UNIVERSITY OF CALIFORNIA, SAN DIEGO

Chronic Stress and Extended Access to Cocaine Produce Increased Drug Intake: Involvement of κ-opioid Receptor Activation

A Thesis submitted in partial satisfaction for the requirements for the degree

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in

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by

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2013

The Thesis of Sharon Chaing is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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University of California, San Diego
2013

DEDICATION

This is dedicated to the following:

Dr. George F. Koob and Dr. Olivier George for giving me the opportunity to work in Koob Lab and earn my Master's degree.

Dr. Tim Whitfield for guiding me through the norBNI experiment and providing laughter and amusement.

Dr. Scott Edwards for guiding me through the stress-cocaine experiment, giving me resources, and answering my incessant questions.

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EPIGRAPH

"A smooth sea never made a skilled sailor."

English Proverb

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Figure 10 is a reprint of material as it appears in "Emergence of Context-associated GluR1 and ERK Phosphorylation in the Nucleus Accumbens Core during Withdrawal from Cocaine Self-administration." Edwards, Scott, Ryan K. Bachtell, Daniel Guzman, Kimberly N. Whisler, and David W. Self. Addiction Biology 16.3 (2011): 450-57. Dr. Scott Edwards was first author of this paper.

Graphs 1, 2, and 3 were produced by Dr. Tim Whitfield.

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ABSTRACT OF THE THESIS

Chronic Stress and Extended Access to Cocaine Produce Increased Drug Intake: Involvement of κ-opioid Receptor Activation

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The transition from recreational cocaine use to drug addiction (aka "drug dependence") is thought to be a result of the dysregulation of brain reward and stress

systems. The present studies examined the participation of κ-opioid receptor activation in the escalation of cocaine intake produced by either extended-access to cocaine or stress.

Part 1: Dynorphin is a neuropeptide that activates κ-opioid receptors to produce negative emotional states (e.g., dysphoria) associated with cocaine dependence. Injection of norbinaltorphimine (norBNI), a κ-receptor antagonist, attenuated cocaine intake in rats allowed extended access (6 h/day). These findings indicate a functional role of κ-receptors in cocaine dependence and implicate κ-receptors as a potential therapeutic target.

Part 2: Chronic mild stress (footshocks: 0.5 mA, 20 min/session: 10 min after each hour of self-administration) during cocaine self-administration sessions (2 h/day) produced a significant escalation in cocaine intake in rats. This data suggests that stress is a factor that contributes to drug addiction. Western blot analysis revealed that in the nucleus accumbens (NAc) core, phosphorylation of GluR1at serine 845 was significantly decreased in stressed subjects in comparison to non-stressed subjects and in the NAc shell, phosphorylation of ERK1/2 was increased in subjects undergoing cocaine withdrawal.

Extended access to cocaine as well as exposure to stress are environmental factors that have each been shown to cause increased cocaine intake, which can be indicative of a transition to drug addiction.

Introduction

1. Cocaine and Addiction

Cocaine is a potent reinforcer with a high abuse potential. Like many drugs of abuse, addiction to cocaine can result in an individual seeking and taking the drug even at a high cost to the addicted individual, his family, and to society. In the European Union, cocaine use has increased dramatically in the past decade (Hoare and Moon, 2010). In the United States, there are approximately 1.4 million current (previous month) cocaine users (National Survey on Drug Use and Health , 2011). In 2009, it was estimated that 422, 896 emergency department visits were related to cocaine (Substance Abuse and Mental Health Services Administration, 2011). However, most recreational cocaine users do not become cocaine addicts. Studies have approximated that 15.6% (29 million) of the U.S. adult population will engage in nonmedical or illicit drug use at some time in their lives but approximately 19% (5.4 million) of those who had used drugs will go on to acquire addiction to illicit drugs (Grant and Dawson, 1998; Grant et al, 2004). In the transition from recreational cocaine use to addiction, cocaine users will spend progressively more time and effort to chase the euphoria associated with cocaine that continually decreases with repeated use (Ahmed et al, 2002). In order to be able to properly treat and, possibly, prevent cocaine addiction, it is necessary to know how cocaine administration affects and dysregulates brain neurocircuitry.

Drug addiction (also referred to as "substance dependence") is defined as a maladaptive pattern of substance abuse leading to clinically significant impairment or

distress, as manifested by three (or more) of the following occurring at any time in the same 12-month period ("Diagnostic and Statistical Manual of Mental Disorders: DSM-IV-TR," 2000):

1. Tolerance, which is defined by either of the following:

    (a) the progressive need for higher drug doses to produce a desired effect which results in an escalation of drug use

    (b) markedly diminished effect with continued use of the same amount of substance.

2. Withdrawal, as manifested by either of the following:

    (a) emergence of characteristic withdrawal symptoms for the substance, or

    (b) the same (or closely related) substance is taken to avoid or relieve withdrawal symptoms.

3. The substance is often taken in larger amounts and/or over a longer period than intended.

4. Unsuccessful efforts to cut down or control substance use.

5. A great deal of time is spent in trying to obtain the substance, use the substance, or recover from its effects.

6. Important social, occupational, or recreational activities are reduced or sacrificed due to substance use.

7. Substance use is continued despite understanding of the persistent physical or psychological problems that are likely to have been caused or exacerbated by the substance.

Cocaine is a psychostimulant drug that blocks dopamine reuptake on the central nervous system and leads to increases in synaptic dopamine levels. Dopamine is a neurotransmitter originally considered to mediate reward, but recent research has shown that it is not only associated with appetitive stimuli but also aversive stimuli and that instead of processing reward, it might be more involved in the attribution of incentive salience and learning processes associated with positive and negative reinforcement (Berridge, 2007). When dopamine is released by neurons in response to salient stimuli (e.g., the taste of palatable food), it is recycled back into the pre-synaptic neuron through the dopamine transporter (DAT). DATs and dopamine D2 autoreceptors work together to limit synaptic dopamine levels via rapid dopamine reuptake and negative feedback regulation, respectively (Bello et al., 2011). However, cocaine prevents dopamine from being recycled by blocking DATs, which leads to excessive amounts of dopamine in the synapse, which amplifies the message to and response of the post-synaptic neuron. It is believed that this excess of synaptic dopamine is responsible for the hedonic effect (the "high") of cocaine ("Drug Facts: Cocaine," 2010). With chronic use, cocaine has been shown to cause long-term changes in the brain reward and stress systems, and these neuroadaptations are hypothesized to cause the development and maintenance of escalated drug intake associated with cocaine addiction (Edwards and Koob, 2010).

Addiction can be viewed as the end result of a transition from recreational drug use to excessive, compulsive drug use, which is a defining feature of addiction (Ahmed and Koob, 1998). Many factors can contribute to this transition: route of drug administration, availability, genetic makeup, history of drug use, and stress (Koob and Le Moal, 1997). Thus, it is critical to understand which neuroadaptive changes are associated with recreational drug use and the loss of behavioral control over drug-seeking and drug-taking seen in addiction. Identifying these neuroadaptive changes is critical, not only in understanding addiction but also for the development of therapeutic targets for the treatment of the addiction.

1.1 Studying Drug Addiction with the Rat Model

It is difficult to study the cause of drug addiction using only human subjects because of polydrug use, failure to comply, and individual differences. With these limitations in mind, it has been advantageous to model certain aspects of addiction using animal models (Hyman, Malenka, and Nestler, 2006). Drug self-administration in animals is often used to investigate the reinforcing properties of drugs of abuse and has shown that cocaine is an effective reinforcer in both humans and animals (Goeders and Guerin, 1994). Cocaine is readily self-administered by humans, monkeys, and rats (Koob and Bloom, 1998).

In these models, drug availability is a determining factor in the transition from stable drug intake to excessive drug intake. When rats are maintained on a restricted 1-hour access (short access; ShA) to self-administer intravenous cocaine, drug intake remained low and stable. In contrast, rats that are given extended drug availability by

being maintained on a 6-hour access (long-access; LgA) to cocaine display escalation of drug intake over days (Ahmed and Koob, 1998). LgA rats also display increased intake early in the session, sustained intake over the session, and an upward shift in the dose-effect function, suggesting an increase in the hedonic set point (Koob and Kreek, 2007). This phenomenon is also observed in humans where increasing drug availability can trigger escalation of stimulant consumption (Wikler, 1952). Human cocaine addicts have been shown to self-administer cocaine to the extent of their funds and drug availability and within the limits of tolerable side effects (ex. jitteriness, nervousness, dysphoria, etc.). Therefore, an extended access model of cocaine availability, rather than short-access, is believed to more closely reflect the human condition of drug addiction (Koob and Kreek, 2007).

Stress has also been found to be an integral factor in the transition from drug use to drug dependence. There is evidence that mild foot-shock, which is a stressor to animals, increases cocaine escalation in comparison to subjects that are not exposed to the stress. (Mantsch and Katz, 2007; Goeders and Guerin, 1994). Social stress has also been shown to increase cocaine intravenous (IV) self-administration in rats (Miczek and Mutschler, 1996). In cocaine dependent humans, the incidence of a number of stress disorders, including post-traumatic stress disorder (PTSD; Najavits et al, 1998) and panic disorders (Anthony, et al, 1989) is higher than the normal population. In a subpopulation of cocaine users, stressful life events may be a factor that leads to later cocaine use, abuse, and/or addiction (Sinha et al., 1999; 2001).

It has also been observed that both human and animal cocaine users adjust their intake to maintain a specific level of cocaine reward. Koob et al. (1994) has shown that rats trained to self-administer IV cocaine with limited access (3 hours/day) develop a stable and regular drug intake across sessions. This finding has been systematically reproduced in cocaine users (Zittel-Lazarini et al., 2007). These results suggest that both humans and animals actively regulate their cocaine intake in order to experience the positive rewarding effects of the drug at an ideal level, also known as the hedonic or reward set point. Thus we can conceptualize escalation of drug intake, which is indicative of drug addiction, as an alteration in the hedonic set point due to allostatic changes in response to drug availability, environmental stress, etc.

1.2 Allostatic Regulation tied to Cocaine Addiction

Allostasis is defined by "the process of achieving stability through change" (Koob and Kreek, 2007). The difference between homeostatic regulation and allostatic regulation is that homeostatic regulation reacts only to deviations from normal set points, whereas allostatic regulation allows for continuous readjustments to new set points depending on demand (Sterling and Every, 1988). Thus, an allostatic model of brain motivational systems can frame addiction as a cycle of continued dysregulation of the brain reward and stress systems. The brain reward system includes dopamine and opioid peptide networks (Koob and Le Moal, 2001). The stress system includes neurotransmitters such as corticotropin-releasing factor (CRF), norepinephrine, and dynorphin that produce aversive or stress-like states (Edwards and Koob, 2010).

These processes of dysregulation result in the negative emotional state that contributes to the compulsive seeking and taking of drugs, even in the face of detrimental consequences. Chronic use of drugs of abuse will force the brain to maintain stability through epigenetic, biochemical, cellular, and neuroplastic changes. These changes can cause a decreased function of reward circuits and recruitment of brain stress systems thus setting up an elevated reward set point (a new hedonic set point) which leads to the loss of control over cocaine intake, which represents one defining feature of cocaine addiction (Edwards and Koob, 2010).

Because dysregulation of brain systems is linked to increased and escalating patterns of intake, the examination of cocaine self-administration under conditions that promote these patterns of intake should allow for better understanding of the factors that contribute to cocaine addiction. The present study tested the hypothesis that κ-opioid receptor activity increases cocaine intake. To this end, we used two different experimental approaches to explore this possibility. The first experiment tested the effect of a κ-opioid receptor antagonist (norbinaltorphimine, norBNI) on cocaine intake. The second experiment focused on stress-induced escalation and explored whether it was associated with changes in κ-opioid receptor-regulated signaling cascades.

1.3 Dynorphin / k-opioid receptor activation

The norBNI experiment focused on the κ-opioid system that has been shown to play a role in mediating negative reinforcement. The dysregulation of the κ-opioid system is thought to result in a potentiation of brain stress systems that promotes a

dysphoric state during withdrawal and, consequently, motivate cocaine intake via negative reinforcement. Thus, we hypothesized that chronic κ-opioid receptor blockade with norBNI would attenuate cocaine intake in rats with extended access to cocaine.

The activation of the dynorphin system in the nucleus accumbens has been associated with reduced dopamine release as a consequence of chronic administration of opioids and psychostimulants, specifically cocaine and amphetamine (Nestler 2004; Koob 2008; Wee and Koob, 2010). Dynorphins are a class of opioid peptides cleaved from the precursor protein prodynorphin and are endogenous ligands for the κ-opioid receptor, a member of the G-protein coupled receptor family. Dynorphin is produced in many parts of the brain, including the hypothalamus, striatum, hippocampus, and spinal cord where it has a role in many different physiological actions including motor function, cardiovascular function, eating, sensory processing, and pain perception (Fallon and Leslie, 1986). Dynorphin activates κ-opioid receptors which inhibits dopamine release in the mesolimbic pathway. Dynorphin appears to function in an intercellular feedback loop. The release of dynorphin binds to the κ-opioid receptors present on dopaminergic presynaptic nerve terminals in the nucleus accumbens and also on cell bodies and dendrites in the ventral tegmental area to inhibit their functioning (Nestler et al., 1999). Figure 1 and 2 illustrates this feedback loop, motivationally and molecularly, respectively. Chronic cocaine exposure upregulates the activity of this negative feedback loop which results in increased dynorphin peptide levels.

Dynorphin-induced dopamine inhibition produces negative emotional states (e.g. dysphoria) associated with cocaine dependence and decreased reward during cocaine withdrawal, thus increasing the motivation to take cocaine to relieve this aversive state (Spanagel and Shippenberg, 1992; Margolis et al., 2003; Sivam, 1989; Spangler, Unterwald, and Kreek, 1993). This compensation to take more drug to avoid the dysphoric state seen in withdrawal marks a change in the hedonic set point and thus a dysregulation of the dynorphin system (a brain stress system); there is an inability for the drug to be sufficiently rewarding to overcome the dysphoric state without escalating intake (Figure 1).

At the molecular level, the activation of the dynorphin system by chronic cocaine starts with the activation of stimulatory G-protein coupled dopamine D1 receptors that stimulates protein kinase A (PKA) activity to increase cyclic-AMP (cAMP) formation. This leads to an increase in the phosphorylation of cAMP response-element binding protein (CREB) on serine 133 (Edwards and Koob, 2010). Phosphorylation of CREB allows translocation to the nucleus and, in the nucleus, p-CREB binds to specific DNA sequences called cAMP response elements (CRE), which increases the transcription of certain downstream genes (Purves, et al, 2008). CREB is known to increase the transcription of a large number of genes that can alter neuronal function and regulate synaptic plasticity (Frank and Greenberg 1994; Mayr and Montminy 2001) such as c-fos, prodynorphin (the precursor of dynorphin), CRF, and brain-derived neurotrophic factor (BDNF), which have all been implicated in aspects of drug addiction (Nestler et al., 1999, Edwards and Koob, 2010, Itoi et al,

1996, Pandey et al., 2004*).* Thus increased transcription of prodynorphin can lead to an increase in dynorphin peptide levels and, ultimately, dynorphin release. Figure 3 illustrates this process in a non-addicted state and figure 4 illustrates this process in the addicted state.

Studies have shown that activation of the κ-opioid receptors causes place aversion in rodents and dysphoria in humans (Pfeiffer et al. 1986; Shippenberg and Herz, 1986*).* These data suggest that dynorphins, acting through κ-opioid receptors, underlie the dysphoric, aversive, and anxiogenic effects of stress resulting in an overall negative emotional state. This negative emotional state during cocaine withdrawal is thought to promote drug relapse and compulsive patterns of use. It is plausible to hypothesize that dynorphin activation of κ-opioid receptors mediates the intense craving and dysphoria during drug withdrawal in human and animal models of drug addiction (Chavkin, 2012).

Thus, we hypothesized that pretreatment with nor-binaltorphimine (norBNI), a highly selective, long-lasting κ-opioid receptor antagonist, would prevent animals from becoming dependent on cocaine even if given long-access to the drug, by preventing the negative reinforcement associated with the activation of κ-opioid receptors and thus preventing the over-recruitment of the dynorphin system and the change in the hedonic set point.

1.4 Stress & Cocaine

Stress has been identified as a factor in the transition from recreational cocaine use to cocaine dependence. In biological terms, stress is defined as any condition that

alters the homeostasis of an organism (Kim and Diamond, 2002). Exposure to stressors induces behavioral and physiological changes that promote long-term, adaptive responses to such stressful events (Krugers et al., 2010). Such changes may include the alteration of brain reward circuits resulting in a greater sensitivity to the reinforcing properties of drugs thus increasing the motivation for compulsive drug use. Thus, with regards to drug addiction, stress may "prime" brain reward systems making drug use more reinforcing than without stress (Piazza and Le Moal 1998, Koob and Le Moal 1997). It has been suggested that during acute and chronic stress states, there is greater motivation to enhance mood. Since addictive substances provide both negative reinforcement (by reducing negative affect through relief from stress) and positive reinforcement (by enhancing mood), initial drug use may be used to modulate tension or distress enhanced by stress. However, the repeated use of this maladaptive coping strategy to successfully reduce negative affect and increase positive affect may lead to drug intake as the pervasive response for both stress relief and mood enhancement (Shiffman 1982).

The stress-cocaine experiment focused on the effects of stress on cocaine intake and adaptations in brain neurocircuitry related to the κ-opioid receptor system. Neuroadaptations were studied through analysis of phosphoprotein levels between rats with a history of stress-induced escalation of cocaine intake versus rats with a history of stable cocaine intake and rats with no history of exposure to cocaine. Various human and animals studies have found a positive association between chronic stress and drug abuse. Human individuals with early histories of sexual abuse have been

found to be at greater risk for drug abuse and often report an earlier age of onset for drug abuse (Dembo et al. 1988; Harrison et al. 1997; Widom et al. 1999). In fact, in cocaine dependent individuals, the incidence of stress disorders, including post-traumatic stress disorder (PTSD; Najavits et al, 1998) and panic disorder (Anthony, et al, 1989) is higher than the normal population. Studies have suggested that, at least in a subpopulation of cocaine users, stressful life events may be a factor that leads to later cocaine use, abuse, and/or addiction *(*Sinha et al., 1999; 2001).

Laboratory animals exposed to social defeat stress and animals exposed to foot-shock stress also increased cocaine self-administration (Covington and Miczek, 2001*;* Goeders and Guerin, 1994). Stress-induced escalated cocaine intake can be seen in animals even when given a limited access (2 or 3 hours per session) to the drug (Mantsch and Katz, 2007). Human subjects have reported that initial cocaine use produces profound subjective feelings of well-being along with a decrease in anxiety (Gawin and Ellinwood, 1989). On the other hand, cocaine withdrawal often includes symptoms such as severe anxiety, restlessness, agitation and depression (Goeders and Guerin, 1994). In both human and rats, there is a positive correlation between stress and cocaine seeking (Sinha, Capatano, and O'Malley, 1999; Ahmed and Koob, 1997; Erb and Stewart, 1999*).* These findings suggest the possibility that neuroadaptations in brain stress circuits may lead to a greater sensitivity to the reinforcing properties of cocaine.

Changes in glutamate homeostasis may be a possible neuroadapatation in drug addiction. Glutamate is the main excitatory neurotransmitter in the central nervous

system. Glutamate receptors can be divided into two categories: ionotropic glutamate receptors and metabotropic glutamate receptors. Ionotropic glutamate receptors are ligand-gated ion channels and are classified into three major subtypes: *N*-methyl-D-aspartic acid (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and kainic acid (Guo et al., 2009*).* AMPA receptors are responsible for the primary depolarization in glutamate-mediated neurotransmission and play key roles in synaptic plasticity (Santos et al., 2009). They are heterotretrameric protein complexes composed of various subunits referred to as GluR1, GluR2, GluR3, GluR4 which combine in tetramers in different stoichiometries (Hollman and Heinemann, 1994) and determine channel function (i.e. conductance and sensitization/resensitization properties) (Ozawa, Kamiya, and Tsuzuki, 1998) and trafficking to synapses (Malinow, Mainen, and Hayashi, 2000) The key post-translational modification in regulating AMPA receptor function is phosphorylation (Carvalho et al., 2008) which can regulate the physiological properties of the channel as well as protein trafficking (Santos et al., 2009). The GluR1 subunit has four phosphorylation sites but the one of interest in this study is at serine 845 (S845) that is phosphorylated by PKA (Roche et al., 1996) and regulates AMPA receptor opening probability and assists in delivering GluR1-containing AMPA receptors to the synapse (Banke et al., 2000; Esteban, 2003).

Changes to glutamate homeostasis occur after withdrawal from chronic cocaine administration (Figure 5). Reduction of cystine-glutamate exchange results in a decrease in basal extrasynaptic glutamate levels. This results in an upregulation of activator G protein signalling 3 (AGS3) causes a decrease in mGluR2/3 signaling and

results in an increase in synaptic release of glutamate (Kalivas, 2009). This leads to a Ca^{2+} influx through Ca^{2+} permeable AMPA receptors and thus increases intracellular Ca^{2+} levels. Ca^{2+}/calmodulin-dependent kinase II (CamKII) is activated following increases in intracellular Ca^{2+} (Hanson and Schulman, 1992). Ras, acting through the ERK1/2 MAPK signaling pathways, enhances AMPA receptor-mediated synaptic transmission by driving synaptic delivery of AMPA receptors containing long cytoplasmic tails to the membrane (Zhu et al., 2002). In this pathway, the phosphorylation of CREB is dependent on a PI 3-kinase-sensitive activation of the ERK1/2 MAPK cascade (Perkinton, Sihra, and Williams, 1999). The phosphorylation of CREB increases transcription of specific genes that have been implicated in drug addiction. Withdrawal also causes lowered levels of mesolimbic dopamine release and increased PKA activity (Fitzgerald et al., 1996; Lee and Messing, 2008). PKA is implicated in the phosphorylation of CREB and $GluR1^{S845}$. The phosphorylation of GluR1 at serine 845 increases the opening probability of AMPA receptors as well as assists in the delivery of GluR1-containing AMPA receptor to the synapses (Banke et al., 2000; Esteban, 2003).

Behaviorally, the hypothesis was that foot-shock stress would cause cocaine escalation, as seen in other studies. Molecularly, the hypothesis was that stress-induced escalation of cocaine intake is driven by κ-opioid receptor-regulated signaling cascades. Thus, there should be differences in phosphoprotein levels between these three groups of animals (stress-induced cocaine intake, cocaine intake, and naïve) within the mesolimibic circuitry, specifically the nucleus accumbens. The mesolimbic

dopaminergic circuitry (Figure 6) is composed of ventral tegmental area (VTA) dopaminergic neurons and its projections to the nucleus accumbens (NAc), the amygdala (Amy), and the medial prefrontal cortex (mPFC) (Guo et al, 2009). The VTA and the NAc are the nuclei most consistenly implicated in a variety of reward effects (Bjorkland and Dunnet, 2007).

The NAc is involved in the attentional and emotional states that accompany motivated behavior (Muschamp and Carlezon, 2013). The NAc is associated with dopamine and glutamate homeostasis. The NAc and its inputs from other components of the mesolimbic dopamine circuitry are critical in the activation of drug-seeking behavior in response to various drugs of abuse or drug-paired stimuli (Ghitza et al., 2004). The NAc receives excitatory glutamatergic afferents from the prefrontal cortex and certain limbic structures and receives dopaminergic inputs from the ventral tegmental area (McFarland, Lapish, and Kalivas, 2003; Corbit, Muir, and Balleine, 2001).The NAc and its inputs exists as a "limbic-motor" interface by integrating memory input from the amygdala and contextual information from the hippocampus and then coordinating appropriate goal-directed behavior responses through the recruitment of elements of the basal ganglia, such as the globus pallidus, that are critical for motor output (Mogenson, Jones, and Yim, 1980; Redgrave, Vautrelle, and Reynolds, 2011).

The NAc is comprised of two regions, the "shell" and the "core", which differ in connectivity, neurochemistry, and function (Meredith et al., 1996; Heimer et al., 1991; Brog et al., 1991; Corbit, Muir, and Balleine, 2001). The shell is innervated

preferentially by the ventromedial prefrontal and infralimbic cortex, whereas the core is innervated preferentially by the dorsal prefrontal cortex and is more closely connected with structures associated with somatomotor function (Berndse, Graaf, and Groenewegen, 1992). With regards to drug addiction, the medial NAc shell is suggested to be involved in the processing of information regarding the motivational significance of learned drug cues (Ghitza et al., 2003) and the NAc core is suggested to be involved in the execution of drug-seeking responses (McFarland, Lapish, and Kalivas, 2003; Everitt, Dickinson, and Robbins, 2001). Because of these distinctions, this study looked at the NAc shell and core, separately.

Repeated drug exposure causes compensatory neuroadaptations in neurons of the NAc that modulate excess dopamine in a homeostatic fashion by activating the transcription promoting activity of CREB. This attenuates dopamine transmission by increasing expression of dynorphin. The seeking and relapse from abused drugs may be mediated by increased glutamatergic transmission in the NAc (Zhu, Rockhold, and Ho, 1998; Kenny and Markou, 2004). The NAc adapts to glutamate transmission in two ways: 1) promotion of presynaptic glutamate release and 2) alterations in postsynaptic responsiveness to released glutamate.

Extracellular signal-related kinase (ERK1/2) activity was evaluated herein because it is a downstream component of κ-opioid receptor activation and it is an upstream component of dynorphin production. We also looked at the phosphorylation of AMPA receptor subunit, GluR1 at serine 845 because it is an index of PKA activity that is part of D1-like receptor activation that leads to increased dynorphin production.

Also, κ-opioid receptor activation can inhibit both dopamine and glutamate transmission (Nestler et al., 1999; Hjelmstad and Fields; 2001). If glutamate homeostasis is affected by cocaine withdrawal or stress, it may be reflected in the level of p-GluR1^{S845}.

Therefore, this study explores whether cocaine escalation is driven by chronic κ-opioid receptor activity. Specifically, we tested: 1) whether NorBNI persistently antagonizing κ-opioid receptor activity attenuates extended access-induced cocaine escalation and 2) whether stress-induced cocaine escalation is driven by κ-opioid receptor-regulated signaling cascades. Figure 7 shows the connections between all these components. Better understanding of the factors that may promote escalating self-administration patterns in rats and the identification of the neurobiological substrates through which the factors act should assist in further understanding of the mechanisms underlying human cocaine addiction.

Materials and Methods

2. Materials and Methods - *both experiments*

All animal use procedures were approved by The Scripps Research Institute Animal Care and Use Committee and were in accordance with National Institutes of Health guidelines (NIH publication no. 85-23, revised 1996).

2.1 *Animals and apparatus - both experiments*

Thirty-eight male Wistar rats (Charles River, Hollister, CA), each weighing between 270-370 g at the beginning of the study were used. The rats were housed in groups of two or three in plastic cages with a reversed 12:12 hour light/dark cycle (lights on at 6:00AM for the Nor-BNI study and 10:00PM for the cocaine-stress stress study). Food and water were available without restraint outside of self-administration sessions. During self-administration sessions, each rat was placed in a standard operant conditioning chamber, which was situated within a light- and sound-attenuating cubicle (28x26x20x cm; Med Associates Inc., St. Albans, VT). The chamber had two retractable response levels mounted on a sidewall and a stimulus light above each lever. Cocaine injection was delivered by a syringe pump (RazelTM Scientific Instruments, Georgia, VT) located on top of the cubicle. Self-administration sessions were controlled and recorded by a PC computer with custom interface and software in the experimental room.

For the cocaine stress studies, rats were given electric foot-shocks delivered through stainless steel grid floors of the self-administration chambers. Shocks (0.5

mA, 100 ms duration) were delivered an average of every 30 s for 10 min after each hour of self-administration.

2.2 ***Procedure - both experiments***

For catheterization surgery, rats were anesthetized with 2 or 3% isoflurane. They were implanted with a silastic catheter (0.3 mm i.d. x 0.64 mm o.d.; Dow Corning Co. Midland, MI) into either the right or left external jugular vein under aseptic conditions. The other end of the catheter was placed subcutaneously on the back of the rat where it exited the rat via a metal guide cannula (22G; Plastics One Inc., Roanoke, VA) that was anchored onto the back of the rat. At the end of the surgery, rats were given an analgesic (Flunixin, 2.5 mg/kg s.c.) and an antibiotic (Cefazolin, 75 mg, s.c.) once a day for a minimum of two days after surgery. The patency of the catheters in the rats was tested using the short-acting barbituate methohexital sodium (Brevital, 10 mg/ml, 2 mg/rat) around a week after surgery and whenever catheter failure was suspected during the study. Patency of the catheter was indicated by a loss of muscle tone within three s after the methohexital sodium injection.

Self-administration sessions were conducted once a day, five days a week, during the rats' dark (active) cycle. Before the start of each session, the rats were flushed with the antibiotic. Then the rat's catheter was connected to a tube that exited the chamber via a protected metal tether, and was connected to a syringe pump. At the start of each session, the two response levers would come out of the sidewall and be present in the chamber. Pressing the right (active) lever resulted in the delivery of 0.1

ml of cocaine solution over 4 s. During an injection, the stimulus light above the right lever was illuminated and lasted throughout a drug time out period (20 s) that began after each injection. Pressing the left (inactive) lever was counted but had no other consequences. When the session ended, the levers would retract into the chamber. After the session, the rat's catheter was flushed again with heparinzied saline (0.9% saline containing 30 U/ml of heparin) and the rat would be returned to its home cage.

2.3 NorBNI Experiment

2.3.1 *Animals*

Thirteen male Wistar rats were used for the Nor-BNI study, of which seven received norBNI (30 mg/kg, s.c.) and the other six received saline.

2.3.2 *Procedure*

Around a week after catheterization surgery, rats were tested in self-administration sessions once a day, five days a week. Rats were trained to press the active lever for cocaine (0.5 mg/kg per injection) under a fixed-ratio 1 (FR1) schedule of reinforcement, when every lever press was reinforced with a cocaine infusion. After active lever responses became stable (<15% variation in response in three consecutive days), rats were subdivided into four groups balanced by the number of injections/session on the last baseline session. Of the eight rats that were injected with norBNI, four were put in 1 hour (short access, ShA) sessions and four were put in 6 hours (long access, LgA) sessions. Of the eight that were injected with saline, four were put in ShA sessions and four were put in LgA sessions.

The norBNI was subcutaneously injected at the end of baseline before beginning long-access self-administration. Saline was used as a control. Previous studies indicate that norBNI antagonism of the κ-opioid receptors for at least 21 days across several species (Butelman et al. 1993; Horan et al. 1992; Jones and Holtzman, 1992). For this reason, only one injection was given.

The rats were tested for 18 sessions. On the 19th session, a progressive-ratio (PR) schedule was implemented, in which the "price" (number of lever presses) required for the next reinforcement increases progressively (measure of motivation) (Table 1).

2.4 Cocaine-Stress Experiment

2.4.1 *Animals*

Twenty-two male Wistar rats were used. Six rats were allowed to self-administer cocaine and experienced foot-shocks. Eight rats were allowed to self-administer cocaine and never experienced foot-shocks. Eight were used as naïve controls that had no experience with cocaine self-administration nor foot-shocks.

2.4.2 *Procedure*

After a week or two after catheterization surgery, rats were ran in self-administration sessions once a day, five days a week. Rats were trained to press the active lever for cocaine under a FR1 schedule of reinforcement where the rats self-administered 0.5 mg/kg per injection with no time out period after injection. Each session lasted two hours and twenty min: one hour of self-administration followed by ten min of no self-administration (both levers retract into the chamber) and a house-

light illuminating the chamber, then another hour of self-administration and ending with another ten min of no self-administration and a house light on. After active lever responses became stable, training session ended.

In each session afterwards, the rats self-administered 0.5 mg/kg per injection of cocaine on a FR1 schedule with a 20 second time-out period after each injection. Once the rats reached a baseline responding (< 15% variation in response in three consecutive days), the rats were randomly subdivided into two groups balanced by the number of injections/session on the last baseline session. Six were to be foot-shocked during the two 10 minute no self-administration periods while eight did not have foot-shocks added into those 10 minute periods. The foot-shocks were at 0.5 mA and lasting 100 ms and occurred around every 30 s.

2.4.3 ***Western Blotting***

The rats were anesthetized quickly in a bell jar with isoflurane and decapitated immediately after loss of consciousness. The decapitation occurred around 22 hours after the final operant session. The brains were subsequently removed and frozen in isopentane at -30°C, and stored in a -80°C freezer. Sections were serially cut using a cryostat kept at -20°C. Tissue punches were obtain from the nucleus accumbens shell and core (1.5 mm, +2.5-+1.0 bregma) which were removed with a glass pipette (Figure 10). All tissue samples were transferred into homogenization buffer containing (mM) 10M Tris-HCl, pH 7.4, 5M NaF, 1M Na_3VO_4, 1M EDTA and 1M EGTA then sonicated on ice for 30 s and centrifuged at 13,000 rpm for 30 min at 4°C. The pellet which was composed primarily of nuclei and large debris was discarded while the

remaining supernatant which contained the cyotplasmic proteins was collected and centrifuged at 13,000 rpm for another 30 min at 4°C. The pellet (P2) containing the synaptosomoal membrane fraction of proteins was then solubilized.

The protein samples were resolved using a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-page) then the separated proteins from each gel were transferred to a nitrocellulose membrane (company and origin) at 0.3 A overnight.. Each was blocked with a blocking buffer containing 5% milk (5 grams of blotting-grade blocker per 100 ml of 1X TTBS) for an hour at room temperature. The membranes were then probed with primary antibodies against p-GluR1 S845 (1:500), Glur1 (1:500), b-tubulin (1:50,000), p-ERK1/2 (1:500), ERK 1/2 (1:1,000 for the NAc core, 1:10,000 for NAc shell), overnight at 4°C on a shaker. Antibodies for the β-tubulin were purchased from Santa Cruz Biotechnology (Santa Cruz,CA, USA). The rest of the antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA).

After incubation with primary antibodies, the membranes were washed 3 times with 1X TTBS (200 ml 10X TBS, 1800 ml milliq water, 2ml Tween-20) for 15 min per each wash, then each membrane was incubated with the appropriate secondary antiserum for 1 hour at room temperature. The secondary antiserum was from Bio-rad (Hercules, CA). After incubation with secondary antiserum, the membranes were again washed three times with 1X TTBS, then the membranes were developed using enhanced chemiluminescense (ECL) reagents from Thermo Scientific (Rockford, IL). Luminescence from the blots was detected using Hyblot CL autoradiography film

from Denville Scientific (Metuchen, NJ) followed by film development using a Kodak X-OMAT 2000A processor.

Immunoreactive protein bands visualized on film were scanned into a computer and the band intensities were quantified using the ImageJ application (National Institutes of Health). Band intensities from the samples were compared to the corresponding background intensities of the film. For each protein, phosphorylated protein levels were normalized to the total protein levels, therefore, total GluR1 was probed after stripping the same membrane that was confirmed to contain p-GluR1 S845; the same was done for total ERK1/2 with the membrane that was confirmed to contain p-ERK1/2 and β-tubulin was also re-probed on the membrane that corresponded to GluR1. Thus, changes in phosphorylation state within each brain region were able to be determined as in Edwards et al. (2011).

Results

3.1a *NorBNI Results - In LgA rats, NorBNI injection attenuated cocaine intake in comparison to saline injection*

Figure 8 illustrates the cocaine treatment timeline for the experimental groups used in the analysis of norBNI on cocaine self-administration. Self-administering animals developed stable cocaine intake by the end of the 11 days of training with a mean daily cocaine intake (sessions 9-11) averaging 18.3 cocaine-reinforced lever presses (9.15 mg/kg) per hour for all groups of animals. Animals were separated into long-access (LgA; 6 hour access of cocaine daily) and short-access (ShA; 1 hour access of cocaine daily) groups, and injected with either saline or norBNI. The saline-injected LgA animals had a significantly cocaine intake in comparison to the norBNI-injected LgA animals (two way ANOVA, F=6.989, P= 0.0203, Graph 1). There was no effect of norBNI between ShA animals (Graph 2).

3.1b *NorBNI Results - In LgA rats, NorBNI injection decreased motivation for cocaine in comparison to saline injection*

NorBNI (30 mg/kg) decreased cocaine self-administration in LgA rats under a progressive-ratio schedule (Graph 3) in comparison to saline in LgA rats. However, there was no difference in effect when comparing nor-BNI injected ShA rats versus saline injected ShA rats. Saline-ShA rats took an average of 3.5 ± 0.5 injections. NorBNI-ShA rats took an average of 3.7 ± 0.7 injections. Saline-LgA rats took an average of 10.8 ± 0.6 injections. NorBNI-LgA rats took an average of 7.0 ± 0.4 injections. The breakpoint for norBNI-LgA rats was around 15 presses whereas the

breakpoint for saline-LgA rats was around 32 presses and the breakpoint for ShA rats was around 6 presses.

3.2a *Cocaine-Stress Results - Exposure to foot-shock stress increase cocaine self-administration*

Figure 9 illustrates the cocaine treatment regimen for the behavioral groups used in the analysis of protein phosphorylation levels between naive animals, cocaine and foot-shock stress animals, and cocaine and non foot-shock stressed animals. The self-administering animals developed stable cocaine intake by the end of by the end of the 20 sessions of training with mean daily cocaine intake (sessions 18-20) averaging 20 cocaine-reinforced lever presses (10 mg/kg) per hour for all groups of animals. After reaching stable intake, the animals were split into groups of either no foot-shock (each session was identical to the training session) or foot-shock (where foot-shock was added to both 10 minute intervals). Cocaine self-administration behavior for the foot-shock group trended significantly higher than that of the non-foot-shock group (Graph 4, $F_{1,7}$=8.929; P=0.0203). Before withdrawal and decapitation, the mean daily cocaine intake (days 30-34) for the foot-shock group was 45.1 ± 9.1 cocaine-reinforced presses (22.55 ± 4.6 mg/kg) for the whole session and 24.4 ± 5.2 cocaine-reinforced presses (12.2 ± 2.6 mg/kg) for the first hour while for the no foot-shock group, the whole session had a mean of 24.8 ±7.1 cocaine-reinforced presses (12.4 ± 3.6 mg/kg) for the whole session and 13.6 ± 3.7 cocaine-reinforced presses (6.8 ± 1.8 mg/kg) for the first hour. The naive animals were rats that had no cocaine exposure within their lifetimes.

Tissue punches of the nucleus accumbens (NAc) core and shell were collected from coronal brain slices as depicted in Figure 10. All observed changes involved regulation of protein phosporylation status since there were no significant alterations in the total amount of GluR1 or ERK in any brain region studied.

3.2b Cocaine-Stress Results - Phosphorylation of GluR1 S845 in the NAc core was significantly decreased in foot-shock (stressed) animals in comparison to no foot-shock (non-stressed) animals

There was a significant decrease in GluR1^{S845} phosphorylation found in the NAc core after one day of withdrawal in animals exposed to foot-shock stress in comparison to animals not exposed to the foot-shock stress (Graph 5 and Figure 11, $F_{2,19}$= 3.574, p=0.0481). However, there was no such change in GluR1^{S845} phosphorylation in the NAc shell between groups.

3.2c Cocaine-Stress Results - Phosphorylation of ERK1/2 in the NAc core was increased in no foot-shock (non-stressed) animals in comparison to foot-shock (stressed) animals

There was a trend for an increase in ERK1/2 phosphorylation found in the NAc core after one day of withdrawal in cocaine self-administration animals not exposed to foot-shock stress in comparison to animals that were exposed to foot-shock stress (Figure 11). However, this increase was not enough to be considered significant (P=0.06). There was no such change in ERK1/2 phosphorylation in the NAc shell between groups.

Discussion

The negative reinforcement due to the dysregulation of reward and stress systems in drug addiction is thought to be attributed to two processes: 1) function in the reward system where positive motivational circuits of the mesolimbic dopaminergic circuitry (the "reward pathway") is diminished and 2) brain stress systems such as cortictropin-releasing factor (CRF), norepinephrine, and dynorphin are recruited, producing aversive or stress-like states (Edwards and Koob, 2010). This study showed that the activation of the κ-opioid receptor/dynorphin brain stress system drives extended access-induced cocaine escalation. The norBNI experiment indicated that antagonizing κ-opioid receptor activity attenuated extended access-induced increased cocaine intake.

In contrast, the stress-cocaine experiment showed that certain κ-opioid receptor activity, specifically the ERK pathway activity in the NAc shell, is present in cocaine withdrawal but is not altered in the presence of a stress history. However, this experiment showed a significant decrease in p-GluR1^{S845} in the NAc core in rats with both stress history and cocaine withdrawal which may indicate that stress-induced escalation may not be driven by κ-opioid receptor activity, but may instead be driven by the dysregulation of glutamate homeostasis.

4.1 The norBNI Experiment

To test the hypothesis that the persistent antagonism of κ-opioid receptor activity should attenuate cocaine intake, rats were treated with 30 mg/kg of norBNI, a long-acting, selective κ-opioid receptor antagonist before being given extended access

(LgA; 6 hours/session) to cocaine. On a fixed-ratio (FR1) schedule, cocaine intake in LgA rats injected with norBNI was attenuated in comparison to LgA rats injected with saline (Graph 1). On a progressive-ratio (PR) schedule, norBNI-LgA rats showed a significantly decreased breakpoint in comparison to saline-LgA rats but norBNI-LgA rats still had an average breakpoint significantly higher than both groups of ShA (short-access; 1 hour/session) rats (Graph 3). It has been reported that compulsive drug use emerges when daily access to cocaine for self-administration is prolonged and is associated with increased motivation to engage in cocaine-seeking behavior (Ahmed and Koob, 1998; Mantsch et al., 2001). The progressive-ratio schedule demonstrates that prolonged access to cocaine self-administration increases motivation for cocaine intake (as seen in saline-LgA rats) but this motivation is selectively mediated by κ-opioid receptor activity (as seen in norBNI-LgA rats).

NorBNI has a very long-duration of action. In this experiment, one injection of 30 mg/kg norBNI was sufficient to create this long-lasting attenuation of cocaine intake in LgA rats. Horan et al. (1991) showed that action of norBNI at κ-opioid receptors gradually increased to a maximum over 24 hours and lasted 21 days in mice. Melief et al. (2011) showed that norBNI lasts more than three weeks in mice following a single dose. Also, Broadbear et al. (1994) showed norBNI activity after 1 hour, peaking at 4 hours, and was present for at least 4 weeks.

NorBNI is a highly selective κ-receptor antagonist. Takemore et al. (1988) demonstrated that up to 20 mg/kg of norBNI (subcutaneously injected, just like in this study) selectively antagonized κ-opioid antinociceptive effects, without inhibition of μ

and δ receptor agonists. Broadbear et al. (1994) showed that at 32 mg/kg, norBNI was effective against selective κ-receptor agonists but it did not affect a δ receptor agonist and had an attenuated effect on agonists that had an affinity for both μ and κ receptors but antagonized such agonists when used in conjunction with a selective μ antagonist. Thus, an injection of 30 mg/kg should be selective for κ-opioid receptors in rats, especially after the initial hours of treatment.

The lack of effect of norBNI on short-access cocaine self-administration has been previously reported in rats and monkeys (Wee et al., 2009; Glick et al., 1995; Negus et al., 1997) and was seen in this study (Graph 2). Also, although norBNI (3 mg/kg) has been shown to inhibit the acquisition of cocaine self-administration at a very low dose (approximately 0.075 mg/kg per injection), norBNI had no effect on a higher dose of cocaine (approximately 0.15 mg/kg per injection), which is lower than the 0.5 mg/kg per injection used in this study (Kuzmin, Gerrits, and Ree, 1998). Considering the lack of difference in FR and PR schedule intake between norBNI-ShA and saline-ShA rats, cocaine self-administration in ShA rats probably does not involve κ-opioid receptor activity. This supports the notion that it takes chronic cocaine use to cause the dysregulation of κ-opioid receptor activity resulting in the negative emotional state (e.g., dysphoria, etc.) seen in cocaine withdrawal and addiction. This negative emotional state motivates the individual to take more drug in order to alleviate the withdrawal-associated symptoms (negative reinforcement). Thus it is possible that persistent antagonism of the κ-opioid receptor may prevent the formation of this negative emotional state caused by extended access-induced cocaine escalation.

Injection of norBNI attenuated cocaine intake in LgA rats by blocking the effects of increased dynorphin (a brain stress neurotransmitter) on this system. With norBNI preventing the effects of an increase in a brain stress neurotransmission, the dysregulation of the brain reward and stress systems usually caused by chronic cocaine intake cannot be achieved. Thus, we see that although the norBNI prevented LgA animals from reaching the intake levels seen in the saline-LgA animals, the norBNI-LgA rats still had a higher level of intake for cocaine than the ShA animals. Therefore, with extended access to cocaine, norBNI decreased the effect of activation in brain stress neurotransmission, but probably did not prevent loss of positive reward function caused by acute cocaine intake, so norBNI-LgA rats still had more motivation to take cocaine than ShA rats but less motivation than saline-LgA rats (as seen in the PR schedule data, Graph 3).

4.2 Stress-cocaine Experiment

This experiment demonstrated that repeated exposure to an uncontrollable, mild foot-shock escalates the drug intake of cocaine self-administering rats. Thus chronic stress during a period of cocaine availability can escalate cocaine intake, similarly to how extended access to cocaine can escalate cocaine intake, which we observed in the norBNI experiment.

A number of studies have explored exposure to different types of stress and its facilitation of cocaine intake. Miczek and Mutschler (1996) showed that repeated social-defeat stress increased the rate of cocaine intake. Covington and Miczek (2001) also showed that social-defeat stress increased cocaine intake during a 24-hour binge.

Goeders and Guerin (1993) showed that repeated non-contingent foot-shock (0.2 increasing to 0.6 mA, 100 ms duration, around 50 shocks per session) facilitated the acquisition of intravenous cocaine self-administration. Mantsch and Katz (2007) also showed that foot-shock (sequences of three 0.6 mA, 100 ms shock administered at 1-s intervals, around 29 shocks per session) produced a significant escalation of cocaine self-administration. Erb, Shaham, and Stewart (1996) demonstrated that foot-shock stress induced reinstatement of cocaine-taking behavior after prolonged extinction sessions and after a 4- to 6-week drug-free period; the foot-shock was at least as effective as a priming injection of cocaine. These finding indicate that stress can influence a number of aspects of the addiction process, including acquisition and relapse. Thus, stress may serve as a therapeutic target during the management of cocaine addiction.

It is plausible that stress may facilitate cocaine escalation by facilitating the progression of cocaine-induced neuroplasticity. This study hypothesized that stress induced escalation may be driven by κ-opioid receptor-regulated signaling cascades since the norBNI study confirmed that extended access-induced escalation was driven by chronic κ-opioid receptor activity. We focused on the NAc since it is critically involved in aversive behaviors associated with dynorphin and κ-opioid receptor activation (Shirayama and Chaki, 2006). This study measured changes in phosphoprotein in comparison to total protein levels between rats undergoing cocaine withdrawal (with and without stress history) and rats with no cocaine history. Extracellular signal-related kinase (ERK) activity was measured since it is a

downstream component of κ-opioid receptor activation and it is an upstream component of dynorphin production (Figure 7). Striatal ERK phosphorylation is hypothesized to play a central role in the persistence of cocaine craving in withdrawal (Lu et al., 2006). Studies have shown that in cocaine-sensitized rats, ERK2 phosphorylation is increased in the NAc during withdrawal, with the increases being significant at seven and twelve days in comparison to one day withdrawal (Boudreau et al., 2007; Steketee and Kalivas, 2011). Cocaine context-associated ERK1/2 phosphorylation was found in the NAc core of cocaine self-administration rats and in the shell of both cocaine self-administration and yoked animals after three weeks of withdrawal (Edwards et al., 2011). In addition, acute and repeated swim-stress have also been shown to increase κ-opioid mediated ERK activation (Shen et al., 2004; Bruchas and Chavkin, 2008). These studies suggest that cocaine withdrawal should result in increased phosphorylation of ERK1/2 (compared to total ERK1/2), especially in rats with a stress history. However, this experiment showed that there was no difference in phosphorylation of ERK1/2 in the NAc core, although in the NAc shell, there was a trend for increased ($P = 0.06$) p-ERK1/2 in rats undergoing cocaine withdrawal in comparison to naïve rats. Also, stress history did not affect ERK1/2 phosphorylation in the NAc shell.

The results from this experiment suggest: 1) ERK activation during withdrawal is specific to the shell, 2) ERK activation occurs during cocaine withdrawal, and 3) ERK activation is not affected by stress history. Since ERK activation is not affected by stress history, it is possible that the mechanism in which stress increases cocaine

escalation is not the same mechanism in which extended access increases cocaine escalation. Stress-induced cocaine escalation may be κ-opioid receptor activity independent.

This study chose to assay for the phosphorylation of the AMPA receptor subunit GluR1at serine 845 as it is an index of PKA activity which is part of D1-like receptor activation that leads to increased dynorphin production (Figure 11). Also, κ-opioid receptor activation can inhibit both dopamine transmission and glutamate transmission (Nestler et al., 1999; Hjelmstad and Fields; 2001). If glutamate homeostasis is affected by cocaine withdrawal or stress, it may be reflected in the levels of p-GluR1^{S845}.

Studies have shown that cocaine withdrawal results in lowered levels of mesolimbic dopamine and increased PKA activity (Fitzgerald, 1996; Lee and Messing, 2008). PKA is implicated in the phosphorylation of CREB and GluR1^{S845} and increases in p-GluR1^{S845} has been seen in cocaine withdrawal (Ferrario et al, 2011). This would suggest that cocaine withdrawal should result in increased p-GluR1^{S845}, regardless of stress history. However, our results showed that p-GluR1^{S845} levels did not differ significantly in the NAc shell but, in the NAc core, levels actually decreased in rats that were both in cocaine withdrawal and had a stress history.

During cocaine withdrawal, there is an increase in synaptically-released glutamate (Kalivas, 2009). In addition, stress-induced cocaine seeking is associated with a significant overflow of synaptic glutamate into the NAc core from pre-limbic afferents (McFarland, Lapish, and Kalivas, 2003). To add, p-GluR1^{S845} functions to

potentiate AMPA receptor response to glutamate and AMPA receptor trafficking to the neuronal membrane (Banke et al., 2000; Esteban, 2003). Therefore, it is likely that in rats with both cocaine withdrawal and stress history, there are very high levels of glutamate in the NAc core that may be excitotoxic. This glutamate excitotoxicity can activate endoplasmic reticulum (ER) stress (Snyder et al., 200) in order to bring neurons back to glutmate homeostasis. ER stress leads to the increased activation of calcineurin, a phosphatase specific to p-GluR1 at serine 845 (Mukherjee and Soto, 2011). A theoretical increased activation of calcineurin would increase the dephosphorylation of p-GluR1^{S845}. Thus, it is likely that the reason why there is a decrease in p-GluR1^{S845} in the NAc core of stressed cocaine-withdrawal rats is because they have a higher level of calcineurin activity in the NAc core. By dephosphorylating p-GluR1^{S845}, calcineurin is also preventing further potentiation and trafficking of AMPA receptors to the cell surface which may also help bring the neuron back to glutamate homeostasis. Somewhat in accordance with this study's cocaine withdrawal data, tolerance to cocaine-stimulated p-GluR1^{S845} develops in the NAc core (but not shell) between (1 day) and chronic (18 day) cocaine exposure (Edwards et al., 2007), further suggesting a homeostatic balance between cocaine and glutamate signaling. Dysregulation of this balance may promote escalation of cocaine intake.

The region-specificity of the phosphoprotein changes further supports the notion that the NAc shell and the NAc core differ in function. The NAc exists as a "limbic-motor" interface. The NAc core is suggested to influence motor activity, specifically the execution of cocaine-seeking responses and these responses have been

shown to be mediated by prefrontal glutamate release (McFarland, Lapish, and Kalivas, 2003). This glutamate release to the core would explain why there is decreased GluR1^{S845} phosphorylation in the core instead of the shell since glutamate release would be higher in the NAc core than in the NAc shell.

This region-specificity is also supported by the evidence that ERK1/2 activation is NAc shell-specific in cocaine withdrawal. The NAc shell is implicated in the processing of information regarding the motivational significance of learned drug cues (Ghitza et al., 2003) and is also hypothesized to play a central role in the persistence of cocaine craving in withdrawal (Lu et al., 2006). Since ERK1/2 activation only correlated with cocaine withdrawal, it makes sense that it was only seen in the NAc shell and its activation likely has a role in cocaine seeking behavior.

4. Future Directions

In order to have a better understanding of what is occurring in the NAc core during stress-induced escalation, more experiments should be done. Performing microdialysis in the NAc core in a replication of the stress-cocaine study and measuring glutamate levels would help confirm if the dysregulation of glutamate homeostasis is what is contributing to stress-induced cocaine escalation.

Also, replicating the stress-cocaine study with an extra cohort of animals who do not self-administer but are still exposed to the same foot-shock stress paradigm can reveal the effects of stress itself on neuroadaptations. This would give a better understanding of what neuroadaptations may be driven by stress, driven by cocaine withdrawal, and driven synergiestically by both.

The ERK pathway activation in cocaine withdrawal in this study showed that κ-opioid receptor activation may mediate cocaine withdrawal but may not be necessary for facilitating stress-induced cocaine escalation. However, injection of norBNI into rats before they are exposed to foot-shock stress could help confirm this suggestion. If κ-opioid receptor activation is necessary for stress-induced cocaine escalation, then such escalation would be attenuated with norBNI pre-treatment.

In addition, since rats were pre-treated with norBNI in the norBNI experiment, this study showed that κ-opioid receptor activation facilitates increased intake in extended access cocaine self-administration. However, how would cocaine intake be affected if κ-opioid receptors were chronically activated? Would the dysphoria drive intake? Or would the dysphoria reduce overall behavior? Injection of a selective κ-opioid receptor agonist (such as salvinorin A) in LgA rats may help answer these questions.

Escalating patterns of drug self-administration have been proposed to reflect compulsive drug use (Mantsch and Katz, 2007). In conclusion, Extended access to cocaine as well as exposure to stress are environmental factors that have each been proven to cause increased cocaine intake, which is indicative of a transition from drug use to drug addiction. Understanding the molecular mechanisms underlying these contributing environmental factors will widen general understanding of the multifaceted study of drug addiction, as well as lead to the development of new therapeutic targets

Figures, Graphs, and Tables

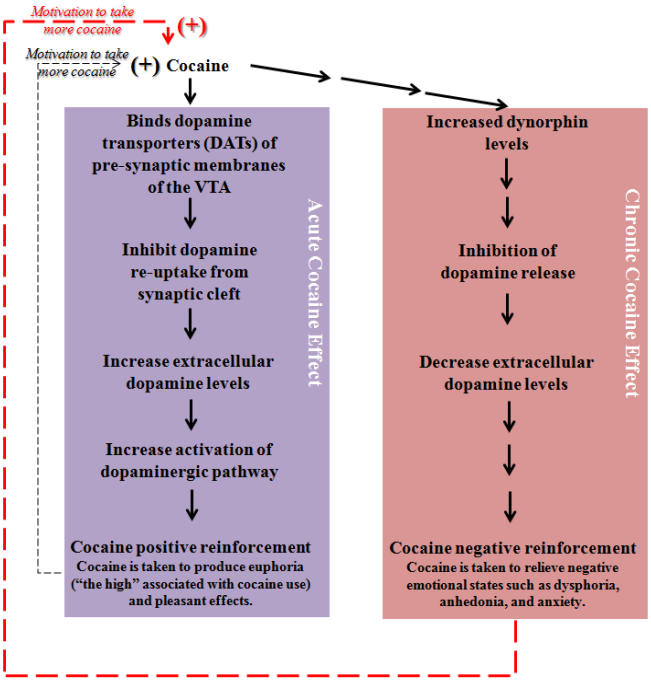


Figure 1: Dynorphin is a brain stress neurotransmitter that activates κ-opioid receptors and is conjectured to produce negative emotional states (e.g., dysphoria) associated with cocaine dependence and thus increases the motivation to administer cocaine to compensate for these aversive effects (Edwards and Koob, 2010).

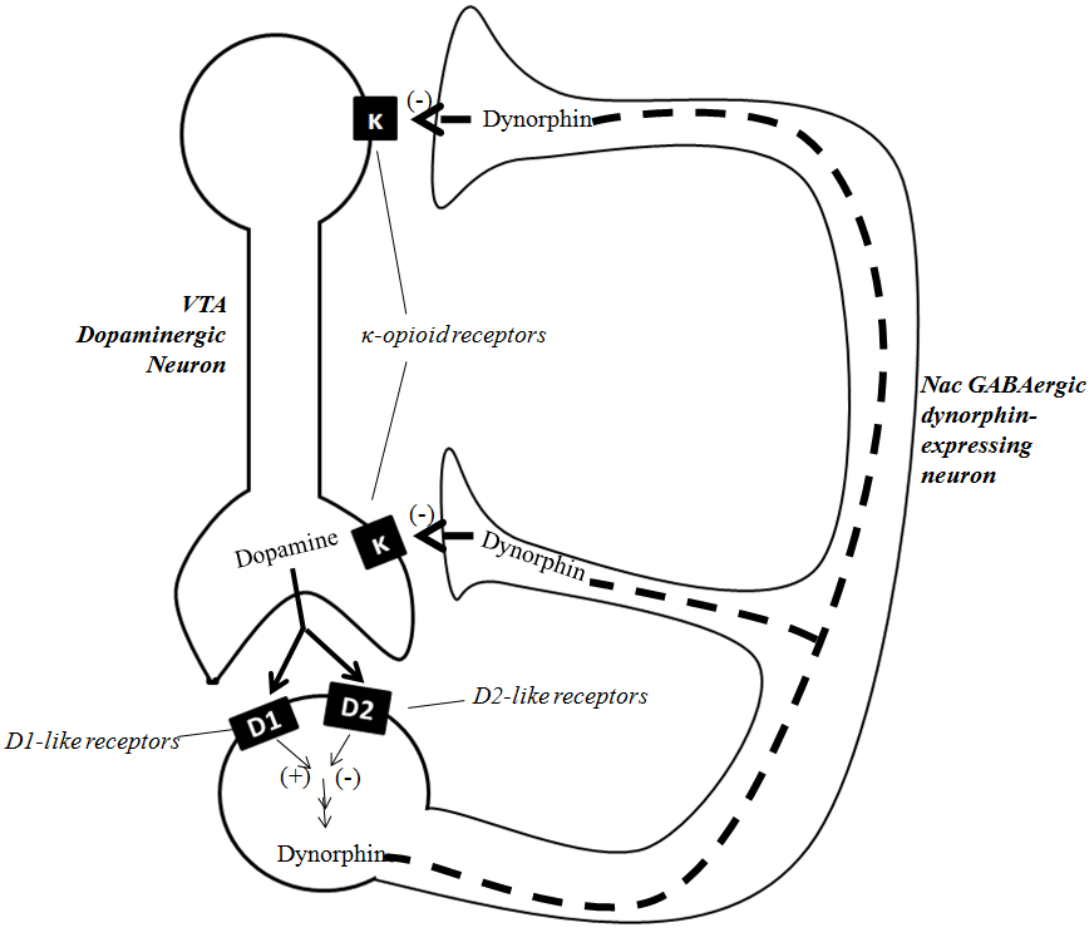


Figure 2: Dynorphin appears to function in an intercellular feedback loop. The release of dynorphin binds to the k-opioid receptors present on dopaminergic nerve terminals in the nucleus accumbens and also on cell bodies and dendrites in the ventral tegmental area (Nestler et al., 1999).

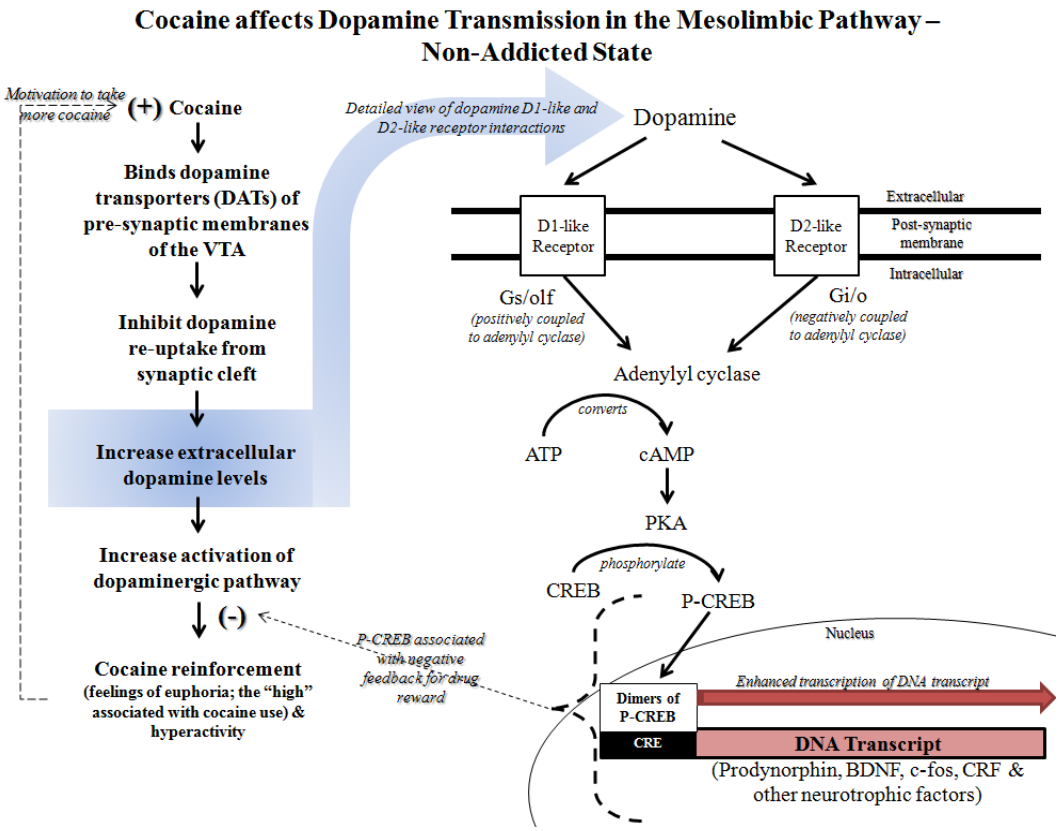


Figure 3: The effect of cocaine intake on dopamine transmission in the mesolimbic pathway in the non-addicted state.

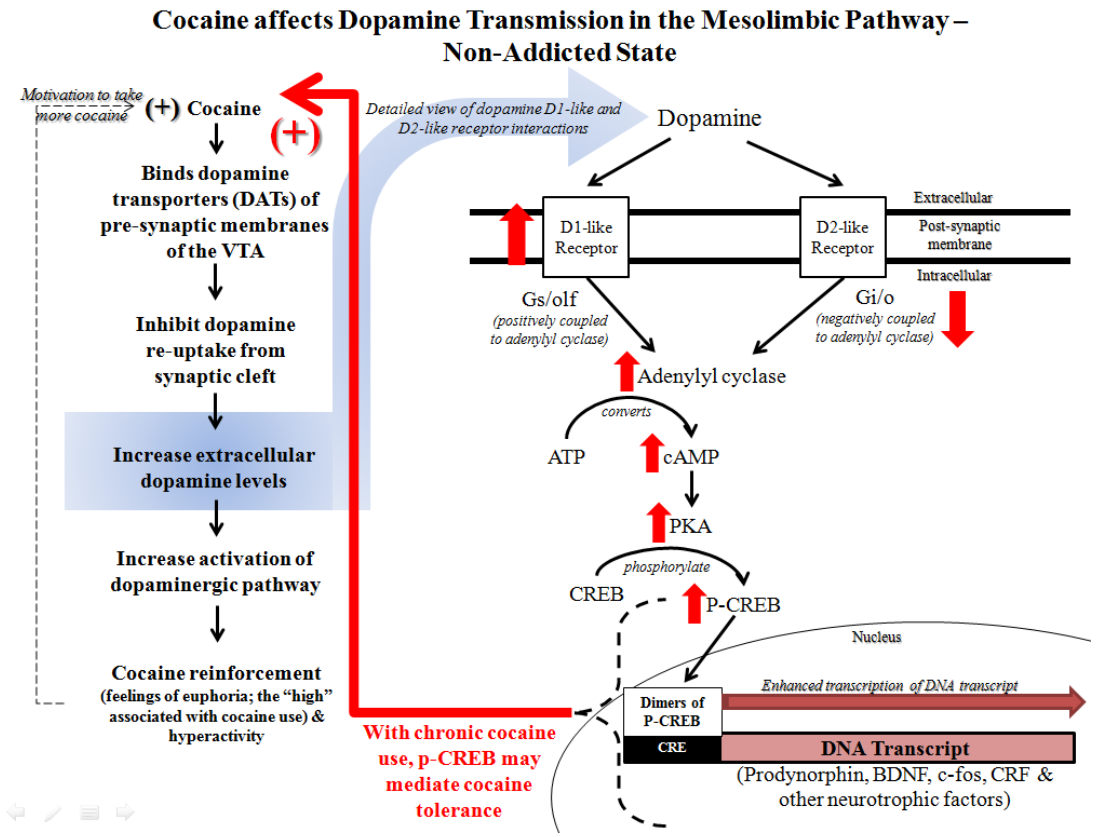


Figure 4: The effect of chronic cocaine intake on dopamine transmission in the mesolimbic pathway.

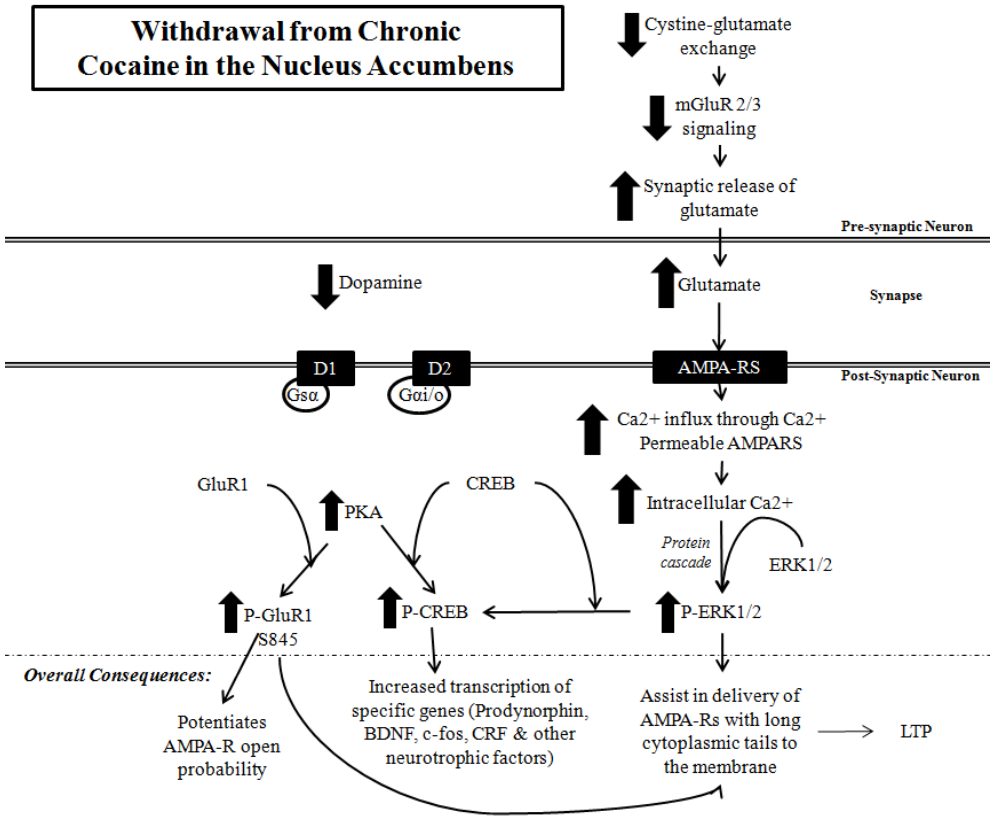


Figure 5:. Changes to glutamate homeostasis occurs after withdrawal from chronic cocaine administration. Reduction of cystine-glutamate exchange results in a decrease in basal extrasynaptic glutamate levels. This and upregulation of activator G protein signalling 3 (AGS3) cause a decrease in mGluR2/3 signaling which results in an increase in synaptically released glutamate (Kalivas, 2009). The rest of this can be read in Figure 7.

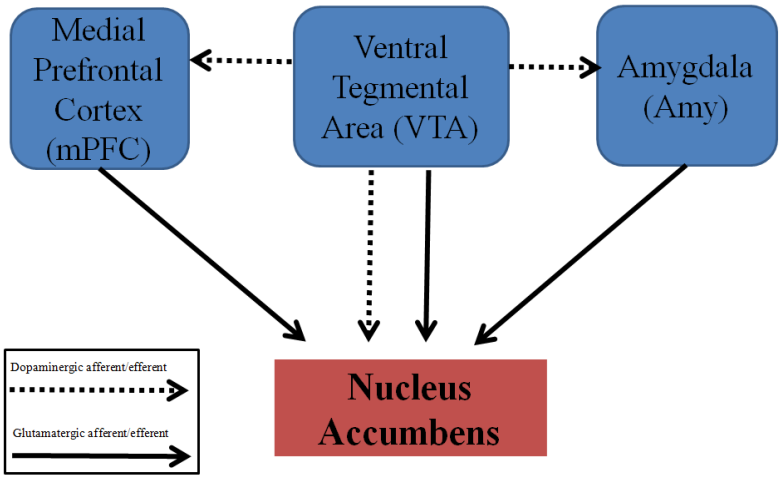


Figure 6: . The mesolimbic dopaminergic circuitry is composed of VTA dopaminergic neurons and its projections, the nucleus accumbens (NAc), the amygdala (Amy), and the medial prefrontal cortex (mPFC) (Guo et al., 2009).

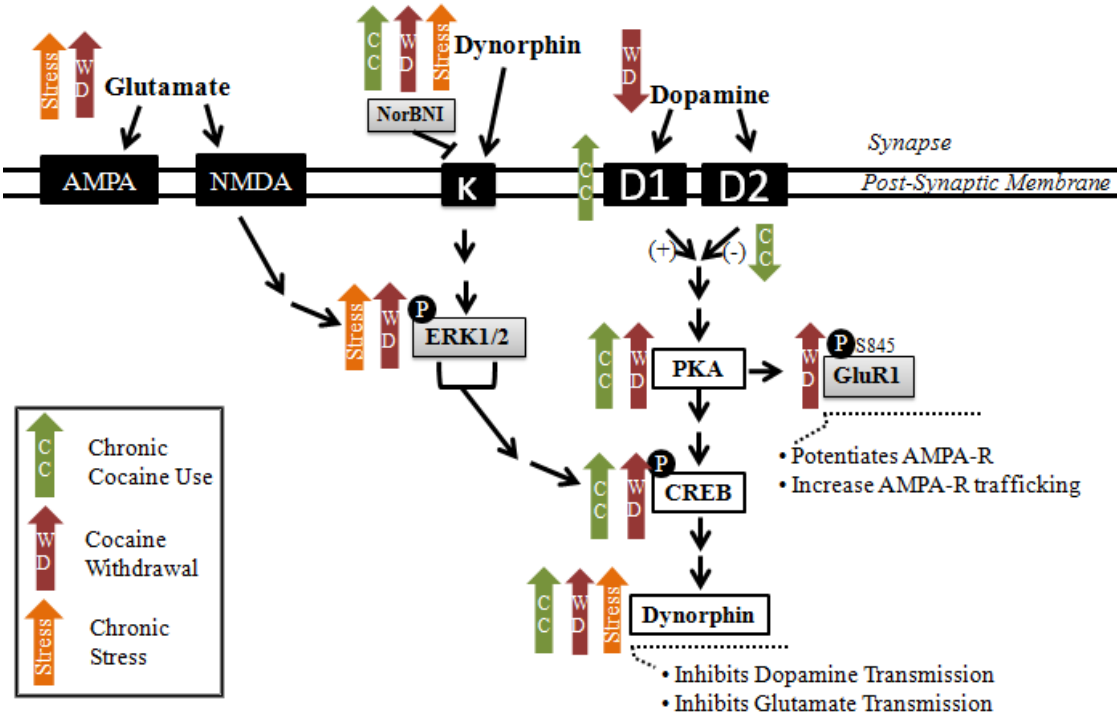


Figure 7: Neuroadaptation Overview

Chronic Cocaine Use: Chronic cocaine causes the over-activation of dopamine D1-like receptors which stimulates PKA activity and increases cAMP activity. This leads to an increase in the phosphorylation of CREB (Edwards and Koob, 2010) which activates it, allowing it to be translocated and to bind to specific DNA sequences, which increases the transcription of certain downstream genes (Purves, et al, 2008) leading to an increase in dynorphin production. Dynorphin can inhibit both dopamine and glutamate transmission (Nestler et al., 1999; Hjelmstad and Fields; 2001).

Cocaine Withdrawal: During cocaine withdrawal, there is an increase in synaptically released glutamate (Kalivas, 2009). The NMDA receptor activates the ERK pathway (Kaprivinsky, 2003). ERK1/2 MAPK cascade leads to the phosphorylation of CREB (Perkinton, Sihra, and Williams, 1999).

Withdrawal also causes lowered levels of mesolimbic dopamine release and increased PKA activity (Fitzgerald, 1996; Lee and Messing, 2008). PKA is implicated in the phosphorylation of CREB as well as GluR1^{S845}. Increase in the phosphorylation of GluR1^{S845} has been seen in cocaine withdrawal (Ferrario et al, 2011). The phosphorylation of GluR1 at serine 845 increase the opening probability of AMPA-Rs as well as assists in the delivery of GluR1-containing AMPA-R to the synapses (Banke et al., 2000; Esteban, 2003).

Stress: Stress increases dynorphin (Land et al., 2008) while stress-induced cocaine seeking is associated with a remarkable overflow of synaptic glutamate into the NAc core from pre-limbic afferents (McFarland, Lapish, and Kalivas, 2003). Stress has also been shown to increase kappa-opioid mediated ERK activation (Bruchas and Chavkin, 2008; Shen et al., 2004).

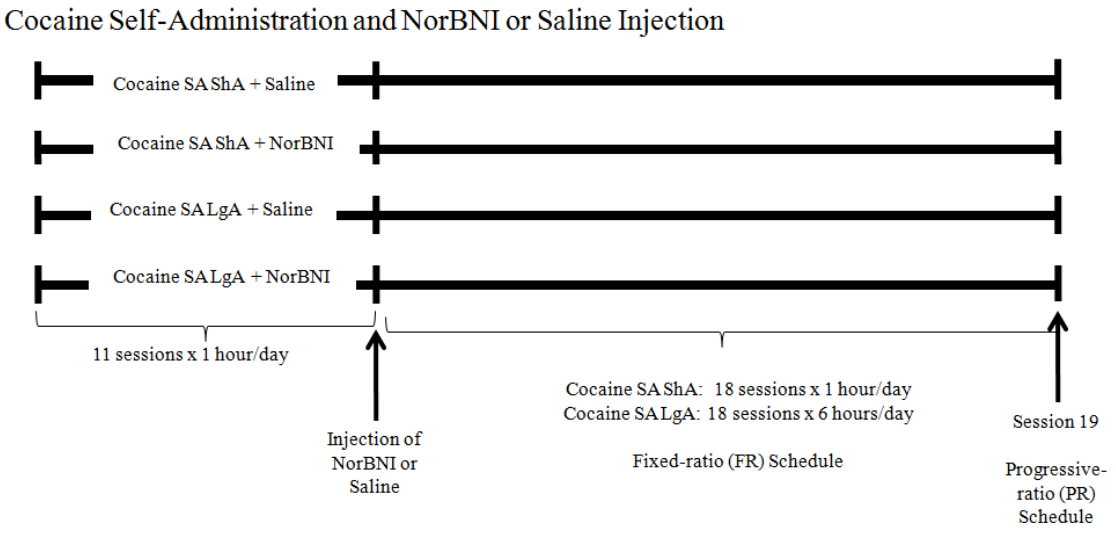


Figure 8: Timeline for NorBNI Experiment: cocaine self-administration regimens and injections (NorBNI or Saline).

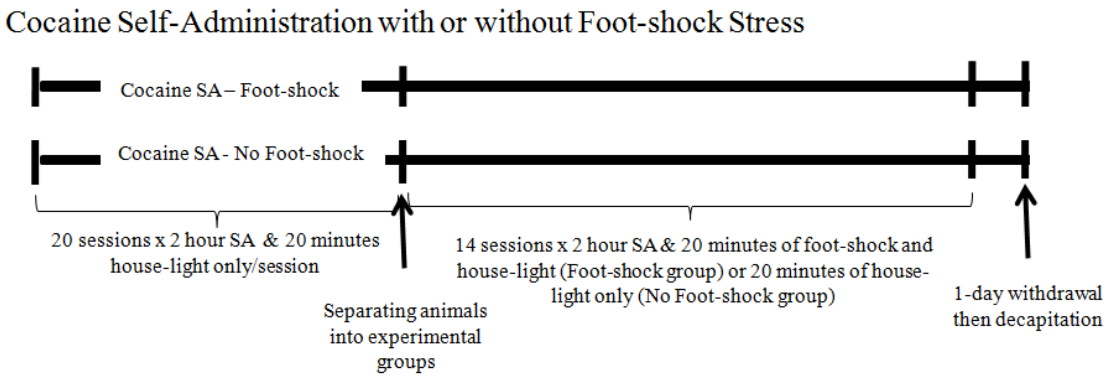


Figure 9: Timeline for Stress-Cocaine Experiment: cocaine-self-administration regimens and withdrawal times. Animals were euthanized by decapitation after one day of cocaine withdrawal.

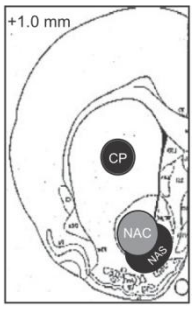


Figure 10: Regional Dissections for Stress-Cocaine Experiment: schematic representation of regional brain dissections collected from 1.5-mm thick coronal slices. NAc core (NAC) and shell (NAS) in anterior-posterior coordinates depicting the posterior side of brain slices. Taken from "Emergence of context-associated GluR1 and ERK phosphorylation in the nucleus accumbens core during withdrawal from cocaine self-administration" (2011) with permission from Scott Edwards who was the primary author of the study.

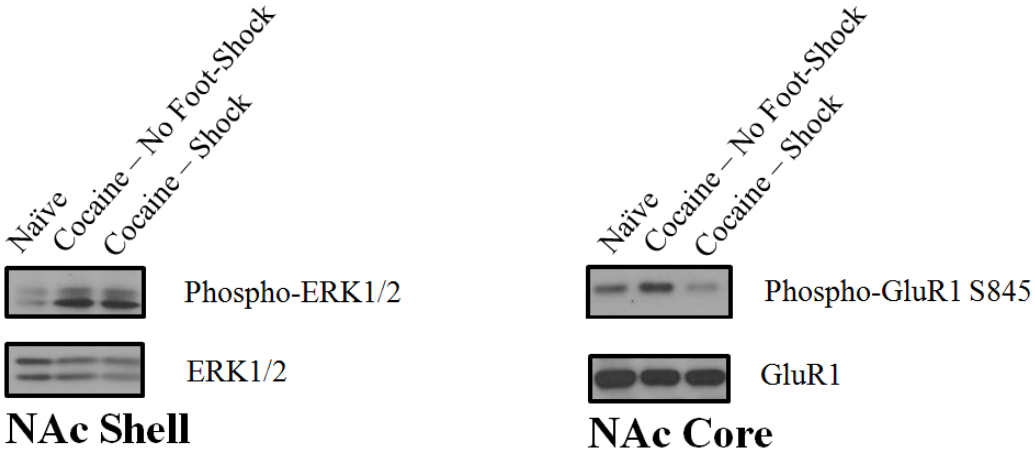
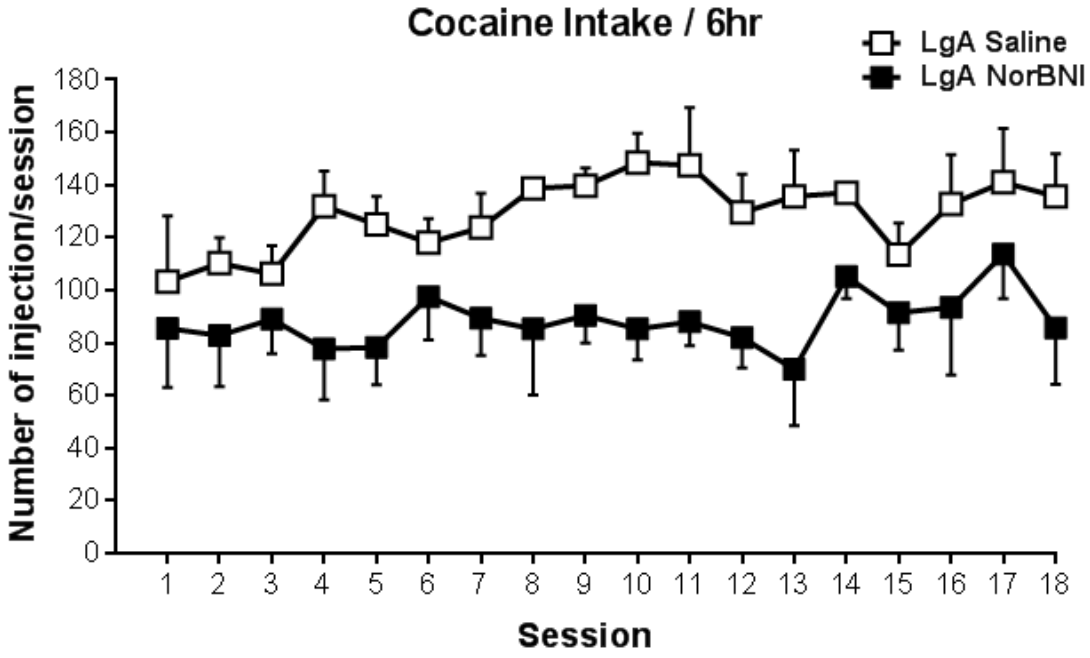


Figure 11: Significantly decreased NAc Core GluR1 S845 phosphorylation following one day cocaine withdrawal in animals that have been exposed to both cocaine self-administration and footshock stress. Increased (*P=0.06*) NAc Shell ERK1/2 phosphorylation following one day cocaine withdrawal in animals that have been exposed to cocaine self-administration but not footshock stress.

Graph 1: The κ-opioid receptor antagonist norBNI attenuates cocaine intake in long-access subjects

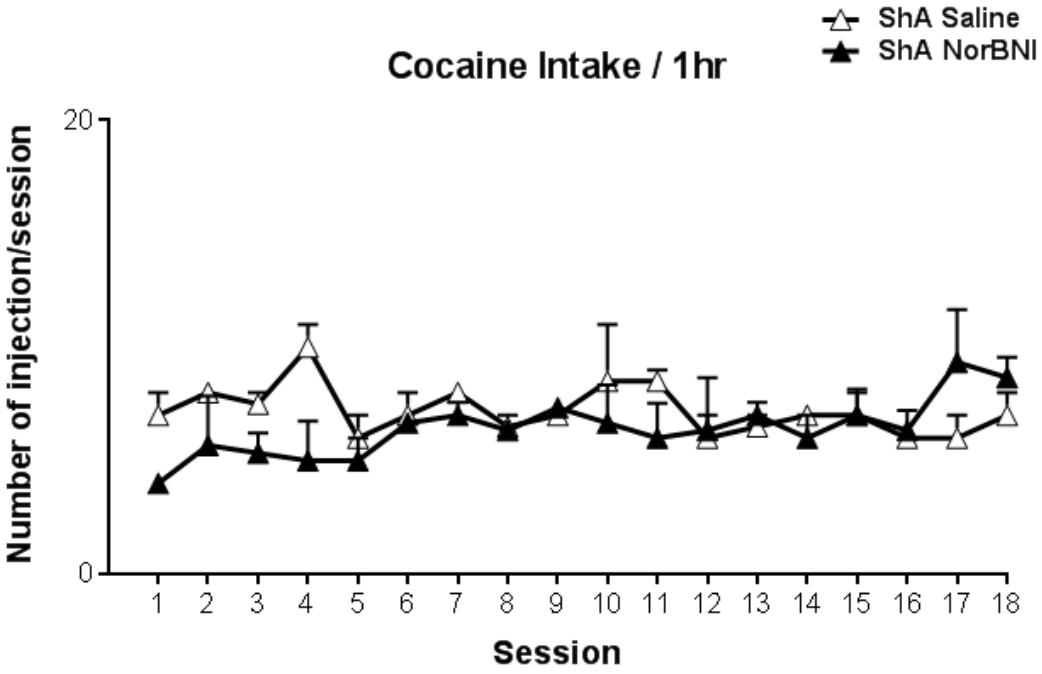
Norbinaltorphimine (norBNI) is a κ-opioid receptor antagonist (i.e. it blocks the effects of dynorphin). It was injected into a subgroup of rats before allowing them extended-access (6 h/day) to cocaine. The control rats were injected with saline before extended-access to cocaine. In comparison to saline-injected LgA rats (n=4), norBNI-injected rats (n=4) had lower cocaine intake ($F_{1,7}$=6.989; P=0.0203).

This graph was produced in part by Tim Whitfield, Ph.D.



Graph 2: The κ-opioid receptor antagonist norBNI showed no effect on ShA cocaine intake.

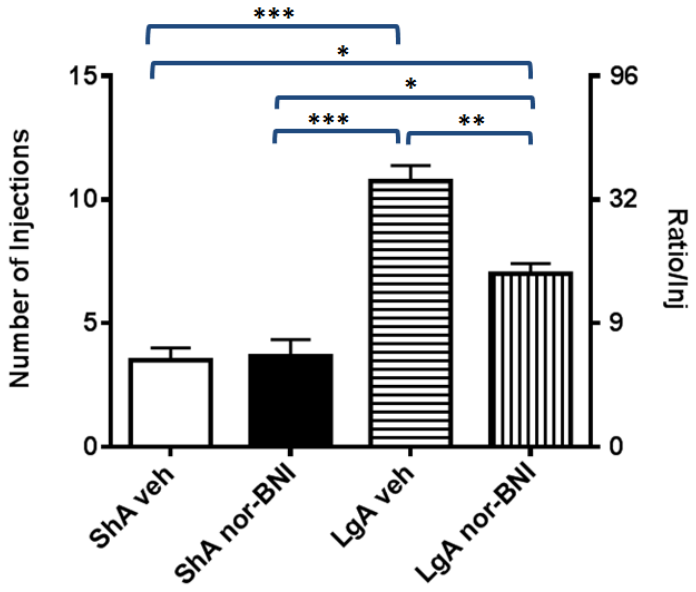
This graph was produced in part by Tim Whitfield, Ph.D.



Graph 3: The κ-opioid receptor antagonist norBNI decreased cocaine self-administration in LgA rats under a progressive-ratio schedule.

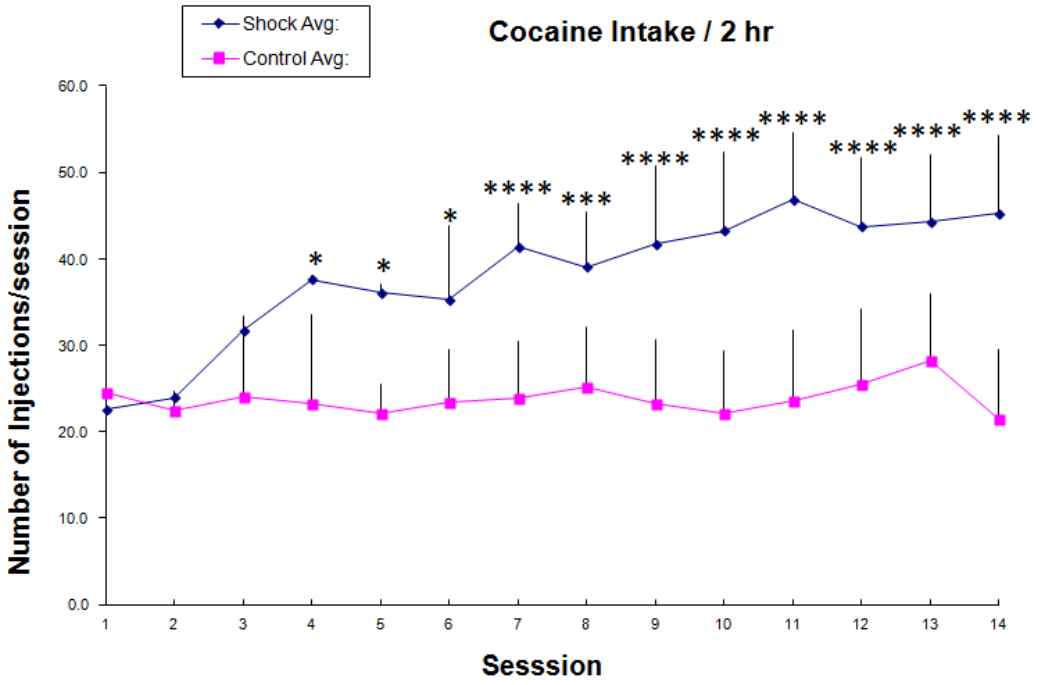
Rates of cocaine intake compared across groups in the norBNI experiment. Saline was used as the vehicle (veh). Saline-ShA rats took an average of 3.5 ± 0.5 injections. NorBNI-ShA rats took an average of 3.7 ± 0.7 injections. Saline-LgA rats took an average of 10.8 ± 0.6 injections. NorBNI-LgA rats took an average of 7.0 ± 0.4 injections. The breakpoint for norBNI-LgA rats was around 15 presses whereas the breakpoint for saline-LgA rats was around 32 presses and the breakpoint for ShA rats was around 6 presses. (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; **** = $P \leq 0.0001$).

This graph was produced in part by Tim Whitfield, Ph.D.



Graph 4: Mild foot-shock stress induced cocaine escalation.

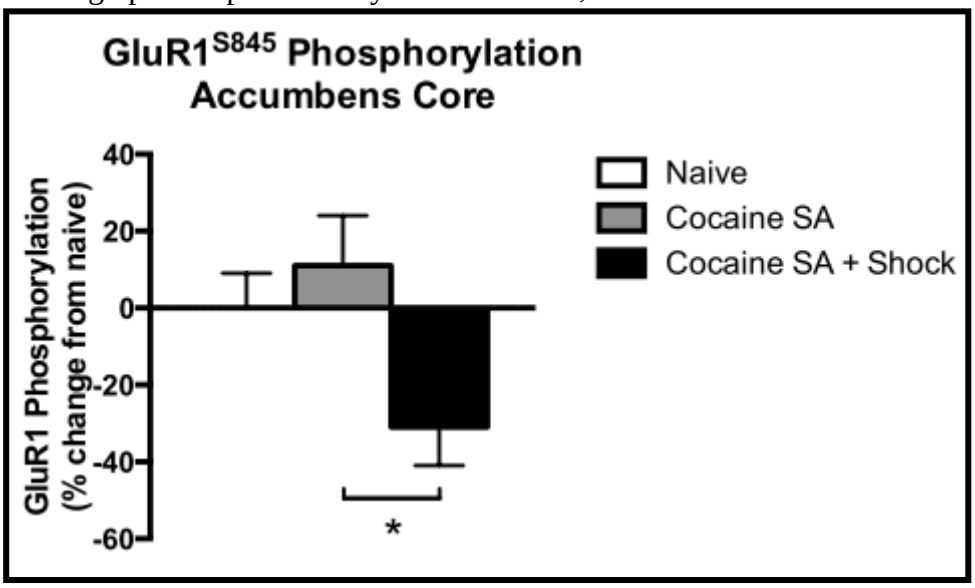
Animals that received mild intermittent foot-shocks (0.5mA, 20 min/day: 10 min after each hour of self-administration) during cocaine self-administration sessions (2 h/day) displayed a significant escalation ($F_{1,13}$=1.3; $P < 0.001$) in cocaine intake in comparison to animals that had no such stressor. Each session's *P*-value was compared to session 1. This data suggests that stress is a factor that contributes to drug addiction. The following graph illustrates the results of cocaine intake in animals chronically exposed to stress versus animals not subjected to stress. (* = $P \leq 0.05$; ** = $P \leq 0.01$; *** = $P \leq 0.001$; **** = $P \leq 0.0001$).



Graph 5: GluR1^{S845} phosphorylation was significantly decreased in rats with both cocaine withdrawal and stress history.

Following cocaine self-administration (1d WD). Western blot analysis compared phophoprotein level variation between foot-shock subjects, non foot-shock subjects, and cocaine-naive subjects. Results showed that in the nucleus accumbens (NAc) core, phosphorylation of GluR1 at serine 845 was significantly decreased during withdrawal from cocaine self-administration in stressed subjects in comparison to non-stressed subjects. Phosphoprotein was compared to total protein.

This graph was produced by Scott Edwards, Ph.D.



Graph 6: ERK1/2 phosphorylation was increased in rats undergoing withdrawal.

Following cocaine self-administration (1d WD). Western blot analysis compared phosphoprotein level variation between foot-shock subjects, non-foot-shock subjects, and cocaine-naive subjects. Results showed that in the nucleus accumbens (NAc) shell, phosphorylation of ERK1/2 was increased during withdrawal from cocaine self-administration. Phosphoprotein was compared to total protein.

This graph was produced by Scott Edwards, Ph.D.

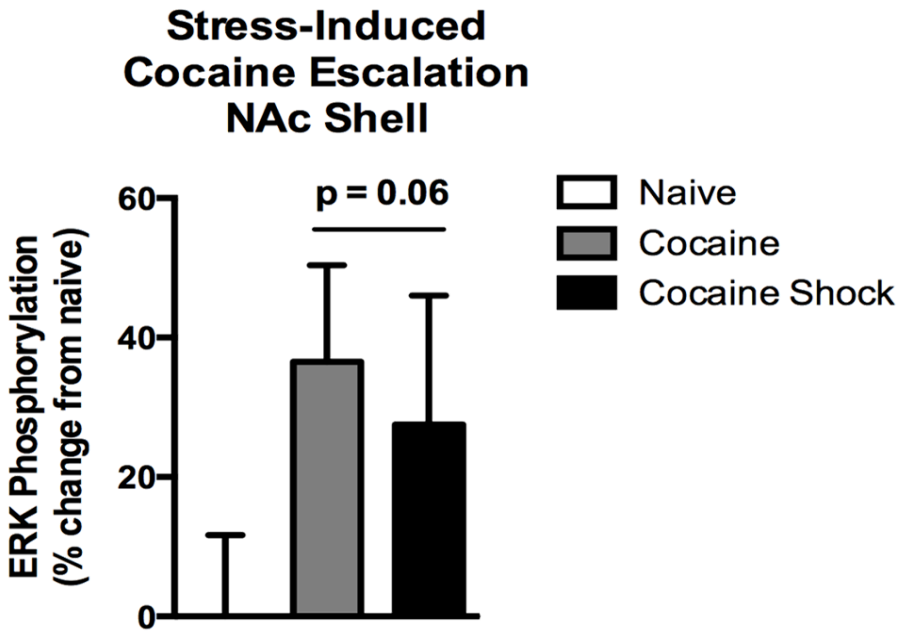


Table 1: Progressive Ratio Schedule. The number of lever presses needed for each cocaine injection.

Progressive Ratio (PR) Schedule

Cocaine Injections	Lever Presses Needed
1st	1
2nd	2
3rd	4
4th	6
5th	9
6th	12
7th	15
8th	20
9th	25
10th	32
11th	40
12th	50

Figure 10 is a reprint of material as it appears in "Emergence of Context-associated GluR1 and ERK Phosphorylation in the Nucleus Accumbens Core during Withdrawal from Cocaine Self-administration." Edwards, Scott, Ryan K. Bachtell, Daniel Guzman, Kimberly N. Whisler, and David W. Self. Addiction Biology 16.3 (2011): 450-57. Dr. Scott Edwards was first author of this paper.

Graphs 1, 2, and 3 were produced by Dr. Tim Whitfield.

Graphs 5 and 6 were produced by Dr. Scott Edwards.

References

Carvalho, A. L., M. V. Caldeira, S. D. Santos, and C. B. Duarte. "Role of the Brain-derived Neurotrophic Factor at Glutamatergic Synapses." *British Journal of Pharmacology*153.S1 (2008): S310-324.

Chao, Steven Z., Marjorie A. Ariano, Daniel A. Peterson, and Marina E. Wolf. "D1 Dopamine Receptor Stimulation Increases GluR1 Surface Expression in Nucleus Accumbens Neurons." Journal of Neurochemistry 83.3 (2002): 704-12.

Chavkin, Charles. "Dynorphin – Still an Extraordinarily Potent Opioid Peptide." Molecular Pharmacology (2012): n. pag. 14 Nov. 2012. Web. 18 Feb. 2013. <http://molpharm.aspetjournals.org/content/early/2012/11/14/mol.112.083337.long>.

Churchill, Lynn, Chad J. Swanson, Mary Urbina, and Peter W. Kalivas. "Repeated Cocaine Alters Glutamate Receptor Subunit Levels in the NucleusAccumbens and Ventral Tegmental Area of Rats That Develop BehavioralSensitization." *Journal of Neurochemistry* 72.6 (1999): 2397-403.

Conrad, Kelly L., Kuei Y. Tseng, Jamie L. Uejima, Jeremy M. Reimers, Li-Jun Heng, Yavin Shaham, Michela Marinelli, and Marina E. Wolf. "Formation of Accumbens GluR2-lacking AMPA Receptors Mediates Incubation of Cocaine Craving." *Nature*454.7200 (2008): 118-21.

Cooke, S. F., and T. V. P. Bliss. "Plasticity in the Human Central Nervous System." Brain 129.7 (2006): 1659-673.

Corbit, Laura H., Janice L. Muir, and Bernard W. Balleine. "The Role of the Nucleus Accumbens in Instrumental Conditioning: Evidence of a Functional Dissociation between Accumbens Core and Shell." *The Journal of Neuroscience* 21.9 (2001): 3251-260.

Cornish, Jennifer L., and Peter W. Kalivas. "Glutamate Transmission in the Nucleus Accumbens Mediates Relapse in Cocaine Addiction." *The Journal of Neuroscience*20 (2000): 1-5.

Covington, Herbert E., III, and Klaus A. Miczek. "Repeated Social-defeat Stress, Cocaine or Morphine. Effects on Behavioral Sensitization and Intravenous Cocaine Self-administration "binges"." *Psychopharmacology* 158 (2001): 388-98.

Covington, Herbert, and Klaus Miczek. "Repeated Social-defeat Stress, Cocaine or Morphine." *Psychopharmacology* 158.4 (2001): 399.

Delgado, J. Y., M. Coba, C. N. G. Anderson, K. R. Thompson, E. E. Gray, C. L. Heusner, K. C. Martin, S. G. N. Grant, and T. J. O'Dell. "NMDA Receptor Activation Dephosphorylates AMPA Receptor Glutamate Receptor 1 Subunits at Threonine 840." *Journal of Neuroscience* 27.48 (2007): 13210-3221.

Dembo, Richard, Linda Williams, Eric Wish, Max Dertke, Estrellita Berry, Alan Getreu, Mark Washburn, and James Schmeidler. "The Relationship between Physical and Sexual Abuse and Illicit Drug Use: A Replication among a New Sample of Youths Entering a Juvenile Detention Center." *Substance Use & Misuse* 23.11 (1988): 1101-123.

Derkach, Victor, Andres Barria, and Thomas R. Soderling. "Ca2+/calmodulin-kinase II Enhances Channel Conductance of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate Type Glutamate Receptors." *Proceedings of the National Academy of Sciences of the United States of America* 96.6 (1999): 3269-274.

Di Ciano, Patricia, and Barry J. Everitt. "Dissociable Effects of Antagonism of NMDA and AMPA/KA Receptors in the Nucleus Accumbens Core and Shell on Cocaine-seeking Behavior." Neuropsychopharmacology 25.3 (2001): 341-60.

Diagnostic and Statistical Manual of Mental Disorders: DSM-IV-TR. Washington, DC: American Psychiatric Association, 2000.

Edwards, Scott, and George F. Koob. "Neurobiology of Dysregulated Motivational Systems in Drug Addiction." Future Neurology 5.3 (2010): 393-410.

Edwards, Scott, Ryan K. Bachtell, Daniel Guzman, Kimberly N. Whisler, and David W. Self. "Emergence of Context-associated GluR1 and ERK Phosphorylation in the Nucleus Accumbens Core during Withdrawal from Cocaine Self-administration." *Addiction Biology* 16.3 (2011): 450-57.

Erb, Suzanne, and Jane Stewart. "A Role for the Bed Nucleus of the Stria Terminalis, but Not the Amygdala, in the Effects of Corticotropin-releasing Factor on Stress-induced Reinstatement of Cocaine Seeking." The Journal of Neuroscience 19 (1999): 1-6.

Erb, Suzanne, Y. Shaham, and J. Stewart. "Stress Reinstates Cocaine-seeking Behavior after Prolonged Extinction and a Drug-free Period." *Psychopharmacology* 128.4 (1996): 408-12.

Esteban, J. A. "AMPA Receptor Trafficking: A Road Map for Synaptic Plasticity." *Molecular Interventions* 3.7 (2003): 375-85.

Everitt, Barry J., Anthony Dickinson, and Trevor W. Robbins. "The Neuropsychological Basis of Addictive Behaviour." Brain Research Reviews 36 (2001): 129-38.

Fallon, James H., and Frances M. Leslie. "Distribution of Dynorphin and Enkephalin Peptides in the Rat Brain." The Journal of Comparative Neurology 249.3 (1986): 293-336.

Ferrario, Carrie R., Jessica A. Loweth, Mike Milovanovic, Kerstin A. Ford, Gregorio L. Galinanes, Li-Jun Heng, Kuei Y. Tseng, and Marina E. Wolf. "Alterations in AMPA Receptor Subunits and TARPs in the Rat Nucleus Accumbens Related to the Formation of Ca2+-permeable AMPA Receptors during the Incubation of Cocaine Craving." *Neuropharmacology* 61 (2011): 1141-151.

Fitzgerald, Lawrence W., Jordi Ortiz, Azita G. Hamedani, and Eric J. Nestler. "Drugs of Abuse and Stress Increase the Expression of GluR1 and NMDAR1 Glutamate Receptor Subunits in the Rat Ventral Tegmental Area: Common Adaptations among Cross-Sensitizing Agents." *The Journal of Neuroscience* 16.1 (1996): 274-82.

Frank, D. A., and M. E. Greenberg. "CREB: A Mediator of Long-term Memory from Mollusks to Mammals." Cell 79.1 (1994): 5-8.

Ghitza, Udi E., Anthony T. Fabbricatore, Volodymyr F. Prokopenko, and Mark O. West. "Differences Between Accumbens Core and Shell Neurons Exhibiting Phasic Firing Patterns Related to Drug-Seeking Behavior During a Discriminative-Stimulus Task."*Journal of Neurophysiology* 92.3 (2004): 1608-614.

Ghitza, Udi E., Anthony T. Fabbricatore, Volodymyr Prokopenko, Anthony P. Pawlak, and Mark O. West. "Persistent Cue-evoked Activity of Accumbens Neurons following Prolonged Abstinence from Self-administered Cocaine." *The Journal of Neuroscience* 23.19 (2003): 7239-245

Glick, Stanley D., Isabelle M. Maisonneuve, John Raucci, and Archer Sydney. "Kappa Opioid Inhibition of Morphine and Cocaine Self-administration in Rats." *Brain Research* 681.1-2 (1995): 147-52.

Goeders, Nick E., and Glenn F. Guerin. "Non-contingent Electric Footshock Facilitates the Acquisition of Intravenous Cocaine Self-administration in Rats." Psychopharmacology 114.1 (1994): 63-70.

Grant, B., D. Dawson, F. Stinson, S. Chou, M. Dufour, and R. Pickering. "The 12-month Prevalence and Trends in DSM-IV Alcohol Abuse and Dependence: United States, 1991–1992 and 2001–2002." Drug and Alcohol Dependence 74.3 (2004): 223-34.

Grant, Bridget F., and Deborah A. Dawson. "Age of Onset of Drug Use and Its Association with DSM-IV Drug Abuse and Dependence: Results from the National Longitudinal Alcohol Epidemiologic Survey." Journal of Substance Abuse 10.2 (1998): 163-73.

Greger, I., and J. Esteban. "AMPA Receptor Biogenesis and Trafficking." *Current Opinion in Neurobiology* 17.3 (2007): 289-97.

Guo, Yuan, Hui-Ling Wang, Xiao-Hui Xiang, and Yan Zhao. "The Role of Glutamate and Its Receptors in Mesocorticolimbic Dopaminergic Regions in Opioid Addiction." Neuroscience & Biobehavioral Reviews 33.6 (2009): 864-73.

Hanson, Phyllis I., and Howard Schulman. "Neuronal Ca2+/Calmodulin-Dependent Protein Kinases." *Annual Review of Biochemistry* 61.1 (1992): 559-601.

Harrison, Patricia A., Jayne A. Fulkerson, and Timothy J. Beebe. "Multiple Substance Use among Adolescent Physical and Sexual Abuse Victims." *Child Abuse & Neglect* 21.6 (1997): 529-39.

Heimer, L., D. Zahm, L. Churchill, P. Kalivas, and C. Wohltmann. "Specificity in the Projection Patterns of Accumbal Core and Shell in the Rat." *Neuroscience* 41.1 (1991): 89-125.

Herz, A., and T. S. Shippenberg. "Differential Effects of Mu and Kappa Opioid Systems on Motivational Processes." Progress in Opioid Research: Proceedings of the 1986 International Narcotics Research Conference. By John W. Holaday, Ping-Yee Law, and Albert Herz. Rockville, MD: U.S. Dept. of Health and Human Services, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute on Drug Abuse, Office of Science, 1986. 563-66.

Hjelmstad, Gregory O., and Howard L. Fields. "Kappa Opioid Receptor Inhibition of Glutamatergic Transmission in the Nucleus Accumbens Shell." *Journal of Neurophysiology* 85 (2001): 1153-158.

Hoare, Jacqueline, and Debbie Moon, eds. Drug Misuse Declared: Findings from the 2009/10 British Crime Survey. July 2010.

Horan, Peter, Jill Taylor, Henry I. Yamamura, and Frank Porreca. "Extremely Long-lasting Antagonistic Actions of Nor-binaltorphimine (nor-BNI) in the Mouse Tail-flick Test."*The Journal of Pharmacology and Experimental Therapeutics* 260.3 (1991): 1237-243.

Hyman, Steven E., Robert C. Malenka, and Eric J. Nestler. "NEURAL MECHANISMS OF ADDICTION: The Role of Reward-Related Learning and Memory." Annual Review of Neuroscience 29.1 (2006): 565-98.

Itoi, K., N. Horiba, F. Tozawa, Y. Sakal, K. Sakal, K. Abe, H. Demura, and T. Suda. "Major Role of 3',5'-cyclic Adenosine Monophosphate-dependent Protein Kinase A Pathway in Corticotropin-releasing Factor Gene Expression in the Rat Hypothalamus in Vivo." Endocrinology 137.6 (1996): 2389-396.

Kalivas, Peter W. "The Glutamate Homeostasis Hypothesis of Addiction." *Nature Reviews Neuroscience* 10.8 (2009): 561-72.

Kalivas, Peter W., and Nora D. Volkow. "The Neural Basis of Addiction: A Pathology of Motivation and Choice." FOCUS 5 (2007): 208-19.

Karler, R., L. I. Calder, I. A. Chaudhry, and S. A. Turkanis. "Blockade of “Reverse Tolerance” to Cocaine and Amphetamine by MK-801." Life Sciences 45.7 (1989): 599-606.

Kauer, Julie A. "Learning Mechanisms in Addiction: Synaptic Plasticity in the Ventral Tegmental Area as a Result of Exposure to Drugs of Abuse." *Annual Review of Physiology* 66 (2004): 447-75.

Kauer, Julie A., and Robert C. Malenka. "Synaptic Plasticity and Addiction." *Nature Reviews Neuroscience* 8.11 (2007): 844-58.

Kenny, P., and A. Markou. "The Ups and Downs of Addiction: Role of Metabotropic Glutamate Receptors." *Trends in Pharmacological Sciences* 25.5 (2004): 265-72.

Kim, Jeansok J., and David M. Diamond. "The Stressed Hippocampus, Synaptic Plasticity and Lost Memories." *Nature Reviews Neuroscience* 3 (2002): 453-62.

Koob, G., and F. Bloom. "Cellular and Molecular Mechanisms of Drug Dependence." Science 242.4879 (1988): 715-23.

Koob, G., and M. J. Kreek. "Stress, Dysregulation of Drug Reward Pathways, and the Transition to Drug Dependence." American Journal of Psychiatry 164.8 (2007): 1149-159.

Koob, George F. "A Role for Brain Stress Systems in Addiction." Neuron 59.1 (2008): 11-34.

Koob, George F. "Animal Models of Drug Addiction." Animal Models of Drug Addiction. American College of Neuropsychopharmacology, 2000. Web. 09 Feb. 2013. <http://www.acnp.org/g4/gn401000072/ch072.html>.

Koob, George F., and Michael Le Moal. "Drug Abuse: Hedonic Homeostatic Dysregulation." Science 278.5335 (1997): 52-58.

Koob, George F., and Michael Le Moal. "Drug Abuse: Hedonic Homeostatic Dysregulation." Science 278.5335 (1997): 52-58.

Koob, George F., and Michel Le Moal. "Drug Addiction, Dysregulation of Reward, and Allostasis." *Neuropsychopharmacology* 24.2 (2001): 97-129.

Koob, George F., Barak Caine, Athina Markou, Luigi Pulvirenti, and Freidbert Weiss. "Role for the Mesocortical Dopamine System in the Motivating Effects of Cocaine." Neurobiological Models for Evaluating Mechanisms Underlying Cocaine Addiction. Comp. Lynda Erinoff and Roger M. Brown. Rockville, MD (5600 Fishers Lane, Rockville 20857): U.S. Dept. of Health and Human Services, Public Health Service, National Institutes of Health, National Institute on Drug Abuse, 1994

Krapivinsky, Grigory, Luba Krapivinsky, Yunona Manasian, Anton Ivanov, Roman Tyzio, Christophe Pellegrino, Yehezkel Ben-Ari, David E. Clapham, and Igor Medina. "The NMDA Receptor Is Coupled to the ERK Pathway by a Direct Interaction between NR2B and RasGRF1." *Neuron* 40.4 (2003): 775-84.

Krugers, Harmen J., Casper C. Hoogenraad, and Laurent Groc. "Stress Hormones and AMPA Receptor Trafficking in Synaptic Plasticity and Memory." *Nature Reviews Neuroscience* 11.10 (2010): 675-81.

Kuzmin, Alexander V., Mirjam A.F.M. Gerrits, and Jan M. Van Ree. "K-Opioid Receptor Blockade with Nor-binaltorphimine Modulates Cocaine Self-administration in Drug-naive Rats." *European Journal of Pharmacology* 358 (1998): 197-202.

Land, Benjamin B., Michael R. Bruchas, Julia C. Lemos, Mei Xu, Erica J. Melief, and Charles Chavkin. "The Dysphoric Component of Stress Is Encoded by Activation of the Dynorphin κ-Opioid System." *Journal of Neuroscience* 2nd ser. 9.28 (2008): 407-14.

Lee, Anna M., and Robert O. Messing. "Protein Kinases and Addiction." *Annals of the New York Academy of Sciences* 1141.1 (2008): 22-57.

Lu, L., E. Koya, H. Zhai, B. Hope, and Y. Shaham. "Role of ERK in Cocaine Addiction."*Trends in Neurosciences* 29.12 (2006): 695-703.

Malinow, Roberto, Zachary F. Mainen, and Yasunori Hayashi. "LTP Mechanisms: From Silence to Four-lane Traffic." *Current Opinion in Neurobiology* 10.3 (2000): 352-57.

Mammen, Andrew L., Kimihiko Kameyama, Katherine W. Roche, and Richard L. Huganir. "Phosphorylation of the α-Amino-3-hydroxy-5-methylisoxazole4-propionic Acid Receptor GluR1 Subunit by Calcium/ Calmodulin-dependent Kinase II." *The Journal of Biological Chemistry* 272.51 (1997): 32528-2533.

Mantsch, John R., and Eric S. Katz. "Elevation of Glucocorticoids Is Necessary but Not Sufficient for the Escalation of Cocaine Self-Administration by Chronic Electric Footshock Stress in Rats." Neuropsychopharmacology 32.2 (2006): 367-76.

Mantsch, John, Ann Ho, Stefan Schlussman, and Mary Kreek. "Predictable Individual Differences in the Initiation of Cocaine Self-administration by Rats under Extended-access Conditions Are Dose-dependent." *Psychopharmacology* 157.1 (2001): 31-39.

Margolis, Elyssa B., Gregory O. Hjelmstad, Antonello Bonci, and Howard L. Fields. "κ-Opioid Agonists Directly Inhibit Midbrain Dopaminergic Neurons." The Journal of Neuroscience 23.31 (2003): 9981-986.

Martin, S. J., P. D. Grimwood, and R. G. M. Morris. "Synaptic Plasticity and Memory: An Evaluation of the Hypothesis." *Annual Review of Neuroscience* 23.1 (2000): 649-711.

Mayr, Berhard, and Marc Montminy. "Transcriptional Regulation by the Phosphorylation-Dependent Factor CREB." Nature Reviews Molecular Cell Biology 2.8 (2001): 599-609.

McFarland, Krista, Christopher C. Lapish, and Peter W. Kalivas. "Prefrontal Glutamate Release into the Core of the Nucleus Accumbens Mediates Cocaine-Induced Reinstatement of Drug-Seeking Behavior." *The Journal of Neuroscience* 23.8 (2003): 3531-537.

Melief, Erica J., Mayumi Miyatake, F. Ivy Carroll, Cecile Beguin, William A. Carlezon, Jr., Bruce M. Cohen, Sarah Grimwood, Charles H. Mitch, Linda Rorick-Kehn, and Charles Chavkin. "Duration of Action of a Broad Range of Selective κ-Opioid Receptor Antagonists Is Positively Correlated with C-Jun N-Terminal Kinase-1 Activation." Molecular Pharmacology 80.5 (2011): 920-29.

Meredith, G. E., A. Pattiselanno, H. J. Groenewegen, and S. N. Haber. "Shell and Core in Monkey and Human Nucleus Accumbens Identified with Antibodies to Calbindin-D28k." The Journal of Comparative Neurology 365 (1996): 628-39.

Miczek, K. A., and Nicole H. Mutschler. "Activational Effects of Social Stress on IV Cocaine Self-administration in Rats." *Psychopharmacology* 128.3 (1996): 256-64.

Miczek, K. A., and Nicole H. Mutschler. "Activational Effects of Social Stress on IV Cocaine Self-administration in Rats." *Psychopharmacology* 128.3 (1996): 256-64.

Mogenson, Gordon J., Douglas L. Jones, and Chi Yiu Yim. "From Motivation to Action: Functional Interface between the Limbic System and the Motor System." *Progress in Neurobiology* 14.2-3 (1980): 69-97.

Morris, R. G. M. "Elements of a Neurobiological Theory of Hippocampal Function: The Role of Synaptic Plasticity, Synaptic Tagging and Schemas." *European Journal of Neuroscience* 23 (2006): 2829-846.

Mukherjee, Abhisek, and Claudio Soto. "Role of Calfineurin in Neurodegeneration Produced by Misfolded Proteins and Endoplasmic Reticulum Stress." *Current Opinion in Cell Biology* 23 (2011): 223-30.

Muschamp, John W., and William A. Carlezon, Jr. "Roles of Nucleus Accumbens CREB and Dynorphin in Dysregulation of Motivation." *Cold Spring Harbor Perspectives in Medicine* (2013): 1-16. 4 Jan. 2013. Web. 23 Feb. 2013. <http://perspectivesinmedicine.cshlp.org/content/3/2/a012005.full>.

Najavits, Lisa M., David R. Gastfriend, Jacques P. Barber, Sharon Reif, Larry R. Muenz, Jack Blaine, Arlene Frank, Paul Crits-Christoph, Michael Thase, and Roger D. Weiss. "Cocaine Dependence With and Without PTSD Among Subjects in the National Institute on Drug Abuse Collaborative Cocaine Treatment Study." The American Journal of Psychiatry 155.2 (1998): 214-19.

National Survey on Drug Use and Health:Summary of National Findings." *Results from the 2011 NSDUH: Summary of National Findings, SAMHSA, CBHSQ*. U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Substance Abuse and Mental Health Services Administration, Sept. 2012. Web. 09 Feb. 2013. <http://www.samhsa.gov/data/NSDUH/2k11Results/NSDUHresults2011.htm>.

Negus, S. Stevens, Nancy K. Mello, Philip S. Portoghese, and Chai-en Lin. "Effects of Kappa Opioids on Cocaine Self-administration by Rhesus Monkeys." *The Journal of Pharmacology and Experimental Therapeutics* 282 (1997): 44-55.

Nestler, E. "Molecular Mechanisms of Drug Addiction." Neuropharmacology 47 (2004): 24-32.

Nestler, Eric J., Max B. Kelz, and Jingshan Chen. "ΔFosB: A Molecular Mediator of Long-term Neural and Behavioral Plasticity." Brain Research 835.1 (1999): 10-17.

Ozawa, Seiji, Haruyuki Kamiya, and Keisuke Tsuzuki. "Glutamate Receptors in the Mammalian Central Nervous System." *Progress in Neurobiology* 54 (1998): 581-618.

Pan, Chunliu, Howard Prentice, Allison L. Price, and Jang-Yen Wu. "Beneficial Effect of Taurine on Hypoxia- and Glutamate-induced Endoplasmic Reticulum Stress Pathways in Primary Neuronal Culture." *Amino Acids* 43 (2012): 845-55.

Pandey, Subhash C., Adip Roy, Huaibo Zhang, and Tiejun Xu. "Partial Deletion of the CAMP Response Element-Binding Protein Gene Promotes Alcohol-Drinking Behaviors." The Journal of Neuroscience 24.21 (2004): 5022-030.

Perkinton, Michael S., Talvinder S. Sihra, and Robert J. Williams. "Ca(2+)-permeable AMPA Receptors Induce Phosphorylation of CAMP Response Element-binding Protein through a Phosphatidylinositol 3-kinase-dependent Stimulation of the Mitogen-activated Protein Kinase Signaling Cascade in Neurons." *The Journal of Neuroscience* 19.14 (1999): 5861-874.

Pfeiffer, A., V. Brantl, A. Herz, and H. Emrich. "Psychotomimesis Mediated by Kappa Opiate Receptors." Science 233.4765 (1986): 774-76.

Phillipson, O., and A. Griffiths. "The Topographic Order of Inputs to Nucleus Accumbens in the Rat." Neuroscience 16.2 (1985): 275-96.

Piazza, P. V., and M. Le Moal. "The Role of Stress in Drug Self-administration." TiPS 19 (1998): 67-74.

Redgrave, P., N. Vautrelle, and J.N.J. Reynolds. "Functional Properties of the Basal Ganglia's Re-entrant Loop Architecture: Selection and Reinforcement." *Neuroscience* 198 (2011): 138-51.

Roche, K., R. Obrien, A. Mammen, J. Bernhardt, and R. Huganir. "Characterization of Multiple Phosphorylation Sites on the AMPA Receptor GluR1 Subunit." *Neuron* 16.6 (1996): 1179-188.

Saal, Daniel, Yan Dong, Antonello Bonci, and Robert C. Malenka. "Drugs of Abuse and Stress Trigger a Common Synaptic Adaptation in Dopamine Neurons." *Neuron* 37.4 (2003): 577-82.

Santos, S., A. Carvalho, M. Caldeira, and C. Duarte. "Regulation of AMPA Receptors and Synaptic Plasticity." *Neuroscience* 158.1 (2009): 105-25.

Shaham, Yavin, Suzanne Erb, and Jane Stewart. "Stress-induced Relapse to Heroin and Cocaine Seeking in Rats: A Review." *Brain Research Reviews* 33 (2000): 13-33.

Shen, Chang-peng, Yelena Tsimberg, Christopher Salvadore, and Emanuel Meller. "Activation of Erk and JNK MAPK Pathways by Acute Swim Stress in Rat Brain Regions."*BMC Neuroscience* 5.36 (2004): n. pag. 20 Sept. 2004. Web. 24 Feb. 2013. <http://www.biomedcentral.com/1471-2202/5/36>.

Shiffman, Saul. "Relapse following Smoking Cessation: A Situational Analysis." *Journal of Consulting and Clinical Psychology* 50.1 (1982): 71-86.

Shirayama, Yukihiko, and Shigeyuki Chaki. "Neurochemistry of the Nucleus Accumbens and Its Relevance to Depression and Antidepressant Action in Rodents." *Current Neuropharmacology* 4.4 (2006): 277-91.

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

Sinha, R., Dana Catapano, and Stephanie O'Malley. "Stress-induced Craving and Stress Response in Cocaine Dependent Individuals." Psychopharmacology 142.4 (1999): 343-51.

Sinha, R., Dana Catapano, and Stephanie O'Malley. "Stress-induced Craving and Stress Response in Cocaine Dependent Individuals." *Psychopharmacology* 142.4 (1999): 343-51.

Sivam, S. P. "Cocaine Selectively Increases Striatonigral Dynorphin Levels by a Dopaminergic Mechanism." The Journal of Pharmacology and Experimental Therapeutics 250.3 (1989): 818-24.

Snyder, Gretchen L., Patrick B. Allen, Allen A. Feinberg, Carmina G. Valle, Richard L. Huganir, Angus C. Nairn, and Paul Greengard. "Neostriatum by Dopamine and Psychostimulants In Vivo." *The Journal of Neuroscience* 20.12 (2000): 4480-488.

Snyder, Gretchen L., Stacey Galdi, Allen A. Fienberg, Patrick Allen, Angus C. Nairn, and Paul Greengard. "Regulation of AMPA Receptor Dephosphorylation by Glutamate Receptor Agonists." *Neuropharmacology* 45.6 (2003): 703-13.

Spanagel, R., A. Herz, and T. S. Shippenberg. "Opposing Tonically Active Endogenous Opioid Systems Modulate the Mesolimbic Dopaminergic Pathway." Proceedings of the National Academy of Sciences 89.6 (1992): 2046-050.

Spangler, R., E. M. Unterwald, and M. J. Kreek. "'Binge' Cocaine Administration Induces a Sustained Increase of Prodynorphin MRNA in Rat Caudate-putamen." Molecular Brain Research 19.4 (1993): 323-27.

Steketee, Jeffery D., and Peter W. Kalivas. "Drug Wanting: Behavioral Sensitization and Relapse to Drug-Seeking Behavior." *Pharmacological Reviews* 63.2 (2011): 348-65.

Sterling, P., and J. Eyer. "Allostasis: A New Paradigm to Explain Arousal Pathology." Handbook of Life Stress, Cognition and Health. Ed. S. Fisher and J. Reason. New York: John Wiley & Sons, 1988. 629-49.

Substance Abuse and Mental Health Services Administration, Center for Behavioral Health Statistics and Quality (formerly the Office of Applied Studies). The DAWN Report: Highlights of the 2009 Drug Abuse Warning Network (DAWN) Findings on Drug-Related Emergency Department Visits. Rockville, MD, December 28, 2011

Ungless, Mark A., Jennifer L. Whistler, Robert C. Malenka, and Antonello Bonci. "Single Cocaine Exposure in Vivo Induces Long-term Potentiation in Dopamine Neurons."*Nature* 411 (2001): 583-87.

Urban, Nina B.L., and Diana Martinez. "Neurobiology of Addiction : Insight from Neurochemical Imaging." Psychiatric Clinics of North America 35.2 (2012): 521-41.

Voorn, Pieter, Louk J.M.J. Vanderschuren, Henk J. Groenewegen, Trevor W. Robbins, and Cyriel M.A. Pennartz. "Putting a Spin on the Dorsal–ventral Divide of the Striatum."*Trends in Neurosciences* 27.8 (2004): 468-74.

Wee, Sunmee, and George F. Koob. "The Role of the Dynorphin–κ Opioid System in the Reinforcing Effects of Drugs of Abuse." *Psychopharmacology* 210.2 (2010): 121-35.

Wee, Sunmee, Laura Orio, Senait Ghirmai, John R. Cashman, and George F. Koob. "Inhibition of Kappa Opioid Receptors Attenuated Increased Cocaine Intake in Rats with Extended Access to Cocaine." *Psychopharmacology* 205.4 (2009): 565-75.

Werling, Linda L., Anne Frattali, P. S. Portoghese, A. E. Takemori, and Brian M. Cox. "Kappa Receptor Regulation of Dopamine Release from Striatum and Cortex of Rats and Guinea Pigs." *The Journal of Pharmacology and Experimental Therapeutics*246.1 (1988): 282-86.

Widom, Cathy Spatz. "Post Traumatic Stress Disorder in Abused and Neglected Children Grown up." *The American Journal of Psychiatry* 156 (1999): 1223-229.

Wikler, Abraham. "A Psychodynamic Study of a Patient during Experimental Self-regulated Re-addiction to Morphine." The Psychiatric Quarterly 26.1-4 (1952): 270-93.

Wolf, Marina E. "Addiction and Glutamate-Dependent Plasticity." Glutamate and Addiction. By Barbara H. Herman and Jerry Frankenheim. Totowa, NJ: Humana, 2003a. 127-41

Wolf, Marina E. "LTP May Trigger Addiction." Molecular Interventions 3.5 (2003b): 248-52.

Wolf, Marina E. "The Role of Excitatory Amino Acids in Behavioral Sensitization to Psychomotor Stimulants." *Progress in Neurobiology* 54 (1998): 679-720.

Zhu, Hong, Robin W. Rockhold, and Ing K. Ho. "The Role of Glutamate in Physical Dependence on Opioids." The Japanese Journal of Pharmacology 76.1 (1998): 1-14.

Zhu, J.Julius, Yi Qin, Mingming Zhao, Linda Van Aelst, and Roberto Malinow. "Ras and Rap Control AMPA Receptor Trafficking during Synaptic Plasticity." *Cell* 110.4 (2002): 443-55.

Zittel-Lazarini, Amandine, Martine Cador, and Serge H. Ahmed. "A Critical Transition in Cocaine Self-administration: Behavioral and Neurobiological Implications." Psychopharmacology 192.3 (2007): 337-46.