem

behaviors (problem gambling and drinking) whereas factors such as sensation seeking are

perhaps more related to risk taking in general (Fischer et al., 2004; Smith et al., 2007).

Impulsivity as it refers to pathological behavior has been well studied in current

and former substance users. In humans, opiate addicts are more impulsive than non-

addicts as measured by an increase in the discounting of delayed rewards (i.e. the devaluation of rewards as a function of time) (Madden et al., 1997) and by a reduction in reflection (i.e. the tendency to use information when making a decision) (Clark et al., 2006). In animals, it has been shown that in rodents a preference for immediate reward over larger, delayed reward predicted the development of nicotine self-administration and maintenance of use (Diergaarde et al., 2008), suggesting that this is a trait that predisposes to drug dependence. This form of impulsive choice in rats has also been demonstrated to predict cocaine self-administration (Perry et al., 2008). Other clinical populations are also characterized by or associated with impulsive behavior such as Attention-Deficit Hyperactivity Disorder (ADHD), Borderline Personality Disorder, eating disorders and pathological gambling (e.g. ADHD, (Swanson et al., 1998); Borderline Personality, (American Psychiatric Association, 2000); Eating Disorders, (Fahy and Eisler, 1993); gambling, (Alessi and Petry, 2003)). Whether impulsivity represents a predisposing factor or is at least partially the result of prior exposure to drugs or ongoing disease is a matter of current debate.

Impulsive characteristics probably do not affect behavior in isolation but are also likely to interact with other factors such as stress. Stressors have a negative impact on drug initiation, maintenance, craving and relapse (see review (Sinha, 2001)). High impulsive gamblers also show neuroendocrine stress axis and cardiovascular responses to gambling situations relative to their low impulsive counterparts (Krueger et al., 2005). In addition, stressors, particularly when combined with substance abuse, are thought to be modulated by individual impulsivity traits to increase the risk of completed suicides (Mann et al., 1999), particularly in younger individuals (Zouk et al., 2006).

While it is increasingly clear that impulsivity, and stress responses confer vulnerability to substance abuse and other risky behaviors, the neurobiological processes underlying these effects are still poorly understood, particularly in humans. DA mechanisms appear involved, for instance, utilizing another animal of impulsivity that utilizes anticipatory responses to a food reward as proxy, Dalley and colleagues observed rats demonstrating greater impulsivity prior to drug exposure exhibited lower dopamine (DA) D2/D3 receptor concentrations in the nucleus accumbens, increased escalation and maintenance of drug self-administration relative to their lower impulsivity counterparts (Dalley et al., 2007). Though DA function in the ventral basal ganglia is thought to have an important role (Thierry et al., 1976; Rouge-Pont et al., 1993; Rouge-Pont et al., 1998) it is unlikely to take place in isolation. The nucleus accumbens lies at the interface of sensorimotor and limbic systems, and through its connections with the ventral pallidum and the amygdala, forms part of a circuit involved in the integration of cognitive, affective and motor responses in animal models (Mogenson and Yang, 1991; Kalivas et al., 1999). This pathway and interconnected regions (e.g., insular and prefrontal cortex, medial thalamus) are heavily modulated by the endogenous opioid system and μ-opioid receptors. For example, this neurotransmitter system is recruited when drug-induced DA release takes place in the context of environmental novelty and stressors (Badiani et al., 1998, 1999; Napier and Mitrovic, 1999; Day et al., 2001; Uslaner et al., 2001). Further, the motivated pursuit and positive behavioral responses to rewards (Pecina and Berridge, 2005; Smith and Berridge, 2007) are enhanced by the selective administration of μ-opioid receptor agonists in the nucleus accumbens and ventral pallidum, nuclei that are central to the regulation of motivated behavior.

The present report examined two orthogonal behavioral traits, impulsiveness (IMP) and deliberation (DLB), as defined by the NEO Personality Inventory Revised (NEO PI-R) (Costa et al., 1992) as a function of in vivo measures of μ-opioid receptor neurotransmission in humans. As defined by the NEO PI-R, the IMP dimension refers to the tendency to act without careful consideration for consequences of delayed gratification, and maps on to urgency, which appears related to problem behaviors such as drug use (Smith et al., 2007). DLB, which corresponds to the lack of planning dimension, is thought to act as a moderating, opposing trait (Fisher and Smith, 2004).

We used positron emission tomography (PET) and the μ-opioid selective radiotracer [11C]carfentanil at rest and during the experience of a physical and emotional stressor, moderate levels of sustained pain. Under these experimental conditions, reductions in the availability of μ-opioid receptors during the stress challenge reflect the activation of endogenous opioid neurotransmission and μ-opioid receptors (Zubieta et al., 2001). It was hypothesized that individual levels of IMP and DBL would be positively and negatively associated, respectively, with the functional response of the μ-opioid system during the stressor. Furthermore, that these effects would take place in motivational circuits modulated by this neurotransmitter system, namely the rostral anterior cingulate and adjacent medial prefrontal cortex, nucleus accumbens/ventral pallidum, medial thalamus and amygdala.

Materials and Methods

Subjects

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Nineteen (19) healthy right-handed, non-smoking men (age range 20-30 years; (mean ± SD, 23 ± 3 years) were recruited via advertisement. In addition to completing physical and neurological examinations, subjects were screened using the Structured Clinical Interview for DSM-IV (non-patient version, SCID-NP). Subjects had no current or past history of medical, neurological, or psychiatric illnesses, including substance abuse or dependence and alcohol intake less than 5 drinks/week. Participants reported no current or recent (within 6 months) history of exposure to centrally active prescription or illicit drugs and were asked to restrain from any alcohol intake for 48 hours prior to scanning. Urine drug screens were performed immediately prior to imaging. Subjects reported no family history of psychiatric disease in first-degree relatives. The sample was restricted to males due to the known sex differences in the regional concentration of μ-opioid receptors (Zubieta et al., 1999), and in the activity of this neurotransmitter system in response to stress (Zubieta et al., 2002), an effect that is influenced by circulating gonadal steroids (Smith et al., 2006). Furthermore, a link between impulsivity and substance use disorders has been shown most conclusively in males (see Discussion).

Protocols were approved by the Investigational Review Boards of the Universities of Michigan and Maryland and the Radioactive Drug Research Committee at the University of Michigan. Written informed consent was obtained in all subjects.

Personality Inventories

Subjects were administered the NEO Personality Inventory Revised (NEO PI-R) (Costa et al., 1992). The facets "Impulsiveness" (IMP), defined as "a lack of control over cravings or desires", and "Deliberation" (DLB), or the "tendency to think carefully before

acting", were utilized as the primary scales of interest. These facets have been previously

demonstrated to reflect the dimensions of impulsivity that have been associated with

negative risk taking (Fischer et al., 2004; Smith et al., 2007). Individuals endorsing

greater behavioral under-control or lack of reflection would display higher IMP and

lower DLB scores. The median scores in population samples of comparable age were

utilized to separate the study sample into high and low scoring groups (population data,

IMP, mean ± SD, 15 ± 4; DLB, mean ± SD, 18 ± 4 (Costa et al., 1992). Study sample

data, IMP, mean ± SD, 15 ± 3; DLB, mean ± SD, 18 ± 3 (Median = 15, High, n = 9

subjects, Low, n = 10; DLB: median = 18, High, n = 9, Low, n = 10).

Stress Challenge

We employed a physical and emotional stressor, moderate levels of sustained pain

of experientially adjusted intensity, to activate endogenous opioid-μ-opioid mediated

neurotransmission, as previously described (Zubieta et al., 2001; Zubieta et al., 2003). In

short, a steady state of moderate muscle pain was maintained 45-65 min after the

radiotracer administration by a computer-controlled delivery system through the infusion

of medication-grade hypertonic saline (5%) into the left masseter muscle. In this model of

sustained deep somatic pain, the intensity of the painful stimulus is standardized across

subjects, as described in detail previously (Zhang et al., 1993; Stohler and Kowalski,

1999). Pain intensity was rated every 15 sec from 0 (no pain) to 100 (most intense pain

imaginable). During the baseline control condition, no infusions took place and the

subject was instructed to lie quietly in the scanner. The pain intensity ratings obtained

every 15 sec were recorded in the computer controller and averaged for statistical analyses.

Integrative measures of the pain experience (sensory and pain affect components) were obtained using the McGill pain questionnaire (MPQ), administered upon completion of the challenge (Melzack and Torgerson, 1971). The Positive and Negative Affectivity Scale (PANAS) (Watson et al., 1988), assessing the internal affective state of the volunteers, was obtained before and after the challenge. The infusion volume required for pain maintenance was also recorded and provided a measure of sustained pain sensitivity for the individual subject.

Scanning Protocols

Two PET scans per subject were acquired with a Siemens HR+ scanner (Knoxville, KN) in 3-D mode (reconstructed FWHM resolution (5.5 mm in-plane and 5.0 mm axially), one at baseline and another using the stress challenge. Radiotracer synthesis and image acquisition, co-registration and reconstruction protocols were identical to those used in previous publications (Zubieta et al., 2001; Zubieta et al., 2002; Zubieta et al., 2003) .

The total activity of [11C]carfentanil administered to each subject in each scan was 14.5 ± 2.7 mCi (535.0 ± 100.9 MBq), with an average mass injected of 0.02 ± 0.01 μg/kg, ensuring that the compound was administered in tracer quantities, i.e., sub-pharmacological doses. Fifty (50) percent of the [11C]carfentanil dose was administered as a bolus with the remainder delivered as a continuous infusion by a computer-controlled automated pump to more rapidly achieve steady-state tracer levels.

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Dynamic image data for each of the receptor scans were transformed, on a voxel-by-voxel basis, into two sets of parametric maps, coregistered to each other: (1) a tracer transport measure (K_1 ratio), proportional to regional cerebral blood flow, and (2) a receptor-related measure, distribution volume ratio at equilibrium. To avoid the need for arterial blood sampling, these parametric images were calculated using a modified Logan graphical analysis (Logan et al., 1996), using the occipital cortex (an area devoid of μ-opioid receptors) as the reference region. The Logan plot became linear by 5-7 minutes after the start of radiotracer administration, with a slope proportional to the $(B_{max}/K_d)+1$ for this receptor site. B_{max}/K_d is the "receptor related" measure (μ-opioid receptor availability or μ-opioid receptor "binding potential", BP_{ND}) (Bmax = receptor concentration; K_d = receptor affinity for the radiotracer).

MRI scans were acquired on a 1.5 Tesla scanner (Signa, General Electric, Milwaukee, WI) for anatomical localization and coregistration to standardized stereotactic coordinates. Acquisition sequences were axial spoiled gradient-recalled (SPGR) MR [echo time (TE)= 5.5; repetition time (TR)= 14; inversion time (TI)= 300; flip angle= 20o; number of excitations (NEX)= 1; 124 contiguous images; 1.5 mm-thick; 24 cm field-of-view; image matrix= 256 x 256 pixels, pixel size= 0.94 mm). T1-weighted MR and PET images of each subject were then co-registered to each other using a mutual information algorithm as previously described (Zubieta et al., 2001; Zubieta et al., 2003).

Image Analyses

Differences between groups (high IMP / low IMP; low DLB / high DLB, two tail, unpaired t tests) were mapped into stereotactic space using z maps of statistical

significance with a modified version of SPM99 (Welcome Department of Cognitive Neurology, University College, London) and Matlab software (MathWorks, Natick, MA), using a general linear model. No global normalization was applied to the data, and therefore the calculations presented are based on absolute B_{max}/K_d estimates. Only regions with specific μ-opioid receptor binding were included in the analyses (voxels with BP_{ND} values > 0.2 as calculated with SPM). A priori hypothesized regions were deemed significant at p < 0.0001 uncorrected for multiple comparisons. For other regions, significant differences were detected using a statistical threshold that controls for a Type-I error rate at p = 0.05 for multiple comparisons (Friston et al., 1994). Numerical values for each region were obtained by averaging the values of voxels contained in each significant cluster, up to a p = 0.001. These data were extracted for quantification of regional changes in BP, graphing, determination of correlation coefficients (Pearson correlations at p < 0.05), rule out the presence of outliers, and further statistical analyses with SPSS for Macintosh 11.0.3 (SPSS Inc., Chicago, IL).

Results

Psychophysical Measures

With the use of the adaptive, experientially-adjusted stimulus delivery system employed, producing comparable perceptions of the pain stressor among participants by individually titrating the rate of infusion of the algesic substance, no significant group differences were obtained in psychophysical measures of pain or affective state during the stress challenge (high vs. low IMP, high vs. low DLB) (Table 1). As would be expected, IMP and DLB scores were negatively correlated (r = -0.55, p = 0.015).

Table 1 Psychophysical Measures by Trait

Impulsiveness	High IMP (n=9)	Low IMP (n=10)	p[†]
Average VAS Intensity	32 ± 16	29 ± 12	0.60
MPQ Total	21.7 ± 8.4	20.4 ± 3.1	0.66
PANAS Positive Affect	16.9 ± 10.1	16.2 ± 9.2	0.90
PANAS Negative Affect	2.6 ± 3.5	3.3 ± 2.9	0.60
Total Volume 0 to 20	2653.56 ± 1567.68	3296.35 ± 878.46	0.28
Deliberation	**Low DLB (n=10)**	**High DLB (n=9)**	**p[†]**
Average VAS Intensity	34 ± 13	26 ± 13	0.20
MPQ Total	23.3 ± 6.41	18.4 ± 4.61	0.08
PANAS Positive Affect	18.8 ± 10.6	14.0 ± 7.6	0.30
PANAS Negative Affect	2.7 ± 3.3	3.2 ± 3.2	0.70
Total Volume 0 to 20	2531.50 ± 1531.91	3503.40 ± 609.10	0.09

Table 1 Psychophysical Measures by Trait Data are expressed as mean ± SD. [†]Calculated using 2-sample *t* tests for differences between high and low IMP and DLB.

Baseline μ-Opioid Receptor BP

Impulsiveness

Significant differences in baseline μ-opioid receptor BP_{ND} were observed between high and low IMP groups. Specifically, greater regional μ-opioid receptor BP_{ND} was observed in the high, compared to low IMP subjects, in the right anterior cingulate and adjacent medial frontal cortex, right ventral basal ganglia (nucleus accumbens, extending into ventral pallidum), and the basolateral area of the right amygdala (Table 2, Figure 2). No effects were obtained in the opposite direction.

Significant positive correlations were obtained between μ-opioid receptor BP_{ND} and IMP scores within all these clusters (right dorsal anterior cingulate, r = 0.59, p < 0.01; right ventral basal ganglia, r = 0.50, p = 0.03; right amygdala, r = 0.49, p = 0.03).

<u>Deliberation</u>

Subjects with high DLB scores showed significantly lower baseline regional μ-opioid receptor BP_{ND} compared to the low DLB group in the right dorsolateral prefrontal cortex, right dorsal anterior cingulate and medial frontal gyrus, left ventral basal ganglia, right thalamus extending inferiorly into hypothalamus and in the right basolateral amygdala (Table 2, Figure 2). No effects were observed in the opposite direction.

Significant negative correlations between μ-opioid receptor BP_{ND} and DLB scores were noted within the right prefrontal cortex ($r = -0.65$, $p = 0.003$), right anterior cingulate (two peaks, x,y,z, coordinates in mm, 16, 10, 39, $r = -0.56$, $p = 0.01$; x, y, z, 8, 14, 26, $r = -0.46$, $p < 0.05$), and right amygdala ($r = -0.54$, $p = 0.02$).

Table 2 Differences in Baseline Regional μ-Opioid Receptor BP			
Region	**Coordinates** **(x, y, z)†**	**Cluster Size‡**	**Z**
High Impulsiveness > Low Impulsiveness			
Right Anterior Cingulate/Medial Frontal Cortex *	15, 3, 47	1294	4.37
Right Ventral Basal Ganglia extending into Ventral Pallidum	7, -3, -3	2660	5.85
Right Basolateral Amygdala	31, -2, -23	440	3.70
Low Deliberation > High Deliberation			
Right Prefrontal Cortex	46, 7, 52	2676	4.84
Right Anterior Cingulate/Medial Frontal Cortex *	16, 10, 39	1294	4.02
Right Thalamus*	12, -9, 7	3218	4.19
Right Ventral Basal Ganglia/Hypothalamus	6, -3, -4	222	5.45
Left Ventral Basal Ganglia	-4, 5, -14	1361	4.18
Right Amygdala	28, 0, -26	416	4.56

Table 2 Differences in Baseline Regional μ-Opioid Receptor BP † Indicates Montreal Neurological Institute coordinates, for significant peaks. ‡ Cluster size is expressed in mm^3. *Multiple peaks were detected in this region. Coordinates indicate center of the largest peak
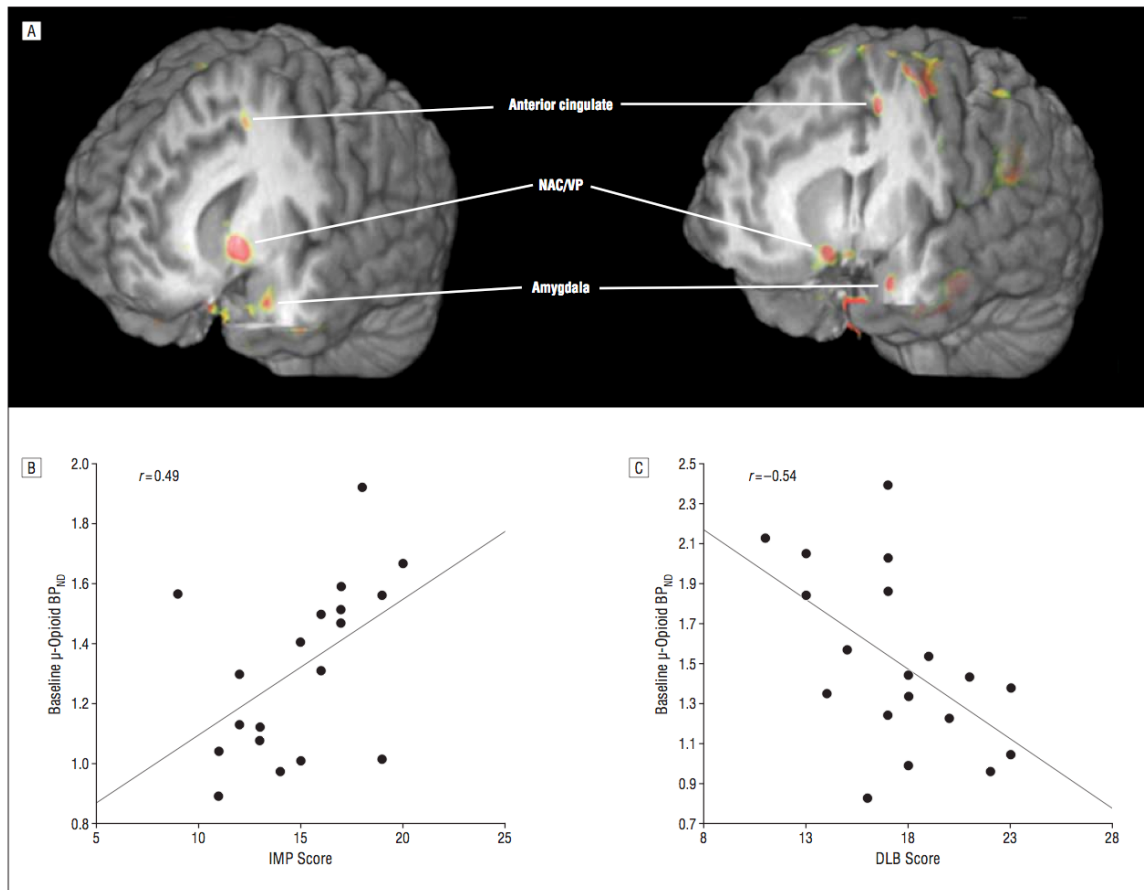
Figure 2 Association between impulsiveness (IMP) and deliberation (DLB) scores and baseline μ-opioid receptor availability. A, Areas in which significant differences in μ-receptor availability in vivo were observed between individuals with high and low IMP scores (left) and DLB scores (right). B and C, Correlations between μ-receptor nondisplaceable binding potential (BP_{ND}) and IMP and DLB scores for the cluster centered in the right amygdala.

Stress – Induced Activation of μ-Opioid Neurotransmission

<u>Impulsiveness</u>

Subjects with higher IMP scores demonstrated significantly greater stress-induced activation of μ-opioid mediated neurotransmission, compared to subjects in the low IMP group, in the left orbitofrontal cortex, right dorsal anterior cingulate, ventral basal ganglia, bilaterally, extending into the hypothalamus, left anterior thalamus and basolateral amygdala bilaterally. (Table 3, Figure 3). There were no regions where the

high IMP group showed significantly lower stress-induced changes in μ-opioid receptor BP_{ND} relative to the low IMP group.

Significant positive correlations between μ-opioid system activation (baseline BP_{ND} – pain BP_{ND}) and IMP scores were noted in the left orbitofrontal cortex ($r = 0.63$, $p < 0.005$), right ventral basal ganglia ($r = 0.49$, $p = 0.03$), left anterior thalamus ($r = 0.61$, $p = 0.006$), and right amygdala ($r = 0.50$, $p < 0.03$).

Deliberation

Significant differences in stress-induced activation of endogenous opioid neurotransmission were also detected between the high DLB and low DLB groups. Opposite to the high IMP group and in a direction similar to that observed for baseline binding measures, the high DLB group showed lower stress-induced activation of the endogenous opioid system compared to the low DLB group in a number of brain regions. These included the left dorsolateral prefrontal cortex, right anterior cingulate/medial frontal cortex, orbitofrontal cortex bilaterally, ventral basal ganglia, bilaterally, with extension into the anterior hypothalamus, and basolateral amygdala bilaterally. No effects were obtained in the opposite direction (Table 3, Figure 3).

Significant negative correlations between μ-opioid system activation and DLB scores were observed within the left dorsolateral prefrontal cortex ($r = - 0.69$, $p = 0.001$), right anterior cingulate ($r = - 0.61$, $p = 0.005$), right and left ventral basal ganglia ($r = - 0.54$, $p < 0.02$ and $r = - 0.60$, $p < 0.01$, respectively), and right amygdala ($r = - 0.70$, $p =$

0.001). The left amygdala cluster followed a similar pattern at trend levels of correlation

(r = - 0.41, p = 0.08).

Table 3 Differences in Stress-Induced Changes in Regional μ-Opioid Receptor BP

Region	Coordinates (x, y, z)†	Cluster Size‡	Z	Baseline BP High IMP/DLB	Baseline BP Low IMP/DLB	Pain BP High IMP/DLB	Pain BP Low IMP/DLB	% Change High IMP/DLB	% Change Low IMP/DLB
High IMP (Baseline-Pain) > Low IMP (Baseline-Pain)									
L Orbitofrontal Cortex	-13, 29, -25	434	3.87	0.87 ± 0.32	0.76 ± 0.21	0.62 ± 0.17	0.82 ± 0.19	-33.4%	7.3%
R Anterior Cingulate	15, 2, 47	661	4.59	0.76 ± 0.29	0.41 ± 0.13	0.55 ± 0.18	0.53 ± 0.13	-32.0%	24.5%
L Thalamus	-10, 0, 5	629	3.98	1.10 ± 0.22	1.02 ± 0.10	0.98 ± 0.17	1.24 ± 0.20	-11.9%	18.9%
R Ventral Basal Ganglia extending into Diencephalon*	6, -3, -4	2776	7.46	1.97 ± 0.47	1.54 ± 0.19	1.41 ± 0.15	1.57 ± 0.20	-33.3%	1.8%
L Ventral Basal Ganglia	-8, 15, -5	233	3.69	2.32 ± 0.34	2.06 ± 0.20	2.01 ± 0.32	2.16 ± 0.34	-14.3%	4.9%
R Basolateral Amygdala	29, -1, -27	466	4.83	1.47 ± 0.28	1.18 ± 0.25	1.09 ± 0.24	1.19 ± 0.11	-29.5%	0.6%
L Basolateral Amygdala	-20, 3, -30	337	5.61	1.16 ± 0.35	0.86 ± 0.27	0.86 ± 0.22	0.97 ± 0.24	-29.4%	12.1%
Low DLB (Baseline-Pain) > High DLB (Baseline-Pain)									
B Orbitofrontal Cortex*	-7, 34, -26	2206	3.73	0.69 ± 0.21	0.88 ± 0.13	0.83 ± 0.09	0.66 ± 0.09	18.5%	-27.7%
L Prefrontal Cortex	-33, 7, 38	2266	3.90	0.42 ± 0.12	0.59 ± 0.22	0.59 ± 0.15	0.44 ± 0.13	34.1%	-30.0%
R Anterior Cingulate	16, 10, 39	3596	4.48	0.54 ± 0.09	0.76 ± 0.18	0.63 ± 0.12	0.56 ± 0.14	25.8%	-44.2%
R Ventral Basal Ganglia extending into Diencephalon*	6, -3, -4	2449	4.64	1.25 ± 0.11	1.57 ± 0.23	1.41 ± 0.15	1.26 ± 0.11	12.0%	-22.0%
L Ventral Basal Ganglia	-2, 4, -15	578	4.57	0.84 ± 0.27	1.15 ± 0.24	1.03 ± 0.25	0.85 ± 0.28	19.9%	-29.0%
R Basolateral Amygdala	28, 0, -26	599	4.40	1.21 ± 0.18	1.55 ± 0.39	1.30 ± 0.11	1.17 ± 0.23	7.0%	-28.3%
L Basolateral Amygdala	-21, 6, -30	145	4.61	0.86 ± 0.19	1.03 ± 0.36	1.12 ± 0.26	0.74 ± 0.19	26.4%	-32.9%

Table 3 Differences in Stress-Induced Changes in Regional μ-Opioid Receptor BP Data are shown as the mean ± S.D. † Coordinates (x,y,z) refer to ICBM coordinates, in mm. ‡ Cluster size is expressed in mm^3. *Multiple peaks detected in this region. Diencephalic regions included thalamus and hypothalamus. % Change represents the mean percent change in regional μ-opioid receptor binding potential between baseline and stress conditions.
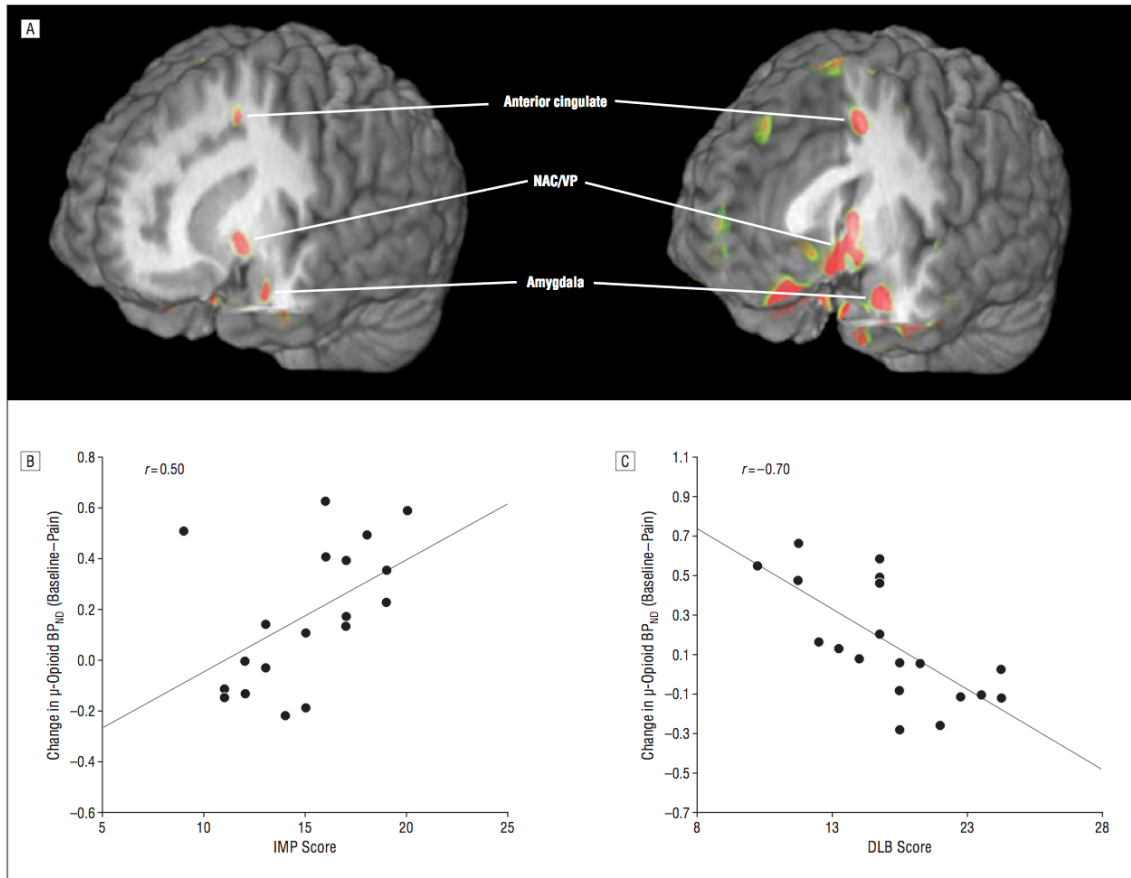
Figure 3. Association between impulsiveness (IMP) and deliberation (DLB) scores and stress-induced activation of μ-opioid receptor (μ-receptor)–mediated neurotransmission. A, Areas in which significant differences in endogenous opioid activity during stress were observed between individuals with high and low IMP scores (left) and DLB scores (right). B and C, Correlations between opioid system activation and IMP and DLB scores for the cluster centered in the right amygdala. BP_{ND} indicates nondisplaceable binding potential.

Interactions Among Measures: Conjunction Analyses

The above data suggested the presence of some, but not complete regional overlap

for the effects of the related traits IMP and DLB, with neurochemical findings in opposite

directions, as would be expected for opposing traits. In an additional analysis, we sought

to determine how the individual combination of these two behavioral traits segregated at

the levels of anatomical and neurochemical substrates (μ-opioid receptor availability and

neurotransmitter responses to stress). Individuals were divided into "behavioral risk"

groups based on their IMP and DLB classifications, resulting in three groups with

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relatively high (High IMP/Low DLB, n=7), low (Low IMP/High DLB, n=7) or

intermediate (High IMP/High DLB, Low IMP/Low DLB, n=5) behavioral trait

vulnerability. Intermediate groups were not separated because of the small sample sizes

in those cells. Then, for both the baseline and activation conditions, we identified brain

areas of coincidence, where BP_{ND} and stress-induced release were greater in both the

high IMP and low DLB groups. For this purpose, the ImCalc function within SPM was

utilized to generate a "mask" that contained only those voxels that were significantly

different above a p =0.007 (T=1.99) in both of the contrasts (Contrast1 = High vs. Low

IMP, Contrast 2 = Low vs. High IMP; Formula: [(Contrast 1 T score > 1.99)*(Contrast 2

T score > 1.99)]. The resulting area of coincidence contained voxels that were

independently significant in each and both of the contrasts, whose joint probability is

given by multiplying the probabilities for each contrast: 0.007 x 0.007 = $p \le 0.000049$

(e.g. (Dolcos et al., 2004)). Measurement values for the regions identified were then

extracted for quantification of regional changes in BP_{ND}, graphing, and statistical

analyses.

Baseline

Three regions showed significant overlap among the two traits: right anterior

cingulate, right ventral pallidum, and right amygdala. The baseline BP_{ND} for each of the

regions analyzed increased in a stepwise progression from the group with the lowest

vulnerability traits (Low IMP/High DLB) to the highest (High IMP/Low DLB) (Figure 4,

Table 4). This effect was tested statistically with ANOVA, which showed significant

effects of "risk" classification for each of the regions ([right anterior cingulate ($F(2,16)$ =

8.009, p = 0.004), right nucleus accumbens/ventral pallidum (F(2,16) = 8.157, p=0.004), right amygdala (F(2,16) = 5.280, p = 0.017)]). Post-hoc tests (Tukey HSD) are shown in Table 4. Of note, both intermediate groups High IMP/High DLB and Low IMP/Low DLB showed similar results for these regions (data not shown).

Stress –Induced Activation of μ-Opioid Neurotransmission

　　　Five regions showed significant activation overlap among traits: right anterior cingulate, right and left nucleus accumbens/ventral pallidum, and right and left amygdala. Again, we observed a stepwise progression in stress-induced endogenous opioid system activation, with the smallest change in the group with the fewest vulnerable behavioral traits (Low IMP/High DLB) and the greatest from the group with the most (High IMP/Low DLB) (Figure 4, Table 4). A significant main effect of group on stress-induced activation of μ-opioid neurotransmission was present for each of the regions ([right anterior cingulate (F(2,16) = 10.53, p=0.001), right ventral pallidum (F(2,16) = 40.91, p<0.0001), left ventral pallidum (F(2,16) = 5.10, p=0.019), right amygdala (F(2,16) = 23.54, p <0.001), left amygdala (F(2,16) = 5.05, p=0.020)]). Post-hoc tests results are shown in Table 4. As with the baseline data, intermediate groups showed similar results for the overlapping regions.

Table 4 Conjunction Analyses

Baseline

	Baseline BP		
Region	High IMP/Low DLB	Intermediate Groups	Low IMP/High DLB
R Anterior Cingulate	0.87 ± 0.09	0.63 ± 0.12	0.50 ± 0.05
R Ventral Basal Ganglia extending into Diencephalon*	1.76 ± 0.11	1.54 ± 0.11	1.29 ± 0.08
R Basolateral Amygdala	1.58 ± 0.14	1.52 ± 0.11	1.13 ± 0.06

Stress-Induced Changes

	Baseline BP			Pain BP		
Region	High IMP/Low DLB	Intermediate Groups	Low IMP/High DLB	High IMP/Low DLB	Intermediate Groups	Low IMP/High DLB
L Orbitofrontal Cortex*	0.98 ± 0.12	0.75 ± 0.06	0.92 ± 0.05	0.67 ± 0.07	0.70 ± 0.07	0.92 ± 0.04
R Anterior Cingulate	0.79 ± 0.09	0.53 ± 0.07	0.43 ± 0.05	0.53 ± 0.07	0.51 ± 0.07	0.56 ± 0.05
R Ventral Basal Ganglia extending into Diencephalon*	1.42 ± 0.05	1.32 ± 0.06	1.20 ± 0.06	1.06 ± 0.04	1.31 ± 0.04	1.28 ± 0.07
L Ventral Basal Ganglia	2.26 ± 0.13	2.04 ± 0.09	1.94 ± 0.08	1.88 ± 0.13	2.03 ± 0.16	2.09 ± 0.10
R Basolateral Amygdala	1.33 ± 0.09	1.31 ± 0.10	1.08 ± 0.04	0.97 ± 0.06	1.14 ± 0.05	1.14 ± 0.02
L Basolateral Amygdala	1.75 ± 0.11	1.66 ± 0.28	1.69 ± 0.08	1.51 ± 0.12	1.65 ± 0.22	1.90 ± 0.16

Table 4 Conjunction Analyses Data are shown as the mean ± S.E.M. † Coordinates (x,y,z) refer to ICBM coordinates, in mm. ‡ Cluster size is expressed in mm³. *Multiple peaks detected in this region. Coordinates indicate center of largest peak. Diencephalic regions included thalamus and hypothalamus. % Change represents the mean percent change in regional μ-opioid receptor binding potential between baseline and stress conditions. R = right, L = left. IMP = NEO PI-R impulsiveness facet; DBL = NEO PI-R deliberation facet.
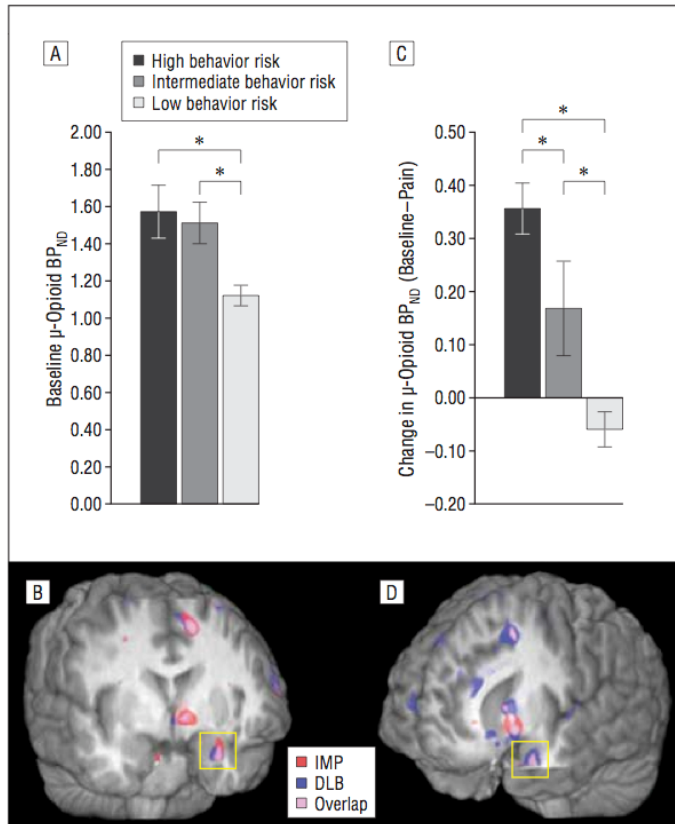
Figure 4 Conjunction analysis of impulsiveness (IMP) and deliberation (DLB) effects on μ-opioid receptor BP$_{ND}$ and stress-induced endogenous opioid system activity. A, The μ-receptor BP$_{ND}$ for high (high IMP/low DLB), intermediate (low IMP/low DLB or high IMP/high DLB), and low (low IMP/high DLB) behavioral risk groups in the amygdala. *P<0.05. Bars indicate mean values; error bars, standard error of the mean. B, Areas with color overlap show coincidence regions for IMP and DLB effects on μ-receptor BP$_{ND}$. The amygdala (the baseline μ-opioid BP$_{ND}$ that is shown in A) is highlighted by the yellow square. C, Stress-induced μ-opioid system activation for high, intermediate, and low behavioral risk groups in the amygdala. *P <0.05. D, Areas with color overlap show coincidence regions for IMP and DLB effects on stress-induced μ-opioid system activation. The amygdala (the stress-induced μ-opioid system activation that is shown in C) is highlighted by the yellow square.

Discussion

The present study demonstrates that impulsiveness and deliberation are highly predicted by measures of endogenous opioid function in limbic regions. The personality facets studied here refer to the tendency to act rashly and without forethought, and have been associated with various psychopathologies and risky phenotypes (e.g., drug consumption, pathological gambling, personality disorders) (American Psychiatric Association, 2000; Alessi and Petry, 2003; Fischer et al., 2004; Smith et al., 2007). Our

major findings are threefold: First, we find that individuals displaying these risky

phenotypes (e.g. high IMP or low DBL) have higher μ-opioid receptor BP_{ND} at rest

within regions implicated in decision making, reward seeking and emotional responsivity.

This higher BP_{ND} reflects a greater availability of μ-opioid receptors in a high affinity

state (e.g., binding to an agonist radiotracer at low, tracer concentrations) (Narendran et

al., 2004). Second, following a pain stress challenge we find larger reductions in BP_{ND}

from baseline in individuals displaying high IMP/low DLB in overlapping regions. These

reductions reflect processes related to the release of endogenous opioid interacting with

μ-opioid receptors, so these receptors are no longer available for binding to the

radioligand (Innis et al., 1992; Narendran et al., 2004). Third, we demonstrate a

cumulative effect of personality traits on in vivo measures of μ-opioid neurotransmission.

We found that individuals exhibiting extreme traits (high IMP/low DLB and low

IMP/high DLB) display the greatest and smallest, respectively, baseline μ-opioid receptor

availability and endogenous opioid system responses to the pain stressor employed.

Personality traits, like impulsiveness, likely manifest as a result of a variety of

factors, both biological and genetic. Converging lines of evidence indicate the opioid

system as one candidate system involved in the expression of the non-planning dimension

of impulsiveness described here. Previous research on a measure related to the non-

planning dimension of impulsiveness, delayed discounting, which refers to the

devaluation of rewards as a function of time, has indicated prominent roles for several

neurotransmitters: serotonin (e.g. (Winstanley et al., 2005)), dopamine (e.g. (Winstanley

et al., 2005)) and based upon the present results, opioids. Manipulation of the opioid

system affects preferences for immediate rewards; for instance, and in animal models,

Kieres and colleagues demonstrated that morphine could increase the rate of delayed discounting among rats, an effect blocked by naloxone (Kieres et al., 2004). Few human studies have directly addressed this issue, however, multiple studies have shown that several psychiatric groups show steeper discounting of delayed rewards such as pathological gamblers (e.g. (Dixon et al., 2004)and drug addicts (e.g. opiate, (Madden et al., 1997). In addition, opiate addicts show a greater preference to immediate monetary rewards relative to non-addicts (Madden et al., 1997), a preference that is potentiated following mild opiate deprivation (Giordano et al., 2002).

In the present work we show that individuals displaying risky personality traits (high IMP, low DLB) showed significantly greater regional μ-opioid receptor availability at baseline and stress-induced regional μ-opioid system activation when compared to individuals endorsing low IMP, high DLB. These effects were observed in multiple brain regions including the orbitofrontal, medial prefrontal and cingulate cortex, nucleus accumbens/ventral pallidum and amygdala. Individually, these regions are known to be involved in impulsive choice, reward seeking, and cognitive-emotional integration and are heavily modulated by μ-opioid receptors (Khachaturian and Watson, 1982; Mansour et al., 1990; Vogt et al., 2001). Many of these regions, particularly the prefrontal cortex and nucleus accumbens have been implicated in disorders characterized by or associated with impulsive behavior such as ADHD, substance abuse disorder, and pathological gambling (ADHD, see review (Spencer et al., 2002); Substance Abuse, see review (Volkow et al., 2004); Gambling, (Reuter et al., 2005)). Manipulation of nucleus accumbens activity can directly influence impulsive behavior, i.e., stimulation of the nucleus accumbens core has been shown to decrease impulsive choice (Sesia et al.,

2008), whereas lesions increase impulsive choice (Cardinal et al., 2001). Similar roles have been ascribed to the prefrontal cortex, orbitofrontal and amygdala, thought to contribute to decision making by the cognitive and emotional evaluation of future consequences (Bechara et al., 1994; Bechara et al., 1996). Collectively, these regions are thought to be involved in the pursuit and receipt of natural rewards, decision-making and, more generally, motivated behavior (Austin and Kalivas, 1991; Napier, 1992; Hiroi and White, 1993; Johnson et al., 1993; Berridge, 1996; Gong et al., 1996; Napier and Mitrovic, 1999; Tom et al., 2007). Neurobiologically this modulation of motivated behavior is thought to take place as a result of their extensive reciprocal connections, well-described between the nucleus accumbens, ventral pallidum, mediodorsal nucleus of the thalamus, prefrontal cortex and amygdala (Haber et al., 1985; Kelley et al., 2005).

We also observed greater stress-induced activation of this neurotransmitter system in subjects scoring above the population average of NEO IMP scores, compared with subjects scoring below, in regions at least partially overlapping with those where baseline differences in μ-opioid receptor BP_{ND} were observed. Opposite effects (lower stress-induced opioid system activity in high scoring subjects) were observed for the orthogonal domain, DLB. These data then supports the contention that there are interactions between neurobiological processes related to stress responsiveness and impulsivity. Physiological stress responses seem greater in more impulsive individuals even among risky populations (e.g., pathological gamblers, Krueger et al., 2005) and therefore point to factors that may contribute to interindividual variations in risky behavior even among pathological states. Outbred rats exposed to the mild stress of a novel environment may show high (HR) or low (LR) rates of exploratory locomotion, and HR rats learned to self-

administer psychostimulants faster than LRs (Piazza et al., 1989; Marinelli and White, 2000; Piazza et al., 2000; Kabbaj et al., 2001). It has been proposed that activation of DA neurotransmission and stress responses during risky behavior are the critical variable underlying the reinforcement of this behavior in the more impulsive individuals (Dellu et al., 1996), an effect that may be mediated by the increase in corticosterone induced by the stressor (Deroche et al., 1992; Deroche et al., 1993, 1994; Rouge-Pont et al., 1995). Relevant to the results presented here, HR rats, more prone to acquire drug-self administration also show increased nucleus accumbens proenkephalin gene expression (Lucas et al., 1998).

A conjunction analysis more formally determined the overlap in the processes and brain regions where IMP and DLB effects were obtained. It demonstrated a cumulative effect of personality risk factors on measures of μ-opioid neurotransmission. Extreme traits (high IMP/low DLB, low IMP/high DLB) demonstrated greatest and smallest, respectively, endogenous opioid system responses to a standardized stressor and μ-opioid receptor availability at baseline. "Intermediate", compounded traits (high IMP/high DLB, low IMP/low DLB) showed intermediate effects for both measures. This is consistent with the observation that the accumulation of risky traits is associated with a greater probability of problem behaviors and substance use problems (Bry, 1982). The coalescence of IMP and DLB effects were observed in the dorsal anterior cingulate, nucleus accumbens/ventral pallidum and amygdala, centrally implicated in decision-making and motivated behavior, as noted above (Mogenson and Yang, 1991; Kalivas et al., 1999; Bush et al., 2002).

Regional μ-opioid receptor availability and μ-opioid system activation during the stressor accounted for 24 to 40% of the variance in IMP scores, and 17 to 49% of the variance in DBL scores. In contrast, no significant relationships have been reported between NEO impulsiveness and dopamine D2/3 receptor binding in the basal ganglia as measured with [11C]raclopride (Oswald et al., 2007) or with dopamine turnover as measured with [18F]fluorodopa (Laakso et al., 2003). Amphetamine-induced dopamine release in the ventral basal ganglia accounted for 9-20% of the variance in NEO impulsiveness scores in a healthy sample otherwise similar to the one studied in the present report (Oswald et al., 2007).

Because the study sample was restricted to males to reduce experimental complexity, additional questions remain that will need to be addressed in subsequent work. Effects of gender, gonadal steroids and age by gender interactions have been described for the μ-opioid receptors and stress-induced μ-opioid system activation (Zubieta et al., 1999; Zubieta et al., 2002; Smith et al., 2006). These effects may or may not be related to IMP and DBL traits and will require specific studies addressing their effects. From a different perspective, impulsive behavior has been suggested to be a result of prefrontal cortex dysfunction. For instance, Bechara and others reported problems with decision making, specifically insensitivity to future consequences, following damage to the ventromedial prefrontal cortex (Bechara et al., 1994; Bechara et al., 1996). The relationship between ventral prefrontal cortex function and endogenous opioid system activity measures are presently unexplored.

It is also unlikely that complex personality domains are solely related to a single neurotransmitter system. Indeed, DA D2/3 receptor concentration within the ventral basal

ganglia has been demonstrated to predict impulsive anticipatory responses to food reward in an animal model of impulsivity (Dalley et al., 2007) . The involvement of dopaminergic mechanisms, however, is not exclusionary of the involvement of other systems, such as the endogenous opioid. DA-opioid interactions have been described in the striatopallidal pathway and interconnected regions, where acute and chronic DA receptor stimulation induce opposite effects on the functional capacity of the μ-opioid system in animal models (George and Kertesz, 1987; Unterwald et al., 1989; Unterwald et al., 1992; Chen et al., 1993; Unterwald et al., 1994; Steiner and Gerfen, 1998) and in humans (Zubieta et al., 1996; Zubieta et al., 2003). These and other, not yet described neurotransmitter systems may underlie the psychophysical differences, such as heart rate and pupilary responses, classically noted between otherwise healthy shy and uninhibited children (Kagan et al., 1988).

The present study provides the first evidence in humans that IMP and DBL, behavioral facets relevant to motivated behavior, the pursuit of reward and risk taking, including the development of substance use disorders, are related to the individual function of the endogenous opioid system. Baseline measures of μ-opioid receptor availability and the capacity to activate this neurotransmitter system in limbic and paralimbic regions accounted for up to half of the variance in trait IMP and DBL scores in a healthy sample.

Chapter III

Sex-Environment Interaction in the Dopaminergic Response to a Pain Stressor[3]

There is considerable variation in how a given individual responds to stress. The factors which influence stress-responsiveness are still being uncovered, however, some evidence suggests that life experiences, like previous exposure to stressful environmental situations, and gender can alter acute stress responses. For example, a recent meta-analysis indicated that exposure to life stress is generally associated with poorer recovery of cardiovascular reactions following an acute stressor (Chida and Hamer, 2008). In addition, high levels of life stress can also induce reductions in immunological reactivity to an acute stressor (e.g. (Brosschot et al., 1994)).

Much evidence suggests that, in general, women and men respond differently to stress. There are well documented sex differences in the prevalence of stress-related disorders such as major depression, posttraumatic stress disorder, and idiopathic pain syndromes such as fibromyalgia and temporomandibular pain disorder (Bush et al., 1993; Kessler et al., 1995; Kornstein, 1997; Yunus, 2001); symptoms of which are often predated and exacerbated by stressful life events (e.g. (McGonagle and Kessler, 1990; Hammen et al., 1992; Kessler, 1997; Dancey et al., 1998; Monroe et al., 2001; Muscatell et al., 2009)). In addition, rates of substance use and dependence vary between men and women which is also heavily moderated by stress (Sinha, 2008; SAMHSA, 2009).

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[3] This work reflects collaboration with Dr. Jon-Kar Zubieta, Dr. Robert Koeppe and Dr. Jill Becker at the University of Michigan and Dr. Christian Stohler at the University of Maryland.

Relative to men, women progress from initiation to addiction faster than men and enroll into treatment programs sooner (Haas and Peters, 2000; Hernandez-Avila et al., 2004; Becker et al., 2007))

Acute responses to stress can differ between men and women; in terms of responses to a painful stressor, men report lower pain ratings and have higher pain thresholds relative to women given the same stimulus (see (Fillingim, 2000). These sex differences, however, have been shown to be impacted by environmental conditions, such as exposure to stress. For example, in animals, exposure to repeated restraint sessions has been shown to reduce acute tail flick latency in male but not female rats (Gamaro et al., 1998). In humans, exposure to sedentary competitive stress prior to cold pressor stress produces analgesia in men but not women (Sternberg et al., 2001). The biological origins of such sex-specific differences have just begun to be revealed but it appears that at the neurochemical interface of stress and sex lays dopamine.

Dopamine, well known for its role in the encoding and modulation of motivated behavior and reward is being increasingly recognized as a player in responses to negative events such as stress and pain (Thierry et al., 1976; Pruessner et al., 2004; Scott et al., 2006). There is great variation in dopaminergic responses to such negative stimuli and some evidence indicates that such responses are influenced by both biological and environmental conditions including sex and life stress (Pruessner et al., 2004; Scott et al., 2006). Recent stressors in animals appear to enhance DA release from the nucleus accumbens and promote psychostimulant drug self-administration in animals (Piazza and Le Moal, 1997). Similarly, individuals experiencing early life stress express an enhanced

dopaminergic response within the ventral striatum in response to a psychosocial stressor (Pruessner et al., 2004).

In terms of sex, in animals, intact female rats show greater DA release within the nucleus accumbens relative to male rats in response to ethanol (Blanchard et al., 1993) and ovariectomized females show enhanced amphetamine-induced DA release in the striatum relative to castrated males (Castner et al., 1993). Human studies have been more equivocal. Greater release of DA has been shown in a small sample of healthy females in response to the psychostimulant amphetamine using positron emission tomography (PET) and the radiotracer [11C] fallypride, localized in the globus pallidus and inferior frontal gyrus (Riccardi et al., 2006a). Munro and collaborators however showed evidence of greater DA release in response to the same challenge in the ventral striatum of males, using PET and [11C] raclopride (Munro et al., 2006). The relatively small samples sizes in the female groups and lack of standardization of menstrual cycle phase may have contributed to this apparent discrepancy in results. Another explanation for these inconsistencies is the interaction of a third uncontrolled variable on dopamine release, such as exposure to life stress (e.g. (Pruessner et al., 2004; Oswald et al., 2007)).

To examine this possibility further, we examined sex differences in the availability of DA D2/3 receptors during a physical and emotional stressor, moderate levels of sustained pain. Prior to scanning, participants were asked to complete a survey, which detailed significant life stressors that may or may not have occurred during the last year. Participants were then segregated into two groups: Recent Life Stress (RLS), which included individuals that reported having experienced some form of significant life stress in the past year, and No Recent Life Stress (NoRLS), where the participants did not detail

any recent life stressors. Analyses were performed to determine the impact of sex and life stress on dopaminergic activity measured using [11C] raclopride PET.

Previous work from our lab has indicated that use of a non-pharmacological stress challenge can induce DA release in both dorsal and ventral basal ganglia regions (Scott et al., 2006). This study sought to extend those previous findings and determine whether sex differences exist in these dopaminergic responses and whether recent life events can make a significant impact. In view of the basic research literature reviewed above, it was expected that DA release would be more pronounced in women than in men during an otherwise comparable stressor and that exposure to recent life stress would influence this response.

Materials and Methods

Subjects

Twenty-two healthy right-handed men (age mean ± SD, 25.7 ± 3.3 years) and twenty-two healthy right-handed women (age mean ± SD, 27.5 ± 6.1 years) were recruited via advertisement. In addition to complete physical and neurological examinations, subjects were screened using the Structured Clinical Interview for DSM-IV (non-patient version, SCID-NP) and had no current or past history of medical, neurological, or psychiatric illness, including substance abuse or dependence. Subjects were not taking any psychotropic medications or hormone treatments, including birth control in women, for at least six months and were non-smokers. Subjects did not exercise in excess of 1 hour three times a week nor were they involved in competitive exercise. All women were studied in the follicular phase of the menstrual cycle,

confirmed with plasma levels of estradiol and progesterone prior to scanning. Protocols were approved by the University of Michigan Investigational Review Board and the Subcommittee for Human Use of Radioisotopes (Ann Arbor, MI). Written informed consent was obtained in all subjects.

Life Stressors

All subjects completed the Health and Daily Living Form. The indices of life change events section, which asks individuals to indicate which if any stressful events recently occurred in their lives and when was used to provide a profile of an individual's life stressors over the last 12 months. We chose to focus on negative life events as these are more strongly associated with problem behavior (i.e. substance abuse) and impaired functioning (i.e. development of depressive episodes, interpersonal problems) than positive life changes (Sarason et al., 1978; Billings and Moos, 1981). Typical significant negative life events included: divorce, death of a spouse, loss of job, etc. In this sample of healthy subjects the number of stressors per individual was low, not allowing for the number of stressful life events to be effectively considered as a variable in the analyses. Further detail is included in the Results section.

Study Protocol

Each subject underwent a 90 minute PET scan with [^{11}C] raclopride, a DA radiotracer with affinity for both D2 and D3 receptors (Seeman et al., 2006). A portion of this sample was previously studied for pain-stress effects on dopaminergic activity (Scott et al., 2006) and details regarding study criteria have been given elsewhere (Scott et al.,

2006). Briefly, we employed a universal physical and emotional stressor, moderate levels of sustained pain, which has been shown to activate DA neurotransmission in the basal ganglia (Scott et al., 2006). A steady state of muscle pain was maintained over 20 min from 45-65 min post-tracer administration. This took place through a computer-controlled infusion of medication-grade hypertonic saline (5%) administered into the left masseter muscle. Pain intensity was rated every 15 seconds from 0 (no pain) to 100 (most intense pain imaginable) using an electronic version of 100mm visual analog scale (VAS). The individual ratings of pain intensity were employed by the computer controller to maintain constant pain in a manner comparable across subjects using a target of 40 VAS units, as previous described (Zhang et al., 1993; Stohler and Kowalski, 1999). Upon completion of the pain challenge, integrative measures of the experience were obtained using the McGill pain questionnaire (MPQ) (Melzack and Torgerson, 1971) and the Positive and Negative Affectivity Scale (PANAS) (Watson et al., 1988), the latter assessing the internal affective state of the participants. Preceding the pain stress challenge, subjects underwent a baseline control condition during which there was no expectation of pain and no infusions took place.

Neuroimaging methods

PET scans were acquired with a Siemens (Knoxville, TN) HR+ scanner in 3-D mode (reconstructed full-width at half maximum (FWHM) resolution (~5.5 mm in-plane and 5.0 mm axially). Radiotracer synthesis and image acquisition, coregistration and reconstruction protocols were identical to those used in previous publications (e.g. (Scott et al., 2006)). Briefly, images were reconstructed using iterative algorithms into a 128 x

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128 pixel matrix in a 28.8 cm diameter field of view. Attenuation correction was performed through a 6 min transmission scan ([68]Ge source) obtained before the PET study. Small head motions during emission scans were corrected by an automated computer algorithm for each subject before analysis, and the images were coregistered to each other with the same software (Minoshima et al., 1993). Time points were then decay-corrected during reconstruction of the PET data.

The total activity of [^{11}C] raclopride administered to each subject was 15.4 ± 1.2 mCi. Fifty percent of the [^{11}C] raclopride dose was administered as a bolus with the remainder delivered as a continuous infusion by a computer-controlled automated pump to more rapidly achieve steady-state tracer levels. Under these conditions, equilibrium conditions across kinetic compartments are achieved approximately 35 min after tracer administration (Carson et al., 1997).

Twenty-eight image frames were acquired over 90 minutes with an increasing duration (30 seconds up to 10 minutes) and were coregistered to each other (Minoshima et al., 1993). Dynamic image data for each of the receptor scans were transformed, on a voxel-by-voxel basis, into two sets of parametric maps, coregistered to each other: (a) a tracer transport measure (K_1 ratio), and (b) a receptor-related measure at equilibrium (DVeq), the latter using data obtained from 35-45 min (control) or 60–80 min (pain stress) after tracer administration. DVeq -1 is proportional to B_{max}/K_d, or binding potential at equilibrium (BPeq). This measure was obtained using the ratio of brain activity to activity in the cerebellum (Carson et al., 1997; Watabe et al., 2000).

MRI scans were acquired on a 3 Tesla scanner (General Electric, Milwaukee, WI) for anatomical localization and coregistration to standardized stereotactic coordinates.

Acquisition sequences were axial spoiled gradient-recalled (SPGR) [echo time (TE), 3.4 ms; repetition time (TR), 10.5; inversion time, 200 ms; flip angle, 25°; number of excitations (NEX), 1; 124 contiguous images; 1.5 mm thickness].

T1-weighted MR and PET images of each subject were then coregistered to each other using a mutual information algorithm as previously described (Meyer et al., 1997). For this purpose, K_1 images (containing anatomical information similar to that of regional cerebral blood flow scans) were first aligned to the MR, and the transformation matrix applied to the co-registered DVR scans of the same series. The MR scans were then anatomically standardized to ICBM brain atlas stereotactic coordinates by linear and non-linear warping, and the resulting transformation matrix applied to K_1 and DVR images (Meyer et al., 1997; Meyer et al., 1998). The accuracy of coregistration and warping algorithms were confirmed for each subject individually by comparing the transformed MRI and PET images to each other and the ICBM atlas template.

Image Analyses

Activation of DA D2/D3 neurotransmission is detected as a reduction in BPeq (i.e. lower levels of *in vivo* DA D2/3 receptor availability during the challenge. This was calculated as the difference between the control state and the activated, pain stress state. Differences between groups for DA activation were mapped into stereotactic space using *F* maps of statistical significance with SPM2 (Welcome Department of Cognitive Neurology, University College, London) and Matlab software (MathWorks, Natick, MA), using a general linear model and correction for multiple comparisons (Friston et al., 1995). No global normalization was applied to the data, and therefore the calculations

presented are based on absolute B_{max}/K_d estimates. Only regions with specific DA D2/D3 receptor binding were included in the analyses (voxels with BPeq values > 0.2). To compensate for small residual anatomic variations across subjects and to improve signal to noise ratios, a three-dimensional Gaussian filter (FWHM 6 mm) was applied to each scan. Voxels of interest were isolated for activation using effects of interest contrasts (i.e. Sex, RLS, Sex x RLS) in a between subjects ANOVA. Significant differences were detected using a statistical threshold that controls for a Type-I error rate at p = 0.05 for multiple comparisons. This was estimated using the Euler characteristic (Worsley, 1994) based on the number of voxels in the gray matter and image smoothness (Friston et al., 1991), corrected for the size of the cluster under consideration (Friston et al., 1994). Numerical values for each region were obtained by averaging the values of voxels contained in each significant cluster up to a p<0.01 level. These data were extracted for quantification of regional changes in BP, rule out the presence of outliers, and examined using further statistical analyses using SPSS for Macintosh 11.0.3 (SPSS Inc., Chicago, IL). To determine group differences post hoc testing was performed for each of the effects of interest (Female x Male, RLS x NoRLS, Female RLS x Female NoRLS, Male RLS x Male NoRLS, Female NoRLS x Male NoRLS, Female RLS x Male RLS). To compensate for multiple comparisons, the p value threshold used was adjusted using the Bonferroni method from the standard 0.05 to 0.008.

Results

No significant differences between sex groups were obtained in psychophysical measures of pain or affective state during the experientially-matched stressor (Table 5). Twenty-six individuals reported having at least one significant negative life event in the

past year (12 men, 14 women). No significant sex differences in the number of stressors were noted: 2.0 ± 1.1 life stressors were reported in males and 1.6 ± 1.0 in females (two-tailed unpaired t-tests, p=0.41).

Table 5 Psychophysical Measures by Sex				
	Male (n=22)	**Female (n=22)**	**t**	**p**
McGill Pain Intensity	40 ± 2	42 ± 3	-0.56	0.58
McGill Pain Unpleasantness	43 ± 2.6	43 ± 4	-0.15	0.88
Δ PANAS Positive Affect	-5.6 ± 1.2	-5.6 ± 1.3	-0.02	0.98
Δ PANAS Negative Affect	-0.4 ± 2.1	-1.0 ± 0.7	0.28	0.78
Average VAS/Total Volume	1.55 ± 0.26	1.55 ± 0.39	-0.01	0.99
Total Volume 5% saline (μL)	2806 ± 241	3159 ± 278	-0.96	0.34

Table 5. Psychophysical Measures by Sex. Data are shown as the mean $\pm$ 1 S.E.

Stress-Induced Activation of DA Neurotransmission

Using an ANOVA to examine the effects of both sex and RLS, significant sex effects were obtained within the nucleus accumbens, bilaterally, left caudate and left putamen (Table 6 & Table 7). Post-hoc testing revealed that for the individuals that did not report any recent life stressors, females demonstrated significantly greater stress-induced activation of DA neurotransmission compared to males in all regions (Table 6 & Table 7).

No significant effects of recent life events (presence/absence) were observed on regional DA activation for the entire sample.

A significant sex by life events interaction was also noted within the left nucleus accumbens (Table 6 & Table 7). In this region, the presence of recent negative life events

was associated with significantly greater DA responses to the experimental stressor in men and significantly lesser DA responses in females.
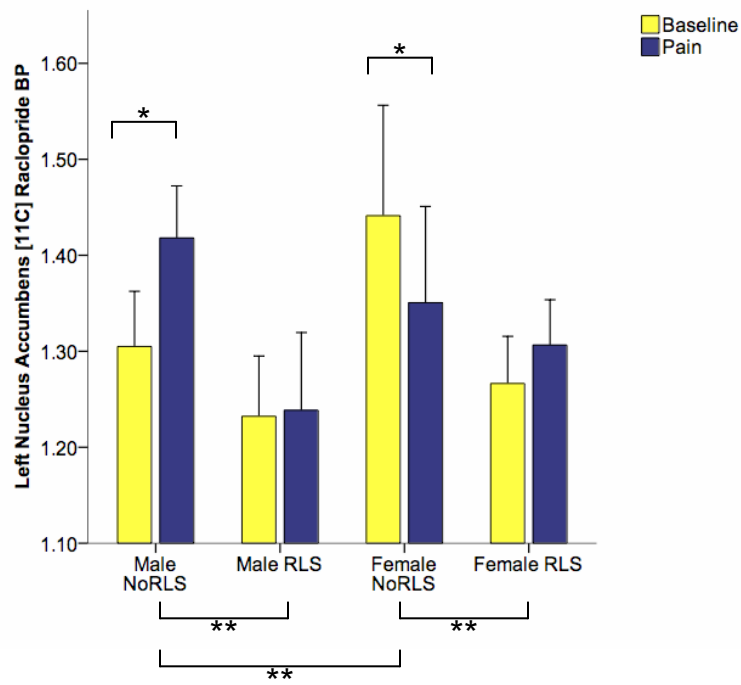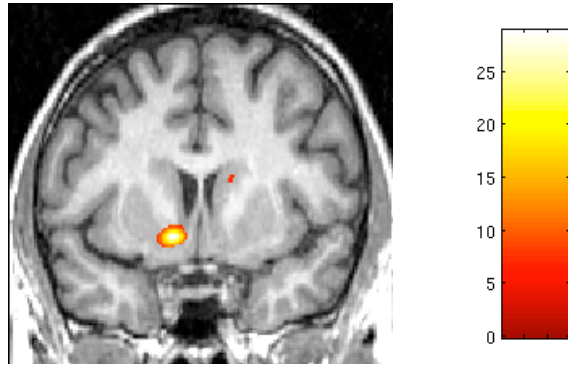
Figure 5. Sex by RLS interaction within the left nucleus accumbens. Above: Location of significant peak in Interaction contrast. Below: Graph depicting binding during the control condition and during the challenge. * Indicates significant differences between Control and Pain conditions as determined by paired t-tests (Male NoRLS, $p<0.0001$, Female NoRLS $p=0.007$). **Indicates significant differences between groups, values given in Table 7).

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Table 6 Differences in Stress-Induced Changes in Regional DA 2/3 Receptor BP

Region	Coordinates (x, y, z)†	Cluster Size‡	NoRLS Male	NoRLS Female	RLS Male	RLS Female	ANOVA Stats F	p
Sex Effects								
R Nucleus Accumbens extending into R Putamen	15, 18, -6	6806	-0.08 ± 0.03	0.13 ± 0.03	-0.02 ± 0.03	0.06 ± 0.03	21.79	0.013
L Nucleus Accumbens	-11, 9, -5	846	-0.12 ± 0.02	0.10 ± 0.03	-0.03 ± 0.03	0.00 ± -0.06	23.47	0.013
L Caudate	-15, 12, 9	708	-0.07 ± 0.04	0.14 ± 0.05	-0.01 ± 0.03	0.06 ± 0.01	19.75	0.013
L Putamen	-31, 1, -2	749	-0.11 ± 0.03	0.15 ± 0.05	-0.03 ± 0.04	0.04 ± -0.03	20.79	0.013
Sex x Stress Interaction								
L Nucleus Accumbens	-11, 8, -9	1160	-0.11 ± 0.02	0.09 ± 0.02	-0.01 ± 0.03	-0.04 ± -0.08	28.83	0.015

Table 6 Differences in Stress-Induced Changes in Regional DA 2/3 Receptor BP. Data are shown as the mean ± S.E. † Coordinates (x,y,z) refer to ICBM coordinates, in mm. ‡ Cluster size is expressed in mm^3. * Change represents the mean difference in regional DA 2/3 binding potential between control and stress conditions ** All regions significant after FDR correction for multiple comparisons. R = right, L = left.

Table 7. Post Hoc Statistics – Stress-Induced Changes in Regional DA 2/3 Receptor BP

Region	Male x Female	RLS x NoRLS	Male NoRLS x Female NoRLS	Male RLS x Female RLS	Male RLS x NoRLS	Female RLS x RLS
Sex Effects						
R Nucleus Accumbens extending into R Putamen	0.000*	0.899	0.000*	0.067	0.172	0.098
L Nucleus Accumbens	0.001*	0.853	0.000*	0.443	0.025	0.018
L Caudate	0.000*	0.990	0.004*	0.048	0.283	0.100
L Putamen	0.000*	0.853	0.000*	0.169	0.095	0.089
Sex x Stress Interaction						
L Nucleus Accumbens	---------	-----------	0.000*	0.368	0.008*	0.001*

Table 7. Post Hoc Statistics – Stress-Induced Changes in Regional DA 2/3 Receptor BP. Data are shown as p values. *Significant following Bonferroni correction of multiple comparisons (see Methods).

Psychophysical Measures

Examining men and women separately, we observed a positive relationship between the change in negative affect and DA response within the right nucleus accumbens (r=0.466, p=0.029), left caudate (r=0.537, p=0.010), and left putamen (r=0.452, p=0.035) in women. In men, however, we observed a negative relationship between the change in negative affect and DA response within the left (r=-0.427, p=0.048) and right nucleus accumbens (r=-0.423, p=0.050), and left putamen (r=-0.442, p=0.040). No other relationships were observed between DA responses and any other psychophysical measures (i.e. McGill, PANAS, Infusion).

Hormonal Measures

No relationships between estradiol, progesterone or testosterone levels and the degree of stress-induced DA response were noted in females or males.

Discussion

This is the first study to demonstrate sex differences in DA activity in response to a painful stress challenge in healthy humans. In addition, we observed an interaction between sex and recent negative life events for stress-induced DA release within the left nucleus accumbens. Positive relationships were found between activation of DA neurotransmission within dorsal and ventral striatal regions and the change in negative affect ratings in women; a similar relationship was observed for men, albeit in the negative direction.

Previous investigations indicated that dopamine neurotransmission is engaged in response to the pain-stress challenge employed here (Scott et al., 2006). Other investigations examining humans have also described increased dopamine release within the striatum as a result of psychological stress (Pruessner et al., 2004). However, sex differences in such dopaminergic responsivity have only received modest attention.

In our sample, women exhibited greater changes in dopaminergic activity within the caudate, putamen and ventral striatum relative to men. The enhanced activity seen within women relative to men may suggest that the dopamine system is more active in females and may be influential in their modulation of stress and pain. This is interesting considering that μ-opioid system mechanisms are thought to play a greater role in mediating pain and stress in men than it does within women during their follicular phase (Zubieta et al., 2002). We did not observe any relationships between any of the gonadal hormone measures and dopaminergic activity however this may be due to our exclusion of women in their luteal phase. Further research is needed to determine if the sex effects on stress-induced dopaminergic neurotransmission are modulated by the menstrual cycle.

We noted a significant positive relationship between negative affective state and stress-induced dopaminergic activity in women; conversely a negative relationship was observed in men. The positive relationship between negative affective state and dopaminergic system activation in females is consistent with previous observations indicating positive correlations with emotional ratings and dopaminergic activity within ventral basal ganglia (Scott et al., 2006). The negative correlation observed in the male sample is somewhat perplexing. However, in this group of subjects, males tended to exhibit decreases in dopaminergic activity in the areas where these negative correlations

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were observed whereas females tended to show increases (and positive correlations with negative affect) in these same regions. It appears that greater stress-induced changes in dopaminergic activity relative to our control condition, whether positive or negative, were associated with greater emotional distress in both groups.

In addition to the sex differences in stress-induced dopamine activity, we also observed a significant interaction between sex and RLS. We found that relative to men and women who did not report RLS in the past year, the exposure to RLS was associated with significantly greater DA responses to the experimental stressor in men but significantly lesser DA activation in females. The activity between the control condition and pain-stress condition in both RLS males and RLS females was not observed to be significantly different in either sex. These data seem to indicate an overall blunting of the dopaminergic response in men and women, albeit in different directions. Indeed, previous reports have indicated exposure to chronic stress can lead to inhibition of dopaminergic responses to amphetamine in humans (Oswald et al., 2007). This is somewhat contrary to Pruessner and colleagues work, demonstrating exaggerated dopaminergic responses in individuals exposed to early life stress (Pruessner et al., 2004). However, in predicting dopaminergic neurotransmission alterations, the nature of the stressor to which the individual is exposed is significant. Though certain stressors can potentiate dopaminergic activity, others can lead to blunted responses (Kalivas and Duffy, 1989; Mangiavacchi et al., 2001; Lucas et al., 2004). Unfortunately, the measure utilized to determine presence of RLS in our subjects does not record how severe or distressing the subjects' perceived the stress to be. It is possible that some of the

differences observed could be a result of sex differences in the perception of controllability or severity. Future studies will be needed to parse out these effects.

In sum, we observe extensive dopaminergic activation differences in response to a painful stressor between men and women, with women showing greater dopaminergic responses in most regions. We find that while sex clearly influences dopaminergic responses to stress, outside influences, such as the stressful life experiences, can be a mediating factor.

Chapter IV

Polymorphisms in Oxytocin Receptor Impact Dopaminergic Responses in Humans in a Sex Dependent Manner: a C[11] Raclopride Study[4]

Dopamine is released in response to both psychological and physiological stressors (e.g. (Thierry et al., 1976; Rouge-Pont et al., 1998; Scott et al., 2006). The magnitude of stress-induced dopamine release varies greatly from individual to individual (Scott et al., 2006). The sources of this variability are likely many, however a portion of the variance is predicted to be the result of heredity. Strain differences in the mesocorticolimbic dopamine response to a forced swim test have been noted in mice whereby the mice of a hypoactive phenotype (DBA/2) show enhanced stress-induced mesoaccumbal DA release relative to their more active counterpart (C57BL/6) (see review (Cabib et al., 2002)). In rats, novelty-seeking phenotypes classified as high responders (HR) or low responders (LR) on the basis of their locomotor response to novelty, a mild stressor, which show differential dopamine responses in response to stress (Rouge-Pont et al., 1998) can be selectively bred for this trait (Stead et al., 2006). Little work has been done to this end in humans, however, given dopamine's key role in psychopathologies, such as addiction, schizophrenia, and depression, highlighting

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[4] This work reflects extensive collaboration with Dr. David Goldman, Dr. Mary-Anne Enoch, and Dr. Colin Hodgkinson at the National Institute of Alcohol Abuse and Alcoholism, Dr. Christian Stohler at the University of Maryland, and Dr. Jon-Kar Zubieta, Dr. Margit Burmeister and Ellen Schmidt at the University of Michigan.

potential gene targets which can impact dopaminergic responsiveness is of significant value.

Recently, several groups have highlighted the important role the neuropeptide oxytocin plays in modulating the stress response. Oxytocin, a nonapeptide synthesized primarily within the hypothalamus, though first described for its ability to stimulate milk-ejection during breastfeeding and smooth muscle contraction during labor (Dale, 1906; Ott and Scott, 1910), appears to have much broader effects including influencing HPA axis activity in response to stress. Oxytocin is released from the pituitary into peripheral circulation in response to a variety of stimuli including physical stressors (e.g. Morris water maze (Engelmann et al., 2006), forced swimming (Lang et al., 1983; Kasting, 1988)) and psychological stressors (e.g. shaker stress (Hashiguchi et al., 1997; Nishioka et al., 1998) and centrally (e.g. forced swimming (Wotjak et al., 1998), shaker stress (Hashiguchi et al., 1997; Nishioka et al., 1998), osmotic stress (see (Jezova et al., 1995)). Multiple lines of evidence suggest oxytocin has anxiolytic and anti-stress effects, for instance acute intracerebral infusion of oxytocin reduces distress vocalization in rat pups (Insel and Winslow, 1991), increases punished crossings in the four plate test (Ring et al., 2006) and increases time spent in the open quadrants of the elevated zero maze (Ring et al., 2006). In addition, a chronic infusion of oxytocin dose dependently lowers plasma corticosterone concentrations following noise stress in ovariectomized female rats (Windle et al., 1997). Also, intracerebral infusion of oxytocin antagonist results in elevated stress-induced ACTH and corticosterone concentrations in both male and female rats (e.g. elevated plus maze (Neumann et al., 2000)). Similarly in humans, administration of oxytocin intranasally also been shown to reduce cortisol responses to

psychosocial stressors (Heinrichs et al., 2003) and couple conflict discussions (Ditzen et al., 2009).

In addition to modulating HPA axis responsiveness, oxytocin is also capable of affecting central nervous system activity, including central dopaminergic functioning. Oxytocin and its binding sites exist in regions outside of the hypothalamus; in rats and primates these regions include the septum, amygdala, ventral tegmental area, nucleus accumbens, and various nuclei within the brainstem (primate: (Fliers et al., 1986; Caffé et al., 1989; Loup et al., 1991), rat: (Brinton et al., 1984; Van Leeuwen et al., 1985; Krémarik et al., 1993)). Injections of oxytocin into the ventral tegmental area, which contains the cell bodies of the dopaminergic neurons which makeup the core of the mesolimbic dopamine system, results in increases in extracellular dopamine within the nucleus accumbens and hypothalamus (Melis et al., 2007). In addition, peripheral and intracerebral ventricular oxytocin administration can affect dopamine utilization within the mesencephalon (e.g. ventral tegmental area, substantia nigra), hypothalamus and striatum (see review (Sarnyai and Kovács, 1994)). Oxytocin administration in rats can also alter central dopaminergic responses associated with a variety of behaviors, such as addictive behaviors (e.g. self-administration, tolerance and dependence) and stress (see (Kovács et al., 1984; Ibragimov et al., 1987; Sarnyai and Kovács, 1994)).

In sum, oxytocin is released in response to stress and is capable of affecting both stress and dopaminergic responsiveness. What effect genetics has on oxytocin's influence on these activities has yet to be studied. Here, we examined genotype in relation to changes in healthy control subjects' dopaminergic response to a physical and psychological stressor, pain, using Positron Emission Tomography (PET). Genome wide

scans previously indicated several common polymorphisms near the oxytocin gene

(Hodgkinson et al., 2008). Blood was taken from the participants and used to genotype

for each of the previously identified oxytocin single nucleotide polymorphisms (SNPs).

We then examined whether these polymorphisms were associated with altered stress-

induced dopaminergic responsiveness among our participants.

Materials and Methods

Subjects

Thirty-two healthy right-handed women (age, mean ± SD, 26.97 ± 6.08) and

twenty-three healthy right-handed men (age, mean ± SD, 25.74 ± 3.19) were recruited via

advertisement. A portion of this sample was previously studied for pain-stress effects on

dopaminergic activity and details regarding study criteria have been given elsewhere

(Scott et al., 2006). Briefly, subjects were screened using Structured Clinical Interview

for DSM-IV (non-patient version, SCID-NP) and had no current or past history of

medical, neurological, or psychiatric illness, including substance abuse or dependence.

Subjects were not taking any psychotropic medications or hormone treatments, including

birth control in women, for at least six months and were non-smokers. Protocols were

approved by the University of Michigan Investigational Review Board and the

Subcommittee for Human Use of Radioisotopes (Ann Arbor, MI). Written informed

consent was obtained in all subjects.

Genotyping

DNA was extracted from blood samples taken at the time of scanning. A genomic region containing sequence 5 kb upstream and 1 kb downstream of *OXT*, the oxytocin gene mapped to chromosome 20p13, was retrieved from NCBI Human Build 35.1. Tagging SNPs were identified using a previously described design pipeline (Hodgkinson et al., 2008). Four tagging SNPs (rs4813625, rs877172, rs3761248, rs2740210), located in noncoding regions just upstream/downstream to *OXT*, were genotyped using the Illumina GoldenGate platform (Hodgkinson et al., 2008). Genotype frequencies and base changes are shown in Table 8. Some genetic data was not available due to blood collection issues during the study (e.g. bad veins) or due to poor DNA quality; final sample sizes are listed in Table 8.

Study Protocol

Given oxytocin's anxiolytic effects and as changes in oxytocin plasma have been previously shown to be related to attachment anxiety (Turner et al., 1999; Turner et al., 2002; Marazziti et al., 2006), subjects were administered the Adult Attachment Questionnaire (Collins and Read, 1990) prior to scanning.

Each subject underwent a 90 minute PET scan with [^{11}C] raclopride, a DA radiotracer with affinity for both D2 and D3 receptors (Seeman et al., 2006). We employed a universal physical and emotional stressor, moderate levels of sustained pain, which has been shown to activate DA neurotransmission in the basal ganglia (Scott et al., 2006). A steady state of muscle pain was maintained over 20 min from 45-65 min post-tracer administration. This took place through a computer-controlled infusion of medication-grade hypertonic saline (5%) administered into the left masseter muscle. Pain

intensity was rated every 15 seconds from 0 (no pain) to 100 (most intense pain imaginable) using an electronic version of 100mm visual analog scale (VAS). The individual ratings of pain intensity were employed by the computer controller to maintain constant pain in a manner comparable across subjects using a target of 40 VAS units, as previous described (Zhang et al., 1993; Stohler and Kowalski, 1999). Upon completion of the pain challenge, integrative measures of the experience were obtained using the McGill pain questionnaire (MPQ) (Melzack and Torgerson, 1971) and the Positive and Negative Affectivity Scale (PANAS) (Watson et al., 1988), the latter assessing the internal affective state of the participants. Preceding the pain stress challenge, subjects underwent a baseline control condition during which there was no expectation of pain and no infusions took place. At the time of scanning, blood was taken to determine hormone levels (i.e. estrogen, progesterone, and testosterone).

Neuroimaging methods

PET scans were acquired with a Siemens (Knoxville, TN) HR+ scanner in 3-D mode (reconstructed full-width at half maximum (FWHM) resolution (~5.5 mm in-plane and 5.0 mm axially). Radiotracer synthesis and image acquisition, coregistration and reconstruction protocols were identical to those used in previous publications (Scott et al., 2006). Briefly, images were reconstructed using iterative algorithms into a 128 x 128 pixel matrix in a 28.8 cm diameter field of view. Attenuation correction was performed through a 6 min transmission scan (^{68}Ge source) obtained before the PET study. Small head motions during emission scans were corrected by an automated computer algorithm for each subject before analysis, and the images were coregistered to each other with the

same software (Minoshima et al., 1993). Time points were then decay-corrected during reconstruction of the PET data.

The total activity of $[^{11}C]$ raclopride administered to each subject was 15.0 ± 2.2 mCi. Fifty percent of the $[^{11}C]$ raclopride dose was administered as a bolus with the remainder delivered as a continuous infusion by a computer-controlled automated pump to more rapidly achieve steady-state tracer levels. Under these conditions, equilibrium conditions across kinetic compartments are achieved approximately 35 min after tracer administration (Carson et al., 1997).

Twenty-eight image frames were acquired over 90 minutes with an increasing duration (30 seconds up to 10 minutes) and were coregistered to each other (Minoshima et al., 1993). Dynamic image data for each of the receptor scans were transformed, on a voxel-by-voxel basis, into two sets of parametric maps, coregistered to each other: (a) a tracer transport measure (K_1 ratio), and (b) a receptor-related measure at equilibrium (DVeq), the latter using data obtained from 35-45 min (control) or 60–80 min (pain stress) after tracer administration. DVeq -1 is proportional to B_{max}/K_d, or binding potential at equilibrium (BPeq). This measure was obtained using the ratio of brain activity to activity in the cerebellum (Carson et al., 1997; Watabe et al., 2000).

MRI scans were acquired on a 3 Tesla scanner (General Electric, Milwaukee, WI) for anatomical localization and coregistration to standardized stereotactic coordinates. Acquisition sequences were axial spoiled gradient-recalled (SPGR) [echo time (TE), 3.4 ms; repetition time (TR), 10.5; inversion time, 200 ms; flip angle, 25°; number of excitations (NEX), 1; 124 contiguous images; 1.5 mm thickness].

T1-weighted MR and PET images of each subject were then coregistered to each other using a mutual information algorithm as previously described (Meyer et al., 1997). For this purpose, K_1 images (containing anatomical information similar to that of regional cerebral blood flow scans) were first aligned to the MR, and the transformation matrix applied to the co-registered DVR scans of the same series. The MR scans were then anatomically standardized to ICBM brain atlas stereotactic coordinates by linear and non-linear warping, and the resulting transformation matrix applied to K_1 and DVR images (Meyer et al., 1997; Meyer et al., 1998). The accuracy of coregistration and warping algorithms were confirmed for each subject individually by comparing the transformed MRI and PET images to each other and the ICBM atlas template.

Image Analyses

Activation of DA D2/D3 neurotransmission is detected as a reduction in BPeq (i.e. lower levels of *in vivo* DA D2/3 receptor availability during the challenge). This was calculated as the difference between the control state and the activated, pain stress state. Differences between groups for DA activation were mapped into stereotactic space using *F* maps of statistical significance with SPM2 (Welcome Department of Cognitive Neurology, University College, London) and Matlab software (MathWorks, Natick, MA), using a general linear model and correction for multiple comparisons (Friston et al., 1995). No global normalization was applied to the data, and therefore the calculations presented are based on absolute B_{max}/K_d estimates. Only regions with specific DA D2/D3 receptor binding were included in the analyses (voxels with BPeq values > 0.2). To compensate for small residual anatomic variations across subjects and to improve signal

to noise ratios, a three-dimensional Gaussian filter (FWHM 6 mm) was applied to each scan.

Given the previously described sex differences in dopaminergic activity (e.g. (Munro et al., 2006; Riccardi et al., 2006a)) and the potential for sex differences in the effects of oxytocin, genotype effects were examined for males and females separately. Given the low frequency for some of the genotypes (rs2740210, TT; rs3761248, CC; rs4813625, CC; rs877172, CC), homozygotes for the rare allele and heterozygotes were combined. Voxels of interest were then isolated using two-sample t-tests. Each SNP was analyzed separately, to compensate for the four SNP comparisons the p value threshold used to detect significant differences was adjusted using the Bonferroni method from the standard 0.05 to 0.0125. This was estimated using the Euler characteristic (Worsley, 1994) based on the number of voxels in the gray matter and image smoothness (Friston et al., 1991). Numerical values for each region were obtained by averaging the values of voxels contained in each significant cluster up to a $p<0.001$ level. These data were extracted for quantification of regional changes in BP, to rule out the presence of outliers, and for further statistical analyses using SPSS for Macintosh 11.0.3 (SPSS Inc., Chicago, IL).

Results

Stress-Induced Activation of DA Neurotransmission

Two sample t-tests were employed to determine the presence of genotype differences in DA neurotransmission in males and females.

Using our conservative p value threshold of $p < 0.0125$ following FDR correction for multiple comparisons, significant differences between carriers of GG vs. GC/CC for SNP rs4813625 were noted within the right ventral striatum in our female sample (Table 8, Figure 6). Examination of the other three SNPs did not reveal any significant differences; however, effects were noted in the same region for SNPs rs3761248 and rs877172 albeit below our stringent *a priori* threshold (Table 1). The similar effects noted for these three SNPs are consistent with the finding that these SNPs appear to be in linkage disequilibrium (Figure 7). No effects were seen in the male sample.
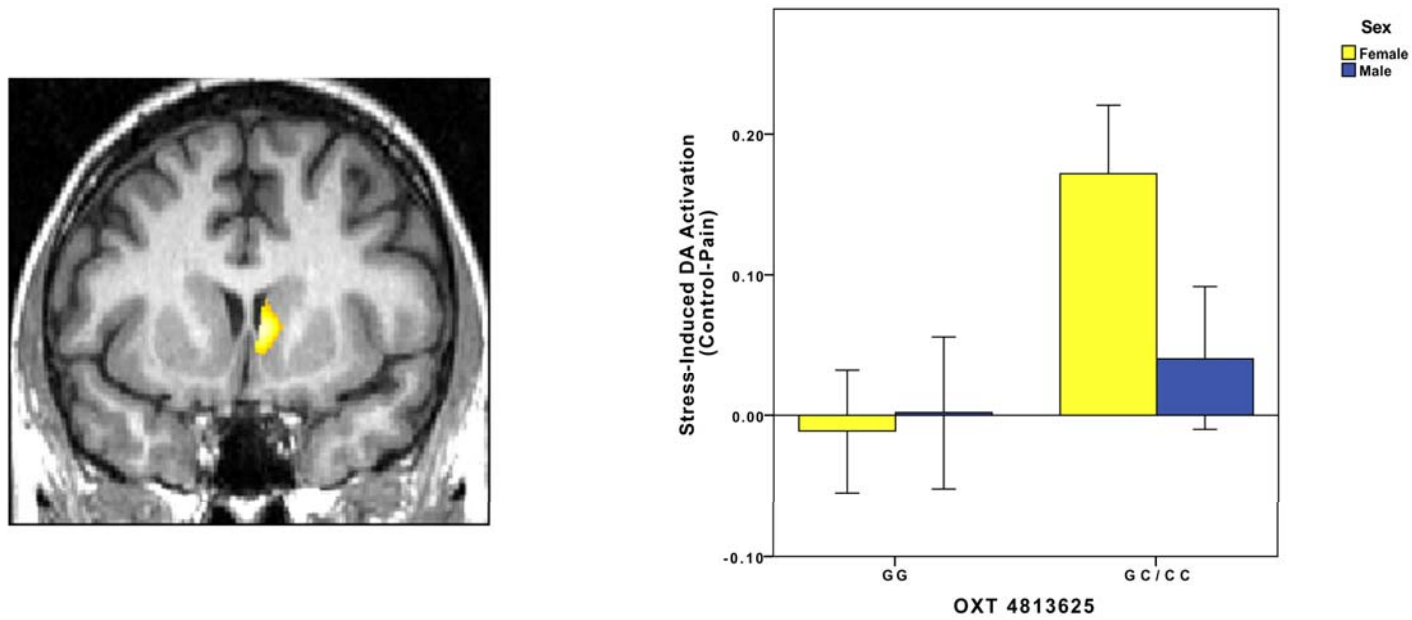
Figure 6. Impact of OXT rs4813625 on Stress-Induced DA Neurotransmission. *Left.* Results of two-sample t-test for the OXT SNP rs4813625 in the female samples showing greater release in the GC/CC group relative to the GG group within the right ventral striatum. *Right.* Bar graph of extracted data illustrating stress-induced DA activation among males and females by genotype.
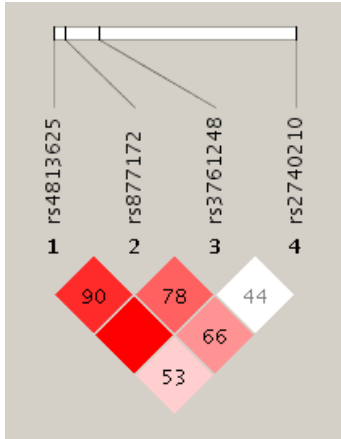
Figure 7. Linkage Disequilibrium. LD expressed as D' in the study population

Table 8. Effects of Genotype on Stress-Induced Dopaminergic Activity

SNP	Allele Info	Females				Males			
		N	x,y,z Coordinates, mm [a]	T score [b]	P [b]	n	x,y,z Coordinates, mm [a]	T score	P Value
rs2740210	G/T	n=23 (TT=4, TG=10, GG=17)	-----	-----	-----	n=17 (TT=3, TG=6, GG=13)	-----	-----	-----
rs3761248	C/T	n=24 (TT=19, TC=13, CC=0)	5, 19, 6	4.80	0.025*	n=18 (TT=14, TC=6, CC=3)	-----	-----	-----
rs4813625	C/G	n=20 (GG=10, GC=12, CC=6)	5, 15, 1	5.48	0.010**	n=18 (GG=8, GC=12, CC=2)	-----	-----	-----
rs877172	A/C	n=24 (AA=13, AC=16, CC=3)	4, 12, 2	3.32	0.001	n=18 (AA=10, AC=8, CC=5)	-----	-----	-----

Table 8. Effects of Genotype on Stress-Induced Dopaminergic Activity a. Indicates the Montreal Neurological Institute coordinates b. Calculated using 2-sample t tests for differences between genotype *P value after multiple comparisons correction (FDR) ** Significant differences detected between genotype groups after multiple comparisons correction (FDR).

Other Measures

Given the significant differences noted for the rs4813625 SNP, GG and GC/CC

carriers were compared across multiple measures using two-sample t-tests to determine if

there were any differences in demographics or their pain experience.

There were no significant differences in any of the demographic or scanning

variables tested (Table 9). In addition, no differences were observed related to menstrual

cycle in females or hormones (Table 9). Two-sample t-tests revealed significant

differences in MPQ Pain Intensity ($p = 0.048$) and MPQ Affect ($p=0.045$) between GG

and GC/CC carriers within our female sample. No other differences in psychophysical

measures were noted in either the males or females (Table 10).

Table 9. Demographics & Scanning Measures - rs4813625

	Females			Males		
	GG (n=10)	*GC/CC (n=18)*	*P Value [b]*	*GG (n=8)*	*GC/CC (n=14)*	*P Value [b]*
Age	26.2 (7.4)	26.6 (5.2)	0.865	26.5 (3.6)	25.2 (3.1)	0.386
Dose (mCi)	15.3 (1.2)	14.8 (2.9)	0.587	14.8 (3.1)	15.2 (1.2)	0.666
Testosterone (ng/mL)	62.8 (21.1)	48.7 (18.7)	0.097	601.2 (288.0)	604.0 (235.0)	0.982
Estradiol (pg/mL)	106.3 (60.4)	94.2 (71.0)	0.669	----	----	----
Progesterone (pg/mL)	4.8 (6.9)	2.8 (4.2)	0.384	----	----	----

Table 9. Demographics & Scanning Measures - rs4813625 a. Data are expressed as mean (SD). b. Calculated using 2-sample t tests for differences between genotype

Table 10. Psychophysical Measures - rs4813625

	Females			Males		
	GG (n=10)	*GC/CC (n=18)*	*P Value*	*GG (n=8)*	*GC/CC (n=14)*	*P Value*
Average VAS intensity	31 (12)	38 (13)	0.153	34 (11)	32 (9)	0.745
Total volume of hypertonic saline solution (5%)	2912 (1260)	2498 (1380)	0.440	2706 (1045)	2677 (1160)	0.955
MPQ Pain Intensity	39.8 (11.8)	49.2 (11.3)	0.048*	43.1 (15.8)	40.6 (9.6)	0.641
MPQ Pain Unpleasantness	46.5 (22.1)	48.6 (16.5)	0.776	47.5 (12.0)	42.8 (14.3)	0.440
MPQ Sensory	15.9 (5.9)	15.4 (5.6)	0.822	16.8 (6.4)	16.1 (8.9)	0.867
MPQ Affect	2.7 (1.8)	1.2 (1.9)	0.045*	2.1 (4.2)	1.6 (2.1)	0.682
PANAS Negative Affect	2.0 (4.0)	1.1 (2.5)	0.474	-3.5 (9.4)	1.2 (11.6)	0.392
PANAS Positive Affect	1.7 (5.7)	5.8 (5.3)	0.066	7.3 (4.1)	5.1 (6.3)	0.432

Table 10. Psychophysical Measures - rs4813625 a. Data are expressed as mean (SD) b. Calculated using 2-sample t tests for differences between genotype *
Significant differences detected between genotype groups.

Attachment Scales

We observed significant differences between GG and GC/CC carriers in attachment anxiety within our female sample (mean ± SD, GG, 46.1 ± 16.7, GC/CC, 63.6 ± 19.5; p=0.038) (Figure 3). We did not detect any differences in attachment avoidance (mean ± SD, Females: GG, 45.6 ± 21.7, GC/CC, 50.7 ± 20.1; p=0.570) nor were any differences observed among the males (mean ± SD, Anxiety: GG, 54.8 ± 16.3, GC/CC, 54.9 ± 18.0, p=0.982); Avoidance: GG, 41.8 ± 20.2, GC/CC, 49.2 ± 22.8, p=0.452).
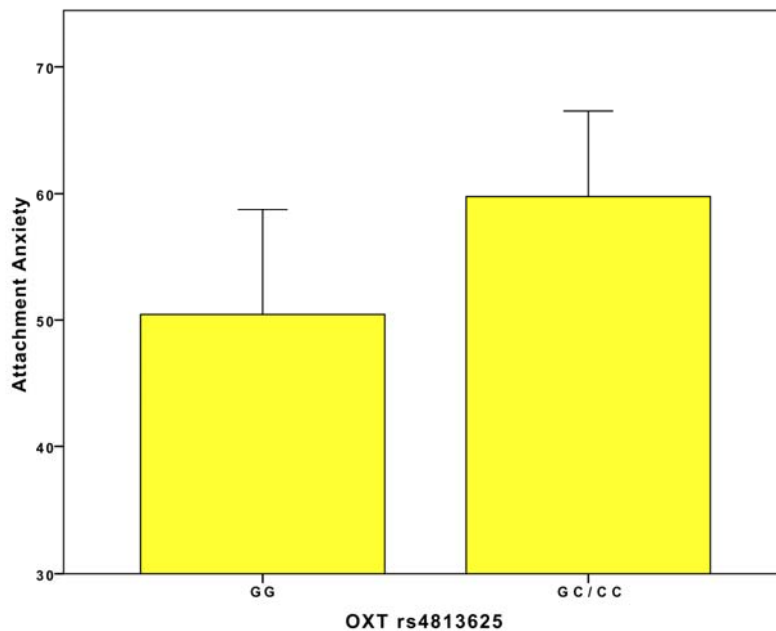

Figure 8. Attachment Anxiety by Genotype in Females. Association of OXT rs4813625 genotype on attachment anxiety in the female sample.

Discussion

This study is the first to associate a gene polymorphism with differential dopaminergic responses to a stressor. We find that in females, carriers of the C allele for SNP rs4813625 of the oxytocin gene show greater DA responses to the pain stress

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challenge relative to G homozygotes. These same individuals, though receiving similar levels of hypertonic saline and reporting comparable VAS ratings during the challenge itself, recall greater pain intensity and ascribe more negative affective qualities to the pain immediately following the pain challenge. In addition, C carriers display increased attachment anxiety. We failed to find similar associations among the males in our sample, which may suggest a sex-specific effect of this gene.

Encoded by the *OXT* gene, oxytocin is initially synthesized as a precursor complex comprised of oxytocin and its carrier peptide neurophysin I. During post-translational processing, the oxytocin-neurophysin prohormone is cleaved to produce oxytocin and neurophysin (Land et al., 1983). The first polypeptide hormone to be synthesized in a laboratory, oxytocin was first noted and utilized for its labor and parturition effects which had been described more than a century ago (Dale, 1906; du Vigneaud et al., 1954)). Further investigations into oxytocin, uncovered widespread effects capable of modulating complex processes including stress responsiveness. In response to stress both central and peripheral mechanisms are engaged and oxytocin appears to affect both processes (e.g. (Lang et al., 1983; Jezova et al., 1995; Wotjak et al., 1998)). Oxytocin seems to be involved in the stress reduction process, having anxiolytic and antinociceptive effects upon release or administration (e.g. (Jezova et al., 1995; Robinson et al., 2002; Onaka, 2004)). This effect of oxytocin has been often considered in the realm of maternal and affiliative behavior (see review (Lee et al., 2009)), whereby reduction of stress may be beneficial to the formation of social bonds; however, researchers have noted oxytocin-related reductions in stress responsiveness in stress paradigms which did not contain social elements such as forced swimming and osmotic

stress (e.g. (Lang et al., 1983; Kasting, 1988; Jezova et al., 1995; Wotjak et al., 1998; Neumann et al., 2000).

Here, we find that polymorphisms of the oxytocin gene are associated with reductions in stress-induced dopamine release in response to pain within the ventral striatum. Increases in dopamine activity within the striatum following this pain challenge have been associated with more negative pain experiences (Scott et al., 2006). Dopamine release within the ventral basal ganglia is particularly related to negative emotional responses; specifically greater stress-induced activity is positively associated with increases in negative affect and fear as reported by the PANAS (Scott et al., 2006). This follows with this report, where female carriers of the C allele for SNP rs4813625 display the greatest dopamine release and report higher pain intensity and assign more affective qualities to the pain when compared to G homozygotes. We did not, however, observe a similar effect in our male sample. There is some evidence to suggest that oxytocin may affect men and women differently, for instance oxytocin and its receptor expression tend to be higher in females, show different distributions in males and females, and appear to be modulated by gonadal steroids in animal models (see (Insel and Shapiro, 1992; Gimpl and Fahrenholz, 2001; Nomura et al., 2002; Carter, 2007; Lee et al., 2009)). Whether oxytocin expression and distribution varies among sexes in humans has not been examined fully, however some data suggest there may be sex differences in its effects in humans. For instance, oxytocin release has been shown to be triggered by empathy, more so in women than in men (Barraza and Zak, 2009). The paucity of data on sex differences in humans and the data presented here suggest that sex differences in oxytocin functioning should be further explored in humans.

Finally, we noted a difference in attachment anxiety between C carriers and G homozygotes for SNP rs4813625, specifically, we note that C carriers display increased attachment anxiety relative to G homozygotes. As mentioned earlier, oxytocin is known to modulate affiliative behavior; for instance, in humans intranasal administration of oxytocin has been shown to increase trust (Kosfeld et al., 2005; Baumgartner et al., 2008), positive communication during couple conflict (Ditzen et al., 2009), social memory (Guastella et al., 2008a; Savaskan et al., 2008; Rimmele et al., 2009), eye gaze (Guastella et al., 2008b), use of social cues to infer mental state (Domes et al., 2007), and maternal behavior (Feldman et al., 2007). Consistent with our findings here, previous studies have noted associations between changes in oxytocin plasma and attachment anxiety in humans (Turner et al., 2002; Marazziti et al., 2006). Oxytocin receptor polymorphisms have been shown to be associated with autistic disorders, where a significant impairment of attachment behavior has been noted (Wu et al., 2005; Jacob et al., 2007; Lerer et al., 2008; Wermter et al., 2009) and with adult attachment style in depressive patients (Costa et al., 2009). In regards to the direction of our findings we note a pattern of antistress: we note lower attachment anxiety in the same group of individuals (G homozygotes) who also exhibited the least stress-induced DA release and recalled the least distress following the pain challenge. Given oxytocin anxiolytic properties, this may suggest greater oxytocin functioning in this group however further studies are needed to determine the exact source of the differences observed here.

This study represents preliminary research into oxytocin genetic influence on pain and stress responses. Our data suggests the oxytocinergic system may be involved in dopaminergic stress mechanisms in humans. We note that oxytocin is much more than a

social and reproductive hormone, rather it has larger more disparate effects and it appears to influence central stress processes in humans. The mechanism by which we see our effects is unknown, however, it is likely a yet undiscovered nearby polymorphism which is in LD with this SNP confers sex-specific variability in DA responses to stress by influencing the expression, posttranslational processing or function of oxytocin or it's carrier protein neurophysin. Further work is needed to determine what processes are involved. This study is of particular interest in regards to psychiatric disorders, such as addiction, whereby stress induced dopamine release is thought to be related to its neuropathology. Interestingly, oxytocin may impart resilience to drug addiction and has been previously observed to inhibit tolerance, physical dependence and attenuate relapse (see (Kovács et al., 1998). To conclude, we find that genetic variation in the OXT gene may be relevant in the formation of individual variability in dopamine functioning; further work needs to be done to uncover the exact mechanism by which this occurs.

Chapter V

Conclusion

It has been hypothesized that drug abusers may be biologically predisposed to use and abuse drugs (Piazza et al., 1998b). How factors typically associated with increased risk relate to this biological predisposition however is not well understood. While some individuals appear to be 'spontaneously predisposed' as perhaps a result of genetic factors others may become vulnerable as a consequence of interacting genetic and environmental agents interfering with key neurobiological processes (Piazza et al., 1998b).

As Piazza, LeMoal and other researchers have described, there are two main theoretical perspectives to describe why individuals are motivated to use drugs: individual-centered, drug-centered. In the drug-centered view, paramount to the cause of addiction are drug-induced alterations to key neural pathways that put an individual at risk for compulsive use. Alternatively, in the individual-centered view, biological characteristics unique to a person leave them in a vulnerable state to the effects of drugs; thus addiction is not solely a result of the drug effects on brain activity but interacts with underlying biological susceptibilities (Piazza and Le Moal, 1996; Piazza et al., 1998b; Piazza et al., 1998a; le Moal, 2009).

Taking individual differences into account, multiple factors, some inherited (i.e. genetics) and some acquired (e.g. stress), interact to produce a vulnerable phenotype that

exists prior to the initiation of drug use. Given this, it should be possible to observe

differences in functioning that are related to an individual's risk. Unfortunately,

identifying such differences in humans prior to the onset of addiction has been hampered

by methodological difficulties and much that we know regarding addiction vulnerabilities

comes from the study of animals or current and former drug addicts. Though not ideal,

studying animals or examining humans diagnosed with substance abuse or dependence

do provide some perspective as to the neurobiological alterations that occurred either as a

result of chronic drug use or that may have predisposed them to drug use initially. These

studies have established that alterations within the stress and motivational circuitry can

contribute to the development of addiction. An alternative approach to studying substance

use vulnerability involves studying a population that carries risk factors that are

hypothesized to place them at a higher risk but who have no history of substance

dependence or abuse. This allows for the characterization of neurobiological activity as a

function of risk without concern for previous chronic drug use.

The work presented here attempted to ascertain whether and how particular

susceptibility characteristics identified as risk factors resulted in variation in

neurobiological functioning in healthy, non-drug using individuals. We demonstrated

relationships between dopaminergic and opioidergic activity with multiple factors

relating to substance abuse vulnerability. Genetic, environmental and trait characteristics

were all observed to influence activity within regions in the motivational circuitry,

particularly within the ventral striatum. In sum, we extended and clarified previously

described differences in dopaminergic activity between men and women, noting a

significant interaction with environmental factors. Next, genetic variation within the

oxytocin receptor was found to predict variation in dopaminergic activity within the ventral striatum. Finally, we demonstrated that trait characteristics, specifically measures of non-planning impulsivity, are associated with μ-opioid receptor activity, expanding upon the former literature previously describing an association with dopaminergic functioning.

Animal work done nearly twenty years ago highlighted alterations within the brain's motivational circuitry and in stress system functioning in animals predisposed to self-administer drugs. Piazza and colleagues recognized that individual differences in stress-reactivity predicted self-administration behavior (e.g. (Piazza et al., 1989; Piazza et al., 1990b; Piazza et al., 1998a)). Later it would be demonstrated that by modulating stress reactivity, self-administration behavior could be manipulated. In addition, stress was shown to have the capacity to sensitize behavioral and neurochemical responses to psychostimulants and vice-versa (Antelman et al., 1980; Deroche et al., 1992; Piazza and Le Moal, 1996; Deroche et al., 1997). Given these observations, specifically that vulnerability to seek and use drugs is related to individual differences in stress reactivity and could be critical to identifying the presence of a vulnerability characteristic, we chose to examine the effects of susceptibility factors on neurochemical activity following a non-pharmacological stress challenge, pain-stress.

Pain is a universal stressor and is frequently used to provoke stress responses in animals and humans alike. For the challenge employed here, pain was administered over twenty minutes by the infusion of 5% hypertonic saline into the masseter muscle within the jaw via a computer-controlled pump. By recording pain intensity every fifteen seconds, we are able to adjust the infusion of hypertonic saline and maintain pain at a

constant level using a target of 40 VAS units (Zhang et al., 1993; Stohler and Kowalski, 1999). Hence, we are able to ensure that all subjects share a similar pain experience despite differences in pain sensitivity. This challenge has been shown to produce robust activations within both the endogenous opioid and dopaminergic systems, though there is a high degree of interindividual variability (e.g. (Zubieta et al., 2001; Scott et al., 2006)). These previous observations are in line with research describing the recruitment of multiple neuronal pathways in response to stress, presumably activated to allow an individual to adapt to the challenge. Research performed to date has provided significant information towards understanding the neurobiological aspects of the stress response, though less is understood of the nature and consequences of individual differences in these responses. In this work, we find a significant portion of this variability can be explained by factors identified for substance abuse risk.

Dopaminergic System Variation

Sex and Stress Effects

Our results obtained in a sample of healthy, college age men and women observed a significant contribution of sex in determining dopaminergic responses to stress. This was the first study to demonstrate sex differences in response to a pain-stress challenge in humans. Utilizing [11C] raclopride labeled PET, we obtained measures of D2/D3 receptor activity during the pain-stress challenge described earlier. Examining dopaminergic activity following our challenge, females exhibited significantly greater stress-induced dopaminergic activity when compared to males in regions throughout the striatum, though notably within the nucleus accumbens. This is interesting given

previous research indicating that enhanced dopaminergic activity within the mesolimbic dopamine system is associated with greater drug use (Rouge-Pont et al., 1993; Piazza and Le Moal, 1997; Piazza et al., 1998b; Rouge-Pont et al., 1998; Marinelli and Piazza, 2002) and that females appear to more rapidly progress from initiation to compulsive drug use compared to men (see (Becker et al., 2007)) . The differences in dopaminergic stress responsivity noted here might be an important underlying factor in determining later substance use risk.

The precise mechanisms whereby our sex differences in dopaminergic responsivity emerged are not clear. Though we did not observe any relationships between gonadal hormone levels and dopaminergic activity, the effect of circulating hormones should not be entirely discounted. Our sample size was somewhat small and excluded females in their luteal phase. Obtaining larger sample sizes and sampling dopaminergic activity over the course of the menstrual cycle would help determine hormonal influence on dopaminergic activity. Certainly such influences would be predicted based on multiple lines of research described earlier. For example, previous research indicates different behavioral responses to dopaminergic drugs across the menstrual cycle (Justice and de Wit, 1999; Sofuoglu et al., 1999; White et al., 2002)).

Continuing in this work, we further uncovered a significant interaction between sex and environment in determining dopaminergic stress responses. Prior to scanning, all subjects were instructed to complete an inventory that presented a series of common life events and asked the subject to indicate which events occurred within the last year. Subjects were divided into those that had experienced recent negative life stress (RLS) and those who had not (NoRLS). We then compared stress-induced dopaminergic activity

between the RLS group and the NoRLS group incorporating sex into the model. What we observed could be interpreted as an overall blunting of the stress response. Whereas significant differences between control and pain conditions were observed in the NoRLS men and NoRLS women, no such differences were recorded between control and pain conditions in either RLS group. A net dampening of dopaminergic responsiveness as a result of persistent or chronic stress has been previously observed. Some chronic stress protocols in animal models have also been shown to elicit significant decreases in dopaminergic activity within accumbens and in certain forebrain areas (e.g. (Gambarana et al., 1999; Mangiavacchi et al., 2001; Scheggi et al., 2002)). We may be observing neurobiological adaptations within the mesolimbic dopamine system as a result of exposure to recent life stress. Previous research has demonstrated that exposure to stress can place an individual at greater risk to use and abuse drugs. This work indicates that exposure to recent stressful life events can influence dopaminergic stress-responsivity measures. It is possible that disruptions in dopaminergic responsivity as a result of historical stress may partially account for increased risk for substance use.

Sex and Genetic Effects

In the next set of experiments, we examined data collected from a larger set of men and women, which had undergone [11C] raclopride scanning during which time blood was collected and genotyped. Polymorphisms in the oxytocin gene were examined to determine if genetic variation at this gene could predict differences in stress induced dopaminergic activity. The choice to study oxytocin gene polymorphisms came as a result of extensive research indicating that oxytocin has broad effects on a wide range of

behavior in reproductive, affiliative and motivational realms. In addition, oxytocin has been shown to alter dopaminergic responses associated with addictive behaviors and stress (e.g. (Pfister and Muir, 1989; Sarnyai and Kovács, 1994)).

We examined oxytocin genotype in relation to changes in dopaminergic activity in response to our pain-stress challenge. Given our previous findings that sex influences dopaminergic activity and the potential for sex differences in the effects of oxytocin, genotype effects were examined for males and females separately. We observed that carriers of the C allele for SNP rs4813625 of the oxytocin gene exhibit greater DA responses to the pain stress challenge relative to G homozygotes – this relationship was only noted in females. Female carriers of the C allele also recall greater pain intensity, assign more negative affective qualities to the pain immediately following the pain challenge and demonstrate higher levels of trait attachment anxiety. Why these effects of oxytocin genotype were only observed in our female sample is not entirely clear, however oxytocin is observed to have sex specific effects on behavior (e.g. maternal behavior). Another possibility is that due to our relatively small sample of males, we lacked sufficient power to resolve such genotype differences in our males.

Since the SNPs identified for the oxytocin gene were outside of the coding region and this is the first investigation examining their relationship to behavior or neurobiological functioning, the means whereby we observe our effects is not known. It is possible that rs4813625 is in linkage disequilibrium with a functional polymorphism that mediates alterations in stress or dopaminergic activity. Given that oxytocin has been frequently demonstrated to possess anxiolytic properties (e.g. (Windle et al., 1997; Neumann et al., 2000; Heinrichs et al., 2003; Ring et al., 2006; Ditzen et al., 2009)) it is

tempting to speculate that our observation may reflect greater or more efficient functioning of oxytocin in our G carriers. Future studies will need to determine the exact genetic mechanisms behind the observations noted here.

Mu-Opioid System Variation

Previous epidemiological studies, as described in detail earlier, have suggested trait characteristics, particularly trait impulsivity, are important factors in determining future drug use and abuse. Given that several reports have previously demonstrated effects of sensation seeking and impulsiveness traits on dopaminergic activity in humans (Leyton et al., 2002; Boileau et al., 2006; Riccardi et al., 2006a; Oswald et al., 2007), we chose to examine the influence of trait impulsivity on a different neurotransmitter system, the opioid system. We examined data collected from a healthy group of college-aged males which had been administered the NEO personality inventory then subsequently underwent [11C] carfentanil imaging. Subjects were grouped based on their impulsivity and deliberation ratings and compared on their μ-opioid system response to the pain challenge described earlier.

Though dopamine is the best studied in regards to reward, motivation and substance abuse, mesolimbic dopamine activity is heavily modulated by other neurotransmitter systems. The μ-opioid system interacts with the mesolimbic dopamine system both directly and indirectly. μ-opioid receptors are present throughout the motivational circuit including on GABAergic interneurons within the VTA and on medium spiny GABAergic neurons within the nucleus accumbens that project back to the VTA. The μ-opioid system can also modulate activity within the mesolimbic circuitry

indirectly through its actions on HPA stress responses. Endogenous opioids can attenuate stress responses, whereas opioid receptor blockade can result in disinhibition of the HPA axis leading to release of cortisol and adrenalcorticotropin hormone (Janssens et al., 1995; Wand and Schumann, 1998; Geer et al., 2005). μ-opioid activity can also modulate the expression of stress-responsive genes within the hypothalamus and anterior pituitary. In addition, μ-opioid activity can inhibit neurons within in the locus coeruleus that normally stimulates hypothalamic CRH (Wand and Schumann, 1998).

The μ-opioid system is capable of modulating the mesolimbic dopamine pathway, and is central to the regulation of motivated behavior. In sum, given the relevance of the μ-opioid system, we explored how individual differences in impulsivity, a known susceptibility factor for substance use and abuse, were associated with μ-opioid system activity.

We examined two orthogonal traits that relate to the non-planning dimension of impulsivity: NEO Impulsiveness (IMP) and NEO deliberation (DLB). We found that individuals displaying high IMP or low DLB had greater μ-opioid receptor availability at rest and greater stress-induced activity within regions involved in motivated behavior and the effects of drugs of abuse: prefrontal cortex, anterior cingulate, thalamus, basolateral amygdala, and, notably, the nucleus accumbens. In order to determine how the combination of the two behavioral traits could influence μ-opioid receptor availability and responses to stress we performed a conjunction analysis; this revealed that individuals exhibiting extreme traits (high IMP/low DLB and low IMP/high DLB) displayed the greatest and smallest baseline μ-opioid receptor availability and stress-

induced activity, respectively. These findings come in absence of any difference in the experience of pain itself between high/low IMP or high/low DLB groups.

Our observations in humans are in line with previous research indicating HR rats, which show greater stress-reactivity, a propensity to self-administer drugs and greater dopaminergic responses to drugs, display increased nucleus accumbens proenkephalin gene expression (Lucas et al., 1998). We would anticipate similar disparate effects between high/low IMP and high/low DLB groups in stress-induced dopaminergic activity. In fact, the differences in dopaminergic activity the HR group have been hypothesized to result at least partially, from the higher enkephalin tone seen within the nucleus accumbens (Lucas et al., 1998). Whether our high IMP/low DLB individuals also differ in dopaminergic activity is not yet known though several investigations have noted positive relationships between novelty seeking and impulsivity with dopaminergic activity in the ventral striatum (Leyton et al., 2002; Riccardi et al., 2006a) however, these findings are somewhat inconsistent, with some reporting negative relationships (Riccardi et al., 2006b; Riccardi et al., 2006a; Oswald et al., 2007). All of the aforementioned studies utilized pharmacological interventions as a means to increase dopaminergic release, so examining the association between these traits and stress-induced dopamine activity would be of some interest. In particular, it would be of much use to see how dopamine and opioid activity in the same individuals are affected by trait characteristics to develop a more complete picture.

In conclusion, this work was the first to provide evidence that μ-opioid system functioning is associated with personality traits identified as susceptibility factors to develop substance use problems.

Common Discussion

There does not appear to be any one factor unique to producing drug abuse. Understanding how susceptibility factors relate to differences in underlying neurobiological functioning may lead to a better understanding of how these elements convey risk to substance abuse and a better comprehension of what is shared among those vulnerable to use. The results from this body of work find that various neurochemical responses to stress within the motivational circuitry are influenced by certain susceptibility factors. Specifically, as a whole we find that individual characteristics related to substance abuse risk are somewhat predictive of dopaminergic and opioidergic responses within the ventral striatum to stress. This region is critical in determining not only the reinforcing effects of drugs but in determining motivated behavior in general.

Enhanced dopaminergic activity within the ventral striatum in response to mild stress has been shown to be predictive of later self-administration behavior (see (Piazza et al., 1998a)). Indeed, Piazza and others have directly postulated that individuals presenting higher dopaminergic activity may be at higher vulnerability to self-administer drugs (Piazza et al., 1998b). Related to this, enhanced enkephalin tone at this region could also be a contributory factor (Lucas et al., 1998). We note within our sample that females in general and particularly carriers of the C allele for rs4213625, demonstrate the highest greater stress-induced dopaminergic activity within the right ventral striatum. Similarly, within this same region males exhibiting high levels of impulsiveness show enhanced stress-induced μ-opioid system activation. Research suggests that increased opioidergic and dopaminergic activity within this region could potentially positively

influence individual assessments of rewarding stimuli like drugs of abuse – could this partially underlie the previous epidemiological demonstrations of increased risk to use drugs in the groups studied here? This may be possible, however, the work presented here represents a preliminary step towards understanding the neurobiological mechanisms underlying vulnerability. It would be of significant interest to determine if variation in ventral striatal opioidergic and dopaminergic functioning is indeed predictive of later drug use in humans, however much more preliminary work is needed before direct connections between susceptibility factors, future drug use and neurobiological activity can be established. First, given the ample evidence suggesting differences in HPA axis functioning among animals predisposed to self-administer drugs of abuse, the relationships between some of the dopaminergic and opioidergic measures obtained here should be linked to cortisol activity during the challenge. Second, future studies should attempt to determine how the presence of multiple susceptibility factors influences functioning in this circuit – Do different factors have distinct effects, or do they, as some data collected here suggests, have additive effects within similar regions? How does the interplay between different neurotransmitter systems reflect the expression of these factors? If risk can be modified (e.g. teaching of coping strategies) how does this affect the previously noted relationships?

There is an important point to consider when linking the observations made here to substance abuse vulnerability – Though we note associations between neurochemical activity and susceptibility characteristics associated with risk for substance abuse problems, these susceptibility factors could also play a role in vulnerability for other psychopathological disorders (e.g. PTSD, major depressive disorder). However, it should

be noted that the neurobiological circuitry disrupted in psychiatric disorders like PTSD and MDD are not entirely separable from the pathways disturbed in addiction; in fact, this may be why mental illness so frequently co-occurs with substance abuse problems (SAMHSA, 2009). Evidence suggests that psychiatric disorders and addiction share common neurobiological variation possibly within the motivational and stress systems (Volkow, 2001; Volkow, 2009). It will be important to discover how susceptibility factors are represented in diseased populations and determine if similar neurobiological variation is observed.

Final Comments

People vary widely in their propensity to initiate and maintain drug use. Understanding the mechanisms that underlie the predisposition to seek out and use drugs is of significant importance in developing strategies to mitigate drug use before and after initial consumption. These investigations extend previous research that outlined the neurobiological pathways associated with the effects of drugs of abuse and which appeared to be disrupted as a result of the addiction process. This work reveals that some of the characteristics identified as risk factors for substance use are associated with biological processes previously identified as playing critical roles in the development of addiction (for summary, see Figure 9).
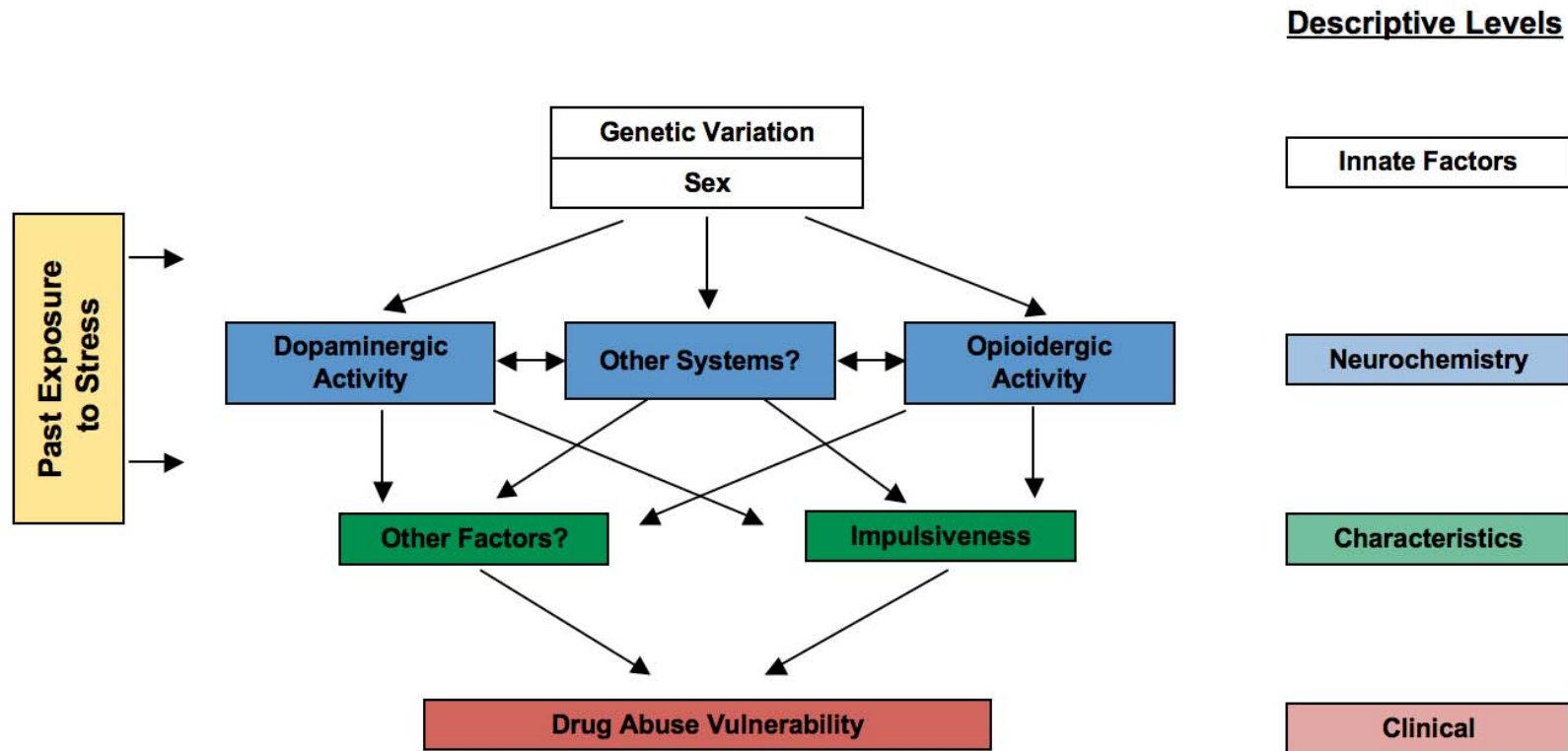
Figure 9. Summary of potential mechanism involved in drug abuse vulnerability. Innate factors in interaction with environmental circumstances such as stress exposure determine responsivity of various neural substrates to external stimuli (e.g. acute stress), which can influence the expression of characteristics like impulsiveness and ultimately moderate drug abuse vulnerability.

Bibliography

Abercrombie ED, Keefe KA, DiFrischia DS, Zigmond MJ (1989) Differential effect of stress on in vivo dopamine release in striatum, nucleus accumbens, and medial frontal cortex. Journal of Neurochemistry. 52:1655-1658.

Adler CM, Elman I, Weisenfeld N, Kestler L, Pickar D, Breier A (2000) Effects of acute metabolic stress on striatal dopamine release in healthy volunteers. Neuropsychopharmacology. 22:545-550.

Alessi SM, Petry NM (2003) Pathological gambling severity is associated with impulsivity in a delay discounting procedure. Behavioural Processes. 64:345-354.

Allen SS, Bade T, Center B, Finstad D, Hatsukami D (2008) Menstrual phase effects on smoking relapse. Addiction. 103:809-821.

Ambroggi F, Turiault M, Milet A, Deroche-Gamonet V, Parnaudeau S, Balado E, Barik J, van der Veen R, Maroteaux G, Lemberger T, Schütz G, Lazar M, Marinelli M, Piazza PV, Tronche F (2009) Stress and addiction: glucocorticoid receptor in dopaminoceptive neurons facilitates cocaine seeking. Nature Neuroscience. 12:247-249.

Ambrose LM, Unterwald EM, Van Bockstaele EJ (2004) Ultrastructural evidence for co-localization of dopamine D2 and micro-opioid receptors in the rat dorsolateral striatum. The Anatomical Record. 279:583-591.

American Psychiatric Association. (2000) Diagnostic and statistical manual of mental disorders : 4th Edition. Washington, DC.

Antelman SM, Eichler AJ, Black CA, Kocan D (1980) Interchangeability of stress and amphetamine in sensitization. Science. 207:329-331.

Anton RF, Oroszi G, O'Malley S, Couper D, Swift R, Pettinati H, Goldman D (2008) An evaluation of mu-opioid receptor (OPRM1) as a predictor of naltrexone response in the treatment of alcohol dependence: results from the Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence (COMBINE) study. Archives of General Psychiatry. 65:135-144.

Arias A, Feinn R, Kranzler HR (2006) Association of an Asn40Asp (A118G) polymorphism in the mu-opioid receptor gene with substance dependence: a meta-analysis. Drug and Alcohol Dependence. 83:262-268.

Audrain-McGovern J, Rodriguez D, Epstein LH, Cuevas J, Rodgers K, Wileyto EP (2009) Does delay discounting play an etiological role in smoking or is it a consequence of smoking? Drug and Alcohol Dependence. 103:99-106.

Austin MC, Kalivas PW (1991) Dopaminergic involvement in locomotion elicited from the ventral pallidum/substantia innominata. Brain Research. 542:123-131.

Badiani A, Oates MM, Day HE, Watson SJ, Akil H, Robinson TE (1998) Amphetamine-induced behavior, dopamine release, and c-fos mRNA expression: modulation by environmental novelty. Journal of Neuroscience. 18:10579-10593.

Badiani A, Oates MM, Day HE, Watson SJ, Akil H, Robinson TE (1999) Environmental modulation of amphetamine-induced c-fos expression in D1 versus D2 striatal neurons. Behavioural Brain Research. 103:203-209.

Barraza JA, Zak PJ (2009) Empathy toward strangers triggers oxytocin release and subsequent generosity. Annals of the New York Academy of Sciences. 1167:182-189.

Barrot M, Marinelli M, Abrous DN, Rougé-Pont F, le Moal M, Piazza PV (2000) The dopaminergic hyper-responsiveness of the shell of the nucleus accumbens is hormone-dependent. European Journal of Neuroscience. 12:973-979.

Barrot M, Abrous DN, Marinelli M, Rougé-Pont F, le Moal M, Piazza PV (2001) Influence of glucocorticoids on dopaminergic transmission in the rat dorsolateral striatum. European Journal of Neuroscience. 13:812-818.

Bart G, Heilig M, LaForge KS, Pollak L, Leal SM, Ott J, Kreek MJ (2004) Substantial attributable risk related to a functional mu-opioid receptor gene polymorphism in association with heroin addiction in central Sweden. Molecular Psychiatry. 9:547-549.

Bart G, Kreek MJ, Ott J, LaForge KS, Proudnikov D, Pollak L, Heilig M (2005) Increased attributable risk related to a functional mu-opioid receptor gene polymorphism in association with alcohol dependence in central Sweden. Neuropsychopharmacology. 30:417-422.

Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E (2008) Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. Neuron. 58:639-650.

Bechara A, Damasio AR, Damasio H, Anderson SW (1994) Insensitivity to future consequences following damage to human prefrontal cortex. Cognition. 50:7-15.

Bechara A, Tranel D, Damasio H, Damasio AR (1996) Failure to respond autonomically to anticipated future outcomes following damage to prefrontal cortex. Cerebral Cortex. 6:215-225.

Becker JB, Taylor JR (2008) Sex Differences in Motivation. In: Sex differences in the brain: from genes to behavior, pp 177-195. New York: Oxyford University Press.

Becker JB, Monteggia LM, Perrot-Sinal TS, Romeo RD, Taylor JR, Yehuda R, Bale TL (2007) Stress and disease: is being female a predisposing factor? Journal of Neuroscience. 27:11851-11855.

Bencherif B, Wand GS, McCaul ME, Kim YK, Ilgin N, Dannals RF, Frost JJ (2004) Mu-opioid receptor binding measured by [11C]carfentanil positron emission tomography is related to craving and mood in alcohol dependence. Biological Psychiatry. 55:255-262.

Berridge KC (1996) Food reward: brain substrates of wanting and liking. Neuroscience and Biobehavioral Reviews. 20:1-25.

Bigelow J (1822) A Treatise on the Materia Medica, A sequel to the Pharmacopoeia of the United States. In, p 275. Boston: Charles Ewer.

Billings AG, Moos RH (1981) The role of coping responses and social resources in attenuating the stress of life events. Journal of Behavioral Medicine. 4:139-157.

Blanchard BA, Steindorf S, Wang S, Glick SD (1993) Sex differences in ethanol-induced dopamine release in nucleus accumbens and in ethanol consumption in rats. Alcoholism, Clinical and Experimental Research. 17:968-973.

Blanchard MM, Mendelsohn D, Stamp JA (2009) The HR/LR model: Further evidence as an animal model of sensation seeking. Neuroscience and Biobehavioral Reviews. 33:1145-1154.

Bloom F, Battenberg E, Rossier J, Ling N, Guillemin R (1978) Neurons containing beta-endorphin in rat brain exist separately from those containing enkephalin: immunocytochemical studies. Proceedings of the National Academy of Sciences of the United States of America. 75:1591-1595.

Blum K, Chen ALC, Chen TJH, Braverman ER, Reinking J, Blum SH, Cassel K, Downs BW, Waite RL, Williams L, Prihoda TJ, Kerner MM, Palomo T, Comings DE, Tung H, Rhoades P, Oscar-Berman M (2008) Activation instead of blocking mesolimbic dopaminergic reward circuitry is a preferred modality in the long term treatment of reward deficiency syndrome (RDS): a commentary. Theoretical Biology & Medical Modelling. 5:24.

Boileau I, Dagher A, Leyton M, Gunn RN, Baker GB, Diksic M, Benkelfat C (2006) Modeling sensitization to stimulants in humans: an [11C]raclopride/positron emission tomography study in healthy men. Archives of General Psychiatry. 63:1386-1395.

Bond C, LaForge K, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong J, Leal S, Tischfield J, Kreek M, Yu L (1998) Single-nucleotide polymorphism in the

human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. Proceedings of the National Academy of Sciences of the United States of America. 95:9608-9613.

Bouchard TJ (1994) Genes, environment, and personality. Science. 264:1700-1701.

Boyd CJ, Mieczkowski T (1990) Drug use, health, family and social support in "crack" cocaine users. Addictive Behaviors. 15:481-485.

Bradley KA, Badrinath S, Bush K, Boyd-Wickizer J, Anawalt B (1998) Medical risks for women who drink alcohol. Journal of General Internal Medicine. 13:627-639.

Brainerd H (1891) Report of Committee on Diseases of the Mind and Nervous System: Cocaine Addiction. In: The transactions of the medical society of the state of California (California MSotSo, ed), pp 193-201. San Francisco: W.A. Woodward & Co.

Brinton RE, Wamsley JK, Gee KW, Wan YP, Yamamura HI (1984) [3H]oxytocin binding sites in the rat brain demonstrated by quantitative light microscopic autoradiography. European Journal of Pharmacology. 102:365-367.

Brosschot JF, Benschop RJ, Godaert GL, Olff M, De Smet M, Heijnen CJ, Ballieux RE (1994) Influence of life stress on immunological reactivity to mild psychological stress. Psychosomatic Medicine. 56:216-224.

Bry BH (1982) Reducing the incidence of adolescent problems through preventive intervention: one- and five-year follow-up. American Journal of Community Psychology. 10:265-276.

Burnett FE, Scott LV, Weaver MG, Medbak SH, Dinan TG (1999) The effect of naloxone on adrenocorticotropin and cortisol release: evidence for a reduced response in depression. Journal of Affective Disorders. 53:263-268.

Burton RP, Johnson RJ, Ritter C, Clayton RR (1996) The effects of role socialization on the initiation of cocaine use: an event history analysis from adolescence into middle adulthood. Journal of Health and Social Behavior. 37:75-90.

Bush FM, Harkins SW, Harrington WG, Price DD (1993) Analysis of gender effects on pain perception and symptom presentation in temporomandibular pain. Pain. 53:73-80.

Bush G, Vogt BA, Holmes J, Dale AM, Greve D, Jenike MA, Rosen BR (2002) Dorsal anterior cingulate cortex: a role in reward-based decision making. Proceedings of the National Academy of Sciences of the United States of America. 99:523-528.

Butters JE (2002) Family stressors and adolescent cannabis use: a pathway to problem use. Journal of Adolescence. 25:645-654.

Cabib S, Puglisi-Allegra S, Ventura R (2002) The contribution of comparative studies in inbred strains of mice to the understanding of the hyperactive phenotype. Behavioural Brain Research. 130:103-109.

Caffé AR, Van Ryen PC, Van der Woude TP, Van Leeuwen FW (1989) Vasopressin and oxytocin systems in the brain and upper spinal cord of Macaca fascicularis. The Journal of Comparative Neurology. 287:302-325.

Cardinal RN, Pennicott DR, Sugathapala CL, Robbins TW, Everitt BJ (2001) Impulsive choice induced in rats by lesions of the nucleus accumbens core. Science. 292:2499-2501.

Carson RE, Breier A, de Bartolomeis A, Saunders RC, Su TP, Schmall B, Der MG, Pickar D, Eckelman WC (1997) Quantification of amphetamine-induced changes in [11C]raclopride binding with continuous infusion. Journal of Cerebral Blood Flow and Metabolism. 17:437-447.

Carter CS (2007) Sex differences in oxytocin and vasopressin: implications for autism spectrum disorders? Behavioural Brain Research. 176:170-186.

Caspi A, Moffitt T, Newman D, Silva P (1996) Behavioral observations at age 3 years predict adult psychiatric disorders. Longitudinal evidence from a birth cohort. Archives of General Psychiatry. 53:1033-1039.

Castner SA, Xiao L, Becker JB (1993) Sex differences in striatal dopamine: in vivo microdialysis and behavioral studies. Brain Research. 610:127-134.

Chalmers DT, Lovenberg TW, Grigoriadis DE, Behan DP, De Souza EB (1996) Corticotrophin-releasing factor receptors: from molecular biology to drug design. Trends in Pharmacological Sciences. 17:166-172.

Chang L, Alicata D, Ernst T, Volkow N (2007) Structural and metabolic brain changes in the striatum associated with methamphetamine abuse. Addiction. 102 Suppl 1:16-32.

Chen JF, Aloyo VJ, Weiss B (1993) Continuous treatment with the D2 dopamine receptor agonist quinpirole decreases D2 dopamine receptors, D2 dopamine receptor messenger RNA and proenkephalin messenger RNA, and increases mu opioid receptors in mouse striatum. Neuroscience. 54:669-680.

Chida Y, Hamer M (2008) Chronic psychosocial factors and acute physiological responses to laboratory-induced stress in healthy populations: a quantitative review of 30 years of investigations. Psychological Bulletin. 134:829-885.

Chilcoat HD, Breslau N (1998a) Posttraumatic stress disorder and drug disorders: testing causal pathways. Archives of General Psychiatry. 55:913-917.

Chilcoat HD, Breslau N (1998b) Investigations of causal pathways between PTSD and drug use disorders. Addictive Behaviors. 23:827-840.

Clark L, Robbins TW, Ersche KD, Sahakian BJ (2006) Reflection impulsivity in current and former substance users. Biological Psychiatry. 60:515-522.

Clinton S, Miller S, Watson SJ, Akil H (2008) Prenatal stress does not alter innate novelty-seeking behavioral traits, but differentially affects individual differences in neuroendocrine stress responsivity. Psychoneuroendocrinology. 33:162-177.

Cloninger C, Sigvardsson S, Bohman M (1988) Childhood personality predicts alcohol abuse in young adults. Alcohol, Clinical and Experimental Research. 12:494-505.

Cohen P, Chen H, Crawford TN, Brook JS, Gordon K (2007) Personality disorders in early adolescence and the development of later substance use disorders in the general population. Drug and Alcohol Dependence. 88 Suppl 1:S71-84.

Collins NL, Read SJ (1990) Adult attachment, working models, and relationship quality in dating couples. Journal of Personality and Social Psychology. 58:644-663.

Conaglen JV, Donald RA, Espiner EA, Livesey JH, Nicholls MG (1985) Effect of naloxone on the hormone response to CRF in normal man. Endocrine Research. 11:39-44.

Contini V, Marques FZC, Garcia CED, Hutz MH, Bau CHD (2006) MAOA-uVNTR polymorphism in a Brazilian sample: further support for the association with impulsive behaviors and alcohol dependence. American Journal of Medical Genetics Part B, Neuropsychiatric Genetics. 141B:305-308.

Costa B, Pini S, Gabelloni P, Abelli M, Lari L, Cardini A, Muti M, Gesi C, Landi S, Galderisi S, Mucci A, Lucacchini A, Cassano GB, Martini C (2009) Oxytocin receptor polymorphisms and adult attachment style in patients with depression. Psychoneuroendocrinology. 34:1506-1514.

Costa P, McRae R (1992) Normal personality assessment in clinical practice: The NEO Personality Inventory. Psychological Assessment. 4:5-13.

Crothers T (1893) The disease of inebriety from alcohol, opium and other narcotic drugs: its etiology, pathology, treatment and medico-legal relations. In: (Inebriety AAftSaCo, ed). New York: E.B. Treat, Publisher.

Crowley JJ, Oslin DW, Patkar AA, Gottheil E, DeMaria PA, O'Brien CP, Berrettini WH, Grice DE (2003) A genetic association study of the mu opioid receptor and severe opioid dependence. Psychiatric Genetics. 13:169-173.

Dale HH (1906) On some physiological actions of ergot. The Journal of Physiology. 34:163-206.

Dalley JW, Fryer TD, Brichard L, Robinson ES, Theobald DE, Laane K, Pena Y, Murphy ER, Shah Y, Probst K, Abakumova I, Aigbirhio FI, Richards HK, Hong Y, Baron JC, Everitt BJ, Robbins TW (2007) Nucleus accumbens D2/3 receptors predict trait impulsivity and cocaine reinforcement. Science. 315:1267-1270.

Dancey CP, Taghavi M, Fox RJ (1998) The relationship between daily stress and symptoms of irritable bowel: a time-series approach. Journal of Psychosomatic Research. 44:537-545.

Daughters SB, Richards JM, Gorka SM, Sinha R (2009) HPA axis response to psychological stress and treatment retention in residential substance abuse treatment: a prospective study. Drug and Alcohol Dependence. 105:202-208.

Davis BA, Clinton SM, Akil H, Becker JB (2008) The effects of novelty-seeking phenotypes and sex differences on acquisition of cocaine self-administration in selectively bred High-Responder and Low-Responder rats. Pharmacology, Biochemistry, and Behavior. 90:331-338.

Dawe S, Loxton NJ (2004) The role of impulsivity in the development of substance use and eating disorders. Neuroscience and Biobehavioral Reviews. 28:343-351.

Dawson DA (2006) The link between family history and early onset alcoholism: earlier initiation of drinking or more rapid development of dependence? Journal of Studies on Alcohol. 61:637-646.

Day HE, Badiani A, Uslaner JM, Oates MM, Vittoz NM, Robinson TE, Watson SJ, Akil H (2001) Environmental novelty differentially affects c-fos mRNA expression induced by amphetamine or cocaine in subregions of the bed nucleus of the stria terminalis and amygdala. Journal of Neuroscience. 21:732-740.

de Wit H, Söderpalm AHV, Nikolayev L, Young E (2003) Effects of acute social stress on alcohol consumption in healthy subjects. Alcoholism, Clinical and Experimental Research. 27:1270-1277.

Dellu F, Piazza PV, Mayo W, Le Moal M, Simon H (1996) Novelty-seeking in rats-- biobehavioral characteristics and possible relationship with the sensation-seeking trait in man. Neuropsychobiology. 34:136-145.

Deminière JM, Piazza PV, Guegan G, Abrous N, Maccari S, le Moal M, Simon H (1992) Increased locomotor response to novelty and propensity to intravenous amphetamine self-administration in adult offspring of stressed mothers. Brain Research. 586:135-139.

Deroche V, Piazza PV, Le Moal M, Simon H (1993) Individual differences in the psychomotor effects of morphine are predicted by reactivity to novelty and influenced by corticosterone secretion. Brain Research. 623:341-344.

Deroche V, Piazza PV, Le Moal M, Simon H (1994) Social isolation-induced enhancement of the psychomotor effects of morphine depends on corticosterone secretion. Brain Research. 640:136-139.

Deroche V, Marinelli M, Le Moal M, Piazza P (1997) Glucocorticoids and behavioral effects of psychostimulants. II: cocaine intravenous self-administration and reinstatement depend on glucocorticoid levels. Journal of Pharmacology and Experimental Therapeutics. 281:1401-1407.

Deroche V, Piazza PV, Casolini P, Maccari S, Le Moal M, Simon H (1992) Stress-induced sensitization to amphetamine and morphine psychomotor effects depend on stress-induced corticosterone secretion. Brain Research. 598:343-348.

Deroche-Gamonet V, Sillaber I, Aouizerate B, Izawa R, Jaber M, Ghozland S, Kellendonk C, Le Moal M, Spanagel R, Schütz G, Tronche F, Piazza PV (2003) The glucocorticoid receptor as a potential target to reduce cocaine abuse. Journal of Neuroscience. 23:4785-4790.

DeWit DJ (1998) Frequent childhood geographic relocation: its impact on drug use initiation and the development of alcohol and other drug-related problems among adolescents and young adults. Addictive Behaviors. 23:623-634.

Diergaarde L, Pattij T, Poortvliet I, Hogenboom F, de Vries W, Schoffelmeer AN, De Vries TJ (2008) Impulsive choice and impulsive action predict vulnerability to distinct stages of nicotine seeking in rats. Biological Psychiatry. 63:301-308.

Ditzen B, Schaer M, Gabriel B, Bodenmann G, Ehlert U, Heinrichs M (2009) Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. Biological Psychiatry. 65:728-731.

Dixon MR, Marley J, Jacobs EA (2004) Delay discounting by pathological gamblers. Journal of Applied Behavior Analysis. 36:449-458.

Dolcos F, LaBar KS, Cabeza R (2004) Dissociable effects of arousal and valence on prefrontal activity indexing emotional evaluation and subsequent memory: an event-related fMRI study. Neuroimage. 23:64-74.

Domes G, Heinrichs M, Michel A, Berger C, Herpertz SC (2007) Oxytocin improves "mind-reading" in humans. Biological Psychiatry. 61:731-733.

Donaldson ZR, Young LJ (2008) Oxytocin, vasopressin, and the neurogenetics of sociality. Science. 322:900-904.

Doran N, Spring B, McChargue D (2007) Effect of impulsivity on craving and behavioral reactivity to smoking cues. Psychopharmacology. 194:279-288.

Doran N, Cook J, McChargue D, Spring B (2009) Impulsivity and cigarette craving: differences across subtypes. Psychopharmacology. in press

Doran N, Spring B, McChargue D, Pergadia M, Richmond M (2004) Impulsivity and smoking relapse. Nicotine and Tobacco Research. 6:641-647.

Drakenberg K, Nikoshkov A, Horváth MC, Fagergren P, Gharibyan A, Saarelainen K, Rahman S, Nylander I, Bakalkin G, Rajs J, Keller E, Hurd YL (2006) Mu opioid receptor A118G polymorphism in association with striatal opioid neuropeptide gene expression in heroin abusers. Proceedings of the National Academy of Sciences of the United States of America. 103:7883-7888.

du Vigneaud V, Ressler C, Swan J, Roberts C, Katsoyannis P (1954) The Synthesis of Oxytocin. Journal of the American Chemical Society. 76:3115-3121.

Duaux E, Gorwood P, Griffon N, Bourdel MC, Sautel F, Sokoloff P, Schwartz JC, Ades J, Lôo H, Poirier MF (1998) Homozygosity at the dopamine D3 receptor gene is associated with opiate dependence. Molecular Psychiatry. 3:333-336.

Dube SR, Felitti VJ, Dong M, Chapman DP, Giles WH, Anda RF (2003) Childhood abuse, neglect, and household dysfunction and the risk of illicit drug use: the adverse childhood experiences study. Pediatrics. 111:564-572.

Elkins IJ, McGue M, Iacono WG (2007) Prospective effects of attention-deficit/hyperactivity disorder, conduct disorder, and sex on adolescent substance use and abuse. Archives of General Psychiatry. 64:1145-1152.

Engelmann M, Ebner K, Landgraf R, Wotjak CT (2006) Effects of Morris water maze testing on the neuroendocrine stress response and intrahypothalamic release of vasopressin and oxytocin in the rat. Hormones and Behavior. 50:496-501.

Ernst M, Luckenbaugh DA, Moolchan ET, Leff MK, Allen R, Eshel N, London ED, Kimes A (2006) Behavioral predictors of substance-use initiation in adolescents with and without attention-deficit/hyperactivity disorder. Pediatrics. 117:2030-2039.

Fahy T, Eisler I (1993) Impulsivity and eating disorders. The British Journal of Psychiatry. 162:193-197.

Falck B, Hillarp NA (1959) On the cellular localization of catechol amines in the brain. Acta Anatomica. 38:277-279.

Feldman R, Weller A, Zagoory-Sharon O, Levine A (2007) Evidence for a neuroendocrinological foundation of human affiliation: plasma oxytocin levels across

pregnancy and the postpartum period predict mother-infant bonding. Psychological Science. 18:965-970.

Fibiger HC, LePiane FG, Jakubovic A, Phillips AG (1987) The role of dopamine in intracranial self-stimulation of the ventral tegmental area. Journal of Neuroscience. 7:3888-3896.

Fillingim RB (2000) Sex, gender, and pain: women and men really are different. Current Review of Pain. 4:24-30.

Finley JC, Lindström P, Petrusz P (1981) Immunocytochemical localization of beta-endorphin-containing neurons in the rat brain. Neuroendocrinology. 33:28-42.

Fischer S, Anderson KG, Smith GT (2004) Coping with distress by eating or drinking: role of trait urgency and expectancies. Psychology of Addictive Behaviors. 18:269-274.

Fishbein DH, Herman-Stahl M, Eldreth D, Paschall MJ, Hyde C, Hubal R, Hubbard S, Williams J, Ialongo N (2006) Mediators of the stress-substance-use relationship in urban male adolescents. Prevention Science. 7:113-126.

Fisher S, Smith GT (2004) Deliberation affects risk taking beyond sensation seeking Personality and Individual Differences. 36:527-537.

Fliers E, Guldenaar SE, van de Wal N, Swaab DF (1986) Extrahypothalamic vasopressin and oxytocin in the human brain; presence of vasopressin cells in the bed nucleus of the stria terminalis. Brain Research. 375:363-367.

Foltin RW, Fischman MW (1991) Assessment of abuse liability of stimulant drugs in humans: a methodological survey. Drug and Alcohol Dependence. 28:3-48.

Friston K, Frith C, Liddle P, Frackowiak R (1991) Comparing functional (PET) images: the assessment of significant change. Journal of Cerebral Blood Flow and Metabolism. 11:690-699.

Friston K, Worsley K, Frackowiak R, Mazziotta J, Evans A (1994) Assessing the significance of focal activations using their spatial extent. Human Brain Mapping. 1:210-220.

Friston K, Holmes A, Worsley K, Poline J, Frith C, Frackowiak R (1995) Statistical parametric maps in functional imaging: A general linear approach. Human Brain Mapping. 2:189-210.

Frost JJ, Wagner HN, Dannals RF, Ravert HT, Links JM, Wilson AA, Burns HD, Wong DF, McPherson RW, Rosenbaum AE (1985) Imaging opiate receptors in the human brain by positron tomography. Journal of Computer Assisted Tomography. 9:231-236.

Galea S, Nandi A, Vlahov D (2004) The social epidemiology of substance use. Epidemiologic Reviews. 26:36-52.

Gamaro GD, Xavier MH, Denardin JD, Pilger JA, Ely DR, Ferreira MB, Dalmaz C (1998) The effects of acute and repeated restraint stress on the nociceptive response in rats. Physiology & Behavior. 63:693-697.

Gambarana C, Masi F, Tagliamonte A, Scheggi S, Ghiglieri O, De Montis MG (1999) A chronic stress that impairs reactivity in rats also decreases dopaminergic transmission in the nucleus accumbens: a microdialysis study. Journal of Neurochemistry. 72:2039-2046.

Garzón M, Pickel VM (2001) Plasmalemmal mu-opioid receptor distribution mainly in nondopaminergic neurons in the rat ventral tegmental area. Synapse. 41:311-328.

Garzón M, Pickel VM (2002) Ultrastructural localization of enkephalin and mu-opioid receptors in the rat ventral tegmental area. Neuroscience. 114:461-474.

Geer EB, Landman RE, Wardlaw SL, Conwell IM, Freda PU (2005) Stimulation of the hypothalamic-pituitary-adrenal axis with the opioid antagonist nalmefene. Pituitary. 8:115-122.

George SR, Kertesz M (1987) Met-enkephalin concentrations in striatum respond reciprocally to alterations in dopamine neurotransmission. Peptides. 8:487-492.

German DC, Speciale SG, Manaye KF, Sadeq M (1993) Opioid receptors in midbrain dopaminergic regions of the rat. I. Mu receptor autoradiography. Journal of Neural Transmission. General Section. 91:39-52.

Gimpl G, Fahrenholz F (2001) The oxytocin receptor system: structure, function, and regulation. Physiological Reviews. 81:629-683.

Giordano LA, Bickel WK, Loewenstein G, Jacobs EA, Marsch L, Badger GJ (2002) Mild opioid deprivation increases the degree that opioid-dependent outpatients discount delayed heroin and money. Psychopharmacology (Berl). 163:174-182.

Goeders NE, Guerin GF (1994) Non-contingent electric footshock facilitates the acquisition of intravenous cocaine self-administration in rats. Psychopharmacology (Berl). 114:63-70.

Goldman D, Oroszi G, Ducci F (2005) The genetics of addictions: uncovering the genes. Nature Reviews. Genetics. 6:521-532.

Golimbet VE, Alfimova MV, Gritsenko IK, Ebstein RP (2007) Relationship between dopamine system genes and extraversion and novelty seeking. Neuroscience and Behavioral Physiology. 37:601-606.

Gong W, Neill D, Justice JB (1996) Conditioned place preference and locomotor activation produced by injection of psychostimulants into ventral pallidum. Brain Research. 707:64-74.

Gorelick DA, Kim YK, Bencherif B, Boyd SJ, Nelson R, Copersino M, Endres CJ, Dannals RF, Frost JJ (2005) Imaging brain mu-opioid receptors in abstinent cocaine users: time course and relation to cocaine craving. Biological Psychiatry. 57:1573-1582.

Guastella AJ, Mitchell PB, Mathews F (2008a) Oxytocin enhances the encoding of positive social memories in humans. Biological Psychiatry. 64:256-258.

Guastella AJ, Mitchell PB, Dadds MR (2008b) Oxytocin increases gaze to the eye region of human faces. Biological Psychiatry. 63:3-5.

Guillemin R, Vargo T, Rossier J, Minick S, Ling N, Rivier C, Vale W, Bloom F (1977) beta-Endorphin and adrenocorticotropin are selected concomitantly by the pituitary gland. Science. 197:1367-1369.

Guo J, Hill KG, Hawkins JD, Catalano RF, Abbott RD (2002) A developmental analysis of sociodemographic, family, and peer effects on adolescent illicit drug initiation. Journal of the American Academy of Child and Adolescent Psychiatry. 41:838-845.

Haas AL, Peters RH (2000) Development of substance abuse problems among drug-involved offenders. Evidence for the telescoping effect. Journal of Substance Abuse. 12:241-253.

Haber SN, Groenewegen HJ, Grove EA, Nauta WJ (1985) Efferent connections of the ventral pallidum: evidence of a dual striato pallidofugal pathway. Journal of Comparative Neurology. 235:322-335.

Haber SN, Lynd E, Klein C, Groenewegen HJ (1990) Topographic organization of the ventral striatal efferent projections in the rhesus monkey: an anterograde tracing study. Journal of Comparative Neurology. 293:282-298.

Hammen C, Davila J, Brown G, Ellicott A, Gitlin M (1992) Psychiatric history and stress: predictors of severity of unipolar depression. Journal of Abnormal Psychology. 101:45-52.

Hampton T (2006) Interplay of genes and environment found in adolescents' alcohol abuse. JAMA. 295:1760-1762.

Haney M, Maccari S, le Moal M, Simon H, Piazza PV (1995) Social stress increases the acquisition of cocaine self-administration in male and female rats. Brain Research. 698:46-52.

Härfstrand A, Fuxe K, Cintra A, Agnati LF, Zini I, Wikström AC, Okret S, Yu ZY, Goldstein M, Steinbusch H (1986) Glucocorticoid receptor immunoreactivity in monoaminergic neurons of rat brain. Proceedings of the National Academy of Sciences of the United States of America. 83:9779-9783.

Harrison PA, Fulkerson JA, Beebe TJ (1997) Multiple substance use among adolescent physical and sexual abuse victims. Child Abuse and Neglect. 21:529-539.

Hashiguchi H, Ye SH, Morris M, Alexander N (1997) Single and repeated environmental stress: effect on plasma oxytocin, corticosterone, catecholamines, and behavior. Physiology and Behavior. 61:731-736.

Heath AC, Martin NG (1993) Genetic models for the natural history of smoking: evidence for a genetic influence on smoking persistence. Addictive Behaviors. 18:19-34.

Heath RG (1963) Electrical self-stimulation of the brain in man. The American Journal of Psychiatry. 120:571-577.

Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U (2003) Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biological Psychiatry. 54:1389-1398.

Heinz A, Reimold M, Wrase J, Hermann D, Croissant B, Mundle G, Dohmen BM, Braus DF, Braus DH, Schumann G, Machulla H-J, Bares R, Mann K (2005) Correlation of stable elevations in striatal mu-opioid receptor availability in detoxified alcoholic patients with alcohol craving: a positron emission tomography study using carbon 11-labeled carfentanil. Archives of General Psychiatry. 62:57-64.

Hernandez-Avila CA, Rounsaville BJ, Kranzler HR (2004) Opioid-, cannabis- and alcohol-dependent women show more rapid progression to substance abuse treatment. Drug and Alcohol Dependence. 74:265-272.

Hien D, Cohen L, Campbell A (2005) Is traumatic stress a vulnerability factor for women with substance use disorders? Clinical Psychology Review. 25:813-823.

Hietala J, West C, Syvälahti E, Någren K, Lehikoinen P, Sonninen P, Ruotsalainen U (1994) Striatal D2 dopamine receptor binding characteristics in vivo in patients with alcohol dependence. Psychopharmacology (Berl). 116:285-290.

Hiroi N, White NM (1993) The ventral pallidum area is involved in the acquisition but not expression of the amphetamine conditioned place preference. Neuroscience Letters. 156:9-12.

Hodgkinson CA, Yuan Q, Xu K, Shen P-H, Heinz E, Lobos EA, Binder EB, Cubells J, Ehlers CL, Gelernter J, Mann J, Riley B, Roy A, Tabakoff B, Todd RD, Zhou Z,

Goldman D (2008) Addictions biology: haplotype-based analysis for 130 candidate genes on a single array. Alcohol and Alcoholism. 43:505-515.

Hooks M, Jones G, Smith A, Neill D, Justice J (1991) Response to novelty predicts the locomotor and nucleus accumbens dopamine response to cocaine. Synapse. 9:121-128.

Hooks M, Juncos J, Justice J, Meiergerd S, Povlock S, Schenk J, Kalivas P (1994) Individual locomotor response to novelty predicts selective alterations in D1 and D2 receptors and mRNAs. Journal of Neuroscience. 14:6144-6152.

Hussong AM, Chassin L (1997) Substance use initiation among adolescent children of alcoholics: testing protective factors. Journal of Studies on Alcohol. 58:272-279.

Hutchison KE, Wood MD, Swift R (1999) Personality factors moderate subjective and psychophysiological responses to d-amphetamine in humans. Experimental and Clinical Psychopharmacology. 7:493-501.

Ibragimov R, Kovács GL, Szabó G, Telegdy G (1987) Microinjection of oxytocin into limbic-mesolimbic brain structures disrupts heroin self-administration behavior: a receptor-mediated event? Life Sciences. 41:1265-1271.

Innis RB, Malison RT, al-Tikriti M, Hoffer PB, Sybirska EH, Seibyl JP, Zoghbi SS, Baldwin RM, Laruelle M, Smith EO, al., e. (1992) Amphetamine-stimulated dopamine release competes in vivo for [123I]IBZM binding to the D2 receptor in nonhuman primates. Synapse. 10:177-184.

Insel TR, Winslow JT (1991) Central administration of oxytocin modulates the infant rat's response to social isolation. European Journal of Pharmacology. 203:149-152.

Insel TR, Shapiro LE (1992) Oxytocin receptors and maternal behavior. Annals of the New York Academy of Sciences. 652:122-141.

Insel TR, Hulihan TJ (1995) A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles. Behavioral Neuroscience. 109:782-790.

Jaber M, Robinson SW, Missale C, Caron MG (1996) Dopamine receptors and brain function. Neuropharmacology. 35:1503-1519.

Jacob S, Brune CW, Carter CS, Leventhal BL, Lord C, Cook EH (2007) Association of the oxytocin receptor gene (OXTR) in Caucasian children and adolescents with autism. Neuroscience Letters. 417:6-9.

Janssens CJ, Helmond FA, Loyens LW, Schouten WG, Wiegant VM (1995) Chronic stress increases the opioid-mediated inhibition of the pituitary-adrenocortical response to acute stress in pigs. Endocrinology. 136:1468-1473.

Jezova D, Skultetyova I, Tokarev DI, Bakos P, Vigas M (1995) Vasopressin and oxytocin in stress. Annals of the New York Academy of Sciences. 771:192-203.

Johnson PI, Stellar JR, Paul AD (1993) Regional reward differences within the ventral pallidum are revealed by microinjections of a mu opiate receptor agonist. Neuropharmacology. 32:1305-1314.

Johnson SW, North RA (1992) Opioids excite dopamine neurons by hyperpolarization of local interneurons. Journal of Neuroscience. 12:483-488.

Johnston L, O'Malley P, Bachman J, Schulenberg J (2009) Monitoring the Future national survey results on drug use: Overview of key findings, 2008, NIH Publication No. 09-7401 Edition. Bethesda, MD: National Institute on Drug Abuse.

Jones J (1700) The Mysteries of Opium Reveal'd. London: Printed for Richard Smith.

Justice AJ, de Wit H (1999) Acute effects of d-amphetamine during the follicular and luteal phases of the menstrual cycle in women. Psychopharmacology. 145:67-75.

Kabbaj M (2004) Neurobiological bases of individual differences in emotional and stress responsiveness: high responders-low responders model. Archives of Neurology. 61:1009-1012.

Kabbaj M, Devine D, Savage V, Akil H (2000) Neurobiological correlates of individual differences in novelty-seeking behavior in the rat: differential expression of stress-related molecules. Journal of Neuroscience. 20:6983-6988.

Kabbaj M, Norton C, Kollack-Walker S, Watson S, Robinson T, Akil H (2001) Social defeat alters the acquisition of cocaine self-administration in rats: role of individual differences in cocaine-taking behavior. Psychopharmacology (Berl). 158:382-387.

Kagan J, Reznick JS, Snidman N (1988) Biological bases of childhood shyness. Science. 240:167-171.

Kalivas PW, Duffy P (1989) Similar effects of daily cocaine and stress on mesocorticolimbic dopamine neurotransmission in the rat. Biological Psychiatry. 25:913-928.

Kalivas PW, Duffy P (1995) Selective activation of dopamine transmission in the shell of the nucleus accumbens by stress. Brain Research. 675:325-328.

Kalivas PW, Nakamura M (1999) Neural systems for behavioral activation and reward. Current Opinion in Neurobiology. 9:223-227.

Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology of motivation and choice. American Journal of Psychiatry. 162:1403-1413.

Kalivas PW, Churchill L, Klitenick MA (1993) GABA and enkephalin projection from the nucleus accumbens and ventral pallidum to the ventral tegmental area. Neuroscience. 57:1047-1060.

Kalivas PW, Churchill L, Romanides A (1999) Involvement of the pallidal-thalamocortical circuit in adaptive behavior. Annals of the New York Academy of Science. 877:64-70.

Kaplan HB, Martin SS, Johnson RJ, Robbins CA (1986) Escalation of marijuana use: application of a general theory of deviant behavior. Journal of Health and Social Behavior. 27:44-61.

Kaplow JB, Curran PJ, Dodge KA, Group CPPR (2002) Child, parent, and peer predictors of early-onset substance use: a multisite longitudinal study. Journal of Abnormal Child Psychology. 30:199-216.

Kasting NW (1988) Simultaneous and independent release of vasopressin and oxytocin in the rat. Canadian Journal of Physiology Pharmacology. 66:22-26.

Kauhanen J, Hallikainen T, Tuomainen TP, Koulu M, Karvonen MK, Salonen JT, Tiihonen J (2000) Association between the functional polymorphism of catechol-O-methyltransferase gene and alcohol consumption among social drinkers. Alcoholism, Clinical and Experimental Research. 24:135-139.

Kelley AE, Baldo BA, Pratt WE, Will MJ (2005) Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. Physiology and Behavior. 86:773-795.

Kelly TH, Robbins G, Martin CA, Fillmore MT, Lane SD, Harrington NG, Rush CR (2006) Individual differences in drug abuse vulnerability: d-amphetamine and sensation-seeking status. Psychopharmacology. 189:17-25.

Kendler KS, Neale MC, Sullivan P, Corey LA, Gardner CO, Prescott CA (1999) A population-based twin study in women of smoking initiation and nicotine dependence. Psychological Medicine. 29:299-308.

Kessler RC (1997) The effects of stressful life events on depression. Annual Review of Psychology. 48:191-214.

Kessler RC, Sonnega A, Bromet E, Hughes M, Nelson CB (1995) Posttraumatic stress disorder in the National Comorbidity Survey. Archives of General Psychiatry. 52:1048-1060.

Khachaturian H, Watson SJ (1982) Some perspectives on monoamine-opioid peptide interaction in rat central nervous system. Brain Research Bulletin. 9:441-462.

Kieres AK, Hausknecht KA, Farrar AM, Acheson A, de Wit H, Richards JB (2004) Effects of morphine and naltrexone on impulsive decision making in rats. Psychopharmacology (Berl). 173:167-174.

Kim S-G, Kim C-M, Choi S-W, Jae Y-M, Lee H-G, Son B-K, Kim J-G, Choi Y-S, Kim H-O, Kim S-Y, Oslin DW (2009) A micro opioid receptor gene polymorphism (A118G) and naltrexone treatment response in adherent Korean alcohol-dependent patients. Psychopharmacology. 201:611-618.

King KM, Chassin L (2004) Mediating and moderated effects of adolescent behavioral undercontrol and parenting in the prediction of drug use disorders in emerging adulthood. Psychology of Addictive Behaviors. 18:239-249.

Kollins SH (2003) Delay discounting is associated with substance use in college students. Addictive Behaviors. 28:1167-1173.

Kornstein SG (1997) Gender differences in depression: implications for treatment. The Journal of Clinical Psychiatry. 58 Suppl 15:12-18.

Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E (2005) Oxytocin increases trust in humans. Nature. 435:673-676.

Kosten TR, Kosten TA, McDougle CJ, Hameedi FA, McCance EF, Rosen MI, Oliveto AH, Price LH (1996) Gender differences in response to intranasal cocaine administration to humans. Biological Psychiatry. 39:147-148.

Kovács GL, Sarnyai Z, Szabó G (1998) Oxytocin and addiction: a review. Psychoneuroendocrinology. 23:945-962.

Kovács GL, Izbéki F, Horváth Z, Telegdy G (1984) Effects of oxytocin and a derivative (Z-prolyl-D-leucine) on morphine tolerance/withdrawal are mediated by the limbic system. Behavioural Brain Research. 14:1-8.

Kreek M, Nielsen D, Butelman E, LaForge K (2005) Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nature Neuroscience. 8:1450-1457.

Krémarik P, Freund-Mercier MJ, Stoeckel ME (1993) Histoautoradiographic detection of oxytocin- and vasopressin-binding sites in the telencephalon of the rat. Journal of Comparative Neurology. 333:343-359.

Krishnan-Sarin S, Reynolds B, Duhig AM, Smith A, Liss T, McFetridge A, Cavallo DA, Carroll KM, Potenza MN (2007) Behavioral impulsivity predicts treatment outcome in a smoking cessation program for adolescent smokers. Drug and Alcohol Dependence. 88:79-82.

Krueger TH, Schedlowski M, Meyer G (2005) Cortisol and heart rate measures during casino gambling in relation to impulsivity. Neuropsychobiology. 52:206-211.

Laakso A, Wallius E, Kajander J, Bergman J, Eskola O, Solin O, Ilonen T, Salokangas RK, Syvalahti E, Hietala J (2003) Personality traits and striatal dopamine synthesis capacity in healthy subjects. American Journal of Psychiatry. 160:904-910.

Land H, Grez M, Ruppert S, Schmale H, Rehbein M, Richter D, Schütz G (1983) Deduced amino acid sequence from the bovine oxytocin-neurophysin I precursor cDNA. Nature. 302:342-344.

Lang RE, Heil JW, Ganten D, Hermann K, Unger T, Rascher W (1983) Oxytocin unlike vasopressin is a stress hormone in the rat. Neuroendocrinology. 37:314-316.

Laucht M, Becker K, Frank J, Schmidt MH, Esser G, Treutlein J, Skowronek MH, Schumann G (2008) Genetic variation in dopamine pathways differentially associated with smoking progression in adolescence. Journal of the American Academy of Child and Adolescent Psychiatry. 47:673-681.

Le Foll B, Gallo A, Le Strat Y, Lu L, Gorwood P (2009) Genetics of dopamine receptors and drug addiction: a comprehensive review. Behavioural Pharmacology. 20:1-17.

le Moal M (2009) Drug abuse: vulnerability and transition to addiction. Pharmacopsychiatry. 42 Suppl 1:S42-55.

Lee H-J, Macbeth AH, Pagani JH, Young WS (2009) Oxytocin: the great facilitator of life. Progress in Neurobiology. 88:127-151.

Lerer E, Levi S, Salomon S, Darvasi A, Yirmiya N, Ebstein RP (2008) Association between the oxytocin receptor (OXTR) gene and autism: relationship to Vineland Adaptive Behavior Scales and cognition. Molecular Psychiatry. 13:980-988.

Levran O, Londono D, O'hara K, Nielsen DA, Peles E, Rotrosen J, Casadonte P, Linzy S, Randesi M, Ott J, Adelson M, Kreek MJ (2008) Genetic susceptibility to heroin addiction: a candidate gene association study. Genes, Brain and Behavior. 7:720-729.

Levy SJ, Pierce JP (1990) Predictors of marijuana use and uptake among teenagers in Sydney, Australia. The International Journal of the Addictions. 25:1179-1193.

Leyton M, Boileau I, Benkelfat C, Diksic M, Baker G, Dagher A (2002) Amphetamine-induced increases in extracellular dopamine, drug wanting, and novelty seeking: a PET/[11C]raclopride study in healthy men. Neuropsychopharmacology. 27:1027-1035.

Li MD, Burmeister M (2009) New insights into the genetics of addiction. Nature Reviews. Genetics. 10:225-231.

Limosin F, Loze J-Y, Rouillon F, Adès J, Gorwood P (2003) Association between dopamine receptor D1 gene DdeI polymorphism and sensation seeking in alcohol-dependent men. Alcoholism, Clinical and Experimental Research. 27:1226-1228.

Lodge DJ, Grace AA (2005) Acute and chronic corticotropin-releasing factor 1 receptor blockade inhibits cocaine-induced dopamine release: correlation with dopamine neuron activity. Journal of Pharmacology and Experimental Therapeutics. 314:201-206.

Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL (1996) Distribution volume ratios without blood sampling from graphical analysis of PET data. Journal of Cerebral Blood Flow and Metabolism. 16:834-840.

Loup F, Tribollet E, Dubois-Dauphin M, Dreifuss JJ (1991) Localization of high-affinity binding sites for oxytocin and vasopressin in the human brain. An autoradiographic study. Brain Research. 555:220-232.

Love TM, Stohler CS, Zubieta JK (2009) Positron emission tomography measures of endogenous opioid neurotransmission and impulsiveness traits in humans. Archives of General Psychiatry. 66:1124-1134.

Lucas LR, Angulo JA, Le Moal M, McEwen BS, Piazza PV (1998) Neurochemical characterization of individual vulnerability to addictive drugs in rats. European Journal of Neuroscience. 10:3153-3163.

Lucas LR, Celen Z, Tamashiro KLK, Blanchard RJ, Blanchard DC, Markham C, Sakai RR, McEwen BS (2004) Repeated exposure to social stress has long-term effects on indirect markers of dopaminergic activity in brain regions associated with motivated behavior. Neuroscience. 124:449-457.

Lukas SE, Sholar M, Lundahl LH, Lamas X, Kouri E, Wines JD, Kragie L, Mendelson JH (1996) Sex differences in plasma cocaine levels and subjective effects after acute cocaine administration in human volunteers. Psychopharmacology. 125:346-354.

Madden GJ, Petry NM, Badger GJ, Bickel WK (1997) Impulsive and self-control choices in opioid-dependent patients and non-drug-using control participants: drug and monetary rewards. Experimental and Clinical Psychopharmacology. 5:256-262.

Mangiavacchi S, Masi F, Scheggi S, Leggio B, De Montis MG, Gambarana C (2001) Long-term behavioral and neurochemical effects of chronic stress exposure in rats. Journal of Neurochemistry. 79:1113-1121.

Mann JJ, Waternaux C, Haas GL, Malone KM (1999) Toward a clinical model of suicidal behavior in psychiatric patients. American Journal of Psychiatry. 156:181-189.

Mansour A, Meador-Woodruff JH, Camp DM, Robinson TE, Bunzow J, Van Tol H, Civelli O, Akil H, Watson SJ (1990) The effects of nigrostriatal 6-hydroxydopamine

lesions on dopamine D2 receptor mRNA and opioid systems. Progress in Clinical and Biological Research. 328:227-230.

Manuck SB, Flory JD, Ferrell RE, Mann JJ, Muldoon MF (2000) A regulatory polymorphism of the monoamine oxidase-A gene may be associated with variability in aggression, impulsivity, and central nervous system serotonergic responsivity. Psychiatry Research. 95:9-23.

Marazziti D, Dell'Osso B, Baroni S, Mungai F, Catena M, Rucci P, Albanese F, Giannaccini G, Betti L, Fabbrini L, Italiani P, Del Debbio A, Lucacchini A, Dell'Osso L (2006) A relationship between oxytocin and anxiety of romantic attachment. Clinical Practice and Epidemiology in Mental Health. 2:28.

Marinelli M, White FJ (2000) Enhanced vulnerability to cocaine self-administration is associated with elevated impulse activity of midbrain dopamine neurons. Journal of Neuroscience. 20:8876-8885.

Marinelli M, Piazza PV (2002) Interaction between glucocorticoid hormones, stress and psychostimulant drugs. The European Journal of Neuroscience. 16:387-394.

Masse L, Tremblay R (1997) Behavior of boys in kindergarten and the onset of substance use during adolescence. Archives of General Psychiatry. 54:62-68.

McEwen BS, De Kloet ER, Rostene W (1986) Adrenal steroid receptors and actions in the nervous system. Physiological Reviews. 66:1121-1188.

McGonagle KA, Kessler RC (1990) Chronic stress, acute stress, and depressive symptoms. American Journal of Community Psychology. 18:681-706.

McGue M (1999) The Behavioral Genetics of Alcoholism. Current Directions in Psychological Science. 8:109-115.

McMahon RC (2001) Personality, stress, and social support in cocaine relapse prediction. Journal of Substance Abuse Treatment. 21:77-87.

Melis MR, Melis T, Cocco C, Succu S, Sanna F, Pillolla G, Boi A, Ferri G-L, Argiolas A (2007) Oxytocin injected into the ventral tegmental area induces penile erection and increases extracellular dopamine in the nucleus accumbens and paraventricular nucleus of the hypothalamus of male rats. The European Journal of Neuroscience. 26:1026-1035.

Melzack R, Torgerson W (1971) On the language of pain. Anesthesiology. 34:50-59.

Meyer C, Boes J, Kim B, Bland P, Frey K (1998) Warping normal patients into the ICBM atlas by maximizing MI. In: International Conference of Functional Mapping of the Human Brain.

Meyer C, Boes J, Kim B, Bland P, Zasadny K, Kison P, Koral K, Frey K, Wahl R (1997) Demonstration of accuracy and clinical versatility of mutual information for automatic multimodality image fusion using affine and thin-plate spline warped geometric deformations. Medical Image Analysis. 1:195-206.

Mikus G, Mörike K, Griese EU, Klotz U (1998) Relevance of deficient CYP2D6 in opiate dependence. Pharmacogenetics. 8:565-568.

Miller DS, Miller TQ (1997) A test of socioeconomic status as a predictor of initial marijuana use. Addictive Behaviors. 22:479-489.

Miller PM, Hersen M, Eisler RM, Hilsman G (1974) Effects of social stress on operant drinking of alcoholics and social drinkers. Behaviour Research and Therapy. 12:67-72.

Milner PM (1989) The discovery of self-stimulation and other stories. Neuroscience and Biobehavioral Reviews. 13:61-67.

Minoshima S, Koeppe R, Mintun M, Berger K, Taylor S, Frey K, Kuhl D (1993) Automated detection of the intercommissural line for stereotactic localization of functional brain images. Journal of Nuclear Medicine. 34:322-329.

Mitchell SH (1999) Measures of impulsivity in cigarette smokers and non-smokers. Psychopharmacology. 146:455-464.

Mogenson G, Yang C (1991) The contribution of basal forebrain to limbic-motor integration and the mediation of motivation to action. Advances in Experimental Medicine and Biology. 295:267-290.

Monroe SM, Harkness K, Simons AD, Thase ME (2001) Life stress and the symptoms of major depression. Journal of Nervous Mental Disease. 189:168-175.

Montgomery AJ, Mehta MA, Grasby PM (2006) Is psychological stress in man associated with increased striatal dopamine levels?: A [11C]raclopride PET study. Synapse. 60:124-131.

Mumenthaler MS, Taylor JL, O'Hara R, Yesavage JA (1999) Gender differences in moderate drinking effects. Alcohol Research and Health. 23:55-64.

Munafò MR, Yalcin B, Willis-Owen SA, Flint J (2008a) Association of the dopamine D4 receptor (DRD4) gene and approach-related personality traits: meta-analysis and new data. Biological Psychiatry. 63:197-206.

Munafò MR, Hitsman B, Rende R, Metcalfe C, Niaura R (2008b) Effects of progression to cigarette smoking on depressed mood in adolescents: evidence from the National Longitudinal Study of Adolescent Health. Addiction. 103:162-171.

Munro CA, McCaul ME, Wong DF, Oswald LM, Zhou Y, Brasic J, Kuwabara H, Kumar A, Alexander M, Ye W, Wand GS (2006) Sex differences in striatal dopamine release in healthy adults. Biological Psychiatry. 59:966-974.

Muscatell KA, Slavich GM, Monroe SM, Gotlib IH (2009) Stressful life events, chronic difficulties, and the symptoms of clinical depression. Journal of Nervous Mental Disease. 197:154-160.

Napier TC (1992) Dopamine receptors in the ventral pallidum regulate circling induced by opioids injected into the ventral pallidum. Neuropharmacology. 31:1127-1136.

Napier TC, Mitrovic I (1999) Opioid modulation of ventral pallidal inputs. Annals of New York Academy of Sciences. 877:176-201.

Narendran R, Hwang DR, Slifstein M, Talbot PS, Erritzoe D, Huang Y, Cooper TB, Martinez D, Kegeles LS, Abi-Dargham A, Laruelle M (2004) In vivo vulnerability to competition by endogenous dopamine: comparison of the D2 receptor agonist radiotracer (-)-N-[11C]propyl-norapomorphine ([11C]NPA) with the D2 receptor antagonist radiotracer [11C]-raclopride. Synapse. 52:188-208.

Nestler EJ (2004) Historical review: Molecular and cellular mechanisms of opiate and cocaine addiction. Trends in Pharmacological Sciences. 25:210-218.

Neumann ID, Krömer SA, Toschi N, Ebner K (2000) Brain oxytocin inhibits the (re)activity of the hypothalamo-pituitary-adrenal axis in male rats: involvement of hypothalamic and limbic brain regions. Regulatory Peptides. 96:31-38.

Newcomb MD, Maddahian E, Bentler PM (1986) Risk factors for drug use among adolescents: concurrent and longitudinal analyses. American Journal of Public Health. 76:525-531.

Nishioka T, Anselmo-Franci JA, Li P, Callahan MF, Morris M (1998) Stress increases oxytocin release within the hypothalamic paraventricular nucleus. Brain Research. 781:56-60.

Nomura M, McKenna E, Korach KS, Pfaff DW, Ogawa S (2002) Estrogen receptor-beta regulates transcript levels for oxytocin and arginine vasopressin in the hypothalamic paraventricular nucleus of male mice. Brain Research. Molecular Brain Research. 109:84-94.

Olds J, Milner P (1954) Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. Journal of Comparative and Physiological Psychology. 47:419-427.

Onaka T (2004) Neural pathways controlling central and peripheral oxytocin release during stress. Journal of Neuroendocrinology. 16:308-312.

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Oslin DW, Berrettini W, Kranzler HR, Pettinati H, Gelernter J, Volpicelli JR, O'Brien CP (2003) A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. Neuropsychopharmacology. 28:1546-1552.

Oswald L, Wong D, Zhou Y, Kumar A, Brasic J, Alexander M, Ye W, Kuwabara H, Hilton J, Wand G (2007) Impulsivity and chronic stress are associated with amphetamine-induced striatal dopamine release. Neuroimage. 36:153-166.

Oswald LM, Wong DF, McCaul M, Zhou Y, Kuwabara H, Choi L, Brasic J, Wand GS (2005) Relationships among ventral striatal dopamine release, cortisol secretion, and subjective responses to amphetamine. Neuropsychopharmacology. 30:821-832.

Ott I, Scott JC (1910) The galactogogue action of the thymus and corpus luteum. Proceedings of the Society for Experimental Biology and Medicine. 8:49-50.

Pecina S, Berridge KC (2005) Hedonic hot spot in nucleus accumbens shell: where do mu-opioids cause increased hedonic impact of sweetness? Journal of Neuroscience. 25:11777-11786.

Pedersen CA, Caldwell JD, Walker C, Ayers G, Mason GA (1994) Oxytocin activates the postpartum onset of rat maternal behavior in the ventral tegmental and medial preoptic areas. Behavioral Neuroscience. 108:1163-1171.

Perkins KA, Gerlach D, Broge M, Grobe JE, Wilson A (2000) Greater sensitivity to subjective effects of nicotine in nonsmokers high in sensation seeking. Experimental and Clinical Psychopharmacology. 8:462-471.

Perry JL, Nelson SE, Carroll ME (2008) Impulsive choice as a predictor of acquisition of IV cocaine self- administration and reinstatement of cocaine-seeking behavior in male and female rats. Experimental and Clinical Psychopharmacology. 16:165-177.

Pfister HP, Muir JL (1989) Influence of exogenously administered oxytocin on central noradrenaline, dopamine and serotonin levels following psychological stress in nulliparous female rats (Rattus norvegicus). International Journal of Neuroscience. 45:221-229.

Piazza P, Le Moal M (1997) Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. Brain Research Reviews. 25:359-372.

Piazza P, Le Moal M (1998a) The role of stress in drug self-administration. Trends in Pharmacological Sciences. 19:67-74.

Piazza P, Deminiere J, le Moal M, Simon H (1990a) Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. Brain Research. 514:22-26.

Piazza P, Deroche-Gamonent V, Rouge-Pont F, Le Moal M (2000) Vertical shifts in self-administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. Journal of Neuroscience. 20:4226-4232.

Piazza P, Deminiere J, Le Moal M, Simon H (1989) Factors that predict individual vulnerability to amphetamine self-administration. Science. 245:1511-1513.

Piazza P, Deroche V, Rouge-Pont F, Le Moal M (1998b) Behavioral and biological factors associated with individual vulnerability to psychostimulant abuse. NIDA Research Monograph. 169:105-133.

Piazza P, Maccari S, Deminiere J, Le Moal M, Mormede P, Simon H (1991a) Corticosterone levels determine individual vulnerability to amphetamine self-administration. Proceedings of the National Academy of Sciences of the United States of America. 88:2088-2092.

Piazza P, Rouge-Pont F, Deminiere J, Kharoubi M, Le Moal M, Simon H (1991b) Dopaminergic activity is reduced in the prefrontal cortex and increased in the nucleus accumbens of rats predisposed to develop amphetamine self-administration. Brain Research. 567:169-174.

Piazza P, Rouge-Pont F, Deroche V, Maccari S, Simon H, Le Moal M (1996) Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission. Proceedings of the National Academy of Sciences of the United States of America. 93:8716-8720.

Piazza P, Deminiere J, Maccari S, Mormede P, Le Moal M, Simon H (1990b) Individual reactivity to novelty predicts probability of amphetamine self-administration. Behavioural Pharmacology. 1:339-345.

Piazza PV, Le Moal ML (1996) Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Annual Review of Pharmacology and Toxicology. 36:359-378.

Pruessner JC, Champagne F, Meaney MJ, Dagher A (2004) Dopamine release in response to a psychological stress in humans and its relationship to early life maternal care: a positron emission tomography study using [11C]raclopride. Journal of Neuroscience. 24:2825-2831.

Ray R, Tyndale RF, Lerman C (2009) Nicotine dependence pharmacogenetics: role of genetic variation in nicotine-metabolizing enzymes. Journal of Neurogenetics. 23:252-261.

Reuter J, Raedler T, Rose M, Hand I, Gläscher J, Büchel C (2005) Pathological gambling is linked to reduced activation of the mesolimbic reward system. Nature Neuroscience. 8:147-148.

Reynolds B (2006) A review of delay-discounting research with humans: relations to drug use and gambling. Behavioural Pharmacology. 17:651-667.

Riccardi P, Zald D, Li R, Park S, Ansari MS, Dawant B, Anderson S, Woodward N, Schmidt D, Baldwin R, Kessler R (2006a) Sex differences in amphetamine-induced displacement of [(18)F]fallypride in striatal and extrastriatal regions: a PET study. American Journal of Psychiatry. 163:1639-1641.

Riccardi P, Li R, Ansari MS, Zald D, Park S, Dawant B, Anderson S, Doop M, Woodward N, Schoenberg E, Schmidt D, Baldwin R, Kessler R (2006b) Amphetamine-induced displacement of [18F] fallypride in striatum and extrastriatal regions in humans. Neuropsychopharmacology. 31:1016-1026.

Rimmele U, Hediger K, Heinrichs M, Klaver P (2009) Oxytocin makes a face in memory familiar. Journal of Neuroscience. 29:38-42.

Ring RH, Malberg JE, Potestio L, Ping J, Boikess S, Luo B, Schechter LE, Rizzo S, Rahman Z, Rosenzweig-Lipson S (2006) Anxiolytic-like activity of oxytocin in male mice: behavioral and autonomic evidence, therapeutic implications. Psychopharmacology. 185:218-225.

Robbins SJ, Ehrman RN, Childress AR, O'Brien CP (1999) Comparing levels of cocaine cue reactivity in male and female outpatients. Drug and Alcohol Dependence. 53:223-230.

Robinson DA, Wei F, Wang GD, Li P, Kim SJ, Vogt SK, Muglia LJ, Zhuo M (2002) Oxytocin mediates stress-induced analgesia in adult mice. The Journal of Physiology. 540:593-606.

Robinson TE, Becker JB (1986) Enduring changes in brain and behavior produced by chronic amphetamine administration: a review and evaluation of animal models of amphetamine psychosis. Brain Research. 396:157-198.

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Research Reviews. 18:247-291.

Robinson TE, Berridge KC (2000) The psychology and neurobiology of addiction: an incentive-sensitization view. Addiction. 95 Suppl 2:S91-117.

Robinson TE, Jurson PA, Bennett JA, Bentgen KM (1988) Persistent sensitization of dopamine neurotransmission in ventral striatum (nucleus accumbens) produced by prior experience with (+)-amphetamine: a microdialysis study in freely moving rats. Brain Research. 462:211-222.

Rolls ET, Rolls BJ, Kelly PH, Shaw SG, Wood RJ, Dale R (1974) The relative attenuation of self-stimulation, eating and drinking produced by dopamine-receptor blockade. Psychopharmacology (Berl). 38:219-230.

Rouge-Pont F, Deroche V, Le Moal M, Piazza PV (1998) Individual differences in stress-induced dopamine release in the nucleus accumbens are influenced by corticosterone. European Journal of Neuroscience. 10:3903-3907.

Rouge-Pont F, Piazza P, Kharouby M, Le Moal M, Simon H (1993) Higher and longer stress-induced increase in dopamine concentrations in the nucleus accumbens of animals predisposed to amphetamine self-administration. A microdialysis study. Brain Research. 602:169-174.

Rouge-Pont F, Marinelli M, Le Moal M, Simon H, Piazza PV (1995) Stress-induced sensitization and glucocorticoids. II. Sensitization of the increase in extracellular dopamine induced by cocaine depends on stress-induced corticosterone secretion. Journal of Neuroscience. 15:7189-7195.

Ruiz MA, Pincus AL, Dickinson KA (2003) NEO PI-R predictors of alcohol use and alcohol-related problems. Journal of Personality Assessment. 81:226-236.

SAMHSA (2009) Results from the 2008 National Survey on Drug Use and Health: National Findings. In: Substance Abuse and Mental Health Services Administration., pp 1-304. Rockville, MD.

Sarason IG, Johnson JH, Siegel JM (1978) Assessing the impact of life changes: development of the Life Experiences Survey. Journal of Consulting and Clinical Psychology. 46:932-946.

Sarnyai Z, Kovács GL (1994) Role of oxytocin in the neuroadaptation to drugs of abuse. Psychoneuroendocrinology. 19:85-117.

Saunders JB, Davis M, Williams R (1981) Do women develop alcoholic liver disease more readily than men? British medical journal (Clinical research ed). 282:1140-1143.

Savaskan E, Ehrhardt R, Schulz A, Walter M, Schächinger H (2008) Post-learning intranasal oxytocin modulates human memory for facial identity. Psychoneuroendocrinology. 33:368-374.

Scheggi S, Leggio B, Masi F, Grappi S, Gambarana C, Nanni G, Rauggi R, De Montis MG (2002) Selective modifications in the nucleus accumbens of dopamine synaptic transmission in rats exposed to chronic stress. Journal of Neurochemistry. 83:895-903.

Schreckenberger M, Klega A, Gründer G, Buchholz H-G, Scheurich A, Schirrmacher R, Schirrmacher E, Müller C, Henriksen G, Bartenstein P (2008) Opioid receptor PET

reveals the psychobiologic correlates of reward processing. Journal of Nuclear Medicine. 49:1257-1261.

Schuckit MA (1987) Biological vulnerability to alcoholism. Journal of Consulting and Clinical Psychology. 55:301-309.

Scott D, Heitzeg M, Koeppe R, Stohler C, Zubieta J (2006) Variations in the human pain stress experience mediated by ventral and dorsal basal ganglia dopamine activity. Journal of Neuroscience. 26:10789-10795.

Seckl JR, Dickson KL, Yates C, Fink G (1991) Distribution of glucocorticoid and mineralocorticoid receptor messenger RNA expression in human postmortem hippocampus. Brain Research. 561:332-337.

Seeman P, Wilson A, Gmeiner P, Kapur S (2006) Dopamine D2 and D3 receptors in human putamen, caudate nucleus, and globus pallidus. Synapse. 60:205-211.

Sesia T, Temel Y, Lim LW, Blokland A, Steinbusch HW, Visser-Vandewalle V (2008) Deep brain stimulation of the nucleus accumbens core and shell: Opposite effects on impulsive action. Experimental Neurology. in press.

Sihvola E, Rose RJ, Dick DM, Pulkkinen L, Marttunen M, Kaprio J (2008) Early-onset depressive disorders predict the use of addictive substances in adolescence: a prospective study of adolescent Finnish twins. Addiction. 103:2045-2053.

Sinha R (2001) How does stress increase risk of drug abuse and relapse? Psychopharmacology (Berl). 158:343-359.

Sinha R (2008) Chronic Stress, Drug Use, and Vulnerability to Addiction. Annals of the New York Academy of Sciences. 1141:105-130.

Sinha R, Catapano D, O'Malley S (1999) Stress-induced craving and stress response in cocaine dependent individuals. Psychopharmacology (Berl). 142:343-351.

Sinha R, Fuse T, Aubin LR, O'Malley SS (2000) Psychological stress, drug-related cues and cocaine craving. Psychopharmacology. 152:140-148.

Sinha R, Garcia M, Paliwal P, Kreek MJ, Rounsaville BJ (2006) Stress-induced cocaine craving and hypothalamic-pituitary-adrenal responses are predictive of cocaine relapse outcomes. Archives of General Psychiatry. 63:324-331.

Siqueira LM, Rolnitzky LM, Rickert VI (2001) Smoking cessation in adolescents: the role of nicotine dependence, stress, and coping methods. Archives of pediatrics and Adolescent Medicine. 155:489-495.

Smith GT, Fischer S, Cyders MA, Annus AM, Spillane NS, McCarthy DM (2007) On the validity and utility of discriminating among impulsivity-like traits. Assessment. 14:155-170.

Smith K, Berridge K (2007) Opioid limbic circuit for reward: interaction between hedonic hotspots of nucleus accumbens and ventral pallidum. Journal of Neuroscience. 27:1594-1605.

Smith KS, Tindell AJ, Aldridge JW, Berridge KC (2009) Ventral pallidum roles in reward and motivation. Behavioural Brain Research 196:155-167.

Smith YR, Stohler CS, Nichols TE, Bueller JA, Koeppe RA, Zubieta JK (2006) Pronociceptive and antinociceptive effects of estradiol through endogenous opioid neurotransmission in women. Journal of Neuroscience. 26:5777-5785.

Sofroniew MV (1983) Morphology of vasopressin and oxytocin neurones and their central and vascular projections. Progress in Brain Research. 60:101-114.

Sofuoglu M, Dudish-Poulsen S, Nelson D, Pentel PR, Hatsukami DK (1999) Sex and menstrual cycle differences in the subjective effects from smoked cocaine in humans. Experimental and Clinical Psychopharmacology. 7:274-283.

Spanagel R, Herz A, Shippenberg T (1992) Opposing tonically active endogenous opioid systems modulate the mesolimbic dopaminergic pathway. Proceedings of the National Academy of Sciences of the United States of America. 89:2046-2050.

Spencer TJ, Biederman J, Wilens TE, Faraone SV (2002) Overview and neurobiology of attention-deficit/hyperactivity disorder. The Journal of Clinical Psychiatry. 63 Suppl 12:3-9.

Stead JDH, Clinton S, Neal C, Schneider J, Jama A, Miller S, Vazquez DM, Watson SJ, Akil H (2006) Selective Breeding for Divergence in Novelty-seeking Traits: Heritability and Enrichment in Spontaneous Anxiety-related Behaviors. Behavior Genetics. 36:697-712.

Steiner H, Gerfen CR (1998) Role of dynorphin and enkephalin in the regulation of striatal output pathways and behavior. Experimental Brain Research. 123:60-76.

Sternberg WF, Bokat C, Kass L, Alboyadjian A, Gracely RH (2001) Sex-dependent components of the analgesia produced by athletic competition. The Journal of Pain. 2:65-74.

Stohler C, Kowalski C (1999) Spatial and temporal summation of sensory and affective dimensions of deep somatic pain. Pain. 79:165-173.

Stoops WW, Lile JA, Robbins CG, Martin CA, Rush CR, Kelly TH (2007) The reinforcing, subject-rated, performance, and cardiovascular effects of d-amphetamine: influence of sensation-seeking status. Addictive Behaviors. 32:1177-1188.

Succu S, Sanna F, Melis T, Boi A, Argiolas A, Melis MR (2007) Stimulation of dopamine receptors in the paraventricular nucleus of the hypothalamus of male rats induces penile erection and increases extra-cellular dopamine in the nucleus accumbens: Involvement of central oxytocin. Neuropharmacology. 52:1034-1043.

Sullivan PF, Kendler KS (1999) The genetic epidemiology of smoking. Nicotine and Tobacco Research. 1 Suppl 2:S51-57; discussion S69-70.

Swanson JM, Sergeant JA, Taylor E, Sonuga-Barke EJ, Jensen PS, Cantwell DP (1998) Attention-deficit hyperactivity disorder and hyperkinetic disorder. Lancet. 351:429-433.

Szeto CY, Tang NL, Lee DT, Stadlin A (2001) Association between mu opioid receptor gene polymorphisms and Chinese heroin addicts. Neuroreport. 12:1103-1106.

Thierry A, Tassin J, Blanc G, Glowinski J (1976) Selective activation of mesocortical DA system by stress. Nature. 263:242-244.

Tindell AJ, Smith KS, Berridge KC, Aldridge JW (2009) Dynamic Computation of Incentive Salience: "Wanting" What Was Never "Liked". Journal of Neuroscience. 29:12220-12228.

Tindell AJ, Smith KS, Peciña S, Berridge KC, Aldridge JW (2006) Ventral pallidum firing codes hedonic reward: when a bad taste turns good. Journal of Neurophysiology. 96:2399-2409.

Tom SM, Fox CR, Trepel C, Poldrack RA (2007) The neural basis of loss aversion in decision-making under risk. Science. 315:515-518.

Tupala E, Hall H, Särkioja T, Räsänen P, Tiihonen J (2000) Dopamine-transporter density in nucleus accumbens of type-1 alcoholics. Lancet. 355:380.

Tupala E, Kuikka JT, Hall H, Bergström K, Särkioja T, Räsänen P, Mantere T, Hiltunen J, Vepsäläinen J, Tiihonen J (2001) Measurement of the striatal dopamine transporter density and heterogeneity in type 1 alcoholics using human whole hemisphere autoradiography. Neuroimage. 14:87-94.

Turner RA, Altemus M, Enos T, Cooper B, McGuinness T (1999) Preliminary research on plasma oxytocin in normal cycling women: investigating emotion and interpersonal distress. Psychiatry. 62:97-113.

Turner RA, Altemus M, Yip DN, Kupferman E, Fletcher D, Bostrom A, Lyons DM, Amico JA (2002) Effects of emotion on oxytocin, prolactin, and ACTH in women. Stress. 5:269-276.

Tyndale RF, Droll KP, Sellers EM (1997) Genetically deficient CYP2D6 metabolism provides protection against oral opiate dependence. Pharmacogenetics. 7:375-379.

Ungless MA, Singh V, Crowder TL, Yaka R, Ron D, Bonci A (2003) Corticotropin-releasing factor requires CRF binding protein to potentiate NMDA receptors via CRF receptor 2 in dopamine neurons. Neuron. 39:401-407.

Unterwald EM, Horne-King J, Kreek MJ (1992) Chronic cocaine alters brain mu opioid receptors. Brain Research. 584:314-318.

Unterwald EM, Rubenfeld JM, Kreek MJ (1994) Repeated cocaine administration upregulates kappa and mu, but not delta, opioid receptors. Neuroreport. 5:1613-1616.

Unterwald EM, Tempel A, Koob GF, Zukin RS (1989) Characterization of opioid receptors in rat nucleus accumbens following mesolimbic dopaminergic lesions. Brain Research. 505:111-118.

Uslaner J, Badiani A, Day HE, Watson SJ, Akil H, Robinson TE (2001) Environmental context modulates the ability of cocaine and amphetamine to induce c-fos mRNA expression in the neocortex, caudate nucleus, and nucleus accumbens. Brain Research. 920:106-116.

Van Leeuwen FW, van Heerikhuize J, van der Meulen G, Wolters P (1985) Light microscopic autoradiographic localization of [3H]oxytocin binding sites in the rat brain, pituitary and mammary gland. Brain Research. 359:320-325.

Van Pett K, Viau V, Bittencourt JC, Chan RK, Li HY, Arias C, Prins GS, Perrin M, Vale W, Sawchenko PE (2000) Distribution of mRNAs encoding CRF receptors in brain and pituitary of rat and mouse. The Journal of Comparative Neurology. 428:191-212.

Vogt LJ, Sim-Selley LJ, Childers SR, Wiley RG, Vogt BA (2001) Colocalization of mu-opioid receptors and activated G-proteins in rat cingulate cortex. Journal of Pharmacology and Experimental Therapeutics. 299:840-848.

Volkow N, Fowler J, Wang G (1999a) Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. Journal of Psychopharmacology. 13:337-345.

Volkow N, Wang G, Fowler J, Logan J, Gatley S, Gifford A, Hitzemann R, Ding Y, Pappas N (1999b) Prediction of reinforcing responses to psychostimulants in humans by brain dopamine D2 receptor levels. American Journal of Psychiatry. 156:1440-1443.

Volkow ND (2001) Drug abuse and mental illness: progress in understanding comorbidity. American Journal of Psychiatry. 158:1181-1183.

Volkow ND (2009) Substance Use Disorders in Schizophrenia--Clinical Implications of Comorbidity. Schizophrenia Bulletin. 35:469-472.

Volkow ND, Fowler JS, Wang GJ (2004) The addicted human brain viewed in the light of imaging studies: brain circuits and treatment strategies. Neuropharmacology. 47 Suppl 1:3-13.

Volkow ND, Fowler JS, Wang G-J, Swanson JM, Telang F (2007) Dopamine in drug abuse and addiction: results of imaging studies and treatment implications. Archives of Neurology. 64:1575-1579.

Volkow ND, Fowler JS, Wang GJ, Hitzemann R, Logan J, Schlyer DJ, Dewey SL, Wolf AP (1993) Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. Synapse. 14:169-177.

Volkow ND, Wang GJ, Fowler JS, Logan J, Gatley SJ, Wong C, Hitzemann R, Pappas NR (1999c) Reinforcing effects of psychostimulants in humans are associated with increases in brain dopamine and occupancy of D(2) receptors. Journal of Pharmacology and Experimental Therapeutics. 291:409-415.

Wanat MJ, Hopf FW, Stuber GD, Phillips PEM, Bonci A (2008) Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of Ih. The Journal of Physiology. 586:2157-2170.

Wand GS, Schumann H (1998) Relationship between plasma adrenocorticotropin, hypothalamic opioid tone, and plasma leptin. Journal of Clinical Endocrinology and Metabolism. 83:2138-2142.

Wand GS, Oswald LM, McCaul ME, Wong DF, Johnson E, Zhou Y, Kuwabara H, Kumar A (2007) Association of amphetamine-induced striatal dopamine release and cortisol responses to psychological stress. Neuropsychopharmacology. 32:2310-2320.

Wang B, Shaham Y, Zitzman D, Azari S, Wise RA, You Z-B (2005) Cocaine experience establishes control of midbrain glutamate and dopamine by corticotropin-releasing factor: a role in stress-induced relapse to drug seeking. Journal of Neuroscience. 25:5389-5396.

Wang GJ, Volkow ND, Fowler JS, Logan J, Abumrad NN, Hitzemann RJ, Pappas NS, Pascani K (1997) Dopamine D2 receptor availability in opiate-dependent subjects before and after naloxone-precipitated withdrawal. Neuropsychopharmacology. 16:174-182.

Watabe H, Endres CJ, Breier A, Schmall B, Eckelman WC, Carson RE (2000) Measurement of dopamine release with continuous infusion of [11C]raclopride:

optimization and signal-to-noise considerations. Journal of Nuclear Medicine. 41:522-530.

Watson D, Clark L, Tellegen A (1988) Development and validation of brief measures of positive and negative affect: the PANAS scales. Journal of Personality and Social Psychology. 54:1063-1070.

Wermter A, Kamp-Becker I, Hesse P, Schulte-Körne G, Strauch K, Remschmidt H (2009) Evidence for the involvement of genetic variation in the oxytocin receptor gene (OXTR) in the etiology of autistic disorders on high-functioning level. American Journal of Medical Genetics Part B Neuropsychiatric Genetics, in press.

White TL, Justice AJH, de Wit H (2002) Differential subjective effects of D-amphetamine by gender, hormone levels and menstrual cycle phase. Pharmacology, Biochemistry, and Behavior. 73:729-741.

Whiteside SP, Lynam DR (2001) The Five Factor Model and impulsivity: using a structural model of personality to understand impulsivity Personality and Individual Differences. 30:669-689.

Wigger A, Neumann ID (2002) Endogenous opioid regulation of stress-induced oxytocin release within the hypothalamic paraventricular nucleus is reversed in late pregnancy: a microdialysis study. Neuroscience. 112:121-129.

Williams JR, Carter CS, Insel T (1992) Partner preference development in female prairie voles is facilitated by mating or the central infusion of oxytocin. Annals of the New York Academy Sciences. 652:487-489.

Wills TA, Vaccaro D, McNamara G, Hirky AE (1996) Escalated substance use: a longitudinal grouping analysis from early to middle adolescence. Journal of Abnormal Psychology. 105:166-180.

Wills TA, Walker C, Mendoza D, Ainette MG (2006) Behavioral and emotional self-control: relations to substance use in samples of middle and high school students. Psychology of Addictive Behaviors. 20:265-278.

Wills TA, Sandy JM, Yaeger AM, Cleary SD, Shinar O (2001) Coping dimensions, life stress, and adolescent substance use: a latent growth analysis. Journal of Abnormal Psychology. 110:309-323.

Windle RJ, Shanks N, Lightman SL, Ingram CD (1997) Central oxytocin administration reduces stress-induced corticosterone release and anxiety behavior in rats. Endocrinology. 138:2829-2834.

Winstanley CA, Theobald DE, Dalley JW, Robbins TW (2005) Interactions between serotonin and dopamine in the control of impulsive choice in rats: therapeutic implications for impulse control disorders. Neuropsychopharmacology. 30:669-682.

Wise RA (2004) Dopamine, learning and motivation. Nature Reviews. Neuroscience. 5:483-494.

Worsley K (1994) Local maxima and the expected Euler characteristic of excursion sets of chi(2), F and t fields. Advances in Applied Probability. 26:13-42.

Wotjak CT, Ganster J, Kohl G, Holsboer F, Landgraf R, Engelmann M (1998) Dissociated central and peripheral release of vasopressin, but not oxytocin, in response to repeated swim stress: new insights into the secretory capacities of peptidergic neurons. Neuroscience. 85:1209-1222.

Wu S, Jia M, Ruan Y, Liu J, Guo Y, Shuang M, Gong X, Zhang Y, Yang X, Zhang D (2005) Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. Biological Psychiatry. 58:74-77.

Yunus MB (2001) The role of gender in fibromyalgia syndrome. Current Rheumatology Reports. 3:128-134.

Zhang X, Ashton-Miller J, Stohler C (1993) A closed-loop system for maintaining constant experimental muscle pain in man. IEEE Transactions on Biomedical Engineering. 40:344-352.

Zouk H, Tousignant M, Seguin M, Lesage A, Turecki G (2006) Characterization of impulsivity in suicide completers: clinical, behavioral and psychosocial dimensions. Journal of Affective Disorders. 92:195-204.

Zubieta J, Smith Y, Bueller J, Xu Y, Kilbourn M, Jewett D, Meyer C, Koeppe R, Stohler C (2002) mu-opioid receptor-mediated antinociceptive responses differ in men and women. Journal of Neuroscience. 22:5100-5107.

Zubieta J, Smith Y, Bueller J, Xu Y, Kilbourn M, Jewett D, Meyer C, Koeppe R, Stohler C (2001) Regional mu opioid receptor regulation of sensory and affective dimensions of pain. Science. 293:311-315.

Zubieta JK, Dannals RF, Frost JJ (1999) Gender and age influences on human brain mu-opioid receptor binding measured by PET. American Journal of Psychiatry. 156:842-848.

Zubieta JK, Gorelick DA, Stauffer R, Ravert HT, Dannals RF, Frost JJ (1996) Increased mu opioid receptor binding detected by PET in cocaine-dependent men is associated with cocaine craving. Nature Medicine. 2:1225-1229.

Zubieta JK, Heitzeg MM, Smith YR, Bueller JA, Xu K, Xu Y, Koeppe RA, Stohler CS, Goldman D (2003) COMT val158met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science. 299:1240-1243.

Zuckerman M, Kuhlman DM (2000) Personality and risk-taking: common biosocial factors. Journal of Personality. 68:999-1029.