Mesolimbic GluA1 AMPA Receptor Signaling in Dopaminergic Neurons Plays a Critical Role in the Induction of Cross-Sensitization to Psychostimulants in Response to Social Stress

by

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ABSTRACT

Intermittent social defeat stress induces psychostimulant cross-sensitization, as well as long-lasting social avoidance behavior. Previous data reveal heightened expression of AMPA receptor (AMPAR) GluA1 subunits in rat ventral tegmental area (VTA), which occurs concurrently with social stress-induced amphetamine (AMPH) cross-sensitization. These studies described herein examined whether VTA GluA1 AMPARs are important for the behavioral consequences of social stress and investigated the role of the infralimbic (IL) to VTA pathway in the induction of these responses. Functional inactivation of GluA1 in VTA dopamine (DA) neurons prevented stress-induced AMPH sensitization without affecting social avoidance behavior, while GluA1 overexpression in VTA DA neurons mimicked the effects of stress on AMPH sensitization. Female rats were more sensitive to the effects of stress on AMPH administration than males, specifically during proestrus/estrus, which is characterized by higher circulating estradiol. Fluorescent immunohistochemistry revealed that females expressed higher GluA1 in VTA DA neurons as a result of intermittent social defeat stress, independent of estrus stage; by contrast, females during proestrus/estrus displayed higher tyrosine kinase receptor type 2 (TrkB) expression, which is the receptor for brain derived neurotrophic factor (BDNF), in VTA DA neurons, independent of stress exposure. Functional inactivation of GluA1 in VTA DA neurons prevented stress-induced AMPH sensitization and overexpression mimicked the effects of stress on AMPH sensitization. This suggests that BDNF-TrkB signaling may work concomitantly with GluA1 signaling in the VTA to drive sex-dependent differences in stress-induced locomotor sensitization effects. Optogenetic inhibition of the IL-VTA pathway in male rats prevented stress-induced AMPH sensitization compared to control animals. In addition, fluorescent immunohistochemistry displayed less Fos labeling in the nucleus accumbens (NAc) of rats with IL-VTA light inhibition compared to control animals. This suggests that the IL-VTA pathway plays a critical role in the induction of stress-induced sensitivity to AMPH, and blocking this pathway prevents mesolimbic DA signaling to the NAc. We conclude that IL glutamate projections onto GluA1-homomeric AMPA receptors in VTA DA neurons play a critical role in driving the stress-

induced sensitization response in males and females. Therefore, GluA1 VTA DA neurons could potentially be a therapeutic target to prevent stress-induced drug susceptibility in the future.

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INTRODUCTION AND BACKGROUND

1. Significance

Drug addiction is a debilitating neuropsychiatric disorder, which is most often characterized by excessive drug intake, drug-seeking behavior, and frequently, periods of relapse (Reid & Thakkar, 2009). Additionally, drug addiction is among the top three public health concerns in the United States, and is the costliest neuropsychiatric disorder (Uhl & Grow, 2004). With annual health-, productivity-, and crime-related costs of approximately $740 billion related to substance abuse, drug addiction represents a substantial economic and health concern in the United States (NIDA, 2014). Evidence suggests that behavioral sensitization occurs in humans as well as rodents, and is a model for the intensification of drug craving in humans (Sato *et al.*, 1983; Brady *et al.*, 1991; Satel *et al.*, 1991; Strakowski *et al.*, 1996; Bartlett *et al.*, 1997), which characterizes drug addiction and promotes relapse (Robinson & Berridge, 1993; Di Chiara, 1995; Emmett-Oglesby, 1995). Sensitization is a phenomenon that is easy to study, as it can be observed and measured rapidly, has long-term effects, and does not need to be trained/taught, and can be translated to human addiction studies, which makes it an ethologically relevant measure of addiction. Although extensive research has been devoted to developing more effective treatments for substance abuse disorders, most addicts are susceptible to relapse, which highlights the need for a more comprehensive understanding of neural mechanisms of addiction (Sinha, 2011; NIDA, 2014). This could potentially lead to methods to stop the occurrence of substance abuse entirely.

Vulnerability to substance abuse disorders can be influenced by a combination of genetic and environmental factors (Kreek *et al*., 2005). One common environmental factor that is implicated in the transition from recreational drug use to abuse is stress, which is correlated with increased substance abuse and relapse to addictive drugs (Sinha, 2001, 2008, 2011; NIDA, 2014). Social defeat stress reliably produces social avoidance behavior and heightens sensitization to psychostimulants, and intermittent social defeat stress induces long-lasting cross-

sensitization to amphetamine, which is apparent for months after the last social defeat (Covington & Miczek, 2001; Nikulina *et al.*, 2004). Social defeat stress is a highly salient stressor that causes long-lasting activation of the mesocorticolimbic dopaminergic system, with apparent neurochemical alterations 60 days after the last instance of intermittent social defeat stress (Nikulina et al., 2004). We propose to study the plasticity evoked by the administration of a moderate dose of a psychostimulant after intermittent social defeat stress.

The goal of the experiments described herein is to determine whether VTA GluA1 plays a causative role in the induction of stress-induced psychostimulant cross-sensitization, and to determine the neural pathway(s) that drives this mechanism. Since elevated levels of VTA GluA1 are critical for drug-induced sensitization (Ping et al., 2008; Kalivas, 2009), and are implicated after intermittent social defeat stress (Wang et al., 2014), the first experiment asks whether GluA1 in VTA dopamine (DA) neurons plays a critical role in social stress-induced cross-sensitization to amphetamine in male rats. While it is known that females exhibit more robust operant behavior during acquisition of cocaine and heroin self-administration (Lynch and Carroll, 1999; Lynch et al., 2000) and are more sensitive to lower drug doses than males during conditioned place preference (CPP) (Russo et al., 2003; Zakharova et al., 2009), little is understood about the mechanisms that drive sex-dependent differences in stress-induced sensitivity; thus, the second experiment seeks to explore the mesolimbic mechanisms that drive estrus cycle-dependent enhanced sensitivity to psychostimulants. The third experiment utilizes wireless optogenetics to bidirectionally manipulate the IL-VTA pathway in order to identify whether it is this pathway that drives stress-induced heightened amphetamine (AMPH) sensitivity. The combined results of these experiments will improve our comprehensive understanding of the mechanisms that drive social stress-induced psychostimulant cross-sensitization, and could potentially lead to the development of pharmacotherapeutic agents to aid in the treatment of stress-induced substance abuse vulnerability.

1.1. General Pharmacology of Psychostimulants

Psychostimulants, which include AMPH and cocaine, are extremely addictive drugs that increase activity of the central nervous system by increasing extra neuronal dopamine levels. AMPH is a type of a group of compounds known as amphetamines, which includes AMPH, methamphetamine, and methylenedioxymethamphetamine. Generally, AMPH is characterized as a DA releaser that elevates DA levels by using three major mechanisms: (1) AMPH is structurally similar to DA, and acts as a substrate for the DA transporter (DAT) and competitively inhibits DA uptake; (2) it facilitates DA movement out of vesicles and into the cytoplasm; (3) it promotes DAT-mediated reverse-transport of DA into the synaptic cleft independently of action-potential-induced vesicular release (Fleckenstein et al., 2007). Essentially, AMPH binds with DAT and is transported into the cytosol, where it competitively inhibits DA uptake. In addition, AMPH is lipophilic, so it can diffuse directly into the cells even without the aid of DAT (Gulaboski et al., 2007). AMPH increases intracellular binding sites of DAT for DA once in the cytoplasm, and induces the DAT-mediated reverse transport of DA (Sulzer et al., 1995), through the exchange of extracellular AMPH with intracellular DA (Jones et al., 1999). In addition, intracellular AMPH interferes with the vesicular monoamine transporter 2 (VMAT-2) and impairs active transport of monoamines into synaptic vesicles, which further increases DA concentration in the cytosol (Sulzer et al., 2005). Ultimately, the preceding actions lead to abnormal DA release, which can saturate DA receptors and eventually result in depletion of intracellular DA stores (Schmitz et al., 2001). Contrarily, cocaine works by directly inhibiting the DAT (Brown et al., 2001), which prevents reuptake of DA into nerve terminals, resulting in increased DA levels in the synaptic cleft.

Psychostimulant use can result in a range of severe neurological impairments including epilepsy/seizures, cerebral ischaemia, cerebral atrophy, hemorrhages, cognitive impairment, as well as mood disorders (Koppel et al., 1996; Rodnitzky & Keyser, 1992). In addition, clinical studies have shown that people that are addicted to psychostimulants have higher instances of depressive disorders, attention deficit disorder, and other pathophysiological disorders (Clure et

al., 1999). For these reasons, it is important to gain a better understanding of molecular mechanisms underlying substance abuse and the development of addiction.

1.2. Drug-Induced Sensitization to Psychostimulants

Psychomotor sensitization is generally characterized by the progressive augmented locomotor response to drugs of abuse following repeated exposure, and persists for many weeks after termination of drug use (Vezina, 2004; Robinson & Berridge, 2008; Vanderschuren & Pierce, 2010). Motivational properties and locomotor-activating effects of psychostimulants undergo sensitization. In rodent models, incentive sensitization can be tested as the ability to more rapidly learn to self-administer a low dose of psychostimulant. The most common experimental approach for the examination of behavioral sensitization to drugs of abuse is to administer repeated i.p. injections of the drug of choice and monitor progressive increases in locomotor activity (Wolf & Tseng, 2012). Psychomotor sensitization is mediated by sensitization of the mesocorticolimbic circuit, which involves changes in neurotransmission strength on the circuit level, and physiological changes on the neuronal level.

Importantly, sensitization to drugs of abuse is dose-dependent; for instance, psychostimulants induce exacerbated locomotor activity when administered at a moderate dose, whereas motor stereotypies impeding locomotion occur when psychostimulants are applied at a higher dose. Sensitization has been shown to be an effective and translational measure of neuroadaptations that occur with drug administration, as studies have also shown that humans experience neural and behavioral sensitization in response to drugs of abuse (Di Chiara, 2002; Strakowski et al., 1996), although, in humans, sensitization is not measured in the form of locomotor activity, and is instead measured as physiological changes that van be described as “excitatory”. Strakowski et al. (1996) found that repeated injection of low-dose AMPH (0.25 mg/kg) was sufficient to induce transient physiological changes, including enhanced energy level, a faster heart rate, and augmented speech and eye-blink rates. These data demonstrate that the behavior sensitization model in rodents is a valid model to study neuroadaptations underlying psychostimulant challenge in humans.

Like first time drug administration, the mesolimbic DA system is the primary neural substrate underlying psychostimulant sensitization. Data suggests that the VTA is a critical region in the *induction* of drug-induced sensitization, whereas the NAc is a critical region for the *expression* of drug-induced sensitization; microinfusions of psychostimulants or opioids directly into the VTA induce a sensitized response, whereas they only induce an acute locomotor response when infused into the NAc. By contrast, microinfusions of psychostimulants into the NAc can maintain the expression of sensitization (Kalivas & Weber, 1988). To better understand the mechanism of action of DA in the VTA during sensitization induction, selective DA type 1 (D1) and type 2 (D2) receptor antagonists were microinfused into the VTA. When this was performed, intra-VTA infusion of D1 receptor, but not D2 receptor antagonist prevented repeated AMPH or morphine-induced sensitization (Stewart & Vezina, 1989). This suggests that activation of D1Rs in the VTA is required for the induction of sensitization to drugs of abuse. Glutamatergic transmission to the VTA, originating in the mPFC, is also required for drug sensitization induction (Cador et al., 1999). Studies have shown that intra-VTA glutamate infusions enhances DA transmission in the NAc, and induces psychomotor stimulating effects (Kalivas et al., 1989).

1.3. The Mesocorticolimbic Dopamine Circuit

The mesocorticolimbic pathway (Fig. 1.1) consists of the VTA, where the cell bodies of dopaminergic neurons are located, as well as its projection areas, including the nucleus accumbens (NAc), amygdala (AMY), and the hippocampus, and is heavily implicated in both the effects of stress and the effects of addictive drugs (Sinha, 2008; Morales & Pickel, 2012; Nikulina et al., 2012). While neuroplasticity in the VTA, NAc, and PFC (among others) is important for reward, stress, and reinforcement, enhanced mesolimbic (VTA-NAc) DA transmission is critical for the effects of stress and drugs of abuse (Fields et al., 2007; Nikulina et al., 2014). In support of this, VTA DA transmission and release in the NAc is necessary for the induction of sensitization to psychomotor stimulants, as well as opiates (Joyce & Iversen, 1979; Vezina et al., 1987; Vezina & Stewart, 1989; Pierce & Kumaresan, 2006).

Ventral tegmental area

The VTA is highly heterogeneous in cell type and distribution. While a majority of VTA neurons are dopamine neurons (50-65%), the VTA also has a substantial population of GABA neurons (30-35%; Swanson 1982; Oades and Halliday, 1987; Yamaguchi et al., 2007), as well as a smaller population of glutamate neurons (2-3%; Nair-Roberts *et al.,* 2008). To make matters even more complicated, immunocytochemistry and electron microscopy show that a majority of VTA glutamate neurons express tyrosine hydroxylase (TH), the rate-limiting enzyme in the synthesis of dopamine (Fields et al., 2007; Morales and Pickel, 2012; Morales and Root, 2014; Zhang *et al*., 2015). This implies that VTA neurons can release GABA, DA, glutamate, or they can co-release DA and glutamate.

In addition to cellular heterogeneity, the VTA is also heterogeneous in electrophysiological properties. For instance, one particular subpopulation of dopamine neurons located in the most lateral edge of the VTA (adjacent to the substantia nigra pars compacta (SNC)) contains properties which are classically associated with dopamine neurons, including a large hyperpolarization-activated cation current and long action potential durations. By contrast, other confirmed TH-expressing neurons in rats and mice are heterogeneous in pharmacological and electrophysiological properties (Lammel *et al*., 2008; Berthet *et al*., 2014; Margolis *et al*., 2006; Margolis *et al*., 2012). More advanced neural tracing studies and functional studies have shown that slow-firing DA neurons in the VTA project to the lateral shell of the NAc and the dorsolateral striatum, while the more unconventional fast-firing DA neurons project specifically to the PFC and NAc core (Bjorklund & Dunnett, 2007; Ikemoto, 2007; Lammel et al., 2008).

VTA DA neurons receive excitatory and inhibitory input from a variety of regions. Lammel et al. (2012) showed that NAc-shell projecting DA neurons in the lateral VTA receive glutamatergic input from the laterodorsal tegmentum, whereas GABA neurons in the medial VTA, which inhibit the VTA DA neurons projecting to the medial prefrontal cortex (mPFC), receive excitatory input from lateral habenula glutamatergic neurons. While the VTA is largely known to regulate reward, it is important to note that it can also modulate aversive stimuli. VTA GABA

neuron activation has been shown to be aversive, while optogenetic inhibition of VTA GABA neurons is rewarding; rodents are more likely to self-administer drugs after optogenetic inhibition of VTA GABA neurons (Jennings et al., 2013). Furthermore, the bed nucleus of the stria terminalis (BNST) sends excitatory glutamatergic and inhibitory GABAergic inputs to VTA GABA neurons (Kudo et al., 2012). Optogenetic excitation of BNST glutamatergic projections to the VTA has been shown to be aversive, whereas optogenetic activation of BNST GABAergic neurons that project to the VTA have been shown to be rewarding (Jennings et al., 2013).

Nucleus Accumbens

The NAc, consisting of the core and shell, plays a key role in integrating reward-related information conveyed by dopaminergic and GABAergic inputs from the VTA with glutamatergic inputs from prefrontal cortical (PFC) regions (Jongen-Relo et al., 1994; Zahm, 2000). The NAc core and NAc shell receive projections from different regions and have diverse projection targets (Heimer et al., 1991). Medium spiny neurons (MSNs), which use gamma-aminobutyric acid (GABA) as their primary neurotransmitter, are the most common cell type in the NAc (making up approximately 95% of cells), and they express either D1-like (D1 and D5) or D2-like DA receptors (D2, D3, D4); a large quantity of this dopaminergic input comes from the VTA. D1 receptors are coupled with G_s/G_{olf}; upon activation, this stimulates adenylyl cyclase to produce the second messenger cyclic AMP (cAMP), further activating cAMP-dependent protein kinase (PKA), leading to phosphorylation of downstream enzymes and transcription factors that are implicated in synaptic plasticity. D2 receptors, however, are coupled to G_i/G_o, and inhibit adenylyl cyclase, and thus induce an inwardly rectifying K^+ current, which results in neuron hyperpolarization. It is important to note that activation of D2 auto-receptors on dopamine terminals inhibits dopamine release (Berhow, Hiroi, and Nestler, 1996).

Compared to D2 receptors, studies have shown that D1 receptors have lower binding affinity for dopamine, and are thus more sensitive to reduction of synaptic dopamine levels (Berhow, Hiroi, and Nestler, 1996). NAc MSNs can express predominantly D1R or D2R, or a combination of both, and these subtypes of MSNs exert balanced but antagonistic influences on

reward-related behaviors. For instance, evidence has shown that D2-MSNs in the NAc exert an inhibitory influence on brain rewarding circuitry. Activation of MSNs which contain D2 receptors (D2R-MSNs) reduces reinforcing effects of drugs of abuse and suppresses drug seeking behavior, whereas inhibition of D2R-MSNs enhances cocaine motivation (Bock et al., 2013). Activation of D1R-MSNs has the opposite effect, resulting in heightened cocaine reward and enhanced conditioned place preference (CPP).

Prefrontal Cortex

In rodents, the prefrontal cortex (PFC), which is comprised of the anterior cingulate (ACG), prelimbic (PrL), and infralimbic (IL) cortices, is a major component of the mesocorticolimbic circuit. The PFC plays a critical role in mediating goal-directed behaviors and impulse control. Glutamatergic pyramidal neurons, which project to the NAc and VTA, are the predominant projection neurons in the ACG, PrL, and IL. The loss of control during drug taking is in part due to disruption of normal PFC function, which then dysregulates the mesolimbic reward pathway and higher-order executive function (Goldstein and Volkow, 2011). A majority of studies on cortical control over addiction have focused on the PL, largely because a homologous region in the human PFC (Uylings et al., 2003; Farovik et al., 2008) regulates decision making and inhibitory response control (Gregoire et al., 2012; Hare et al., 2009; Balleine & O'Doherty, 2009). In rodents, the PL has been shown to play a role in modulating cocaine-seeking behavior (Chen et al., 2013; Limpens et al., 2015), and the IL has been shown to play a role in the development of habitual reward seeking and extinction-related behavior (Barker et al., 2014), but little is understood about the role of the IL-VTA pathway in stress-induced addiction susceptibility.

In humans, positron emission tomography (PET) and functional MRI (fMRI) brain imaging studies have shown that multiple sub-regions of the PFC (ACG, the orbitofrontal, and dorsal lateral prefrontal cortices) have significant differences in activity between addicts and healthy individuals during working memory tasks, decision making, inhibitory control, emotion and motivation, and drug administration (Bolla et al., 2004; Ersche et al., 2005; Goldstein et al., 2009).

1.4. The Role of Dopamine in the Mesocorticolimbic System in Substance Abuse and Drug-Induced Sensitization

The role of the mesocorticolimbic DA circuit during the induction and expression of sensitization has been studied extensively. Destruction of dopaminergic neuron terminals in the NAc disrupts cocaine and AMPH self-administration (Roberts et al., 1980). This evidence implies that VTA-NAc DA neurons play a critical role in compulsive drug taking behavior. Like stated earlier, locomotor sensitization to psychostimulants is initiated in the VTA, but is expressed in response to a challenge of the psychostimulant in the NAc (Kalivas & Stewart, 1991; Vezina, 2004).This differentiation of sites for the initiation and expression of sensitization implies a mechanism to "transfer" sensitization from the VTA to the NAc and other forebrain regions (Wolf & Tseng, 2012; Wolf et al., 1993). Evidence suggests that this sensitization "transfer" mechanism involves a transient increase in VTA DA neuron firing rate and bursting that is detected during the first few days after discontinuing repeated non-contingent cocaine or AMPH injections (Kamata & Rebec, 1984; White & Wang, 1984; Henry et al., 1989; Wolf et al., 1993). Much of the enhanced DA cell signaling that occurs during sensitization is driven by increased glutamate signaling, predominantly from cortical regions.

1.5. Types and Structures of Glutamate Receptors

Psychostimulant administration induces heightened glutamate transmission in parts of the mesocorticolimbic pathway that are involved in behavioral locomotor sensitization to psychostimulants, including the NAc, striatum, and VTA (Johnson & North, 1992; Xue *et al.*, 1996; Reid *et al.*, 1997; Del Arco *et al.*, 1998; Wolf & Xue, 1999). This glutamate input, which predominantly stems from medial prefrontal cortical regions, provides excitatory control over VTA dopamine neurons (Sesack & Pickel, 1992; Taber & Fibiger, 1995; Wang *et al.*, 2014). Glutamate then binds to ionotropic glutamate receptors (iGluRs) or metabotropic glutamate receptors (mGluRs).

iGluR dysfunction is implicated in a number of neurological disorders, including dementia, mood disorders, epilepsy, and drug addiction (Bowie, 2008). The three major subtypes of iGluRs-

- α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), N-methyl-D-aspartate (NMDARs), and kainite receptors (KARs)—contribute different components to a synaptic signal (Traynelis et al., 2010; Fig. 1.2). AMPARs are ionic transmemberane receptors which contain four subunits, GluA1-4, which modulate receptor trafficking and channel functions, and mediate the initial depolarization of the postsynaptic membrane (Carlezon & Nestler, 2002; Straub & Tomita, 2012; Wolf & Tseng, 2012). AMPA receptors that are composed of GluA1 homodimers (GluA2-lacking) are Ca^{2+}-permeable, so they allow Ca^{2+} to pass through the channel and amplify intracellular Ca^{2+}-dependent signaling pathways (Straub & Tomita, 2012; Wolf & Tseng, 2012). GluA1 is phosphorylated at Ser^{831} by Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII); this phosphorylation enhances single-channel conductance and open probability (Banke et al., 2001). Plasticity evoked by enhanced Ca^{2+} signaling through GluA1-homomeric AMPARs increases intracellular signal transduction, so evidence suggests that drug-induced elevations of AMPAR subunit GluA1 levels in the VTA could be an early trigger for sensitization (Carlezon & Nestler, 2002).

Contrarily, all NMDA receptors, which are composed of an NR1 subunit and multiple NR2 subunits, are Ca^{2+}-permeable; however, NMDA receptors require membrane depolarization to open with high probability because of a voltage-dependent Mg^{2+} block (Derkach *et al.*, 2007). Essentially, glutamate binds to these receptors, and membrane depolarization must be high enough to release the Mg^{2+}, activating the ion channel and allowing influx of ions into the cytoplasm (Mehta et al., 2012).

Importantly, activation of metabotropic glutamate receptors (mGluRs) is also necessary for drug-induced AMPH sensitization to occur (Kim and Vezina, 1998); they regulate synaptic transmission by moderating potassium and calcium channels, as well as the activity of ionic glutamate receptors, such as AMPARs (Yap et al., 2005). There are eight types of mGluRs (mGluR1 to mGluR8), and they are classified into three groups according to their structure and physiological activity (groups I, II, and III). Group I mGluRs (mGluR1 and 5) are predominantly post-synaptic receptors that are coupled to a Gq heterotrimeric G protein. When glutamate binds to these receptors, PLC is activated, and 1,4,5-inositol triphosphate (IP_3) is produced, which

initiates intracellular signaling cascades (Kelso et al., 1992; Ugolini et al., 1997). Group II and group III mGluRs are coupled to G_i/G_o, which inhibits the actions of adenylate cyclate and reduces intracellular cAMP levels (Kristis et al., 2015). In particular, mGluR1 and mGluR5 have been implicated in various aspects of addiction, mainly because drug exposure impairs mGluR-dependent synaptic plasticity and modulates GluA1 expression in the NAc and VTA, which will be described in more detail.

1.6. The Role of Mesolimbic Glutamate Receptors in Substance Abuse and Psychostimulant Sensitization

Glutamate receptors in the NAc

While it is known that GluA1 in the VTA plays a critical role in drug-induced sensitization, much of the current knowledge and research performed on GluA1 AMPARs involvement in addiction and sensitization have been performed in the NAc. Cocaine self-administration and withdrawal has been shown to increase GluA1 expression in the NAc (Ferrario et al., 2011). In addition, incubation of cocaine-craving has been shown to occur concomitantly with enhanced GluA1 expression in MSNs of the NAc. It is believed that this enhanced GluA1 expression is modulated by a withdrawal-dependent decrease in mGluR1, along with an increase in phosphorylation of GluA1 subunits (at the protein kinase A site) within extrasynaptic homomeric GluA1 receptors, which would promote their insertion and oppose their removal (Loweth et al., 2014).

Glutamate receptors in the VTA

Studies have shown that the induction of sensitization to psychostimulants can be blocked by the AMPAR antagonist administration (Li *et al.*, 1997; Zhang *et al.*, 1997; Vanderschuren & Kalivas, 2000). Moreover, elevated levels of VTA GluA1 are critical for drug-induced sensitization (Ping *et al.*, 2008; Kalivas, 2009), and direct stimulation of GluA1 subunits has been shown to play a role in the reinstatement of cocaine-seeking behavior after withdrawal (Ping *et al.*, 2008), displaying a role for VTA GluA1 in the sensitized locomotor response to psychostimulants. Converging evidence suggests that GluA1 is a trigger for the induction of drug

sensitization in the VTA. A 'feed-forward' hypothesis exists, which proposes that the behavioral sensitization process includes pathophysiological mechanisms triggered by intracellular processes that are induced by GluA1 accumulation in the VTA. This hypothesis proposes that sensitization is initiated by drug administration, which increases extracellular dopamine (DA) concentration in the VTA (Paulson *et al.*, 1991; Yap & Miczek, 2007). The DA then stimulates D1 receptors on glutamate neuron terminals, which results in higher extracellular glutamate concentrations (Kalivas & Duffy, 1998; Koolhaas *et al.*, 1999; Kalivas, 2009). Additional glutamate in the VTA would then stimulate AMPA or NMDARs on VTA DA neurons, increasing Ca^{2+} influx and results in neuroadaptions facilitated by intracellular transduction pathway (Nestler, 2001). This prolonged glutamate-mediated activity in the VTA would recruit AMPA receptors from intracellular compartments to postsynaptic membranes, enhancing the sensitivity of dopaminergic neurons to glutamate (Malenka & Nicoll, 1999; Ungless *et al.*, 2001), which triggers an increase in GluA1 expression levels. Elevated GluA1 levels results in increased formation of GluA1-homomeric Ca^{2+}-permeable AMPARs, making the cells more sensitive to glutamate and increasing intracellular Ca^{2+} signaling (Carlezon et al., 1997). This causes changes in gene expression and transient increases in the excitability of VTA DA neurons, as well as enhanced sensitivity to the actions of drugs of abuse. Essentially, this implies that sensitization to addictive drugs begins in the VTA but is expressed in the NAc.

This hypothesis would account for many of the reported behavioral, pharmacological, and molecular adaptations in drug-sensitized animals (Carlezon & Nestler, 2002). Not only that, but changes in AMPAR GluA1 subunit expression have been observed in the VTA after repeated cocaine treatment, which increased GluA1 expression one day after drug withdrawal (Di Chiara & Imperato, 1988; Churchill *et al.*, 1999). Furthermore, recent western blot data exhibit that social stress induces heightened GluA1 expression in the VTA compared to control rats, which occurs concomitantly with social-stress induced amphetamine sensitization (Wang *et al.*, 2014).

It is important to also briefly discuss the modulatory role of mesolimbic mGluRs in psychostimulant sensitization. Group I mGluR-dependent long-term depression (mGluR-LTD) plays a critical role in diseases including drug addiction (Gerdeman et al., 2003; Grueter et al.,

2007; Luscher & Huber, 2010). Culminating evidence has shown that mGluR1 negatively regulates GluA1-homomeric AMPAR levels in VTA DA neurons. Bellone and Luscher (2005) examined GluA1-homomeric AMPAR-containing synapses in the VTA of drug-naïve rats; they found that mGluR1 agonist application led to a form of mGluR1-LTD that was blocked by an antagonist of mGluR1 but not mGluR5. Inward rectification was no longer evident after mGluR-LTD, indicating that mGluR-LTD necessitated removal of GluA1-homomeric AMPARs (CP) from the synapse. Bellone and Luscher (2006) then later found that mGluR-LTD reversed cocaine-induced accumulation of GluA1 in mouse VTA DA neurons, indicating that these AMPARs were removed from synapse by a positive modulator of mGluR1. Later studies showed that the removal of GluA1-homomeric AMPARs during mGluR-LTD occurs concomitantly with their replacement with GluA2-homomeric (Ca^{2+}-impermeable) AMPARs (Mameli et al., 2007). While it is known and well-understood that GluA1 function in VTA dopamine neurons is associated with drug-induced sensitization (Kalivas, 2009), little is known about the specific glutamatergic pathway involved in *social* stress-induced amphetamine cross-sensitization, and the exact role that VTA GluA1 plays in this process.

1.7. The Effects of Stress on DA in the Mesocorticolimbic Pathway

Stress is considered any external threat that can alter homeostasis, and has been shown to play a substantial role in the development of addiction-related behavior, including increased substance abuse and drug relapse (Sinha, 2001, 2008, 2011; NIDA, 2012; Rivier, 1990). Stress predominantly acts on the hypothalamus, which induces hormone secretion (Brodish and Redgate, 1973). Specifically, stress causes excitatory neurons to synapse on cells in the paraventricular nuclei of the hypothalamus (PVN), causing a release of corticotrophin-releasing factor (CRF) (Brown and Rivier, 1990). CRF is released into hypothalamo-hypophyseal portal vessels and is transported to the anterior pituitary, where it binds to CRF receptor subtypes 1 and 2 ($CRFR_1$ and $CRFR_2$) (Vale et al., 1981). The primary role of CRF is to activate the hypothalamic-pituitary-adrenal (HPA) axis by ultimately increasing the release of glucocorticoids in response to stress (Bale & Vale, 2004). Along with the HPA axis, CRF axons also project to

extrahypothalamic areas including regions of the mesolimbic pathway including the amygdala, bed nucleus of stria terminalis (BNST), and VTA (Swanson et al., 1983; Sawchenko et al., 1993). In the HPA axis, in response to CRF release, the pituitary secretes adrenocorticotrophic hormone (ACTH) into the blood. ACTH is carried to the adrenal cortex, where it induces glucocorticoid synthesis and secretion. In rats, the major glucocorticoid is corticosterone, whereas in humans, it is cortisol (Baxter and Rousseau, 1979). While the body's response to stress can be beneficial by allowing animals to be ready to adapt to the environment, prolonged stress exposure can result in adverse consequences. Chronic stress can result in depression and post-traumatic stress disorders, for instance, and it also impairs the immune system (Craddock, 1978).

When exposed to stress, the mesocorticolimbic DA system is activated; for instance, electric shock, tail pinch, physical restraint, and social defeat stress all increase extracellular DA concentration (Gresch et al., 1994; Imperato et al., 1992; Tidey and Miczek, 1996). Some of these actions are due to actions of CRF on CRF receptors in the VTA. CRF in the VTA has been shown to increase the action potential firing rate of VTA DA neurons through actions on $CRFR_1$ (Wanat et al., 2008). In addition, intermittent social defeat stress has been shown to activate $CRFR_1$ in the VTA, which is crucial for the enhanced behavioral responses to cocaine (Boyson et al., 2011). By contrast, the binding of CRF to $CRFR_2$ can produce a transient, slow-developing of potentiation of NMDARs and mGluR transmission in a subset of dopaminergic VTA neurons (Fiorillo and Williams, 1998; Ungless et al., 2003). Importantly, the magnitude and specificity of the dopaminergic response to stress depend on the intensity, duration, and intermittency of the stress (Deutch et al., 1990; Thierry et al., 1976). For instance, mild stress selectively influences activation of dopaminergic innervation onto prefrontal cortical areas, whereas increases in intensity or duration of footshock stress increases DA utilization in the NAc and PFC (Dunn, 1988; Roth et al., 1988). This suggests that stress-induced DA neuron activation is differentiated according to different states of stress. In addition, stress selectively increases DA metabolism in the VTA but not substantia nigra (Deutch et al., 1985). Furthermore, our recent western blot analysis showed that intermittent social defeat stress induces higher GluA1 expression in the

VTA compared to handled animals, which occurred concomitantly with exacerbated sensitivity to psychostimulants (Wang et al., 2013). This led us to believe that GluA1, likely in VTA DA neurons, may play a crucial role in the development of stress-induced sensitivity to psychostimulants.

1.8. Social Defeat Stress Model

Social Defeat Stress Induces Anxiety-Like Behavior

There are a variety of stress models used in animal studies to study physiological and behavioral changes induced by stress. There are two types that can be used to categorize these stress models: exposure of an aversive stimulus or deprivation of a condition that is necessary for homeostasis (Lu et al., 2003). Some examples of the first type of stressor (aversive stimulus) include electrical foot shock, restraint stress, and social defeat, whereas some examples of the second stressor (deprivation) include food/water deprivation, maternal deprivation, and social isolation. The social defeat model in rodents is a naturalistic paradigm that is used to study stress-induced changes in the behavior and pathology of humans. Although there are a variety of stress models used to increase anxiety and depression-like behaviors, the social defeat paradigm has high face validity in the social nature of its stressor, also because of the lack of habituation that is associated with repeated stress exposure (Covington & Miczek, 2005). The social defeat stress experience consists of a brief confrontation between a submissive “intruder” rat, which is introduced into the home cage of an aggressive “resident” rat. The resident rat attacks the intruder rat, which ultimately leads to postural responses of an appropriate “defeat”, in which the intruder rat displays a supine posture (Tidey & Miczek, 1997; Fig. 1.3). Even a single exposure to social defeat stress significantly reduces exploratory behavior and alters the animal’s sensitivity to a challenge by other types of stress for several weeks after the social stress exposure (Koolhaas et al., 1999). In rodents, social defeat stress has been shown to produce anxiety, which is evidenced by: increased acoustic startle response (Pulliam et al., 2010), ultrasonic vocalizations (van der Poel & Miczek, 1991; Vivian & Miczek, 1999), and time spent freezing (Venzala et al., 2012).

The temporal pattern of the administration of the social defeat plays a critical role in the specific outcomes of the stress administration (Miczek et al., 2008; Miczek et al., 2011a). Repeated instances of social defeat stress increase social avoidance behavior of a novel conspecific (Razolli et al., 2009; Venzala et al., 2012), and induce weight gain deficits (Meerlo et al., 1996; Fanous et al., 2010; Pulliam et al., 2010; Fanous et al., 2011). By contrast, while continuous social defeat reduces preference for sweet rewards, only intermittent social defeat stress induces behavioral sensitization to psychostimulants, which can lead to heightened drug use (Miczek et al., 2011a). Stress-induced social avoidance behavior, and weight deficits are behavioral phenotypes that are representative of anxiety-like behavior, and seem to be regulated by a number of mesolimbic mechanisms, including mu-opioid receptor signaling and brain-derived neurotrophic factor (BDNF) signaling (Johnston et al., 2015; Wang et al., 2014).

Intermittent Social Defeat Stress Enhances Sensitivity to Psychostimulants

While global illicit drug use remains stable, the estimated number of drug users has risen from 6 million to 246 million (owing to population increase), with 0.6% of the population aged 15-64 estimated to suffer from problem drug use (UNODC, 2014). In the United States, an estimated 22.7 million people aged 12 or older have a diagnosable substance abuse disorder (SAMHSA, 2014). In addition, more than 1 out of 10 drug users is a problem drug user (UNODC, 2014). Majority of recovering addicts are likely to relapse (Sinha, 2011; NIDA, 2015), and evidence suggests that exposure to stressful experiences is as potent as drug cues in potentiating relapse; images which depict stressors induce increases in cocaine craving and anxiety in recovering addicts (Sinha et al., 1999; Sinha et al., 2000). To account for such, many experiments have been conducted targeting relapse and addiction treatments, but the main problem is that treatment can be extremely costly and is not readily available, highlighting the importance of studying variables which confer drug abuse vulnerability. In addition, drug addiction vulnerability has both a genetic and environmental component; human twin studies have shown that only 30-60% of the variance is explained by heritability (Kreek et al., 2005), suggesting that environmental factors play a role in susceptibility to drug abuse.

Stress is a factor that influences the transition from recreational drug use to substance abuse. In humans, stress is associated with substance abuse vulnerability, as well as drug relapse (Brewer et al., 1998, Sinha, 2001, 2008, 2011, NIDA, 2015). Accumulating evidence suggests that substance abuse and drug addiction is exacerbated by acute and chronic stress (Sinha, 2008). Furthermore, in rodents, there is a clear correlation between prior stressful events and augmented behavioral responses to psychostimulants. Environmental stressors such as tail pinch, inescapable foot shock, restraint, and food restriction induce enhanced locomotor activity to psychostimulant administration (Carlson et al., 1987; Piazza et al., 1990; Deroche et al., 1995; Rouge-Pont et al., 1995; Marinelli et al., 1996; Pacchioni et al., 2002). In humans, most significant stressors are of a social nature; social defeat stress, in particular, is an etiological factor that has been shown to lead to addiction-related behavior, including drug sensitization and self-administration behavior in rodents (Covington & Miczek, 2001; Nikulina et al., 2004; Covington et al., 2005).

Similar to behavioral sensitization that is apparent for weeks after repeated drug exposure, rodent studies exhibit that intermittent social defeat stress induces prolonged cross-sensitization to AMPH, which is evident even months after the last social defeat, which happens concomitantly with heightened cellular activation (Covington & Miczek, 2001; Nikulina et al., 2004; Covington et al., 2005). This suggests that intermittent social defeat stress is a fast-acting stressor that induces long-term neurobiological adaptations, which promote specific behavioral components of drug addiction.

1.9. Stress-Induced Changes Across the Mesocorticolimbic Circuit

Social defeat stress has a significant long-lasting effect on the mesocorticolimbic system, especially on brain-derived neurotrophic factor (BDNF) and delta FosB expression. DeltaFosB is a transcription factor and is the truncated form of FosB, a member of the Fos family of proteins. Delta FosB has been heavily studied, mainly because repeated stimuli exposure including stress or drug use, causes it to gradually accumulate and persist for lengthy periods of time (see reviews of Nestler et al., 2001, Nestler, 2008, 2014). Repeated social defeat stress induces

prolonged increase of delta FosB throughout the mesocorticolimbic circuit (Nikulina et al., 2008, Nikulina et al., 2012); BDNF is typically associated with brain regions that are rich in DA neurons, and in the VTA, BDNF has been shown to heavily co-localize with TH, which is a reliable marker for DA neurons (Gall et al., 1992, Seroogy et al., 1994). Social defeat stress has been shown to increase BDNF expression and deltaFosB levels in the NAc and PFC, and it has also been shown to increase co-expression of the two molecules (Nikulina et al., 2012). In addition, the time course of social stress-induced deltaFosB expression persists for around 10 days (Nikulina et al., 2012), which is a time period that corresponds to the expression of stress-induced AMPH sensitization. Importantly, different rodent species and stress models display different behavioral and neurochemical outcomes of social stress.

The expression of BDNF is largely implicated in neuropsychiatric illness, including major depression and bipolar disorder. BDNF is synthesized from the precursor proBDNF (Seidah et al., 1996; Lu et al., 2005; Teng et al., 2010), and furin or other proprotein convertases cleave proBDNF to the mature BDNF (Mowla et al., 1999; Lessmann et al., 2003; Greenberg et al., 2009). Mature BDNF then binds to the tropomyosin receptor kinase B (TrkB) receptor, which activates various intracellular signaling cascades, including the Ras/extracellular signal-regulated kinase (ERK) pathway, the phosphatidylinositol-3'-OH-kinase (PI3K)-AKT pathway, and the phospholipase C-g (PLC- g) pathway (Park and Poo, 2013). The PFC contains dense BDNF mRNA-expressing neurons (Conner et al., 1997), which supply BDNF to the NAc and VTA, modulating the functions of these brain regions (Seroogy et al., 1994; Guillin et al., 2001). In the VTA, evidence has shown that approximately 50% of all neurons co-express TH and BDNF (Seroogy et al., 1994), which largely mediates many of the dopamine-dependent alterations that occur as a result of intermittent social defeat stress.

In rats, intermittent social defeat stress causes self-administration behavior, as well as social avoidance (Fanous et al., 2011b). In addition, bilateral knockdown of VTA BDNF prior to intermittent social defeat stress extinguishes the effects of stress on social avoidance behavior. By contrast, in mice, intermittent social defeat stress does not have these same effects, however,

continuous social defeat stress over a period of 10 days does increase BDNF activity within the mesolimbic DA pathway (in VTA and NAc), and induces social avoidance and enhanced susceptibility to stress (Berton et al., 2006; Krishnan et al., 2007). Continuous social defeat stress in rats, however, has the opposite effect; it was shown to reduce sucrose preference and intake, decrease exploratory behavior, and reduce VTA BDNF expression in defeated rats (Blanchard et al., 2003). This inter-species variation across these behaviors suggests the possibility that they are mediated by different mechanisms.

1.10. Sex Differences in Addiction

Human studies

While drug abuse and addiction rates have generally been lower in women than men, prevalence rates show that the number of female drug users have increased substantially (Becker and Hu, 2008; SAMHSA, 2012). Clinical studies have shown that men drink larger amounts of alcohol than women, though recent data suggests that consumption for women and men is becoming increasingly similar (Keyes et al., 2008). In terms of other drugs of abuse, more men use and are addicted to opiates (Lee and Ho, 2013), as well as other drugs of abuse (SAMHSA, 2012); however, women who become addicted to drugs have been shown to progress from initial use to dependence more rapidly than men (Kosten et al., 1993; Brady and Randall, 1999), as well as display higher levels of craving and impulsivity in drug exposure and abstinence, which leads to increased relapse rates than men (Brady and Randall, 1999; Ignjatova and Raleva, 2009; Perry et al., 2014; Schlaugh et al., 2013). While cultural differences, along with sex differences in brain organization may play a role in some of the sex differences in drug addiction, cumulative evidence suggest that sex hormones modulate drug use vulnerability in women. In accordance with this, women have shown to have greater response to psychostimulants during the follicular phase of their menstrual cycle, when estradiol is the predominant hormone compared to the luteal phase, here progesterone is the predominant hormone (Evans and Foltin, 2006; Evans et al., 2002; Justice and de Wit, 1999; Sofuoglu et al., 1999; White et al., 2002). Furthermore, estradiol has been shown to play a role in promoting

drug-induced, reward-seeking behavior in women (Anker and Carroll, 2011; Quinones-Jenab and Jenab, 2010). This is in part because estradiol has been shown to interact with the dopaminergic system and opioid peptides to facilitate drug addiction (Jacobs and D'Esposito, 2011; Segarra et al., 2010).

Animal models

Like clinical studies, animal studies have exhibited that females display more robust operant behavior during cocaine acquisition or during self-administration of heroine, as well as escalation of drug intake and drug reinstatement (Lynch and Carroll, 1999; Lynch et al., 2000; Roth and Carroll, 2004). The robust animal data suggest that females may be more vulnerable than males due to underlying biological predisposition related to ovarian hormones or developmental differences in male/female neurobiology (Becker & Hu, 2008). Female rats develop a conditioned place preference (CPP) for lower doses of cocaine compared to males, while both sexes show equivalent CPP at higher dose of cocaine. In addition, CPP reinstatement is more pronounced in females at higher cocaine doses (Becker et al., 2012). In addition, female rats show more of an "addicted" phenotype, characterized by heightened self-administration of cocaine along with greater motivation for cocaine following forced abstinence (Lynch & Taylor, 2004). Female rats are also more sensitive to the psychomotor activating effects of psychostimulants than male rats, and with repeated drug treatment, they have shown to display greater psychomotor sensitization (Robinson, 1984; Hu et al., 2003)

1.11. Reproductive Cycles

In humans, women's menstrual cycle consists of two phases, follicular and luteal phases, where ovulation occurs during the transition from follicular to luteal phase (Reed & Carr, 2015). The follicular phase is characterized by low (early) or moderate (late) estrogen levels with low progesterone levels throughout. A surge of estrogen then peaks during the follicular phase followed by a rapid decrease in correlation with ovulation. The luteal phase is distinguished by moderate estrogen levels with higher levels of progesterone, which gradually decreases (Justice & de Wit, 1999) (Fig. 1.4A). Like the human reproductive cycle, the rat reproductive cycle follows

similar fluctuating hormonal patterns, but progresses over a 4-5-day period in comparison to the 28 days of the human menstrual cycle (Bobzean et al., 2014). The rat estrous cycle consists of four stages: proestrus, estrus, metestrus, and diestrus (Long & Evans, 1922; Freeman, 1988). Ovulation occurs during the beginning of proestrus to the end of estrus (Young et al., 1941; Schwartz, 1964). From the onset of sexual maturity up to an age of 12 months, the female rats mean cycle length is 4 days (Long & Evans, 1922; Mandl, 1951; Feder, 1981), though some rats with 5-day cycles have an additional day of estrus or diestrus (Freeman, 1994), which may be due to prolonged progesterone secretion (Nequin et al., 1979). This overall short length of the estrous stage makes the rat an ideal animal for studying sex-dependent differences in neurobiological mechanisms of addiction. During the estrous cycle, prolactin, leutinizing hormone (LH), and follicle stimulating hormone (FSH) remain low and increase steadily during proestrus, during which a peak in estradiol occurs. During metestrus and diestrus, progesterone secretion increases, and then reaches its peak toward the end of proestrus, after which ovulation occurs during estrus (Sportnitz et al., 1999; Marcondes et al., 2001).

In order to effectively perform studies that compare the influence of estrous cycle on physiological functions, vaginal cytology is typically used for estrous cycle determination (Long & Evans, 1922; Hoar & Hickman, 1975). The characterization of each phase is based on the proportion of three cell types present within the vaginal smear: nucleated epithelial cells, anucleated cornified cells, and leukocytes (Marcondez et al., 2002). Proestrus is typically characterized by predominantly nucleated cells; estrus is mainly characterized by anucleated cornified cells; metestrus is characterized by a mixture of leukocytes, cornified, and nucleated epithelial cells; diestrus is predominantly characterized by leukocytes (Marcondez et al., 2002; Fig. 1.4B).

1.12. Role of ovarian hormones in stress and addiction-related behaviors

While a majority of studies on repeated social defeat stress and its role in addiction-related behaviors have been conducted in male subjects, there is increasing interest in the effects of ovarian hormones on social stress in females. Converging evidence has shown that biological

basis for enhanced drug abuse vulnerability in *women* may be attributed to female gonadal hormones (Becker & Hu, 2008; Carroll et al., 2004; Festa & Quinones-Jenab, 2004; Lynch et al., 2002; see review article of Anker & Carroll, 2010). In women, enhanced drug-seeking behaviors are associated with higher endogenous levels of estrogen (EST) (Evans, 2007; Terner & de Wit, 2006). In addition, endogenous or exogenous EST (estradiol) facilitates the acquisition, escalation, and reinstatement of cocaine-seeking behavior in female-rats (see Carroll & Anker, 2010). Chronic social defeat stress has been shown to induce disruptions in estrous cycle, as well as attenuated psychostimulant-induced dopamine levels (Shinamoto et al., 2011). In addition, repeated social defeat stress in females has shown to increase social avoidance behaviors, as well as other anxiety-like behavior and weight reduction, which is suggested to be modulated by ovarian hormones (Tahashaki et al., 2017). Studies have shown that estradiol administration in ovariectomized females affects behaviors induced by psychomotor stimulants, including self-administration behavior (Becker et al., 2012). In addition, Larson et al., (2007) found that estradiol escalates cocaine self-administration, whereas progesterone attenuates these affects. Ultimately, females are more sensitive to drugs of abuse than males, and this is largely dependent on the presence of ovarian hormones and their effects on mesolimbic DA signaling.

Female Intermittent Social Defeat Model: Maternal Aggression

One of the major differences between males and females is the social defeat model that can be utilized to observe specific behavioral outcomes. Females have an extremely different social behavior than do males, and do not become territorial over conspecifics in normal circumstances. While social defeat stress is highly effective in inducing potent effects including decreased weight gain and increased corticosterone (CORT) levels in males, the responses are much lower in females (Haller et al., 1999). The maternal aggression model, however, has been shown to be effective in replicating the normal intermittent social defeat stress model we use in males, to induce stress-induced sensitization behavior. In this paradigm, the lactating female Long-Evans aggressor (resident) is pair-housed with a male Long-Evans rat in her home-cage. These lactating females generally show the highest level of aggression during days 3-12 post-

delivery in the presence of their pups (Shimamoto et al., 2011). After the male resident is removed, the experimental “intruder” rat is placed inside the resident’s home-cage with the resident female and her pups, allowing the resident to attack the intruder until she displays a submissive supine posture. The intruder female is then removed and placed under a small protective cage for 20 min to have continued stress exposure with no risk of injury. This occurs every third day over a period of ten days (Holly et al., 2012; Fig. 1.5).

1.13. Sex-dependent differences in mesolimbic signaling

While many studies have been performed to elucidate mesolimbic mechanisms underlying drug addiction and stress-induced susceptibility to drug abuse, little is understood about sex differences is mesolimbic signaling underlying these differential effects. Research suggests that sex differences, as well as estradiol affect neurotransmitter systems that operate within the mesolimbic reward pathway. In the striatum, for instance, there are sex differences in baseline DA tone and activation following drug exposure Becker & Hu, 2008; Self et al., 1996). In mice, recent studies have suggested that estradiol (during proestrus/estrus) enhances the affinity of cocaine for DAT as a result of enhanced VTA DA neuron activity, thus resulting in augmented behavioral response to cocaine and CPP (Calipari et al., 2017; Fig. 1.5). Facilitation by EST on reinforcing effects of psychostimulants may partially be attributed to the interaction between estrogen receptor β (ER- β) and DA neurotransmission in the mesolimbic pathway. ER- β is found in DA neurons (Laflamme et al., 1998), and influences DA receptor expression and neurotransmission in the mesolimbic circuit (Morissette et al., 2008; Schultz et al., 2009). Application of an ER- β antagonist reduces EST’s facilitating effects on cocaine self-administration acquisition in EST-treated ovariectomized (OVX) female rats (Lynch et al., 2001). These findings suggest that EST-induced enhancement of psychostimulant-related behaviors involves an interaction between ER- β and the mesolimbic DA pathway via intracellular mechanisms. In addition, BDNF-TrkB signaling is also different in males compared to females and is clearly influenced by differences in ovarian hormones. TrkB expression in VTA hippocampal neurons fluctuates in *female* mice across the estrous cycle, with peak expression

during proestrus/estrus, (Spencer et al., 2008), and as discussed earlier, BNDF has been shown to increase GluA1 expression in the cell membrane. Because these mesolimbic dynamics underlying sex-dependent differences in substance abuse vulnerability are poorly understood, we sought to elucidate these mechanisms and highlight any interactions between BDNF-TrkB signaling and stress-induced GluA1-AMPAR-evoked plasticity in female rats.

1.14. Using Viral-Mediated Gene Transfer and recombinant TH-Cre rats to study the role of GluA1 in VTA DA Neurons.

While it is extremely important to study neural mechanisms of behavior, and specifically, substance abuse susceptibility, genetic intervention is not easily accomplished in the brain. One such strategy that is frequently utilized is viral-mediated gene transfer, which is a technique in which viral vectors are engineered and delivered into a region of interest (in our case, the VTA), that carry a gene of interest, which allows the virus to insert genetic material into the cells. When these viral vectors are delivered into the brain, they cause infected cells to increase expression of the desired gene of interest, which is especially useful if the goal is to manipulate expression of a single gene (1) in a specific brain region (2) at a particular time (3) in animals that developed normally (Carlezon & Neve, 2003). These viral constructs could be packaged in a variety of viral systems, including retroviral, adenoviral, adeno-associated, herpes simplex, pox viral, or lentiviral constructs (Nayerossadat et al., 2012). Adeno-associated viral (AAV) constructs, in particular, are extremely beneficial for the study of persistent changes and plasticity in the mesocorticolimbic system, as well as long-lasting behavioral changes, because they have long-lasting expression compared to herpes simplex viral (HSV) constructs, which have much shorter expression. This makes them an ideal viral construct for the gene packaging system in our studies.

In order to preferentially manipulate GluA1 in DA neurons, we used TH-Cre rats, which are Sprague Dawley rats that express Cre recombinase under the control of the endogenous TH promotor, enabling specific expression in dopaminergic neurons. When used with a cre-dependent viral construct, it is possible to express specific genes using viral-mediated gene transfer. These particular rats possess a targeted insertion of (IRES)-cre after the translational

stop in the open reading frame of Th, and this strain was previously verified to have limited ectopic expression of cre. Prior to our viral manipulation experiments, we transcardially perfused several TH-Cre rats and performed fluorescent immunohistochemistry using antibodies recognizing Cre and TH. We found specific double-labeling of TH and Cre, which verified limited ectopic expression of Cre in our recombinant rat model (Fig. 1.6).

1.15. Optogenetics as a tool to study stress and drug addiction

Optogenetics is a photoexcitation or photoinhibition technique involving the use of microbial opsins (or related tools) expressed in specific cells, which are activated by particular wavelengths of light illumination, along with precise temporal precision, *in vitro* or *in vivo* (Deisseroth, 2011; Deisseroth et al., 2006; Zhang et al., 2011; Airan et al., 2009; Boyden et al., 2005; Yizhar et al., 2011). This allows researchers to correlate circuit level neuron activity in vivo to study animal behavior (Britt, et al., 2012). Optogenetic genes can be incorporated within the desired animals through several methods, including through electroporation, transgenic animals, or by viral transfection, which is a common method due to its rapidity and simple method of viral delivery. The most commonly used “excitatory” opsin is channelrhodopsin, which is a protein channel that transports cations across the plasma membrane in response to ~473nm blue light stimulation. There are four variants of channelrhodopsin: ChR1 and ChR2 (which is the most commonly-used variant) from the algae *Chlamydomonas reinhardtii* and VChR1 and VChR2 from *Volvox carteri* (Hegemann and Moglich, 2011). Upon blue light stimulation, ChR2 ultimately undergoes a conformational change, in which the channel shuttles from the dark-adapted state and allows cation influx, depolarizing the ChR2-expressing cell (Bamann et al., 2008; Boyden et al., 2005; Fig. 1.7A).

One of the most common opsins used for optogenetic inhibition is halorhodopsin (NpHR), which comes from *Halobacterium halobium*, and is a small retinal protein that functions in the membrane as an inward-directed light-driven chloride pump, which ultimately hyperpolarizes the transfected cells (Schobert and Lanyi, 1982; Zhang et al., 2007; Fig. 1.7B). While NpHR has been quite successful for assessing and manipulating neuronal circuit function, it can be difficult

to feasibly utilize due to the penetrability of light that is used to activate these opsins. A relatively novel opsin, Jaws, which is a red-shifted cruxhalorhodopsin, is capable of powerful optical hyperpolarization and has the benefit of utilizing red light stimulation (~632nm), which has deeper penetrance (Chuong et al., 2014). Compared to other optogenetic inhibitors, Jaws specifically was found to produce higher light induced currents upon red light stimulation through inducing changes in chloride conductance and has the capability to be activated in deeper regions of the brain, and can even be activated transcranially (Fig. 1.7C). This is likely due to longer light wavelengths having less interaction with blood proteins and lipids (Chuong et al., 2014).

Optogenetics has been an extremely successful tool in the study of neuropsychiatric disorders, including the persistent neuroadaptations induced by stress and drug addiction. Voltammetry studies have shown that optogenetic stimulation of VTA DA neurons mirror natural patterns of DA release in the striatum (Bass et al., 2010), allowing for optogenetic studies on the role of VTA DA neurons in reward. In addition, phasic optogenetic stimulation of DA neurons in the VTA induced conditioned place preference (CPP), as well as drug self-administration in mice and rats (Adamantidis et al., 2011; Kim et al., 2012; Witten et al., 2011). Optogenetics has also been used to show that opposing roles of D1 and D2 NAc pathways in reward-related behaviors; optogenetic stimulation of D1 receptor-expressing neurons (D1R) induced reinforcement behavior, whereas stimulation of D2 receptor-expressing neurons (D2R) induced punishment in operant and place conditioning tasks (Kravitz et al., 2012). In addition, optogenetic circuit mapping has been coupled with extensive behavioral experiments to identify and understand neural circuits involved in the stress response; for instance, optogenetic stimulation of BNST-VTA glutamatergic neurons resulted in aversion and anxiety-like behaviors, whereas optogenetic stimulation of BNST-VTA GABAergic terminals promoted reward-related behaviors and attenuated stress-induced anxiety (Sparta et al., 2013). It is clear that the optogenetics toolbox allows for unprecedented precision and limitless possibilities in the elucidation of neural mechanisms of addiction.

Wireless optogenetics

Wireless optogenetics, specifically, offers an unimpeded approach to the study of freely-moving animal behavior and is an excellent tool for the study of stress-induced AMPH sensitization, especially because it improves data from vivo tracking software by removing motion artifacts created by moving fiber optic cables. Amuza's Teleopto wireless optogenetics technology involves the use of light emitting diodes (LED) source with implantable fiber optics to bring the light from the LED to the target region of the brain and keeps any heat generated by the LED outside the rat. A remote sends an infrared (IR) signal to the battery-operated wireless receiver, which sits on top of exposed prongs from the implanted optic fiber and turns the LEDs on and off. For pulsatile light stimulation, a programmable pulse generator is available to program and send pule trains. In addition, an IR emitter is strategically placed inside the behavior (1-2 meters away from the rat being tested) room to send IR signals to the wireless receiver(s) (Fig.1.8).

1.16. Using c-Fos as a marker for neuronal activity

The immediate early gene (IEG), c-Fos and its associated Fos protein have been used as an indirect marker of neuronal activity since it was identified as a proto-oncogene (Curran et al., 1984). The Fos family, including c-Fos, FosB, Fos-related antigen 1, and Fos-related antigen 2, are considered nuclear oncogenes. Fos protein expression is rapid and is expressed within neurons following voltage-gated Ca^{2+} entry into the cell (Chung, 2015; Morgan & Curran, 1986). Immunohistochemistry can be utilized to detect the protein product within neurons approximately 20-90 minutes following neuronal excitation, and disappears 4-16 hours later (Morgan et al., 1987; Menetrey et al., 1989; Mugnaini et al., 1989). Once expressed, c-Fos protein enters the cell nucleus and participates in protein complexes that interact with DNA; importantly, neuronal excitation induces c-*fos*-like immunoreactivity in the nuclei of neurons rather than glial, ependymal, or endothelial cells (Mugnaini et al., 1989). For these reasons, c-Fos is a specific marker for neuronal activity at the single cell level. Furthermore, there are low basal levels of c-Fos, which further makes it an ideal marker for neuronal activation.

1.17. Research Objectives and Dissertation Organization

Substance abuse and drug addiction represent significant economic and health-related problems annually. Several facets of drug addiction that are extensively studied include drug-seeking behavior (motivation), behavioral sensitization, and self-administration. Behavioral sensitization is a phenomenon that refers to the augmented motor-stimulant response to drugs of abuse after preliminary exposure (Robinson & Berridge, 1993) , and is heavily involved in addiction. Studies show that social stress is an etiological factor that leads to addiction-related behavior, including sensitization.

The experiments described in the following chapters utilized the rat model of intermittent social defeat stress to examine whether GluA1 in VTA DA neurons is necessary and sufficient for the behavioral effects of social stress, specifically cross-sensitization and social avoidance behavior (Chapter 2). In addition, this dissertation investigated the sex-dependent mesolimbic mechanisms underlying differences in stress-induced drug addiction vulnerability between male and females (Chapter 3). Finally, experiments described herein utilized wireless optogenetics to bidirectionally manipulate the IL-VTA pathway to determine its causal role in stress-induced AMPH sensitivity (Chapter 4). The final chapter of this dissertation (Chapter 5) is a discussion of the implications drawn from chapters 2-4.

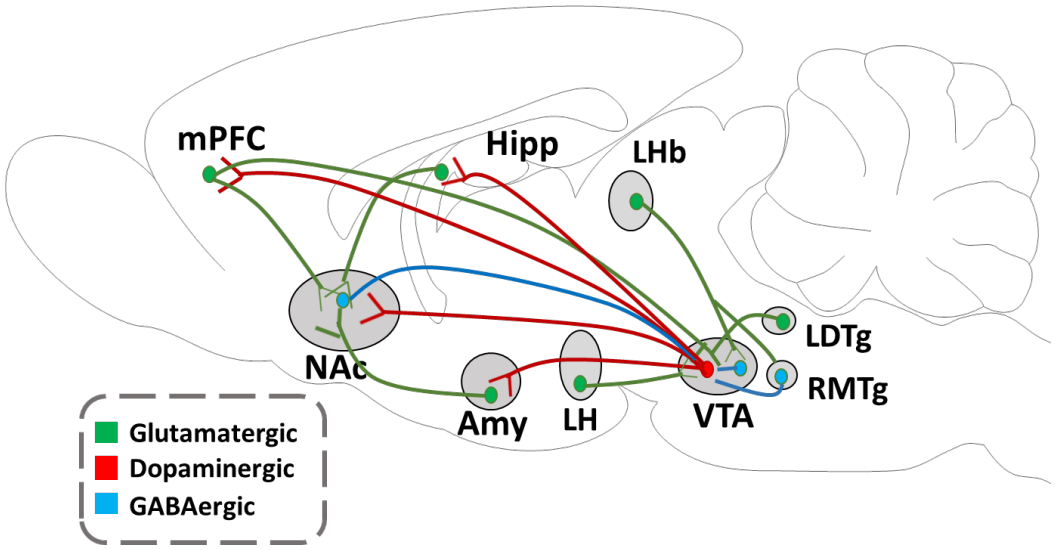

Figure 1.1. Schematic of the mesolimbic reward pathway and its projection sites. The VTA, NAc, and PFC represent major regions in the mesocorticolimbic system and are heavily connected by afferent and efferent projections (Fields et al., 2007, Sesack and Grace, 2010; Morales and Pickel, 2012; Fields and Margolis, 2015).

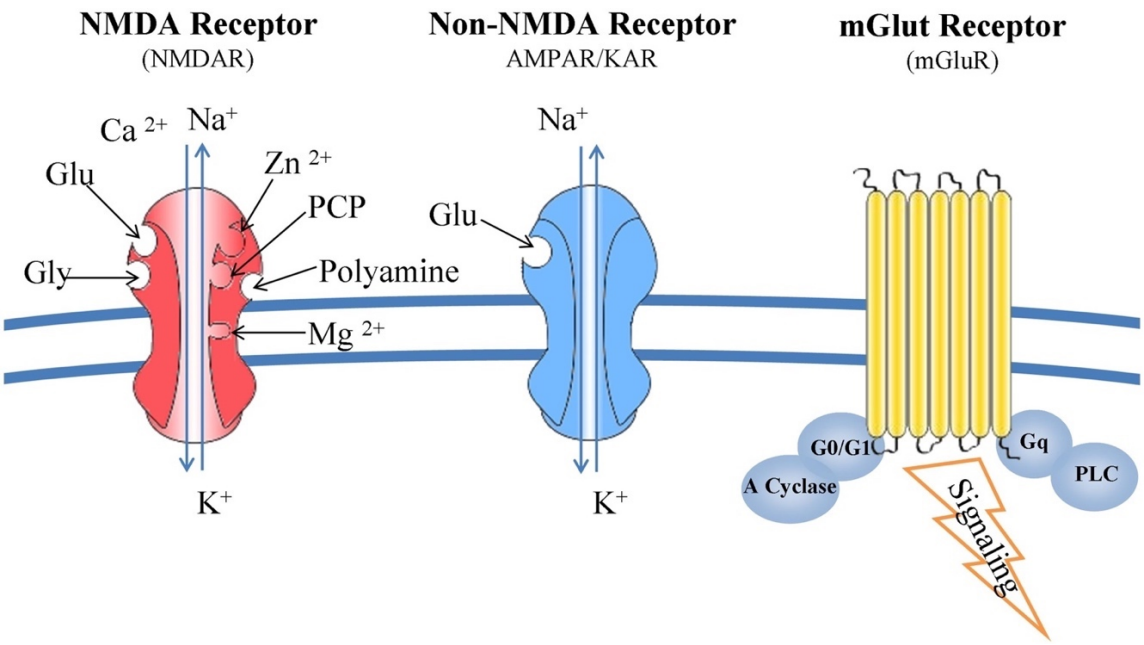

Figure 1.2. Structure and function of glutamate receptors. (Left) NMDARs are a type of iGluR that binds glutamate, Mg^{2+}, Zn^{2+}, as well as polyamines. They are composed of 7 subunits (one NR1, four NR2, and two NR3), but the combination of NR1 and NR2 subunits determines the receptor function. NMDARs require the binding of glutamate, along with the presence of an action potential, to remove the Mg^{2+} block before ion efflux (Ca^{2+}, Na^{+}, or K^{+}) could occur. (Middle) Kainate and AMPARs do not contain an Mg^{2+} block and therefore only interact with glutamate and their specific agonist. These specific channels are also permeable to Na^{+}, K^{+}, and Ca^{2+}. The combination of GluRs determines the Ca^{2+}-permeability in AMPARs, in which GluA2-lacking AMPARs are Ca^{2+}, which gives them higher channel conductance and more open probability. (Right) mGluRs are G-protein coupled receptors that trigger a second-messenger cascade and are found in presynaptic and postsynaptic neurons (from Kritis et al., 2015).

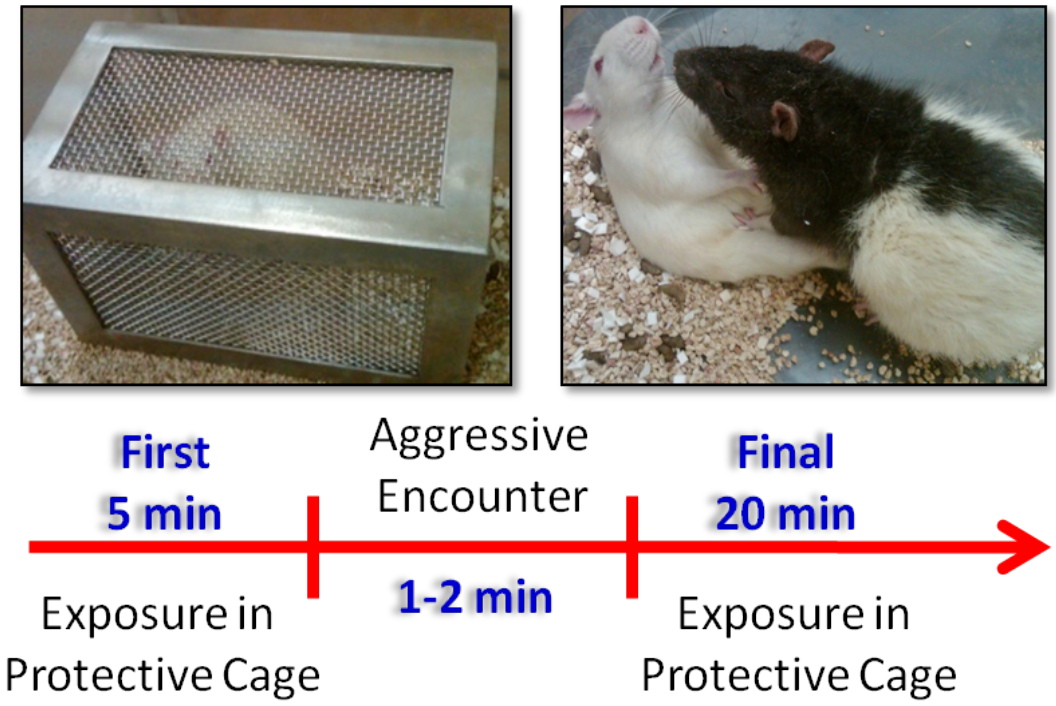

Figure 1.3. Schematic and timeline of intermittent social defeat stress procedures in male rats. In the above schematic, the defeated intruder rat is a docile albino Sprague-Dawley rat, while the aggressive resident in a black and white hooded Long Evans rat, which is specifically bred for aggressive tendencies. To provoke attack by the resident and increase territorial aggression, the intruder is first placed under a small metal protective cage for the first 5 min, which enables sensory contact (left). During the 1-5 min physically aggressive encounter, the protective cage is removed, which allows the aggressive resident to continually attack the intruder, which displays the submissive supine posture as an indicator of a successful defeat (right). After the physical defeat period, the intruder is placed back under the metal protective cage for an additional 20 min for continual stress exposure without any threat of injury. In contrast to continuous models of social defeat, the intruders are placed back in their home cages following defeat, instead of in proximity to the aggressive residents.

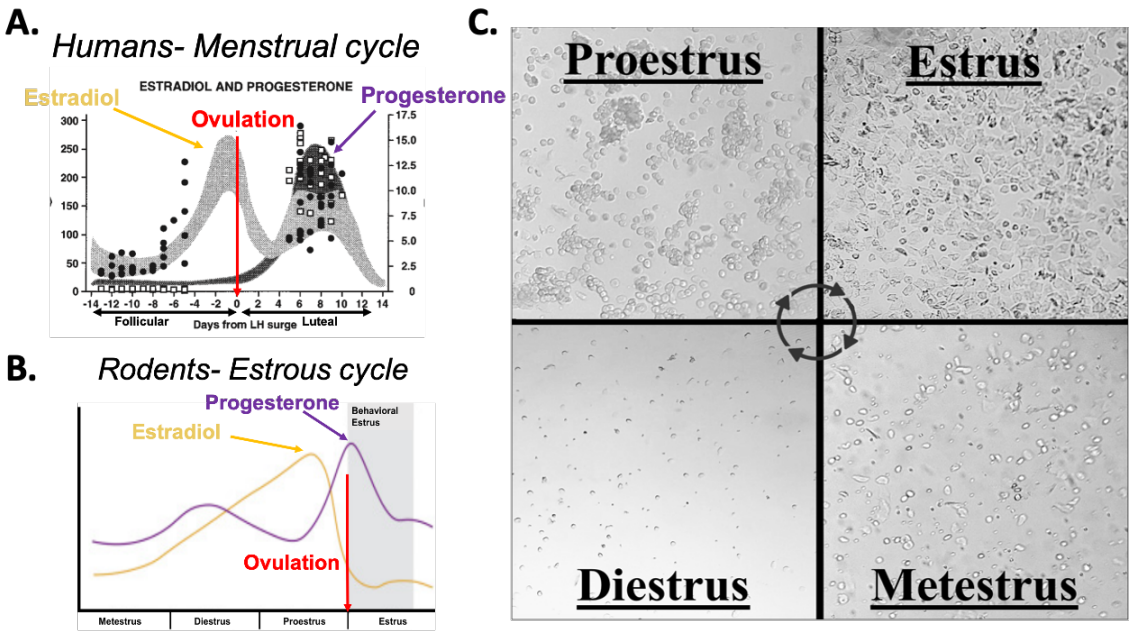

Figure 1.4. Graphical representations of ovarian cycles in humans with corresponding estrus stages in rats, and vaginal cytology showing rat estrus stages. A. (Top) Graph that displays projected plasma estradiol during the 28-day ovarian cycle in humans. During the follicular phase, estradiol levels peak just before ovulation, after which they decline rapidly. The luteal phase is then characterized by the decrease in estradiol and the rapid increase in progesterone to prepare the uterus for implantation (figure modified from Justice and de Wit, 1999); (Bottom) Graph that displays the corresponding 4-5-day rat estrus cycle. Proestrus is the shortest phase and is characterized by a peak in plasma estradiol, after which it declines rapidly and is followed by a rapid rise in progesterone. Ovulation occurs when there is a peak in progesterone during estrus (figure modified from Yoest et al., 2018). B. Unstained vaginal smears taken from female rats and examined under a Zeiss light microscope at 100x magnification. Smears taken from rats cycling in proestrus mainly consists of clumps of nucleated epithelial cells of uniform size; smears taken from rats cycling in estrus consists predominantly of cornified, anucleated cells; metestrus smears are characterized by proportional mixtures of leukocytes, cornified, and nucleated epithelial cells; smears taken from rats cycling in diestrus consist mainly of small leukocytes (Marcondes et al., 2002).

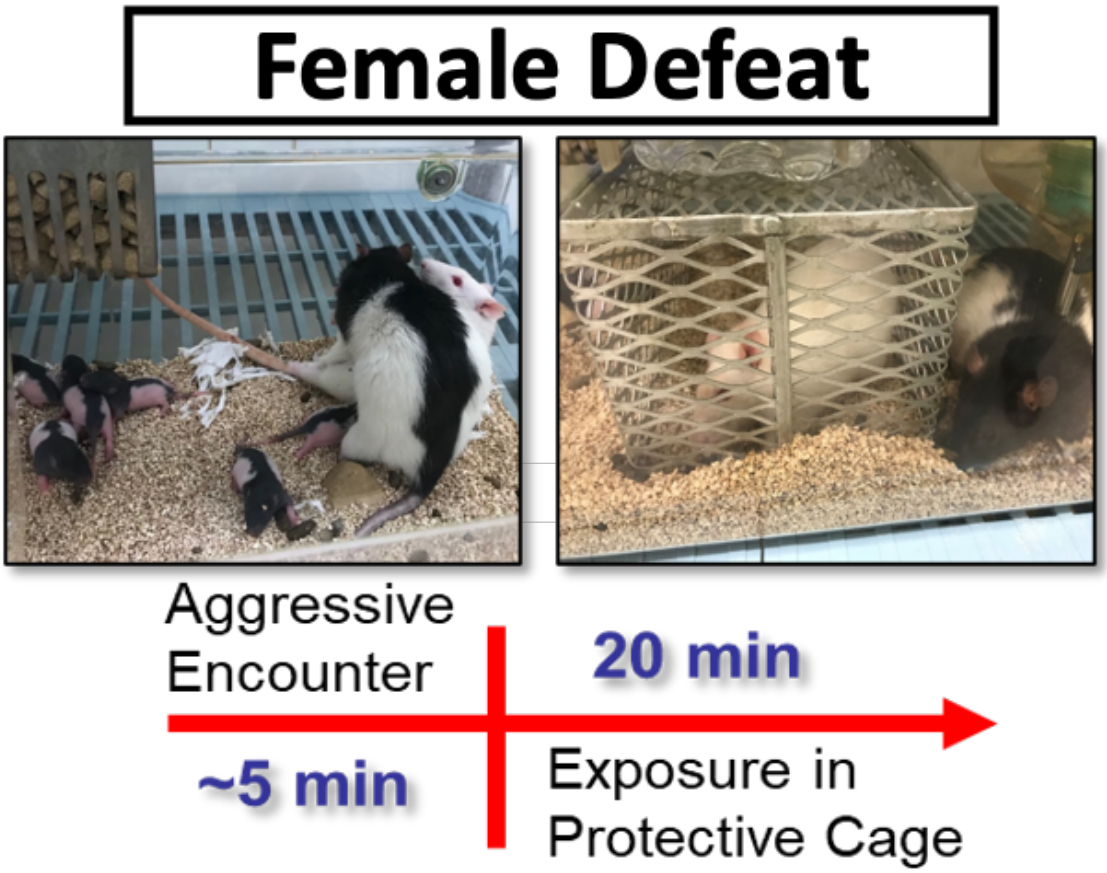

Figure 1.5. Schematic and timeline of intermittent social defeat stress procedures in female rats: maternal aggression model. In the above schematic, the defeated intruder rat is a female docile albino Sprague-Dawley rat, while the aggressive resident in a black and white hooded female lactating Long Evans rat, with 3-13-day-old pups in the cage. The intruder is first placed directly into the resident's cage containing the pups (physically aggressive encounter; left) During the 1-5 min physically aggressive encounter, the aggressive resident continually attacks the intruder, which displays the submissive supine posture as an indicator of a successful defeat (right). After the physical defeat period, the intruder is placed back under the metal protective cage for an additional 20 min for continual stress exposure without any threat of injury. In contrast to continuous models of social defeat, the intruders are placed back in their home cages following defeat, instead of in proximity to the aggressive residents.

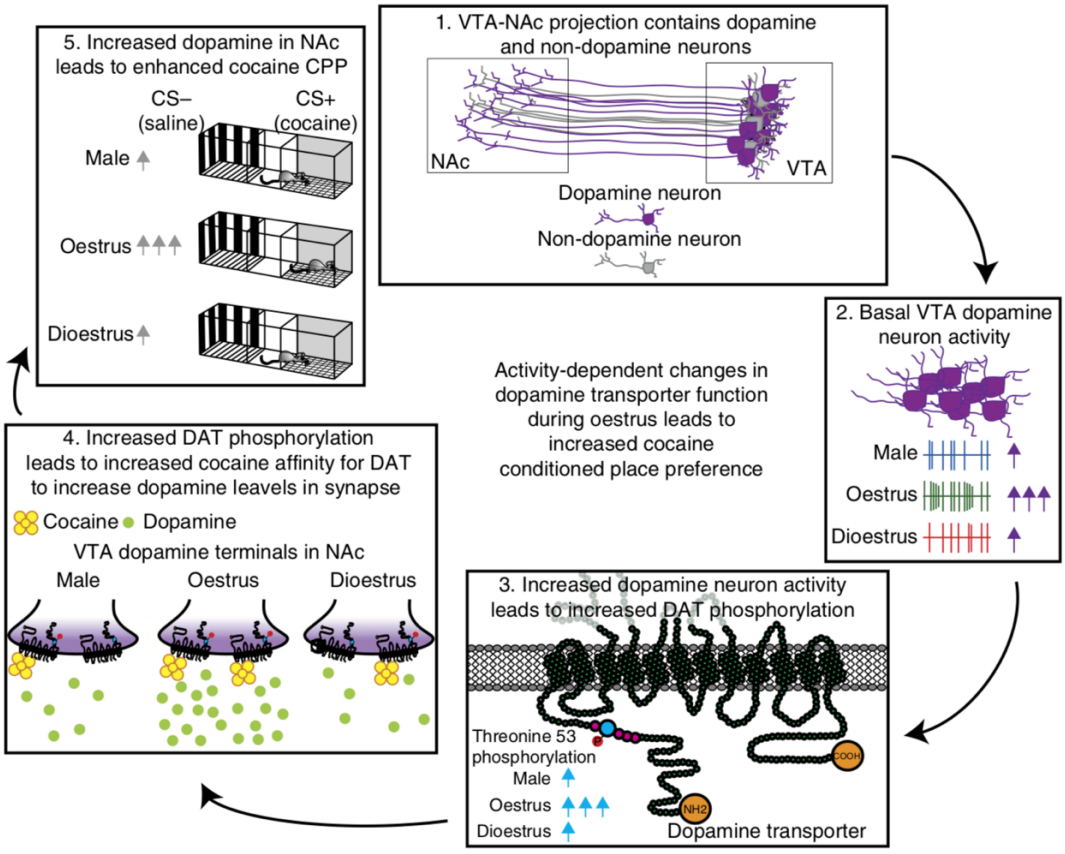

Figure 1.6. Proposed mechanism for sex-dependent mesolimbic regulation of DA signaling and reward processing. (1) VTA contains dopaminergic (purple) and nondopaminergic (grey) neurons that project to the NAc; (2) VTA DA neuron firing is enhanced during proestrus/estrus (high circulating estradiol); (3) Increased DA neuron activity increases ERK activation, as well as phosphorylation of Thr53 (blue) on DAT; (4) This phosphorylation of DAT increases cocaine's affinity, which makes cocaine more able to bind to DAT, ultimately increasing extracellular DA levels. Increased cocaine binding leads to enhanced DA levels in the NAc. (5) Enhanced DA levels due to cycle-related increases in estradiol drives increased associations between cocaine and contextual cue due to differences in the perceived rewarding value of cocaine (Calipari et al., 2017).

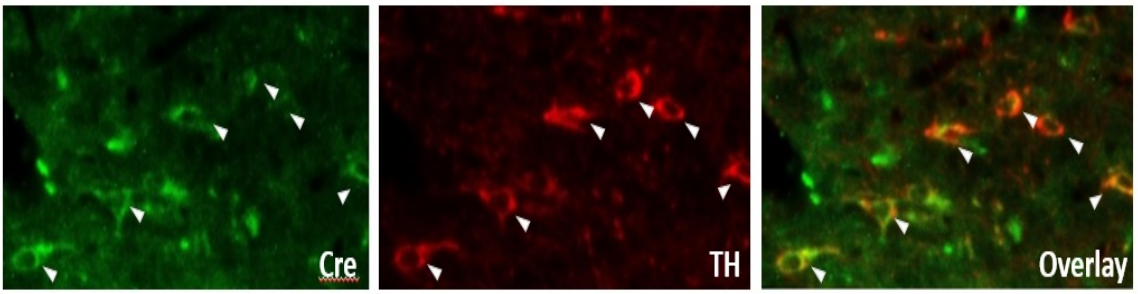

Fig. 1.7. Verification of TH-Cre animals using fluorescent immunohistochemistry.
Representative images of fluorescent Cre labeling (left), TH labeling (center), and Cre/TH double labeling (Right) in handled (top) and stressed (bottom) animals; 20x objective magnification.

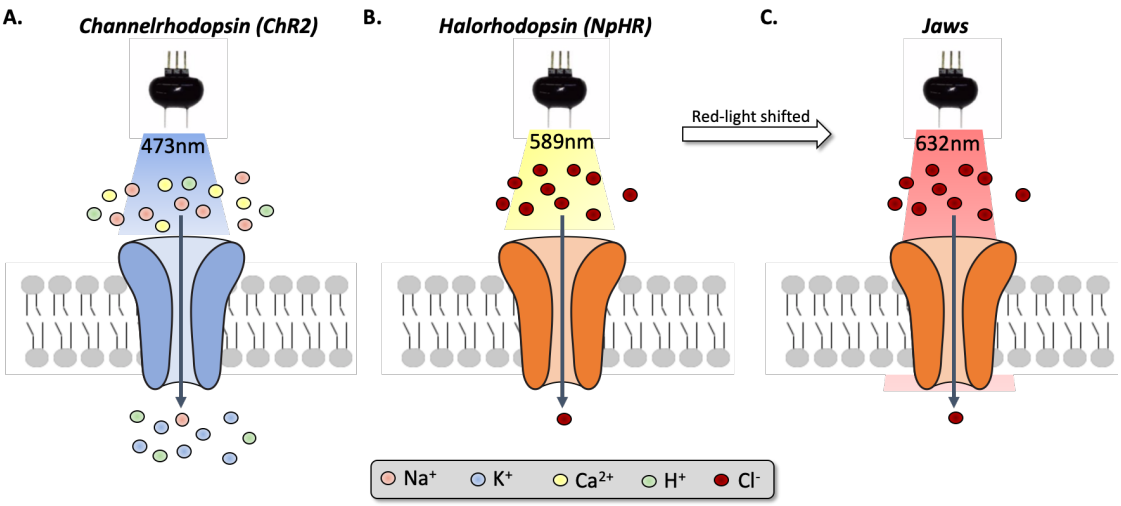

Fig. 1.8. Several optogenetic tools for the stimulation and inhibition of neural pathways. (A) Channelrhodopsin is a 473nm light-gated cation-permeable channel, which changes conformation upon stimulation with 473nm light and allows influx of cations including Na+, Ca2+, ultimately resulting in membrane depolarization and cellular activation. (B) Halorhodopsin is a 589nm light-gated chloride (Cl-) pump, and upon 589nm light stimulation, this channel pumps Cl- ions into the cell, ultimately inducing membrane hyperpolarization and cellular inhibition. (C) Jaws is a spectrally red-light-shifted cruxhalorhodopsin, and is also a Cl- pump like NpHR, but is activated by 632nm red light. This red light has a deeper penetrance, which has the benefit of being able to penetrate deeper brain regions than NpHR (Chuong et al., 2014).

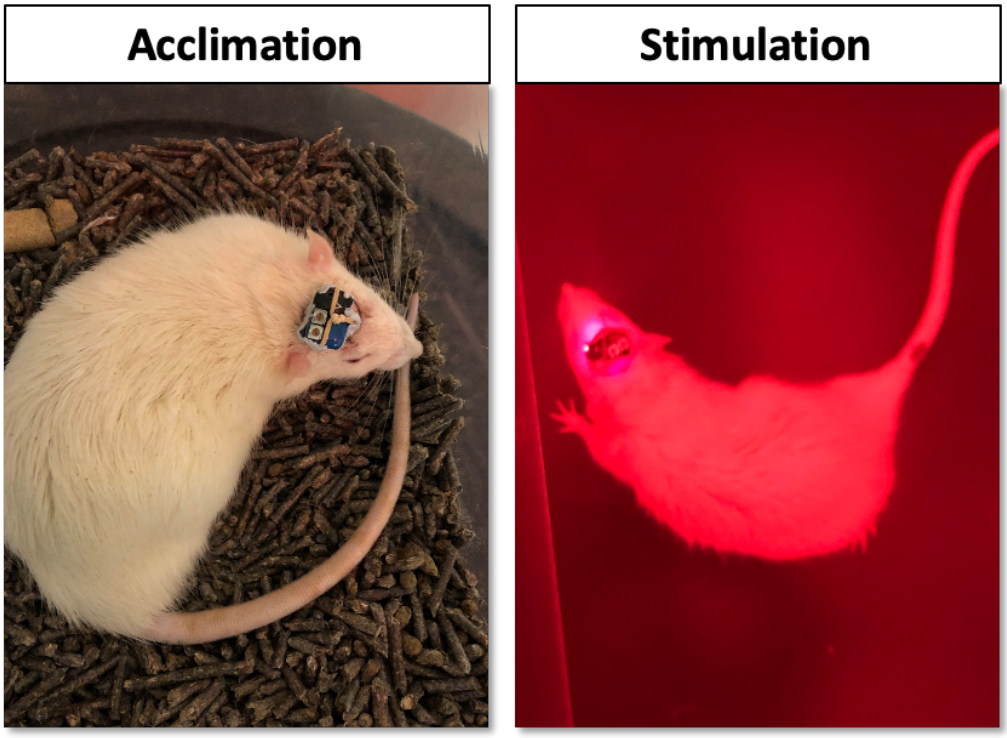

Figure 1.9. Images depicting experimental setup of our wireless optogenetics system. The optic fiber, which contains 3 prongs, is implanted in the IL and attached to the skull using dental acrylic. A 2-g battery-operated wireless receiver attaches to the optic fiber by the three-prongs, and rubber bands are used to securely hold the receiver in place. (Left) Rats are acclimated to a 2-g dummy receiver for several days prior to behavioral testing. (Right) During optogenetic stimulation, the receiver is turned on and the rat is transported into the behavior room, where there is an IR emitter and remote, which communicate to the receiver to turn on the red (or blue) light.

Chapter 2: Enhanced psychostimulant response, but not social avoidance, depends on GluA1 AMPA receptors in VTA dopamine neurons following intermittent social defeat stress in rats[1]

ABSTRACT

Evidence from both human and animal studies demonstrates the importance of social stress in the development of addiction-related behavior. In rats, intermittent social defeat stress causes long-lasting psychostimulant cross-sensitization. Our recent data reveal heightened expression of AMPA receptor (AMPAR) GluA1 subunit in rat ventral tegmental area (VTA), which occurs concurrently with social stress-induced amphetamine (AMPH) cross-sensitization. In addition, social stress in rats induced social avoidance behavior. The present study evaluated the effects of intermittent social defeat stress on GluA1 expression in VTA dopamine (DA) neurons, then utilized Cre-dependent virus-mediated gene transfer to determine the functional role of homomermic GluA1-AMPARs in these neurons. Social defeat stress exposure induced GluA1 expression in VTA DA neurons, as demonstrated by a greater density of GluA1/ tyrosine hydroxylase (TH) double-labeling in VTA neurons in stressed rats. Additionally, functional inactivation of VTA GluA1 AMPARs in DA neurons prevented stress-induced cross-sensitization, or augmented locomotor response to low dose AMPH challenge (1.0 mg/kg, i.p), but had no effect on social stress-induced social avoidance behavior. Furthermore, wildtype overexpression of GluA1 in VTA DA neurons had the opposite effect; locomotor-activating effects of AMPH were significantly augmented, even in the absence of stress. Taken together, these results suggest that stress-induced GluA1 expression in VTA DA neurons is necessary for psychostimulant cross-sensitization, but not for social avoidance. This differential effect suggests that different neural pathways are implicated in these behaviors. These findings could lead to novel pharmacotherapies to help prevent stress-induced susceptibility to substance abuse.

[1] This chapter was accepted as a publication in Rudolph et al. (2020) in *Eur. J. Neurosci*.

1. Introduction

Stress is an influential factor that impacts the transition from recreational drug use to addiction and has been correlated with increased substance abuse susceptibility and relapse (Sinha, 2001; 2008; 2011). Converging evidence from human and animal studies demonstrates that repeated social stress augments the locomotor effect of psychostimulants in a phenomenon referred to as "cross-sensitization" (Covington and Miczek, 2001; Nikulina et al*.,* 2004, 2012), and leads to social avoidance behavior (Berton et al., 2006; Fanous et al., 2011; Komatsu et al., 2011). These social stress-induced behavioral effects are long lasting (Nikulina et al., 2004; Covington et al. 2005), and induce prolonged activation of mesocorticolimbic pathways, which are comprised of dopaminergic neurons that originate in the ventral tegmental area (VTA; Swanson et al., 1982).

In rodents, psychostimulant administration increases mesocorticolimbic glutamate transmission in the nucleus accumbens (NAc), striatum, and VTA (Xue et al., 1996; Reid et al., 1997; Wolf and Xue, 1998, 1999; Del Arco et al., 1999). This glutamate input, mostly originating from cortical regions, provides excitatory control of VTA dopamine (DA) neuronal activity (Johnson et al, 1992; Sesack and Pickel, 1992; Taber and Fibiger, 1995), binding to α-amino-3-hydroxy-5-methyl-4-isoxazoleproionic acid (AMPA) or N-methyl-D-aspartate (NMDA) receptors. AMPA receptors (AMPARs) are ionic transmembrane receptors with four subunits, GluA1-4, which modulate receptor trafficking and channel functions. AMPARs comprised of GluA1 homodimers (i.e., GluA2-lacking) are Ca^{2+}-permeable and have higher single-channel conductance, enhancing Ca^{2+}-dependent intracellular signaling (Wolf, 2010; Straub and Tomita, 2012; Wolf and Tseng, 2012). Induction of psychostimulant sensitization is blocked by AMPAR antagonist administration (Li et al., 1997; Vandershuren and Kalivas, 2000; Zhang et al., 1997), and higher VTA GluA1 levels are implicated in drug-induced sensitization (Carlezon and Nestler, 2002; Kalivas, 2009; Ping et al, 2008). Although evidence has implicated a critical role of GluA1 in drug-induced sensitization, it is unknown whether GluA1 in VTA DA neurons plays a causal role in social stress-induced sensitization to psychostimulants. Western blot data demonstrate that

social stress induces VTA GluA1 expression, which occurs concomitantly with social stress-induced AMPH cross-sensitization (Covington et al, 2008; Wang et al, 2014). However, the cellular localization of induced GluA1 expression by social stress exposure is not known.

To address this question, we first evaluated the effects of intermittent social defeat stress on GluA1 expression in VTA DA neurons. To determine the role of GluA1 in VTA DA neurons in stress-induced AMPH sensitivity and social avoidance behavior, we used TH-Cre rats and utilized Cre-inducible AAV-mediated gene transfer to bidirectionally manipulate GluA1 expression preferentially in VTA DA neurons. We performed intra-VTA infusion of a viral construct that expresses pore-dead GluA1 in a dominant-negative manner to functionally inactivate GluA1 AMPARs. We also used a viral construct that expresses wild-type (wt) GluA1 to overexpress GluA1 AMPARs in VTA DA neurons. By doing so, we were able to test the causal role of GluA1 in VTA DA neurons on social stress-induced sensitization to psychostimulants. To reveal the behavioral specificity of VTA GluA1 signaling for the sensitized response to psychostimulants, we also studied the effect of intra-VTA GluA1 manipulation on intermittent social stress-induced social avoidance behavior.

2. MATERIALS AND METHODS

2.1. Subjects

Experimental subjects were male Sprague Dawley homozygous tyrosine hydroxylase (TH)-Cre rats (Sage Laboratories, MO) weighing 200-250 g at the start of experimentation. Two breeding pairs of TH-Cre Sprague Dawley rats were ordered from SAGE laboratory, where they were verified to be homozygous and to have no observed ectopic expression of Cre (Sage Laboratories, MO). All experimental animals were bred on site at the University of Arizona College of Medicine-Phoenix animal facility and were maintained under a reverse light/dark cycle (12h:12h, lights on at 9:00 am), with *ad libitum* access to food (Purina Rodent Diet, Brentwood, MO) and water. Three days prior to the first social stress exposure, subjects were individually housed in standard plastic cages (25 x 50 x 20 cm^3). Male Long-Evans rats (weighing 550-700 g) termed “residents”, were pair-housed with female Long-Evans rats in larger plastic cages (37 x 50

x 20 cm^3). Residents were screened for aggressive behavior as described previously (Nikulina et al., 2012), and were used to induce social defeat stress in the experimental "intruder" TH-Cre rats as described below. All experimental procedures were approved by the University of Arizona Institutional Animal Care and Use Committees. All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and efforts were made to minimize pain and suffering and reduce the number of animals used.

2.2. Viral Vectors

Rats assigned to the control viral group received bilateral infusions of adeno-associated viral (AAV) constructs that express green fluorescent protein (GFP) and a Cre insert (AAV5-CMV-HI-eGFP-Cre-WPRE-SV40; AAV-GFP; viral titer: 1.864E+13 vg/mL; Penn Vector Core) to identify the injection site. Rats assigned to the VTA GluA1 functional inactivation group received a Cre-dependent AAV that expresses dominant-negative pore-dead GluA1 (AAV5.2-hEF1a-DIO-GluA1-Q58IE-SV40PA; AAV-pd-GluA1; viral titer: 2.24E+13 vg/mL; Gene Technology Core, Massachusetts General Hospital), containing a single point mutation (Q581E) in the pore region, which reduces synaptic AMPA currents through heteromeric interactions with endogenous AMPA subunits (Dingledine et al., 1999; Shi et al., 2001). Rats assigned to the GluA1 overexpression group received a Cre-dependent AAV that expresses wildtype GluA1 (AAV5.2-hEF1a-DIO-Rev-GluA1-wt-SV40PA; AAV-wt-GluA1; viral titer: 2.13E+13 vg/mL; Gene Technology Core, Massachusetts General Hospital). Both viral vectors manipulating GluA1 expression were previously validated *in vitro* and *in vivo* (Bachtell et al, 2008).

2.3. Bilateral intracranial viral infusions

After random group assignment, rats were anesthetized using isoflurane and positioned in a stereotaxic frame (Leica Angle Two; Richmond, IL). The respective viral construct was bilaterally infused (1.0 µl per side) into the VTA (AP -5.0 to -5.3, ML ±2.1, DV -8.2, tilt 10°; Paxinos and Watson, 2007) at a constant flow rate of 0.1 µl/min (Hamilton; Model 7105 KH; 24-gauge tip; Reno, NV). After infusion, the needle remained at the infusion site for 5 min to prevent retrace of

the virus. Animals that were assigned to non-viral control groups received sham surgeries, in which they were anesthetized with isoflurane and were positioned in a stereotaxic frame, but no surgery was performed. Rats recovered in their cages for 2-3 weeks to allow for optimal viral expression before the start of social stress or handling procedures.

Viral infusion site localization was performed using fluorescent immunohistochemistry (described below) after each experiment to ensure accurate viral expression in medial/anterior portions of the VTA, which are known to have a high density of DA neurons (Morales & Margoles, 2017).

2.4. Experimental design

We conducted three separate experiments that were designed to elucidate the mechanisms underlying the involvement of GluA1 localized to VTA DA neurons in stress-induced AMPH sensitization and social interaction (Fig. 1).

Experiment 1: Characterization of VTA neurons in which GluA1 induction occurs after social stress and AMPH administration. Rats were exposed to social stress (n=13) or handling (n=12), then received d-AMPH sulfate (1.0 mg/kg, i.p.; Sigma-Aldrich; St Louis, MO in 0.9% saline) 10 days after the last stress or handling procedure, known to produce long-term behavioral effects of intermittent social defeat stress in rats (Miczek et al., 2011; Covington and Miczek, 2001; Nikulina et al., 2004). Brain tissue was collected and processed 5 days after the AMPH challenge to ensure that the drug was completely removed from the rats' system in order to measure the effects of stress rather than AMPH challenge. GluA1/Tyrosine Hydroxylase (TH) colocalization was assessed in the VTA (Fig. 2.1a).

Experiment 2: Effect of virus-mediated inactivation of GluA1 in VTA DA neurons on behavioral sensitization to AMPH and social interaction. Rats were randomly assigned to one of four groups based on two experimental factors: virus (AAV-pd-GluA1 vs sham infusion) or behavioral treatment (handling vs social stress). The groups were as follows: sham-handled *(n=17*), sham-stressed (*n=15*), AAV-GluA1-pd-handled (*n=15*), and AAV-pd-GluA1-stressed (*n=15*). These rats were subjected to a social interaction test 3 days after termination of social stress and handling

procedures, then were given AMPH challenge 10 days after the last stress or handling procedure. Animals were euthanized 5 days after AMPH challenge, at which time brains were collected for processing. Locomotor activity was analyzed, and immunohistochemistry was performed to examine GluA1/TH double-labeling in the VTA (Fig. 2.1b).

Experiment 3: Effect of viral overexpression of GluA1 in VTA DA neurons on behavioral sensitization to AMPH in handled animals. Rats were randomly assigned to groups based on viral vector (AAV-wt-GluA1, AAV-GFP, or sham), and all animals received handling. The experimental groups were as follows: sham (*n=8*), AAV-GFP (*n=5*), and AAV-wt-GluA1 (*n=14*). These subjects were challenged with AMPH 10 days after the last handling procedure. Rats were euthanized 5 days later, and brains were collected for immunohistochemical processing. Locomotor activity was analyzed, and GluA1/TH double-labeling in the VTA was assessed (Fig. 2.1c).

2.5. Intermittent social defeat stress and handling procedure

Social stress was induced by a brief confrontation between an aggressive resident rat and an experimental intruder rat, as previously described (Nikulina *et al*., 2012). This defeat procedure was performed in a sound-attenuated room to prevent ultrasonic stimuli from affecting unstressed subjects. After removing the female from the resident's home cage, an experimental intruder rat was placed inside the resident's home cage for 5 min under a small metal protective cage (15 x 25 x 15 cm^3). The protective cage was then removed, allowing the resident to attack the experimental rat until it displayed a submissive supine posture for at least 6 s, or for 5 min, whichever came first. The experimental rat was then placed back into the protective cage for an additional 20 min before returning to its home cage. The social stress procedure was performed every third day for 10 days, producing intermittent exposure to social defeat stress. On the same days as social stress, rats in the control groups were handled for 2-3 min and returned to their home cages.

2.6. Social interaction

The social approach/avoidance test was conducted in a three-chamber container (58 x 38 x 41 cm^3), with lightweight metal cages on either side (Fig. 5a). Experimental rats were allowed to habituate to the chamber for 5 min, and then were reintroduced to the “neutral’ zone when a novel Long-Evans rat was placed under the containment cage on one side of the three-chamber container. The behavior of the rat was recorded using Ethovision video tracking software (Noldus; Sacramento, CA), which separated the chamber into three zones: the interaction zone (the side that contains the novel rat), the neutral zone (the middle), and the avoidance zone (the side that contains an empty containment cage). The number of respective entries into the avoidance and interaction zones were recorded, as well as the total distance traveled (cm).

2.7. AMPH challenge

To test for social stress-induced cross-sensitization, a low dose of AMPH was administered as previously described (Nikulina et al., 2012). Rats were injected with vehicle (0.9% sterile saline; 1.0 ml/kg, i.p. daily) on several days prior to the AMPH challenge to acclimate them to i.p. injections. On the day of the AMPH challenge, rats were moved into the procedure room, where locomotor activity measured as total distance traveled (in cm) was recorded in their home cage during sequential 10 min bins using Ethovision. Baseline locomotor activity was recorded for 30 min, after which a 0.9% saline injection (1.0 ml/kg, i.p.) was administered and locomotor activity was recorded for 40 min. Finally, rats received an injection of D-AMPH sulfate (1.0 ml/kg in saline vehicle, i.p.), and locomotor activity was recorded for an additional 60 min.

2.8. Tissue harvesting for immunohistochemistry

As previously described (Fanous et al., 2011), rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.; Euthasol, Virbac Co., St. Louis, MO) and perfused transcardially with 4% paraformaldehyde. Brains were extracted, post-fixed for 90 min at 4°C, and placed into graded sucrose solutions. Frozen brain tissue was sectioned on a cryostat (20 μm) and serial VTA sections (AP = - 5.0 to -5.6, Paxinos and Watson, 2007) were mounted onto slides. Adjacent

VTA slides from each brain were processed for either GluA1/ TH double-labeling or fluorescent localization of GFP expression.

2.9. Immunohistochemistry

To determine the cellular localization of GluA1 expression in VTA DA neurons, we performed fluorescent double-labeling of GluA1 and TH. Sections were first washed in 0.05M potassium phosphate buffered-saline (KPBS), then blocked for 1 h in 10% normal goat serum (NGS) and 0.4% Triton X-100 in 0.05M KPBS. Sections were then incubated simultaneously with rabbit anti-GluA1 (ABN241, 1:500 dilution; Millipore; Temecula, CA), which recognizes both active and inactive forms of GluA1, and mouse anti-TH (SC-7837, 1:500 dilution; Santa Cruz Biotechnology; Santa Cruz, CA) at 4°C for 48 h. Slides were then incubated with biotinylated goat anti-rabbit IgG (1:1000 dilution, Vector Laboratories, CA) for 1 h, and then Alexa Fluor 488 Anti-Rabbit conjugated streptavidin and Alexa Fluor 647 Goat Anti-Mouse (1:1000 dilution; Invitrogen; San Diego, CA) were applied for 2.5 h. After washing with 0.05M KPBS, coverslips were applied with ProLong Diamond Antifade Mountant (Invitrogen, San Diego, CA).

2.10. Modified stereological quantification

Tissue sections were imaged using a Zeiss Axioscope with a 20x objective lens, and were digitalized using a color digital camera. The number of GluA1/TH double-labeled neurons was quantified using ImageJ software (NIH), and the analysis was conducted using a modified stereology counting procedure as described previously (Fanous et al., 2011a; Nikulina et al., 2012). A grid of 30 squares (0.0075 mm^2) was superimposed on 2-3 adjacent VTA sections bilaterally from each subject. Double-labeled neurons were counted in 15 grid random squares such that labeled cells intersecting the bottom or right lines of each square were included, while cells intersecting the top or left lines of the square were excluded from the analysis. Double-labeled cell density (in mm^2) was calculated by averaging the bilateral counts across sections, then dividing the total number of counted cells by the total area that was assessed (0.111 mm^2). Furthermore, total TH neuronal population was approximated by averaging the bilateral counts of

TH-labeled neurons across all sections, then dividing the total number of counted cells by the total area assessed (0.111mm^2). To account for any potential differences in tissue quality, the percentage of GluA1/TH double-labeled neurons out of the entire TH neuronal population was calculated by dividing the number of GluA1/TH double-labeled cells by the corresponding total number of TH-labeled cells, and then multiplying by 100 to obtain the percentage.

2.11. Statistical analyses

Across all experiments, the results are expressed as mean ± standard error (SEM) and a p value ≤ 0.05 was considered significant. GraphPad Prism version 7 (GraphPad Prism; La Jolla, CA) was used to perform all statistical analyses, and Tukey's HSD was the preferred post hoc test across all experiments, except in the AMPH challenge, where we utilized the more conservative Fisher's LSD test. In experiment 1, a two-way ANOVA was used to analyze locomotor activity during the AMPH challenge, and a paired two-tailed t-test was used to analyze double-labeled cell counts in stressed and handled rats. To calculate the average distance traveled during each portion of the AMPH challenge (acclimation, saline, AMPH administration), the average distance traveled between three consecutive timepoints was taken. After AMPH administration, the average distance traveled between the three peak timepoints was taken (20-40min after AMPH administration). A two-way ANOVA was then performed to compare the average distance traveled between experimental groups (stressed and handled) and across timepoints (acclimation, saline, AMPH). In experiment 2, a three-way ANOVA was used to analyze locomotor activity (between-subjects factors: viral vector (AAV-pd-GluA1 or sham), behavioral treatment (handling or stress), and experimental timepoint (acclimation, saline, AMPH), while a two-way ANOVA was used to analyze immunohistochemical labeling density. Average distance traveled across stages of the AMPH challenge were calculated as described in experiment 1. In experiment 3, two-way ANOVA was used to analyze locomotor activity and immunohistochemical labeling density (between-subjects factors: viral vector (AAV-wt-GluA1, AAV-GFP, or sham). Average distance traveled across stages of the AMPH challenge were calculated as described in

experiment 1. In all experiments, data were excluded in the case of video tracking errors (n=3), incorrect viral infusion sites (n=5), or loss of data due to damaged tissue sections (n=6).

3. RESULTS

3.1. Experiment 1

Intermittent social defeat stress induces sensitization to AMPH.

Locomotor response to AMPH challenge was evaluated in naïve rats 10 days after the last exposure to intermittent social defeat stress or handling. A two-way ANOVA revealed that rats exposed to intermittent social defeat stress exhibited significantly more locomotor activity after AMPH than did handled rats (n=27, $F_{12, 325}$=3.16, p=0.0013), reflecting the presence of cross-sensitization (Fig. 2.2A). Specifically, post hoc analysis revealed that stressed rats displayed greater locomotor activity than did handled rats at 10 (p=0.0019), 20 (p=0<0.0001), 30 (p<0.0001), 40 (p=0.0001), 50 (p=0.0005), and 60 (p=0.0021) min after AMPH challenge, but there were no differences across groups before or after saline injection (p>0.05 at all other time points). Additionally, a two-way ANOVA was performed to compare the average distance traveled during acclimation, following saline treatment, and after AMPH administration, in handled versus stressed rats. This two-way ANOVA revealed significant main effects of stage of AMPH challenge (n=27, $F_{2,75}$=32.81, p<0.0001) and stress ($F_{1,75}$=26.5, p<0.0001), and an interaction between the two factors ($F_{2,75}$=8.756, p=0.0004) (Fig. 2.2B).

Intermittent social defeat stress increases GluA1 expression in VTA DA neurons.

To characterize the cell types in which GluA1 induction occurs after intermittent social defeat stress, double-label fluorescent immunohistochemistry was performed in the rostral VTA (Fig. 2.2C, 2.2D). A paired t-test was performed to compare the mean number of GluA1-expressing DA neurons in stressed compared to handled rats. Significantly more GluA1/TH double-labeled cells were present in the rostral VTA in stressed rats compared to handled rats (n=13; t-test: p=0.0021; Fig. 2.2D).

3.2. Experiment 2

Verification of GluA1-pd overexpression in VTA DA neurons using fluorescent immunohistochemistry

One important caveat of this experiment is that the AAV-pd-GluA1 and AAV-wt-GluA1 constructs were too large to insert a fluorescent marker such as GFP, which makes it more difficult to control for the specificity and efficiency of the viral transfections. For this reason, fluorescent immunohistochemistry was used to verify GluA1 expression in VTA DA neurons of sham rats compared to experimental rats. Fluorescent immunohistochemistry revealed that AAV-pd-GluA1 infusions increased GluA1 expression in VTA DA neurons (Fig. 2.3B). A two-way ANOVA demonstrated significant main effects of stress (n=26, $F_{1,22}$= 53.53, p<0.0001) and viral manipulation group ($F_{1,22}$=104.2; p<0.0001), and an interaction between the two factors ($F_{1,22}$=20.56; p=0.0002). Consistent with previous findings, post hoc analysis revealed that stressed rats displayed greater GluA1/TH double-labeling than did handled rats (p<0.0001). Furthermore, AAV-pd-GluA1/stressed rats exhibited significantly more GluA1 labeling in VTA DA neurons than did sham/stressed rats (p=0.0021), and AAV-pd-GluA1/handled rats displayed more GluA1/TH double-labeling than did sham/handled rats (p<0.0001). Thus, bilateral AAV-pd-GluA1 infusions were accurately performed in the VTA (Fig. 2.3A) and viral infusions increased GluA1 expression in DA neurons.

Functional GluA1 inactivation in VTA DA neurons prevents social stress-induced AMPH sensitization, but has no effect on social interaction behavior.

Three-way ANOVA demonstrated significant main effects of stress (n=62, $F_{1,650}$=45.85, p<0.0001) and experimental timepoint ($F_{12,650}$=22.67, p<0.0001), but no significant effect of viral construct ($F_{1,650}$=1.952, p=0.1628); however, there was an interaction between stress and viral construct ($F_{1,650}$=22.58, p<0.0001; Fig. 4B). Specifically, post-hoc analysis revealed that AAV-pd-GluA1/stressed rats exhibited significantly less locomotor activity 40 min (p=0.0024) and 50 min (p=0.0363) after AMPH challenge than did sham/stressed rats (Fig. 4A, B). Additionally, a three-

way ANOVA was performed to compare the average distance traveled during acclimation, following saline treatment, and after AMPH challenge in the different experimental groups. Three-way ANOVA revealed a significant main effect of stress ($F_{1,150}$=15.30, p=0.0001) and experimental time-point ($F_{2,150}$=48.21, p<0.0001), but no significant effect of viral construct ($F_{1,150}$=2.362, p=0.1265) on locomotor activity. In addition, there was an interaction between stress and viral construct on locomotor activity ($F_{1,150}$=8.136, p=0.0050; Fig. 4B).

A social interaction test was performed three days after the last stress or handling procedure in rats with or without AAV-pd-GluA1 viral infusions (Fig. 2.5A-D). A two-way ANOVA revealed a main effect of stress on number of entries into the avoidance zone (n=55, $F_{1,51}$ =22.03, p <0.0001), with stressed animals entering the avoidance zone significantly more times than did handled animals (p <0.05). There was, however, no effect of AAV-pd-GluA1 ($F_{1,51}$ =2.443, p=0.1242), or interaction between factors ($F_{1,51}$ =0.1182, p =0.7552; Fig. 5C-D). A two-way ANOVA also revealed that handled rats trended to enter the social interaction zone more times than did stressed rats (p>0.05), and there was no effect of AAV-pd-GluA1 (p>0.05). In addition, - a two-way ANOVA revealed a main effect of stress on cumulative time spent in interaction zone (s) ($F_{1,51}$=13.91, p=0.0005), with handled animals spending significantly more time in the interaction zone than did stressed animals (p=0.0143). There was, however, no effect of AAV-pd-GluA1 ($F_{1,51}$= 0.2932, p=0.5905) or interaction between the two factors ($F_{1,51}$=0.2131, p=0.6463; Fig. 2.5E-F),

3.3. Experiment 3

Verification of GluA1-wt overexpression in VTA DA neurons using fluorescent immunohistochemistry

Fluorescent immunohistochemistry revealed that AAV-wt-GluA1 infusions increased GluA1 expression in VTA DA neurons (Fig. 2.6C, D). An unpaired t-test was performed to compare the mean number of GluA1-expressing DA neurons in sham compared to GluA1-wt-expressing handled rats. Significantly more GluA1/TH double-labeled cells were present in the rostral VTA in

rats expressing GluA1-wt handled rats compared to sham handled rats (n=12; t(10)=8.845, p=0.0003; Fig. 6D). In addition, an unpaired t-test was performed to compare the percentage of GluA1/TH double-labeled neurons out of the entire TH neuronal population. There was a significantly higher percentage of GluA1/TH double-labeled neurons in GluA1-wt-expressing handled rats compared to sham handled rats (t(10)=10.74, p=0.0001; Fig. 2.6D). Thus, bilateral AAV-wt-GluA1 infusions were accurately performed in the VTA (Fig. 2.6) and viral infusions increased GluA1 expression in DA neurons.

Wildtype GluA1 overexpression in VTA DA neurons mimics the effects of intermittent social defeat stress on AMPH cross-sensitization.

Comparing the effect of AMPH challenge in handled rats with or without prior Cre-dependent AAV-wt-GluA1 infusion revealed a significant main effect of experimental group (n=27, $F_{2,308}$= 47.04, p<0.0001; Fig. 7A) and time-point on locomotor activity ($F_{12,308}$=11.34, p<0.0001), but no interaction between the two factors ($F_{24,308}$=0.8694, p=0.6446). Specifically, rats with GluA1 overexpression had significantly more locomotor activity than control/handled sham and handled/GFP rats at 10 (p=0.0003), 20 (p=0.0009), 30 (p=0.0008), 40 (p=0.0044) and 50 min (p=0.0145) after AMPH challenge. There was no difference in locomotor activity over time in the control sham and handled/GFP groups (p>0.05). Additionally, comparison of the average distance traveled during acclimation, after saline treatment, and after AMPH challenge in the three different experimental groups by two-way ANOVA revealed a significant main effect of experimental group ($F_{2,22}$=6.295, p=0.0069) and time-point ($F_{2,44}$=52.78, p<0.0001; Fig. 7B), as well as an interaction between the two factors ($F_{4,44}$=3.182, p=0.0222).

4. DISCUSSION

In this study, we assessed the role of AMPAR GluA1 subunits expressed by VTA DA neurons on social stress-induced psychostimulant cross-sensitization. The present results demonstrate that virus-mediated functional inactivation of GluA1 expression in VTA DA neurons prevents social stress-induced AMPH cross-sensitization, and overexpression of wildtype

AMPAR GluA1 in VTA DA neurons augments locomotor response to AMPH, mimicking the effect of social stress. These results indicate that GluA1, specifically in VTA DA neurons, is both necessary and sufficient to induce stress-induced sensitization to AMPH in male rats. Furthermore, manipulation of VTA GluA1 did not alter social avoidance behavior, suggesting that the effect of GluA1 in VTA DA neurons is behaviorally-specific.

4.1. Intermittent social defeat stress induces GluA1 expression in VTA DA neurons

The VTA is heterogeneous in cellular composition, and is comprised of 60-65% dopaminergic, approximately 35% GABAergic, and a small percentage of glutamatergic neurons (Nair-Roberts et al., 2008). However, this heterogeneity is further complicated by the fact that VTA DA neurons may co-release glutamate (Stuber et al., 2010; Zhang et al. 2015) or GABA (Tritsch et al., 2012; Stamatakis et al., 2013). In rodents, psychostimulant administration increases glutamate transmission in portions of the mesocorticolimbic pathway involved in behavioral sensitization to psychostimulants, including the NAc and VTA (Xue et al., 1996; Reid et al., 1997; Del Arco et al., 1998; Wolf & Xue, 1999). VTA glutamate input mostly arises from prefrontal cortical regions to modulate VTA DA neurons. Repeated stress has been shown to increase neuronal activity of VTA DA neurons which project to the NAc (Tidey & Miczek, 1997), as well as enhance glutamatergic synaptic plasticity through NMDA receptors (NMDARs) in the VTA (Stelly et al., 2016). The observed elevation of VTA GluA1 expression following exposure to social stress is consistent with previous studies, which revealed increased GluA1 protein expression in the VTA of animals subjected to chronic restraint, unpredictable stress, and repeated social defeat stress (Fitzgerald et al., 1996; Covington et al., 2008; Wang et al., 2014). Glutamatergic synapses on VTA DA neurons undergo LTP that is enhanced by acute stress (Niehaus et al., 2010; Graziane et al., 2013; Luscher & Malenka, 2011), while social stress augments DA release in the NAc (Tidey & Miczek, 1996; Miczek et al, 2011). In addition, cocaine administration induces LTP at excitatory synapses on VTA DA neurons (Ungless et al., 2001; Argilli et al., 2008) Thus, the enhancement of GluA1 expression in VTA DA neurons likely increases dopaminergic activity and plays a critical role in stress-induced sensitization to AMPH.

We did not observe GluA1 labeling in all TH+ neurons, which might suggest that GluA1 expression also occurs in TH-GABA or TH-glutamate co-expressing neurons because there are populations of VTA neurons that co-express TH and GAD or VGlut2 (Morales & Margolis, 2017). In addition, it is possible that GluA1 may also be localized in VTA GABA cells, which could indirectly influence dopaminergic transmission. Further studies must be conducted to analyze GABA/GluA1 labeling density to confirm that GluA1 is selectively localized in VTA DA neurons as a result of intermittent social defeat stress.

4.2. Functional inactivation of GluA1 AMPARs in VTA DA neurons attenuates social stress-induced cross-sensitization, but has no effect on social avoidance behavior

Stressed rats exhibited significantly greater locomotor activity following AMPH challenge compared to handled rats (Fig. 2A), confirming the results of prior studies (Covington and Miczek, 2001; Nikulina et al., 2004). By contrast, viral overexpression of pd-GluA1 in VTA DA neurons significantly attenuated stress-induced AMPH sensitization. GluA1-homomeric AMPARs are Ca^{2+}-permeable and are activated when glutamate occupies at least two of its binding sites (Wolf and Tseng, 2012). Phosphorylation of GluA1 at Ser^{831} by CaMKII and PKC during LTP facilitates delivery of GluA1-containing AMPARs to the synapse (Hayashi et al., 2000), and increases single channel conductance (Derkach et al., 1999). It has been suggested that elevated intracellular Ca^{2+} signaling mediated by an increase of GluA1 in the VTA might be an early trigger for drug sensitization (Carlezon & Nestler, 2002). The induction of psychostimulant sensitization is blocked by systemic AMPAR antagonists (Li et al., 1997; Zhang et al., 1997), and repeated cocaine treatment increases GluA1 expression in the VTA one day later (Di Chiara & Imperato, 1988; Churchill et al., 1999). Although drug sensitization is *initiated* in the VTA by enhanced glutamatergic transmission (Cador et al., 1999), overexpression of wt-GluA1 in the NAc diminishes cocaine sensitization, while functional inactivation of NAc GluA1 enhances sensitization (Bachtell et al., 2008). Thus, these effects of GluA1 in the NAc are opposite to those in the VTA as demonstrated herein and previously (Churchill et al., 1999). Our data suggest that

social stress-induced GluA1 expression specifically in VTA DA neurons plays a critical role in the induction of stress-induced cross-sensitization to AMPH.

Furthermore, stress induces glucocorticoid release, which acts on mesolimbic DA neurons to exacerbate the locomotor effects of addictive drugs (Miczek et al, 2008; Kalivas et al., 2009). It is likely this, along with the glutamatergic potentiation of VTA DA neurons, drives stress-induced sensitization to AMPH. It is important to note that GluA1 AMPARs in the VTA are not the only glutamatergic mediators of social stress-induced sensitization to psychostimulants. Metabotropic glutamate receptors located in the VTA have been shown to modulate cocaine-induced synaptic plasticity (Mameli et al., 2009; Loweth et al., 2013), and NMDA receptors in the VTA are necessary for stress-induced behavioral sensitization in mice (Covington & Miczek, 2008). Additionally, VTA NMDARs have been shown to mediate cocaine-induced VTA GluA1 expression (Guzman et al., 2018) through NMDAR-brain derived neurotrophic factor (BDNF)-TrkB signaling, which mediates LTP of excitatory AMPAR input to VTA DA neurons after systemic cocaine injections (Pu et al., 2006). In addition, increased BDNF expression has been implicated as a long-term mediator of social stress-induced AMPH cross-sensitization (Nikulina et al., 2012), and knockdown of BDNF receptor TrkB in the mesolimbic pathway prevents GluA1 expression in the VTA (Wang et al., 2014). It is likely that corticolimbic glutamate signaling onto GluA1 AMPARs in VTA DA neurons works in parallel with these other mesolimbic mechanisms to induce the stress cross-sensitization.

The present results show that functional inactivation of GluA1 in VTA DA neurons prevents stress-induced sensitization to AMPH, but does not alter social avoidance behavior. While the VTA does regulate stress-induced social avoidance behavior, this could involve different mediators of VTA function, such as mu-opioid receptors (Johnston et al., 2015) or BDNF (Berton et al., 2006; Fanous et al., 2011; Krishnan et al., 2007), whose knockdown in the VTA prevents stress-induced social avoidance behavior. In addition, it was recently shown that the function of VTA dopamine neurons depends on distinct inputs and targets (Lammel et al., 2012;

Edwards et al., 2017) that could reflect a diverse regulation of social stress-induced avoidance behavior and AMPH sensitization.

4.3. Overexpression of wildtype GluA1 AMPARs in VTA DA neurons mimics the effects of stress on AMPH sensitization

Handled rats with VTA GluA1 overexpression exhibited significantly greater locomotor activity after a low dose AMPH challenge compared to handled rats with non-manipulated VTA GluA1. These results are consistent with the idea that overexpression of GluA1 in VTA DA neurons is sufficient to mimic the effects of social stress on sensitization to psychostimulants. Importantly, there is a sharp increase of locomotor activity immediately after low dose AMPH challenge (Fig. 6), which is a more pronounced response than we see in non-manipulated stressed animals. Similarly, wildtype GluA1 overexpression in regions of the VTA rich in DA neurons increases cocaine self-administration (Choi et al., 2011). When GluA1 homomeric, Ca^{2+}-permeable AMPARs are overexpressed in VTA DA cells, there is an enhanced substrate to which glutamate can bind, thereby increasing intracellular Ca^{2+} signaling via CaMKII. CaMKII is heavily implicated in modulating long-term-potentiation and is involved in molecular mechanisms of addiction in the mesolimbic pathway; blocking CaMKII in the VTA has been shown to inhibit the acquisition of cocaine conditioned place preference and cocaine-evoked synaptic plasticity in the NAc (Liu et al., 2014; Kourrich et al., 2007). Additionally, CaMKII activity in the NAc is essential for reinstatement of cocaine-seeking in self-administration, increasing AMPAR phosphorylation at Ser^{831}, which is also obtained through viral overexpression of CaMKII in the NAc (Anderson et al., 2008; Loweth et al., 2010). It could be that overexpression of GluA1 in VTA DA cells drives the activation of intracellular CaMKII-dependent Ca^{2+} pathways, which induces potentiation of VTA-NAc DA neurons. In support of this, studies have shown that potentiation onto hippocampal neurons is largely driven by a CaMKII-mediated augmentation of GluA1 surface expression (Appleby et al., 2011); so not only does GluA1 AMPAR signaling induce heightened CaMKII activity, but CaMKII also drives further insertion of GluA1 into the cell membrane resulting in further synaptic potentiation. Our behavioral results are consistent with previous findings that

indicate that GluA1 plays a role in the induction of drug sensitization, but presents novel findings regarding its involvement in social stress-induced AMPH sensitization.

4.4. Concluding remarks

In summary, functional inactivation of GluA1 in VTA DA neurons prevents social stress-induced cross-sensitization to AMPH but has no effect on social avoidance behavior. In rats, repeated social defeat stress enhances glutamatergic synaptic plasticity in the VTA (Stelly et al., 2016), and GluA1 AMPARs play an essential role in the induction of drug sensitization and self-administration (Carlezon & Nestler, 2002; McCutcheon et al., 2011). Additionally, higher GluA1 expression occurs concomitantly with intermittent social stress-induced AMPH sensitization (Wang et al., 2014). The present results show that GluA1 AMPARs in VTA dopaminergic neurons play an essential role in the induction of social stress-induced psychostimulant sensitization.

Much of the work that has been conducted on the role of GluA1 in drug addiction has been focused on the NAc, which has reciprocal projections to the VTA. Since our bilateral VTA GluA1 inactivation in the present study could have blocked the stress-induced increase of dopaminergic tone, these bidirectional viral manipulations might alter stress-induced changes in the NAc. Further studies are necessary to identify whether VTA GluA1 inactivation or overexpression causes neuronal changes in the NAc (e.g., prolonged ΔFosB expression). Additionally, due to the heterogenous nature of the VTA, GluA1 viral manipulations in other VTA cell type are necessary to determine whether GluA1 effects are specific to DA neurons.

In conclusion, our results present the novel finding that GluA1, specifically in VTA DA neurons, plays a critical role in the expression of social stress-induced AMPH sensitization, thereby providing a potential target for pharmacotherapeutic intervention for drug abuse susceptibility.

Acknowledgements

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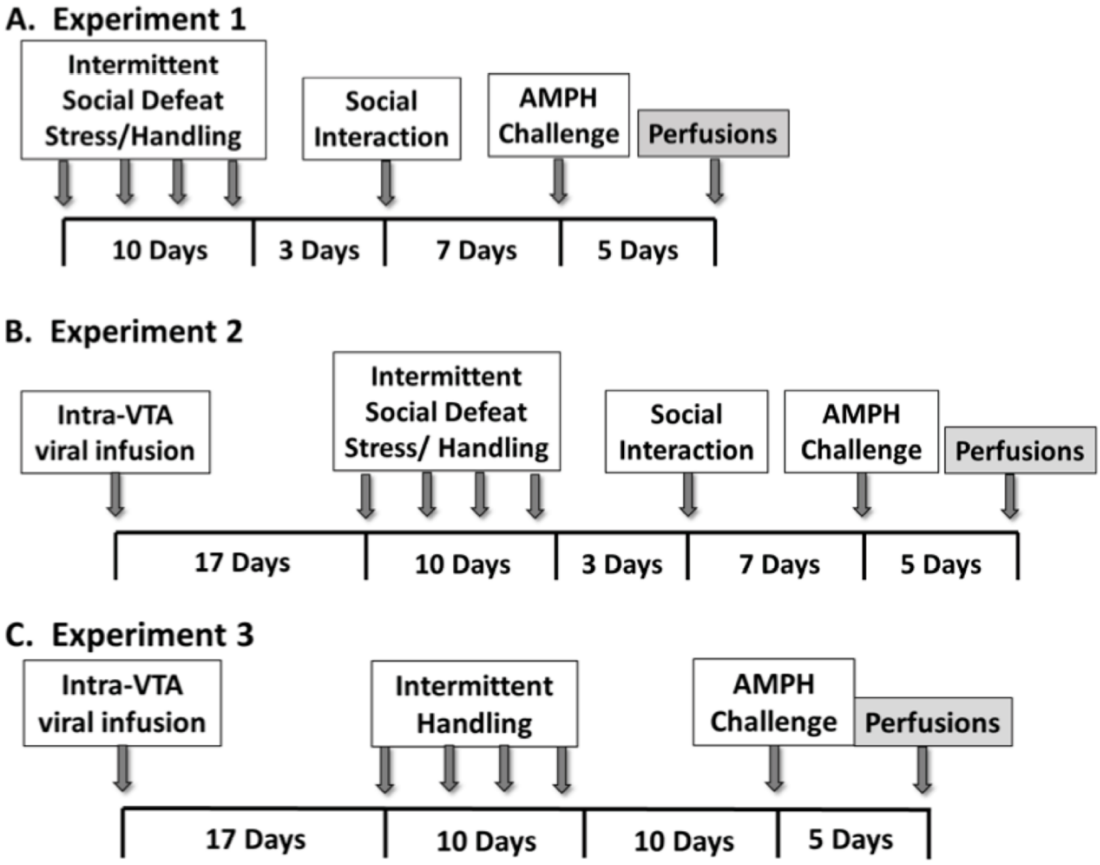

Figure 2.1. **Schematic timelines of experimental design.** (A) Experiment 1: Characterization of VTA neurons in which GluA1 induction occurs after intermittent social defeat stress. (B) Experiment 2: Effect of virus-mediated inactivation of GluA1 in VTA dopamine neurons on behavioral sensitization to AMPH. Experiment 3: Effect of viral overexpression of GluA1 in VTA dopamine neurons on behavioral sensitization to AMPH in handled animals.

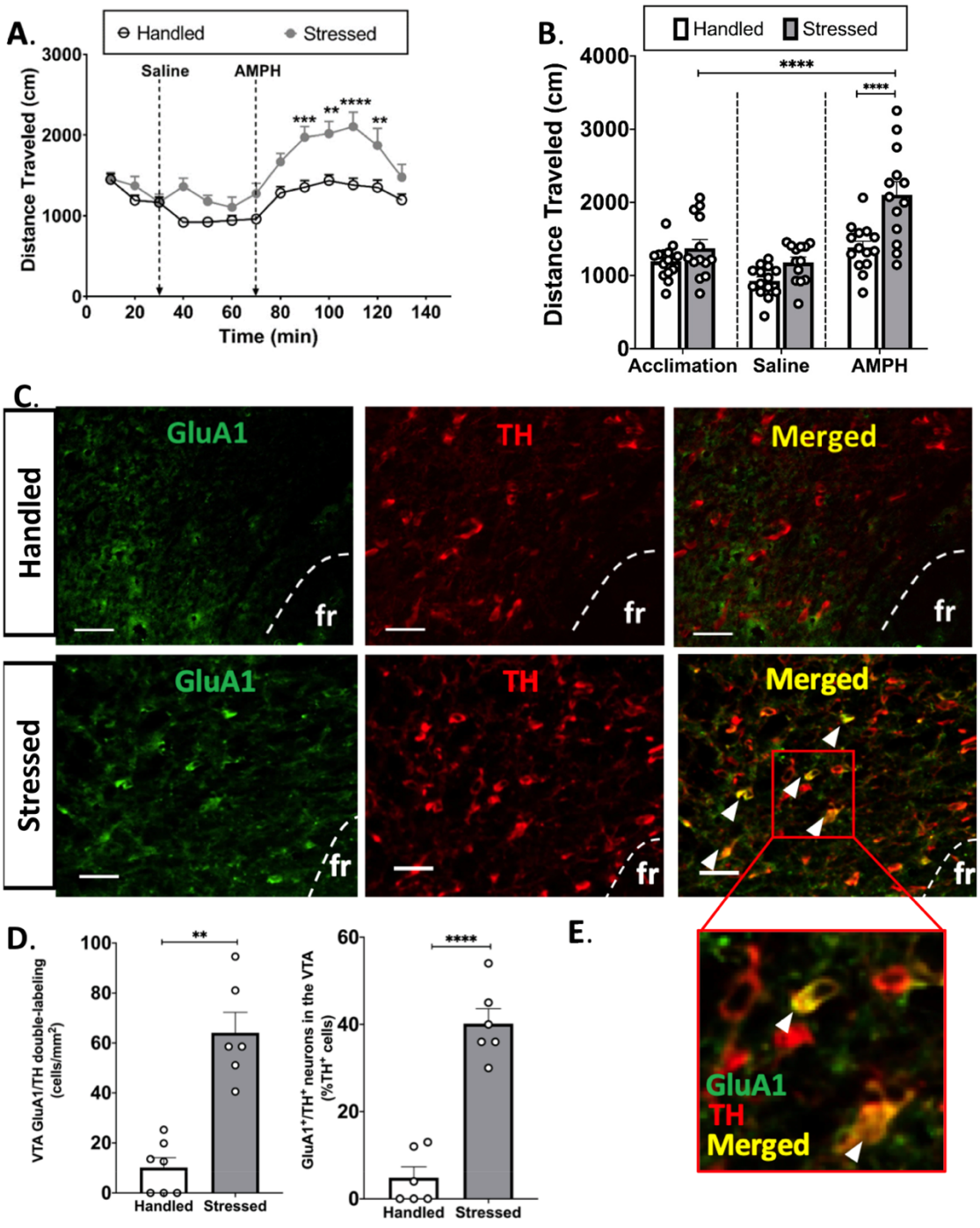
A.
Handled
Stressed
Saline
AMPH
Distance Traveled (cm)
Time (min)
B.
Handled
Stressed
Distance Traveled (cm)
Acclimation
Saline
AMPH
C.
Handled
Stressed
GluA1
TH
Merged
fr
D.
VTA GluA1/TH double-labeling (cells/mm²)
GluA1⁺/TH⁺ neurons in the VTA (%TH⁺ cells)
Handled
Stressed
E.
GluA1
TH
Merged

Figure 2.2. Intermittent social defeat stress induces higher GluA1 expression in VTA dopamine neurons, which occurs concomitantly with stress-induced AMPH cross-sensitization.

(A) Locomotor activity (total distance traveled in cm) over time before and after saline, and following AMPH administration (1.0 mg/kg, i.p.). Injection times are denoted by vertical arrows. Stressed animals had significantly higher locomotor activity compared to handled animals (***$p<0.0005$, **$p<0.005$). (B) There was no difference in locomotor activity between baseline (acclimation) and after saline injections, but stressed rats traveled significantly more than handled rats in response to the AMPH challenge, which was significantly higher than distance traveled during baseline (****$p<0.0001$). (C) Representative images of fluorescent GluA1 labeling (left), TH labeling (center), and GluA1/TH double labeling (right) in handled (top) and stressed (bottom) animals; bar = 50μm; arrow: GluA1/TH double-labeled cell. (D) GluA1/TH double labeling in VTA is significantly higher in animals subjected to intermittent social defeat stress compared to handled animals (**$p<0.005$). (E) Zoomed in fluorescent image of GluA1/TH double-labeling (white arrow: GluA1/TH double-labeled cell).

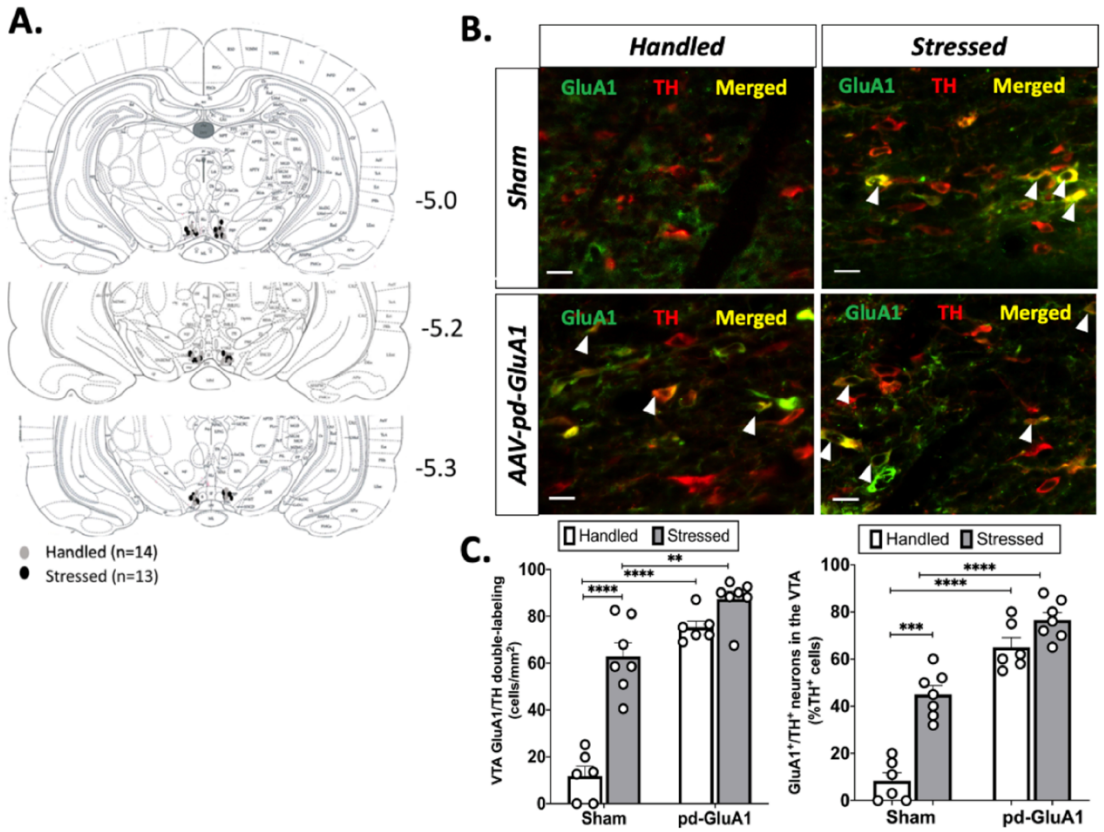

Figure 2.3. Verification of viral infusion sites: bilateral AAV-pd-GluA1 infusions into the VTA increases GluA1 expression in VTA DA neurons.

(A) Schematic depicting all viral infusion sites between -5.0mm and -5.3mm from bregma (n=14 handled rats, n=13 stressed rats). (B) Because GluA1 antibodies recognize both active and inactive GluA1 AMPARs, representative fluorescent images show higher GluA1/TH double-labeling in handled (left) and stressed (right) rats after cre-dependent AAV-pd-GluA1 infusions in TH-Cre rats (bar = 50μm; white arrow: GluA1/TH double-labeled cell). (C) GluA1/TH double labeling in VTA is significantly higher in animals subjected to intermittent social defeat stress compared to handled animals, and rats with AAV-pd-GluA1 infusions showed significantly more GluA1/TH double-labeling than sham animals (**p<0.005, *** p<0.0005, ****p<0.0001).

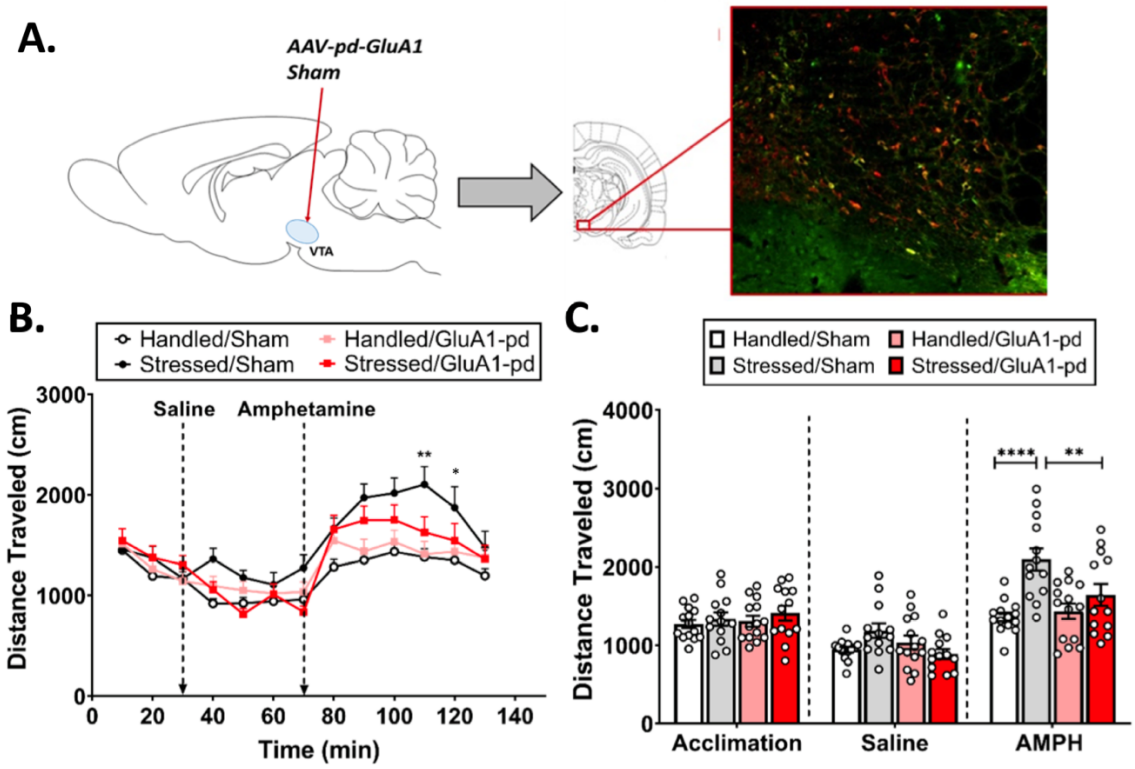

Figure 2.4. Functional inactivation of GluA1 in VTA dopamine neurons prevents stress-induced AMPH sensitization.

(A) Schematic that depicts the viral infusion site (left), and representative fluorescent GluA1/TH double-labeling (right) in rats with viral infusions. (B) Sham-Stressed rats traveled a significantly greater distance at 110 min (**p<0.005) and 120 min (*p<0.05) compared to all other groups. (C) There was no difference in average locomotor activity between groups during the acclimation period or after saline injections, but sham-stressed rats traveled a significantly (****p<0.0005; **p<0.005) greater distance than handled-sham rats and stressed-GluA1-pd rats.

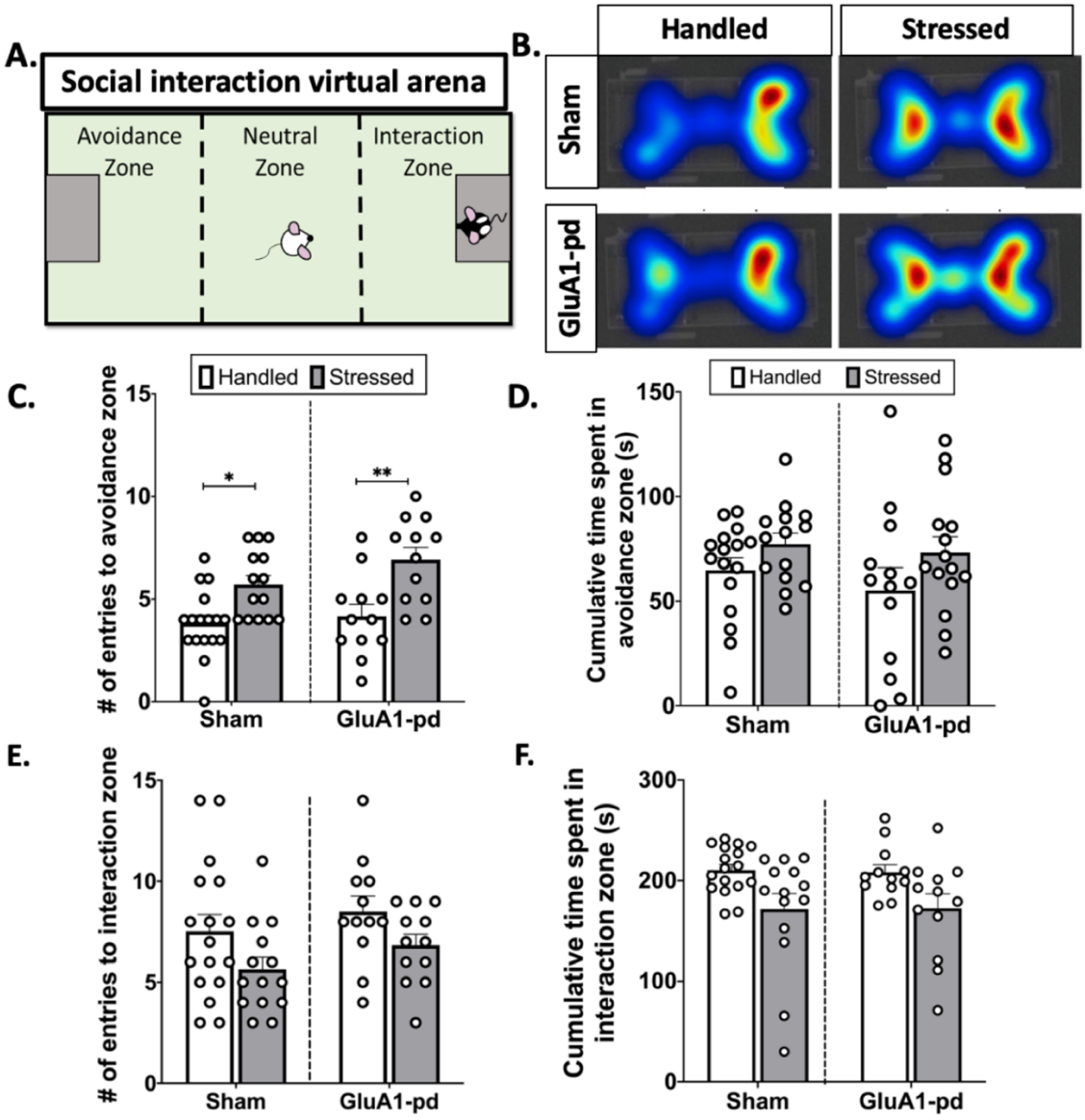

Figure 2.5. Functional GluA1 inactivation has no effect on social stress-induced social avoidance behavior. (A) Schematic of the social interaction test arena, showing the three-chamber cage separated into the avoidance zone (left), neutral zone (middle), and interaction zone (right). The experimental animal was placed into the neutral zone, and then Ethovision software recorded the number of times the animal entered the avoidance zone, neutral zone, and interaction zone, and the total duration of time spent in each respective zone. (B) Representative heat maps during the social interaction test, where warmer colors indicate more time spent. (C) Stressed rats made significantly more entries into the avoidance zone than handled rats (**p<0.005), but there was no difference in the number of entries into the avoidance zone between sham and AAV-pd-GluA1-infused rats (n=12-17 rats per group). (D) There was a trend

for a higher cumulative duration of time spent in the avoidance zone in stressed animals than handled animals, but no effect of AAV-pd-GluA1. (E) There was a trend for a higher frequency of entries into the interaction zone in stressed rats compared to handled rats, but no effect of AAV-pd-GluA1. (F) There was a trend for higher cumulative duration of time spent in the interaction zone in stressed animals than handled animals, but no effect of AAV-pd-GluA1.

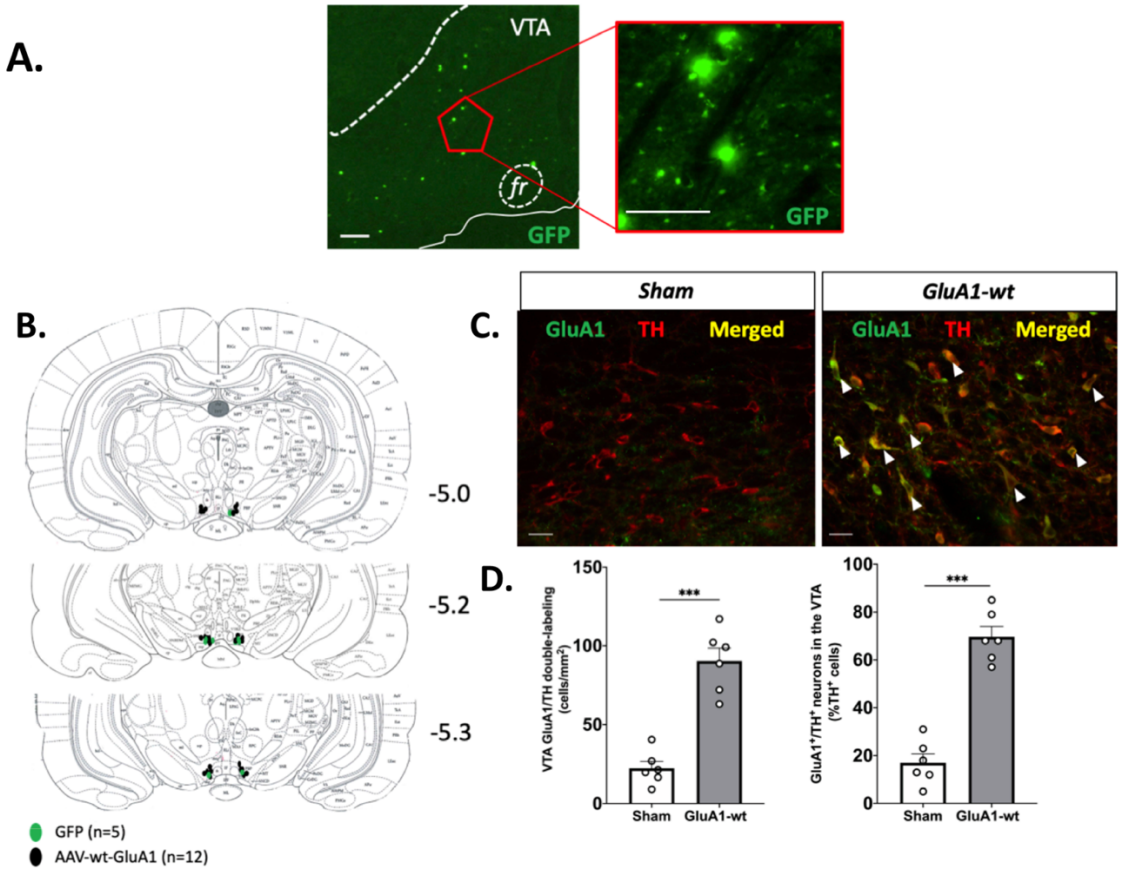

Figure 2.6. Verification of viral infusion sites: bilateral AAV-wt-GluA1 infusions into the VTA increases GluA1 expression in VTA DA neurons. (A) Representative fluorescent images showing GFP-labeled cells in the VTA of a control rat at 5x objective magnification (left; scale bar: 100μm) and 20x objective magnification (right; scale bar: 100μm). (B) Schematic that depicts all viral infusion sites between -5.0mm and -5.3mm from bregma in control (green; n=5) and experimental (black; n=12) rats. (C) Because GluA1 antibodies recognize both active and inactive GluA1 AMPARs, representative fluorescent images show higher GluA1/TH double-labeling in rats with cre-dependent AAV-wt-GluA1 infusions (right) compared to sham (left)TH-Cre rats handled (left) (bar = 50μm; white arrow: GluA1/TH double-labeled cell). (D) GluA1/TH double labeling in VTA is significantly higher in animals with AAV-wt-GluA1 infusions compared to sham rats (*** $p<0.0005$).

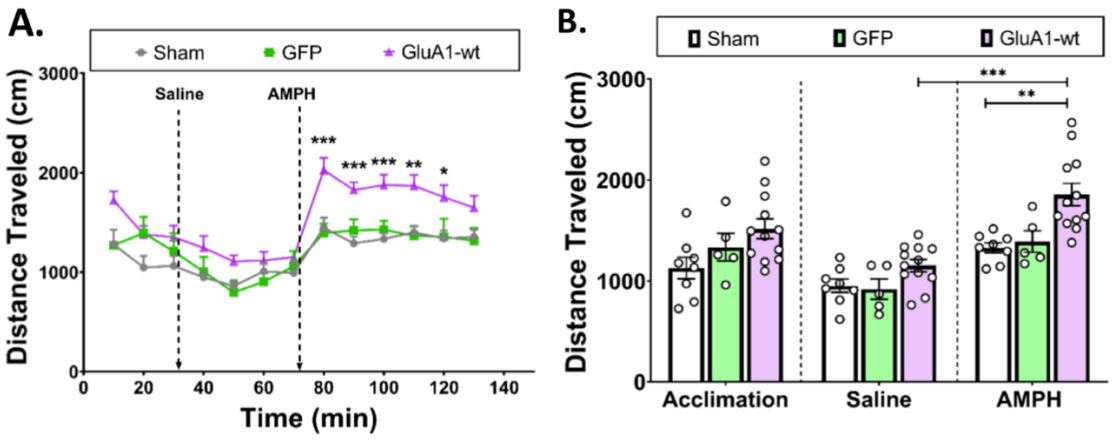

Figure 2.7. Functional GluA1 overexpression in VTA dopamine neurons by AAV-wt-GluA1 induces heightened locomotor activity in response to AMPH administration, even in the absence of intermittent social defeat stress.

(A) AAV-wt-GluA1-infused rats traveled a significantly greater distance than control groups 10-50 min after AMPH injection (***$p<0.0005$, **$p<0.005$, *$p<0.05$). (B) There was no difference in locomotor activity during baseline or after saline injections ($p>0.05$).

Chapter 3: MESOLIMBIC DYNAMICS UNDERLYING ESTROUS CYCLE-DEPENDENT DIFFERENCES IN SOCIAL STRESS-INDUCED AMPHETAMINE CROSS-SENSITIZATION IN FEMALE RATS

ABSTRACT

Studies reveal sex differences in drug use; females progress more rapidly from casual drug use to dependence and have shorter periods of abstinence that are exacerbated by stress. Furthermore, ovarian hormones fluctuate with female reproductive cycles and are thought to cause enhanced sensitivity to psychostimulants. Our recent data suggest that AMPA receptor subunit GluA1 in VTA dopamine neurons plays a critical role in the induction of stress-induced amphetamine (AMPH) cross-sensitization in *male* rats, and that females in proestrus/estrus are more sensitive to psychostimulants than those in metestrus/diestrus. In the present study, we found that social defeat stress exposure induced BDNF receptor TrkB expression in dopamine neurons of the VTA, with a more robust effect during proestrus/estrus, which occurred concomitantly with stress-induced AMPH cross-sensitization. Additionally, social defeat stress induced GluA1 expression in VTA dopamine neurons, as evidenced by a greater density of GluA1/TH double-labeling in VTA neurons of stressed compared to handling rats, independent of estrous stage. Rats with non-manipulated VTA GluA1 expression displayed cross-sensitization, or augmented locomotor response to low dose AMPH challenge (0.5 mg/kg, i.p.), whereas functional inactivation of VTA GluA1 AMPARs in DA neurons prevented stress-induced cross-sensitization to AMPH. By contrast, wildtype overexpression of GluA1 in VTA DA neurons had the opposite effect; locomotor-activating effects of AMPH were significantly augmented, even in the absence of stress. Taken together, these results suggest that there are differential mechanisms that play critical roles in stress-induced amphetamine cross-sensitization of female rats, and that GluA1 and TrkB in VTA dopamine neurons function concomitantly to play induce this sensitized response.

Introduction

Drug addiction is a costly and debilitating neuropsychiatric disorder that is characterized by excessive drug intake, drug seeking behavior, as well as continual cycles of abstinence and relapse (Reid et al., 2012). While both males and females become addicted to drugs, females transition more rapidly from casual drug use to dependence (Becker and Hu, 2008; Hernandez-Avila et al., 2004; Kosten et al., 1985), women have a more difficult time remaining abstinent (Becker & Koob, 2016). In addition, while human studies show that men are more likely to try cocaine than women, both sexes transition to addiction at an equal rate. Furthermore, women are more likely to try drugs at an earlier age (Chen & Kandel, 2002), take drugs in larger quantities (Randall, 1999; Brady & Randall, 1999), and report a more difficult time with relapse (Gallop et al., 2007). Although these sex differences in humans may be caused by a combination of psychosocial and cultural factors, these sex differences are also apparent in animal models, though the precise neurobiological mechanisms that drive these sex-differences in drug-reward processing remain poorly understood.

In addition to females showing higher rates of drug addiction than males, increasing evidence suggest that sex hormones moderate vulnerability to drug use in women. In humans, physiological responses to psychostimulants were reduced during the luteal phase of the menstrual cycle, when progesterone is primarily dominant; conversely, women had higher physiological responses to psychostimulants and reported feeling more “high” during the follicular phase, where estrogen levels are rising (Evans & Foltin, 2010; Evans et al., 2002; Justice & de Wit, 1999; Sofuoglu et al., 1999). In addition, accumulating evidence shows that estradiol is responsible for promoting drug-induced, reward-seeking behavior in women (Anker & Carroll, 2011; Quinones-Jenab & Jenab, 2010). In animal models, evidence shows ovarian hormone-dependent changes in drug reward functioning (Walker et al., 2001). Several studies have shown sex differences in the ability of estradiol to stimulate dopamine release, which could potentially act to drive the increased reward learning that happens during estrous (Becker & Cha, 1989; Becker et al., 1982). In addition, dopamine signaling in the NAc and heightened cocaine potency

are correlated with increased estradiol, not progesterone, indicating that psychostimulant effects are enhanced during proestrus/estrus (when estradiol levels are higher), and not met/diestrus (when progesterone is the predominant hormone) (Calipari et al., 2017). Taken together, females are more vulnerable to substance abuse and addiction, and these apparent sex-differences are largely due to fluctuating hormonal fluctuations, which may drive the differential responses to drugs of abuse.

Stress is also a critical factor that impacts the transition from recreational drug use to addiction, and has been correlated with substance abuse susceptibility, as well as relapse (Sinha, 2001; 2008; 2011). In particular, social stress is an etiological factor that augments the locomotor effect of psychostimulants in a phenomenon referred to as 'cross-sensitization' (Covington & Miczek, 2001; Nikulina et al., 2004, 2012), and leads to social avoidance behavior (Berton et al., 2006; Fanous et al., 2011; Komatsu et al., 2011). Previously, experiments on the effects of repeated social stress on mesolimbic pathways were predominantly conducted in male subjects; however, there is increasing interest in the effects of ovarian hormones on social stress in female rats. Chronic social defeat stress results in estrous cycle disruptions and attenuates psychostimulant-induced dopamine levels (Shinamoto et al., 2011). Furthermore, repeated social defeat stress in females increases susceptibility to social avoidance, anxiety-like behavior, as well as body weight reductions, which is likely facilitated by fluctuations in ovarian hormones (Takahashi et al., 2017).

Repeated social stress induces long-lasting behavioral changes (Nikulina et al., 2004; Covington et al. 2005), and prolonged activation of mesocorticolimbic pathways, which are comprised of dopaminergic neurons that originate in the ventral tegmental area (VTA) (Swanson et al., 1982). In addition, we recently found that intermittent social defeat stress increases α-amino-3-hydroxy-5-methyl-4-isoxazoleproionic acid (AMPA) receptor subunit, GluA1, expression in VTA dopamine neurons of male rats, which is also involved in the induction of social stress-induced amphetamine (AMPH) cross-sensitization (Wang et al., 2014; Rudolph et al., 2020). we know that intermittent social defeat stress increases brain-derived neurotrophic factor (BDNF)

expression in the VTA, which potentiates stress-induced psychostimulant cross-sensitization in male rats (Wang et el., 2013). In male rats, increased BDNF in the VTA is also associated with enhanced drug-seeking behavior, which occurs during withdrawal from cocaine self-administration (Grimm et al., 2003). Intra-NAc infusions of BDNF have been shown to rapidly increase AMPA receptor surface expression in male rats (Li and Wolf, 2011), and in females, BDNF receptor, tropomyosin receptor kinase B (TrkB), expression fluctuates with estrous stage, with highest expression during proestrus (Spencer et al., 2008). For these reasons, it is possible there is some relationship between BDNF/TrkB signaling and AMPAR GluA1 expression in the VTA, and we sought to investigate mesolimbic mechanisms that may drive these sex-dependent differences in stress-induced AMPH sensitivity.

Although evidence has shown a critical role of GluA1 in stress-induced psychostimulant cross-sensitization in male rats, the mesolimbic mechanisms of sex-dependent differences in stress-induced AMPH sensitivity of female rats remains unknown. To address this question, we first characterized the VTA neurons in which GluA1 induction occurs following social stress in female rats across different estrous stages. We then used Cre-inducible AAV-mediated gene transfer to bidirectionally manipulate GluA1 expression in VTA DA neurons, and assessed AMPH cross-sensitization and GluA1/TH and TrkB/TH co-expression in the VTA and compared the effects between different estrous stages to elucidate the role of ovarian hormones in the behavioral and neurochemical response to stress.

MATERIALS AND METHODS

3.1 Subjects

Experimental subjects were female homozygous Sprague Dawley TH-Cre rats (Sage Laboratories, MO) weighing 150-200 g during the start of experimentation. Two breeding pairs of homozygous TH-Cre Sprague Dawley rats were ordered from SAGE laboratory, where they were verified to be homozygous and to have no observed ectopic expression of cre (Sage Laboratories, MO). All experimental animals were bred onsite in the University of Arizona,

College of Medicine-Phoenix animal facility. All animals were maintained under a reverse light/dark cycle (12h: 12h, lights off at 9:00pm), with ad libitum access to food (Purina Rodent Diet, Brentwood, MO) and water. All female rats were subjected to daily vaginal smears, and vaginal cytology was performed to determine estrous cycle phase. Three days prior to the first social stress exposure, subjects were individually housed in standard plastic cages (25 x 50 x 20 cm^3). Female Long-Evans rats (weighing 300-350 g) termed "residents", were pair-housed with resident male Long-Evans rats (weighing 550-700 g) in larger plastic cages (37 x 50 x 20 cm^3). Lactating female residents were used to induce social defeat stress in the female experimental "intruder" TH-Cre rats. All experimental procedures were approved by the Institutional Animal Care and Use Committees at the University of Arizona. All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and all efforts were made to minimize pain and suffering and reduce the number of animals used.

3.2 Vaginal smearing

Daily vaginal smearing began once subjects reached sexual maturity (after 41 days) in order to monitor estrous cycle stages from the beginning of stress/handling procedures until the day of sacrifice. Vaginal secretions were collected by filling the tip of a 3-inch borosilicate glass medicine dropper with approximately 0.2 mL of sterile saline (0.9% NaCl), and inserting it shallowly into the rat's vagina to prevent pseudopregnancy (Hubscher et al., 2005; Marcondes et al, 2002). Sterile saline was then flushed quickly several times in and out of the vagina in order to accumulate the vaginal-secreted cells. Unstained wet vaginal smears were evaluated on a glass microscope slide and viewed under a Zeiss light microscope at 100x magnification to determine estrous stages. For all behavioral and statistical analyses, proestrous and estrous rats were grouped together, as well as metestrous and diestrous rats (Shimamoto et al., 2011), in part due to correlation with the human menstrual cycle. In addition, metestrous and diestrous stages tend to have fewer effects on behavioral and physiological outcomes (Keen-Rhinehart et al., 2009; Quinones-Jenab et al., 1999; Zhang, 2008). Furthermore, the proestrous stage occurs rapidly compared to other estrous stages and was most commonly observed in transition with diestrous or estrous stages.

3.3 Viral Vectors

Rats that were assigned to control viral groups received bilateral infusions of Cre-dependent adeno-associated viral (AAV) constructs that express green fluorescent protein (GFP) (AAV5-CMV-HI-eGFP-Cre-WPRE-SV40; AAV-GFP-Cre; viral titer: 1.864E+13 vg/mL; Penn Vector Core). Rats that were assigned to VTA GluA1 inactivation groups (experiment 2) received a Cre-dependent AAV that expresses dominant-negative pore-dead GluA1 (AAV5.2-hEF1a-GluA1-Q58IE-SV40PA; AAV-pd-GluA1; viral titer: 2.24E+13 vg/mL; Rachel Neve – Gene Technology Core, Massachusetts General Hospital), containing a single point mutation (Q581E) in the pore region. Rats that were assigned to functional GluA1 overexpression groups (experiment 3) received a Cre-dependent AAV that expresses wildtype GluA1 (AAV5.2-hEF1a-DIO-Rev-GluA1-wt-SV40PA; AAV-wt-GluA1; viral titer: 2.13E+13 vg/mL; Rachel Neve – Gene Technology Core, Massachusetts General Hospital). Both viral vectors manipulating GluA1 expression were previously validated in *vitro* and in *vivo* (Bachtell et al., 2008).

3.4 Bilateral intracranial viral infusions

After random group assignment, rats were anesthetized using 4% isofluorane and positioned in a stereotaxic frame (Leica Angle Two; Richmond, IL). The respective viral construct (1.0 µl each) was bilaterally infused into the VTA (AP -5.0, ML ±2.1, DV -8.2, tilt 10°; Paxinos and Watson, 2007) at a constant flow rate of 0.1 µl/min (Hamilton; Model 7105 KH; 24-gauge tip; Reno, NV). After infusion, the syringes remained in the infusion sites for 5 min to prevent retrace of the virus. Animals that were assigned to non-viral control groups received sham surgeries, in which they were anesthetized with 4% isofluorane and were positioned in a stereotaxic frame, but no surgery was performed. Rats recovered in their cages for 2-3 weeks to allow for optimal viral expression before the start of intermittent social stress or handling procedures.

3.5 Experimental design

We conducted three separate experiments that were specifically designed to elucidate the mechanisms underlying the sex-dependent mesolimbic mechanisms in VTA DA neurons in social

stress-induced AMPH sensitivity (N_1=48 subjects, N_2=94 subjects, N_3=26 subjects, N_4=16 subjects) (Fig. 2). To do this, we compared recently obtained data from male rats (Rudolph et al., 2020) to our novel data obtained in female subjects.

Experiment 1: Characterization of VTA neurons in which GluA1 induction occurs after intermittent social defeat stress in female rats across different estrous stages. Female rats were exposed to intermittent social defeat stress (n=13) or handling (n=10), then received d-AMPH sulfate (1.0 mg/kg males, 0.5 mg/kg females, i.p.; Sigma-Aldrich; St Louis, MO) 10 days after the last defeat or handling procedures. Brain tissue was collected and processed 5 days after the AMPH challenge, and GluA1/ Tyrosine Hydroxylase (TH) and TrkB/TH colocalization were assessed in the VTA (Fig. 3.1A).

Experiment 2: Effect of virus-mediated inactivation of GluA1 in VTA DA neurons on behavioral sensitization to AMPH. Rats were randomly assigned into one of four groups based on two experimental factors: virus (AAV-pd-GluA1 vs AAV-GFP) and behavioral treatment (handling vs intermittent social defeat). The groups were as follows: GFP-handled *(n=6),* GFP-stressed (*n=6*), AAV-GluA1-pd-handled *(n=10*), and AAV-pd-GluA1-stressed (*n=10*). These animals were given d-AMPH sulfate (0.5 mg/kg, i.p.; Sigma-Aldrich; St Louis, MO) while in proestrus/estrus, approximately 10 days after the last defeat or handling procedure. Animals were euthanized on day 42 after surgery, whereby brains were collected for processing. Locomotor activity was analyzed for the behavioral studies, and immunohistochemistry was performed to examine GluA1/TH or TrkB/TH double-labeling in the VTA (Fig. 3.1B).

Experiment 3: Effect of viral overexpression of GluA1 in VTA DA neurons on behavioral sensitization to AMPH in handled animals. In experiment 3, animals were randomly assigned into a group based on viral vector (AAV-wt-GluA1, AAV-GFP-Cre, or sham), and all animals were handled intermittently. The experimental groups were as follows: AAV-GFP-Cre (*n=7*), and AAV-wt-GluA1 (*n=6*). These subjects were challenged with d-AMPH sulfate (0.5 mg/kg, i.p.; Sigma-Aldrich; St Louis, MO) 10 days after the last handling procedure. Animals were euthanized on day

42 after surgery, and brains were collected for processing. Locomotor activity was analyzed, and GluA1/TH double-labeling in the VTA was assessed (Fig. 3.1C).

3.6 Intermittent social defeat stress and handling procedure

Female social defeat stress: maternal aggression model

Social defeat stress was induced by a short confrontation between an aggressive lactating female resident rat and an experimental intruder rat, as previously described (Holly et al., 2012). Social defeat stress procedure was performed in a separate room adjacent to the housing location. After removing the male from the resident's home cage, an experimental intruder rat was placed inside the resident's home cage, which contained pups aged 3-12 days postpartum, allowing the resident to attack the experimental rat until it displayed a submissive supine posture for at least 6 s, or for 5 min, whichever came first. Once this submissive posture was exhibited, the experimental rat was placed under a small metal protective cage (15 x 25 x 15 cm^3) for an additional 20 min before returning to its home cage. Intermittent social stress procedures were performed every third day for 10 days. On the same days as social stress, rats in the control groups were handled for 3-4 min and returned to their home cages.

3.7 AMPH challenge

To test for social stress-induced cross-sensitization, a low dose D-AMPH challenge was administered like previously described (Rudolph et al., 2020; Nikulina et al., 2012). Rats were injected with vehicle (0.9% sterile saline; 1.0 ml/kg, i.p.) numerous times prior to the challenge to acclimate them to i.p. injections. On the day of the AMPH challenge, rats remained in their home cage and were moved into the procedure room, where locomotor activity was recorded at 10 min intervals using Ethovision (Noldus; Sacramento, CA) video tracking software. Locomotor activity was recorded as the total distance traveled (cm) across 130 min consisting of 3 phases: Baseline (acclimation), Saline, and AMPH. Baseline locomotor activity was recorded for 30 min, after which a saline injection (1.0 ml/kg, i.p.) was administered, and locomotor activity was recorded for 40

min. Finally, rats received an injection of D-AMPH sulfate (0.1 mg/kg in males, 0.5 mg/kg in females, i.p.), and locomotor activity was recorded for an additional 60 min.

3.8 Tissue harvesting for immunohistochemistry

Estrous stages of female rats were taken 30-60 min prior to sacrifice. As previously described (Fanous et al., 2011), rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.; Euthasol, Virbac Co., St. Louis, MO) and perfused transcardially with 4% paraformaldehyde. Brains were extracted, post-fixed for 90 min at 4°C, and placed into graded sucrose solutions. Frozen brain tissue was sectioned on a cryostat (20 µm) and serial VTA sections (AP = - 5.0 to - 5.6, Paxinos and Watson, 2007) were mounted onto slides. Adjacent VTA slides from each brain were processed for either GluA1/ TH double-labeling, TrkB/TH double-labeling, or fluorescent localization of GFP expression.

3.9 Immunohistochemistry

To examine the cellular localization of GluA1 or TrkB expression in the VTA of rats that showed cross-sensitization, we performed fluorescent double labeling of GluA1 or TrkB and tyrosine hydroxylase. Rat VTA tissue was first washed in 0.05M potassium phosphate buffered-saline (KPBS), then blocked for 1 h in 10% normal goat serum (NGS) and 0.4% Triton X-100 in 0.05M KPBS. Sections were then incubated with primary antibodies; rabbit anti-GluA1 (ABN241, 1:500 dilution; Millipore; Temecula, CA) or rabbit anti-TrkB (R-149-100, 1:400 dilution; Biosensis; Thebarton, South and mouse anti-TH (SC-7837, 1:500 dilution; Santa Cruz Biotechnology; Santa Cruz, CA), were applied simultaneously and incubated at 4°C for 48 h. Slides were then incubated with biotinylated goat anti-rabbit IgG (1:1000 dilution, Vector Laboratories, CA) for 1 h, and then Alexa Fluor 488 Anti-Rabbit conjugated streptavidin and Alexa Fluor 647 Goat Anti-Mouse (1:1000 dilution; Invitrogen; San Diego, CA) were applied for 2.5 h. After washing with 0.05M KPBS, coverslips were applied with ProLong Diamond Antifade Mountant (Invitrogen, San Diego, CA).

3.10 Modified stereological cell counts

Tissue sections were imaged using a motorized Zeiss Axioscope with a 20x objective lens, and were digitalized using a color digital camera. GluA1/TH double-labeled cells were quantified using ImageJ software (NIH), and the analysis was conducted using a modified stereology counting procedure described in Fanous et al. (2011) and Nikulina et al. (2012). A grid of 30 squares (0.0075 mm^2) was superimposed on each of 2-3 VTA sections, bilaterally, from each subject. A random number generator was used to count yellow double-labeled cells in half the squares. Double-labeled cells were counted such that cells crossing the bottom or right lines of each square were included, while cells that crossed the top or left lines of the square were excluded from the analysis. For each subject, estimates of total double-labeling density (mm^2) were calculated by averaging the bilateral counts of double-labeled counts of cells across sections, then dividing the total number of counted cells by the total area that was assessed (0.111 mm^2). Furthermore, total TH neuronal population was approximated by averaging the bilateral counts of TH-labeled neurons across all sections, then dividing the total number of counted cells by the total area assessed (0.111mm^2). To account for any potential differences in tissue quality, the percentage of GluA1/TH or TrkB/TH double-labeled neurons out of the entire TH neuronal population was calculated by dividing the number of GluA1/TH or TrkB/TH double-labeled cells by the corresponding total number of TH-labeled cells, and then multiplying by 100 to attain the percentage.

3.11 Statistical analyses

Across all experiments, the results of each measure are expressed as mean ± standard error (SEM) and a p value ≤ 0.05 was considered significant. GraphPad Prism version 8 (GraphPad Prism for Microsoft; La Jolla, CA) was used to perform all statistical analyses, and Tukey's HSD was the preferred post hoc test across all experiments, except in the AMPH challenge, where we utilized the more conservative Fisher's LSD test. In experiment 1, a one-way ANOVA was used to analyze locomotor activity during the AMPH challenge, and a paired two-tailed t-test was used to analyze the double-labeling cell counts in stressed and handled animals. In experiment 2, a two-

way ANOVA was used to analyze locomotor activity and immunohistochemical data (between-subjects factors: viral vector (AAV-pd-GluA1 or sham) and behavioral treatment (handling or stress)). In experiment 3, two-way ANOVA was used to analyze locomotor activity and immunohistochemical data (between-subjects factors: viral vector (AAV-wt-GluA1, AAV-GFP-Cre, or sham)). For all experiments, data were excluded in the case of video tracking error (n=1), incorrect viral infusion site (n=4), or loss of data due to damaged tissue sections (n=8).

RESULTS

Experiment 1

Intermittent Social Defeat Stress Induces Cross-Sensitization to AMPH During Pro/Estrus, But Not Met/Diestrus.

In females tested during pro/estrus, a two-way ANOVA with multiple comparisons and a Fisher's LSD test revealed a main effect of AMPH (n=23, $F_{12,252}$=33.06, p<0.0001), a main effect of stress ($F_{1,21}$=5.06, p=0.0353), but no interaction between the two factors ($F_{12,252}$=1.097, p=0.3529; Fig. 3.2A). Post hoc analysis revealed that stressed rats displayed greater locomotor activity than did handled rats at 10 (p=0.0161), 20 (p=0.0035), 20 (p=0.0035), 30 (p=0.0042), 40 (p=0.0373), and 60 (p=0.0402) min after low-dose AMPH administration, but there were no differences across groups before or after saline administration (p>0.05 at all other time points). By contrast, in females tested during met/diestrus, a two-way ANOVA revealed a main effect of AMPH (n=18, $F_{12,192}$=21.72, p<0.0001), but no effect of stress ($F_{1,16}$=0.8705, p=0.3647), and an interaction between the two factors ($F_{12,192}$=1.832, p=0.0455; Fig. 3.2B). A significant effect of AMPH on locomotor activity was observed only at one timepoint, 10 min after AMPH administration, but no other effects of stress on locomotor response to AMPH or saline administration was observed in rats tested during met/diestrus (p>0.05 at all other timepoints).

Intermittent Social Defeat Stress Induces GluA1 expression in VTA Dopamine Neurons, Independent of Estrous Stage.

In females tested during pro/estrus and met/diestrus, a two-way ANOVA with multiple comparisons and a Tukey's post-hoc test revealed a significant main effect of stress on GluA1 expression in VTA DA neurons (n=18, $F_{1,14}$=26.64, p=0.0001), but no effect of estrus stage ($F_{1,14}$=0.4417, p=0.5171), or interaction between the two factors ($F_{1,14}$=0.3735, p=0.5509; Fig. 3.3A, 3B). In addition, a two-way ANOVA was performed to compare differences in %GluA1/TH double-labeling between different estrus stages and male rats in the rostral VTA. Again, a two-way ANOVA revealed a significant main effect of stress on %GluA1 neurons double-labeled with VTA DA neurons (n=18; $F_{1,14}$=245.8, p<0.0001), but no effect of estrus stage ($F_{1,14}$=0.3624, p=0.5568), or interaction between the two factors ($F_{1,14}$=0.06909, p=0.7965).

Intermittent social defeat stress has no effect on Tropomyosin-Related Kinase B expression in female rats, which is dependent on estrus stage.

By contrast, a two-way ANOVA with multiple comparisons and a Tukey's multiple comparisons test revealed a significant main effect of estrus stage on TrkB expression in VTA DA neurons (n=17, $F_{1,13}$=13.50, p=0.0028), but no effect of stress ($F_{1,13}$=2.0, p=0.1759), or interaction between the two factors ($F_{1,13}$=0.8, p=0.3743). Specifically, post hoc analysis revealed that handled females cycling during pro/estrus express more TrkB in VTA TH neurons than handled females cycling in met/diestrus (p=0.0242). In addition, there was a trend toward significant interaction in the effects of estrus stage on the total percentage of TH+ neurons that express TrkB (Fig. 3.4A,C).

Experiment 2

Functional GluA1 inactivation in VTA DA neurons prevents social stress-induced AMPH sensitization in female rats

In female rats cycling during pro/estrus, AAV-pd-GluA1/stressed rats exhibited significantly less locomotor activity after AMPH challenge than did sham/stressed rats. A two-way ANOVA revealed significant main effects of experimental group (n=26; $F_{3,273}$=45.07, p<0.0001), and experimental timepoint ($F_{12,273}$=20.77; p<0.0001), but no interaction between the two factors

($F_{36,273}$=1.178, p=0.2328). Specifically, post hoc analysis revealed that stressed rats expressing the control viral construct (AAV-GFP) moved significantly more in response to AMPH compared to stressed rats expressing AAV-pd-GluA1 10 (p=0.0075), 20 (p<0.0001), 30 (p=0.0028), and 40 (p=0.0042) min after AMPH administration. There were no differences across groups before or after saline administration (p>0.05 across all timepoints). Additionally, there were no differences in locomotion in stressed compared to handled females like we would anticipate. To control for this and to verify the effects of stress on AMPH cross-sensitization in this group of rats with viral infusions, we added an additional 2 sham-stressed and 2-handled females to the cohort. A two-way ANOVA revealed significant main effects of experimental group (n=4; $F_{2,26}$=50.06, p<0.0001), experimental timepoint ($F_{12,26}$=17.01, p<0.0001), and an interaction between the two factors ($F_{2,26}$=2.927, p=0.0106). Specifically, post hoc analysis revealed that sham-stressed rats moved significantly more in response to AMPH compared to sham-handled rats 20 (p<0.0001), 30 (p=0.0008), and 40 (p=0.0424) min after AMPH administration (Fig. 3.7).

Experiment 3

Wildtype GluA1 overexpression in VTA DA neurons mimics the effects of intermittent social defeat stress on AMPH cross-sensitization in female rats

Comparing the effect of AMPH challenge in handled rats with or without prior cre-dependent AAV-wt-GluA1 infusions revealed a significant main effect of experimental group (n=27, $F_{2,308}$= 47.04, p<0.0001; Fig. 5B) and time-point on locomotor activity ($F_{12,308}$=11.34, p<0.0001), but no interaction between the two factors ($F_{24,308}$=0.8694, p=0.6446). Specifically, rats with GluA1 overexpression had significantly more locomotor activity than control sham and handled/GFP rats at 10 (p=0.0003), 20 (p=0.0009), 30 (p=0.0008), 40 (p=0.0044) and 50 min (p=0.0145) after AMPH challenge. There was no difference in locomotor activity over time in the control sham and handled/GFP groups (p>0.05). Additionally, comparison of the average distance traveled during acclimation, after saline treatment, and after AMPH challenge in the three different experimental groups by two-way ANOVA revealed a significant main effects of experimental group

($F_{2,22}$=6.295, p=0.0069) and time-point ($F_{2,44}$=52.78, p<0.0001), as well as an interaction between the two factors ($F_{4,44}$=3.182, p=0.0222; Fig. 5C).

DISCUSSION

In our study, we assessed several potential mesolimbic mechanisms underlying sex-dependent differences in stress-induced psychostimulant sensitivity. The present results demonstrate that females are more sensitive to the effects of stress on psychostimulant sensitization than males, and that they have a higher percentage of dopamine neurons that express GluA1 AMPARs, which are independent of estrus stage. By contrast, females express more TrkB in VTA DA neurons than male rats, and across different estrus stages, independent of intermittent social defeat stress. In addition, the virus-mediated knockdown of GluA1 expression in VTA DA neurons prevents social stress-induced AMPH cross-sensitization, and overexpression of wildtype AMPAR GluA1 in VTA DA neurons augments the locomotor response to AMPH, mimicking the effects of social stress, like it does in males. These results suggest that GluA1, specifically in VTA DA neurons, is necessary and sufficient to induce stress-induced sensitization to AMPH in male and female rats, and BDNF-TrkB signaling could potentially play a role in the ovarian hormone-mediated response in females. To our knowledge, this is the first study to investigate sex-specific mesolimbic mechanisms, specifically in the VTA, in social stress-induced psychostimulant sensitivity in rats.

Clinical studies have shown that women are more vulnerable to drug addiction (Haas and Peters, 2000; Lynch et al., 2002; UNODC, 2018), and they are suggested to be more susceptible to cocaine use than men (Caine et al., 2004; Cummings et al., 2011). In addition to women showing higher propensity to drug addiction than men, there are clear menstrual cycle-dependent fluctuations in cocaine effects and the effects of drug craving; women have described a greater “high” from cocaine administration during the follicular phase, when estrogen levels are rising (Evans & Foltin, 2010; Evans et al., 2002). Furthermore, in rats, previous work has shown that dopamine signaling in the NAc and heightened cocaine potency are correlated with increased estradiol, indicating that psychostimulant effects are enhanced during proestrus/estrus (when

estradiol levels are higher), and not met/diestrus (when progesterone is the predominant hormone) (Calipari et al., 2017). In accordance with such results, we observed that female rats showed significantly greater stress-induced locomotor sensitivity to AMPH compared to male rats (despite receiving a lower dose), specifically during proestrus/estrus, thus displaying a more sensitized stress response than males during periods of high estradiol.

In rodents, psychostimulant administration increases glutamate transmission in portions of the mesocorticolimbic pathway involved in behavioral sensitization to psychostimulants, including the NAc and VTA (Xue et al., 1996; Reid et al., 1997; Del Arco et al., 1998; Wolf & Xue, 1999). The VTA is heterogeneous in topography and cellular composition, and is comprised of 60-65% dopaminergic, approximately 35% GABAergic, and a small percentage of glutamatergic neurons (Nair-Roberts et al., 2008). This heterogeneity is further complicated by the fact that VTA DA neurons may co-release glutamate (Stuber et al., 2010; Zhang et al., 2015) or GABA (Tritsch et al., 2012; Stamatakis et al., 2013). VTA glutamate input mostly arises from prefrontal cortical regions to modulate VTA DA neurons. Repeated stress has been shown to increase neuronal activity of VTA DA neurons which project to the NAc (Tidey & Miczek, 1997), as well as enhance glutamatergic synaptic plasticity through NMDA receptors (NMDARs) in the VTA (Stelly et al., 2016). In females,

Our results also show that stressed females display higher GluA1 expression in VTA DA neurons than handled females, exhibited by immunohistochemical data that displays higher GluA1/TH double-labeling in stressed females compared to handled. Because stress induces glucocorticoid release, which acts on mesolimbic DA neurons to exacerbate the locomotor effects of addictive drugs (Miczek et al., 2008), it is likely this, along with glutamatergic potentiation of VTA DA neurons, which drives stress-induced sensitization to AMPH. While there was no difference between males and females in the raw number of GluA1/TH double-labeled cells, females had a higher percentage of GluA1/TH double-labeled cells than males in the VTA. While little is known about mesolimbic dynamics underlying sex differences in stress-induced psychostimulant sensitivity, the higher percentage of GluA1/TH double-labeled cells in the VTA of

female rats is supported by Castro-Zavala et al. (2020), who showed that female mice have higher GluA1 expression in the NAc than male mice in response to cocaine self-administration. This could also be partially due to enhanced long-term potentiation (LTP) in female rats in response to stress and psychostimulant administration. Estrogen enhances LTP in hippocampal slices (Foy et al., 1999); therefore, estrogenic enhancement of glutamatergic circuits onto dopamine pathways could contribute to the enhanced response of proestrus/estrus females to stress-induced AMPH sensitivity and the heightened GluA1 expression in VTA DA neurons.

In addition, it could be that females experience enhanced glutamate signaling onto GluA1 AMPARs in the VTA, regulated by mesolimbic BDNF/TrkB signaling. In male rats, it has been shown that social stress increases VTA BDNF expression, which exerts its effects through binding to the TrkB receptor in the mesolimbic system, as knockdown of TrkB signaling in the NAc prevents cross-sensitization and stress-induced BDNF expression (Wang et al., 2013, 2014). TrkB expression in VTA hippocampal neurons fluctuates in *female* mice across the estrous cycle, with peak expression during proestrus/estrus, (Spencer et al., 2008). VTA BDNF modulates stress-induced psychostimulant sensitivity (Fanous et al., 2010), which supports our data found in male rats, that there is higher TrkB expression in VTA DA neurons as a result of intermittent social defeat stress. Contrarily, there was no effect of social defeat stress on BDNF receptor TrkB expression in female rats; there was, however, a result of estrus stage on BDNF receptor TrkB signaling, with a higher number of TrkB/TH labeled cells in the VTA of rats cycling in proestrus/estrus. In addition, we recently used a viral construct to bilaterally inactivate BDNF TrkB receptors in the VTA of female rats, which prevented cross-sensitization effects (Rudolph et al., 2019, unpublished data), despite a lack of effect of stress on TrkB/TH double-labeling in females. It could be that BDNF/TrkB signaling influences GluA1 expression in the VTA through intracellular signaling pathways, especially since intra-NAc infusions of BDNF rapidly increases GluA1 surface expression in male rats (Li & Wolf, 2011). This could explain why there is a differential expression of GluA1 in VTA DA neurons and TrkB in VTA DA neurons between male and female rats.

To further answer these questions, we preferentially inactivated GluA1 AMPARs in VTA DA neurons of sexually mature female rats, and we found that bilateral intra-VTA inactivation of GluA1 AMPARs prevented stress-induced AMPH sensitization in female rats cycling in pro/estrus (Fig. 3.6). This is supported by our previous data, which showed that social defeat stress increases GluA1 expression in VTA DA neurons of male rats, and this GluA1 is necessary for the induction of stress-induced AMPH sensitization (Wang et al., 2014; Rudolph et al., 2020). GluA1-dominant AMPARs are Ca^{2+}-permeable and are activated when glutamate occupies at least two of its binding sites (Wolf and Tseng, 2012). Phosphorylation of GluA1 at Ser^{831} by CaMKII and PKC during LTP facilitates delivery of GluA1-containing AMPARs to the synapse (Hayashi et al., 2000), and increases single channel conductance (Derkach et al., 1999). We know that GluA1 AMPARs play a critical role in drug-sensitization because the induction of psychostimulant sensitization is blocked by systemic AMPAR antagonists (Li et al., 1997; Zhang et al., 1997), and repeated cocaine treatment increases GluA1 expression in the VTA one day later (Di Chiara & Imperato, 1998; Churchill et al., 1999).

Because estradiol enhances dopaminergic signaling in the mesolimbic pathway of females as a result of stress and/or psychostimulant administration, it would make sense that corticolimbic glutamate signaling onto GluA1 AMPARs plays a critical role in the induction of stress-induced AMPH sensitivity in females as it does in males. We only performed this experiment in female rats cycling in proestrus/estrus because we previously found that there was no cross-sensitization during metestrus/diestrus, so we assume that preferential GluA1 inactivation would have no effect on cross-sensitization during this time. Importantly, while preferential GluA1 inactivation prevented cross-sensitization effects as a result of low-dose AMPH administration, we did not observe differentiation of locomotor response to AMPH between stressed and handled animals. We believe this is because females are exhibiting heightened stress response to surgery, leading to a ceiling effect and thus, no difference in locomotion between stressed and handled animals. To account for this, we included several stressed (n=2) and handled (n=2) females to the last cohort of animals to see if we can observe a stress

response to AMPH. When we did this, we did see that stressed females moved significantly more than handled females when administered a low dose of AMPH (Fig. 3.7). This suggests that female rats (at least during pro/estrus) are more sensitive to the effects of surgery-related stress, which induced a ceiling effect in handled-sham rats, and we could have potentially used a lower dose of AMPH to account for the added stress of surgeries.

When we overexpressed wildtype GluA1 AMPARs in VTA DA, we found that handled females cycling in proestrus/estrus exhibited significantly enhanced locomotor response to AMPH compared to control rats. This implies that the overexpression of GluA1 in VTA DA neurons is sufficient to mimic the effects of social stress on sensitization to psychostimulants in females, as well as males. Similarly, overexpression of wildtype GluA1 in regions of the VTA rich in DA neurons increases cocaine self-administration in male rats (Choi et al., 2011). Glutamate can then bind to these GluA1-homomeric Ca^{2+}-permeable AMPARs, thereby increasing intracellular Ca^{2+} signaling via calcium-calmodulin-dependent protein kinase II (CaMKII). CaMKII mediates LTP and is involved in molecular mechanisms of addiction in the mesolimbic pathway; blocking CaMKII in the VTA inhibits the acquisition of cocaine conditioned place preference, as well as cocaine-evoked synaptic plasticity in the NAc (Liu et al., 2014; Kourrich et al., 2007). It could be that overexpression of GluA1 in VTA DA cells drives the activation of intracellular CaMKII-dependent Ca^{2+} pathways, which induces potentiation of VTA-NAc DA neurons. In support of this, studies have shown that potentiation onto hippocampal neurons is largely driven by a CaMKII-mediated augmentation of GluA1 surface expression (Appleby et al., 2011); so not only does GluA1 AMPAR signaling induce heightened CaMKII activity, but CaMKII also drives further insertion of GluA1 into the cell membrane resulting in further synaptic potentiation. In females, estrogen has been shown to rapidly increase CaMKII activity in the mouse hippocampus, and modulates its activity in rats' dopaminergic pathways, which is involved in behavioral supersensitization to cocaine (Bernier et al., 2002; Zhen et al., 2007). The combined effects of CaMKII on GluA1 AMPARs in the mesolimbic system, and estrogen's additive effects on resulting

plasticity are potential mechanisms for wt-GluA1-induced AMPH sensitivity results, as well as the more robust effects of GluA1 wildtype GluA1 AMPAR expression.

In summary, female rats are more sensitive to the effects of intermittent social stress on AMPH sensitization, specifically during periods of high circulating estradiol. Female rats specifically cycling in proestrus/estrus, are more sensitive to the effects of psychostimulant administration, which is partially due to estrogen's effects on ERβ receptors, which influence dopamine signaling in the mesolimbic pathway (Calipari et al., 2017). We previously showed that GluA1 AMPARs in VTA DA neurons play a critical role in the induction of stress-induced AMPH sensitivity in males, which we believe is due to enhanced enhanced corticolimbic signaling onto VTA DA neurons, which project to the NAc (Wang et al., 2014; submitted in Rudolph et al., 2020). The present results show that GluA1 AMPARs in VTA dopaminergic neurons play an essential role in females' enhanced stress-induced AMPH sensitivity, and it is possible that this is modulated through mesolimbic BDNF-TrkB signaling.

Much of the work on the role of glutamate signaling in drug addiction and sensitization has been focused on the NAc, which has reciprocal projections to the VTA. Furthermore, much of the addiction-related research has been focused on males, so very little is understood about the mesolimbic dynamics underlying stress-induced drug addiction susceptibility in females. Further studies are necessary to identify the precise role of BDNF/TrkB signaling in female rats, potentially through cre-inducible viral mediated gene transfer to preferentially manipulate TrkB function in VTA DA neurons. Additionally, due to the heterogeneous nature of the VTA, GluA1 and BDNF/TrkB viral manipulations in other cell types, such as GABAergic cells, would inform us whether these effects are specific to DA neurons in females.

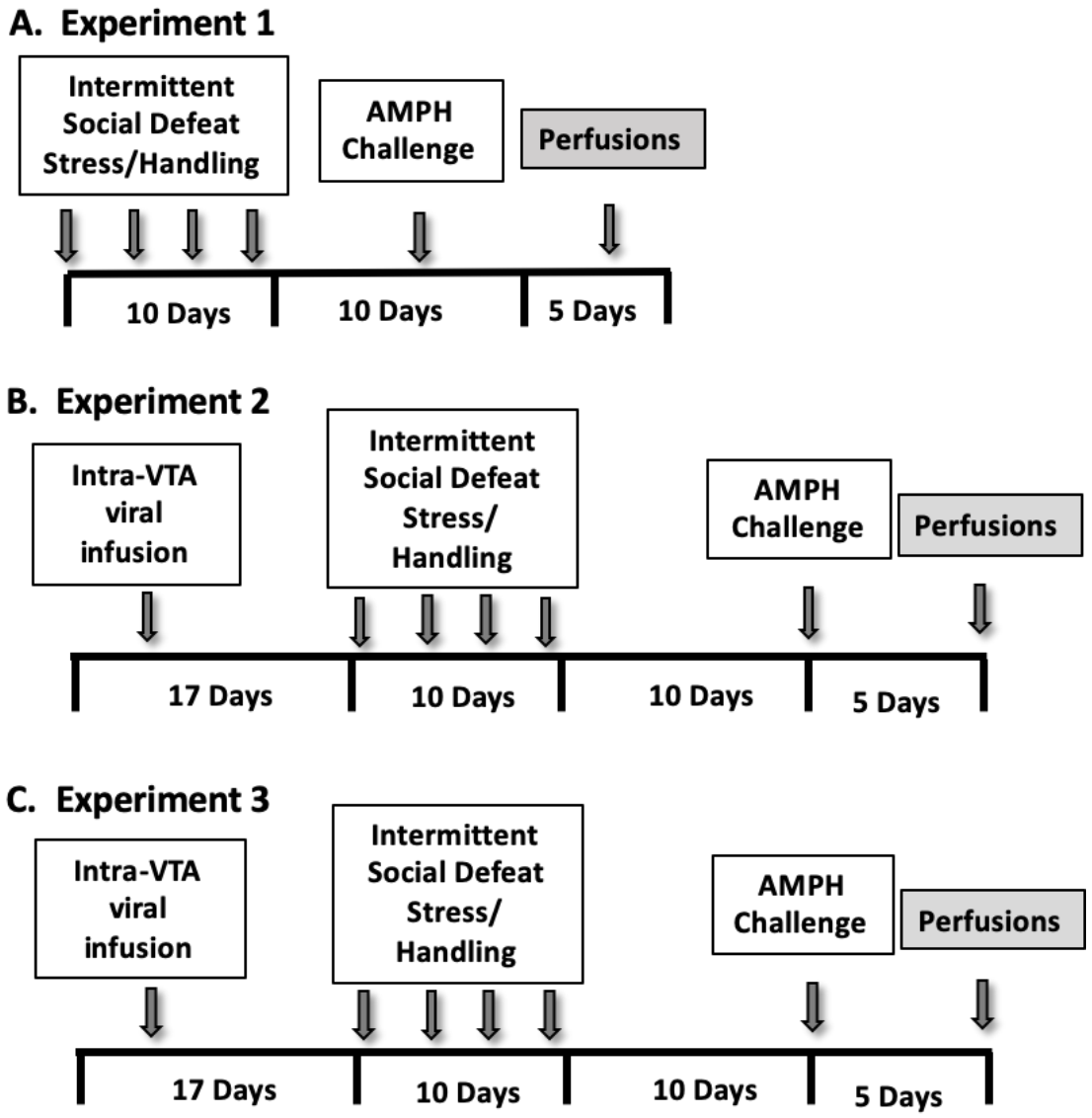

Figure 3.1. Schematic timelines of experimental design. (A) Experiment 1: Characterization of VTA neurons in which GluA1 induction occurs after intermittent social defeat stress. (B) Experiment 2: Effect of virus-mediated inactivation of GluA1 in VTA dopamine neurons on behavioral sensitization to AMPH. Experiment 3: Effect of viral overexpression of GluA1 in VTA dopamine neurons on behavioral sensitization to AMPH in handled animals.

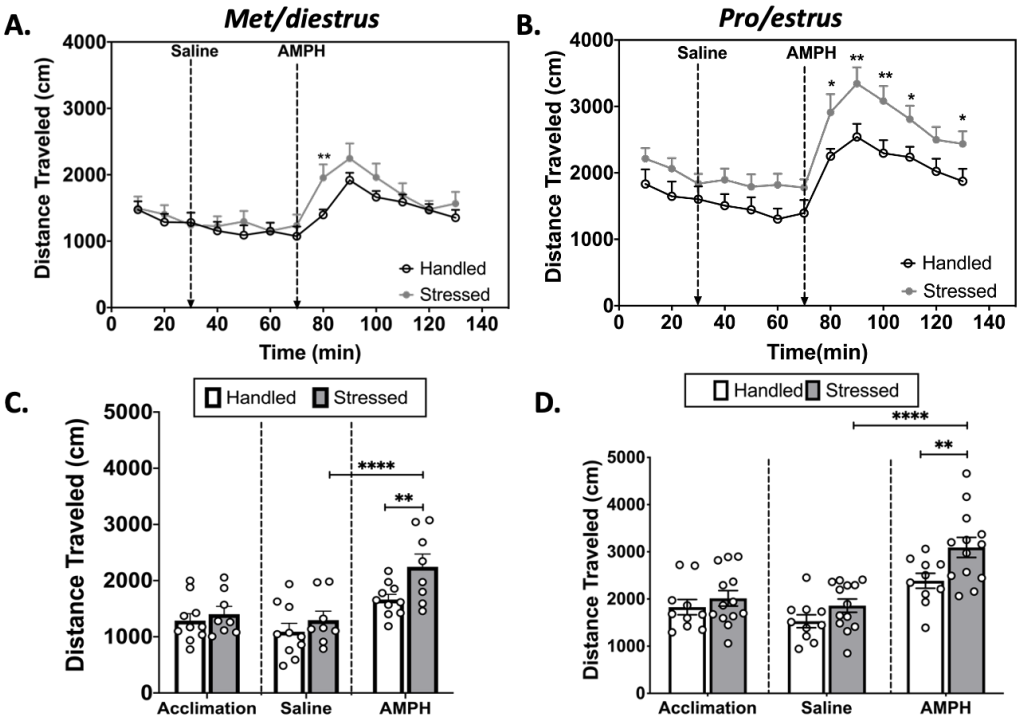

Figure 3.2. Intermittent social defeat stress induces cross-sensitization to low-dose AMPH administration, with a more robust effect during pro/estrus compared to met/diestrus. (A) Locomotor activity (total distance traveled in cm) over time before and after saline, and following AMPH administration (0.5 mg/kg, i.p.) in rats cycling in met/diestrus. Injection times are denoted by vertical arrows. Stressed animals had significantly higher locomotor activity compared to handled animals only 10 min after AMPH administration (**p<0.005). (B) Locomotor activity over time before and after saline, and following AMPH administration (0.5 mg/kg, i.p.) in rats cycling in pro/estrus. Stressed animals traveled significantly more than handled animals after low dose AMPH administration (*p<0.05, **p<0.005). (C) In female rats cycling in met/diestrus, there was no difference in locomotor activity between baseline (acclimation) and after saline injections, but stressed rats traveled significantly more than handled rats in response to the AMPH challenge, which was significantly higher than distance traveled after saline (****p<0.0001). (D) In female rats cycling in met/diestrus, there was no difference in locomotor activity between baseline (acclimation) and after saline injections, but stressed rats traveled significantly more than handled rats in response to the AMPH challenge, which was significantly higher than distance traveled after saline (****p<0.0001).

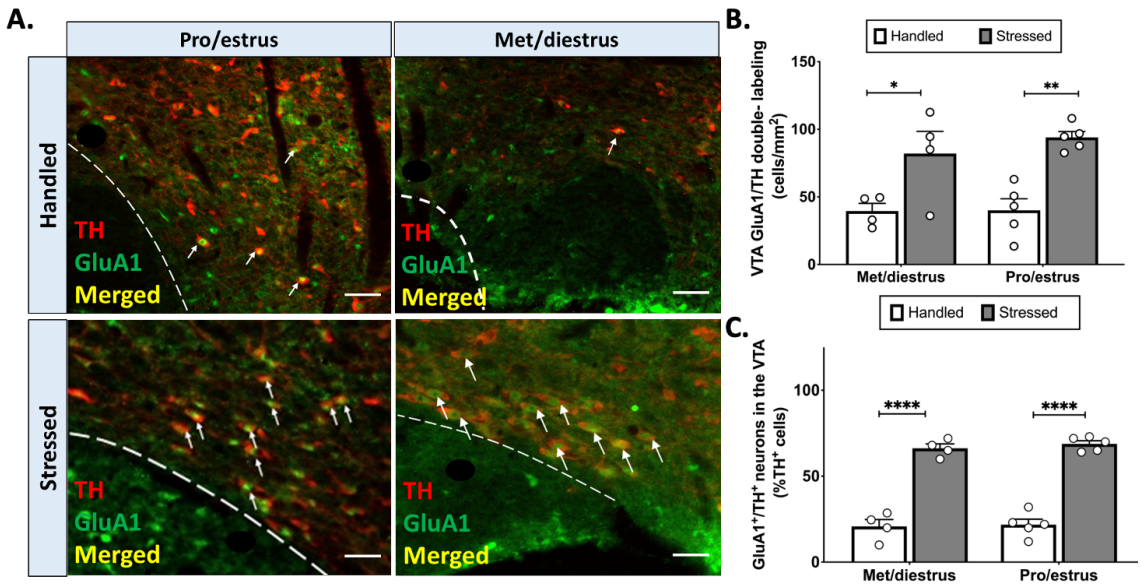

Figure 3.3. Intermittent social defeat stress induces higher GluA1 expression in VTA dopamine neurons, which occurs concomitantly with stress-induced AMPH cross-sensitization, and is independent of estrous stage. A) Representative images of fluorescent GluA1/TH double labeling in handled (top) and stressed (bottom) rats cycling in proestrus/estrus(left) or metestrus/diestrus (right); bar = 50μm; arrow: GluA1/TH double-labeled cell. (B) GluA1/TH double labeling in VTA is significantly higher in animals subjected to intermittent social defeat stress compared to handled animals, but there are no differences between different estrous stages (*p<0.05, **p<0.005). (C) The % of GluA1-expressing neurons that are double-labeled with TH are significantly higher in stressed animals with no difference between estrous stages (****p<0.0001).

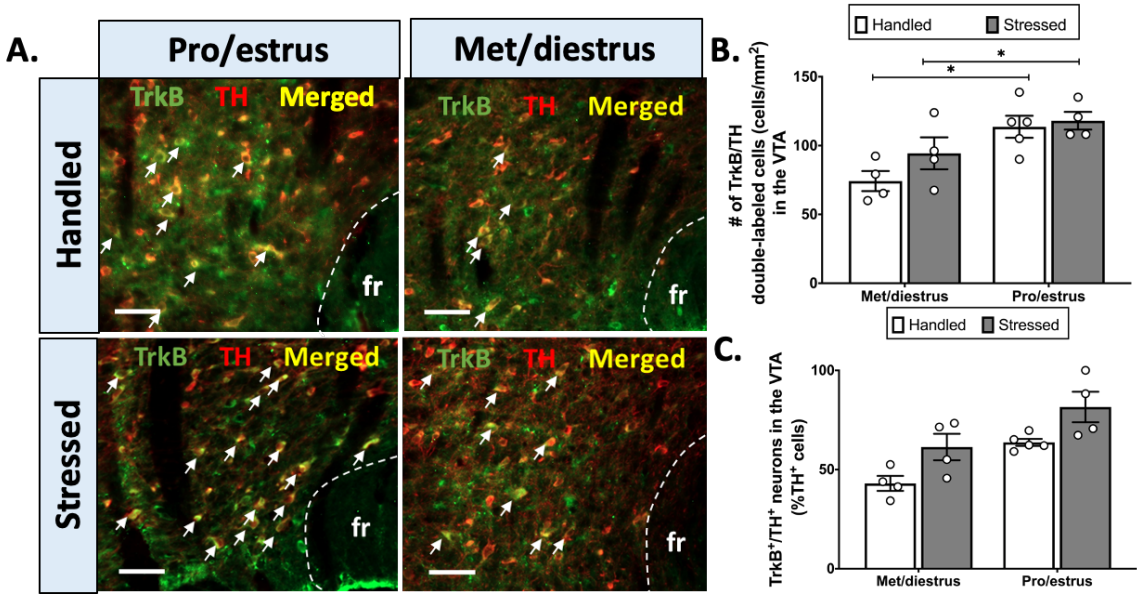

Figure 3.4. Female rats cycling in proestrus/estrus have higher TrkB expression in VTA dopamine neurons, but is independent of intermittent social stress. (A) Representative images of fluorescent GluA1/TH double labeling in handled (top) and stressed (bottom) rats cycling in proestrus/estrus(left) or metestrus/diestrus (right); bar = 50μm; arrow: GluA1/TH double-labeled cell. (B) Trk/TH double labeling in VTA is significantly higher in stressed rats cycling in pro/estrus compared to stressed rats in met/diestrus, as well as in handled rats cycling in pro/estrus compared to handled rats cycling in met/diestrus (*$p<0.05$), but no significant difference between stressed and handled animals ($p>0.06$). (C) The % of TrkB-expressing neurons that are double-labeled with TH have a trend toward being higher in rats cycling in pro/estrus compared to met/diestrus.

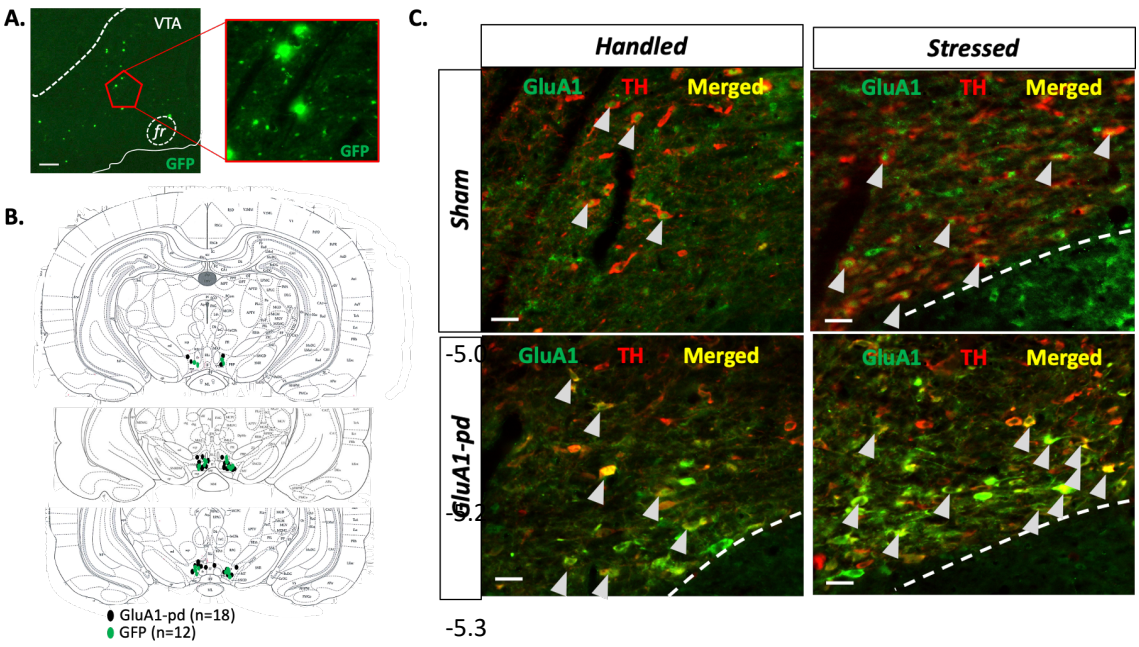

Figure 3.5. Verification of GluA1-pd infusion sites in VTA DA neurons. (A) Representative fluorescent images showing GFP-labeled cells in the VTA of a control rat at 5x objective magnification (left; scale bar: 100μm) and 20x objective magnification (right; scale bar: 100μm). (B) Schematic depicting all viral infusion sites between -5.0mm and -5.3mm from bregma (n=18 GluA1-pd rats, n=12 GFP rats). (B) Because GluA1 antibodies recognize both active and inactive GluA1 AMPARs, representative fluorescent images show higher GluA1/TH double-labeling in handled (left) and stressed (right) rats after cre-dependent AAV-pd-GluA1 infusions in TH-Cre rats (bar = 50μm; white arrow: GluA1/TH double-labeled cell).

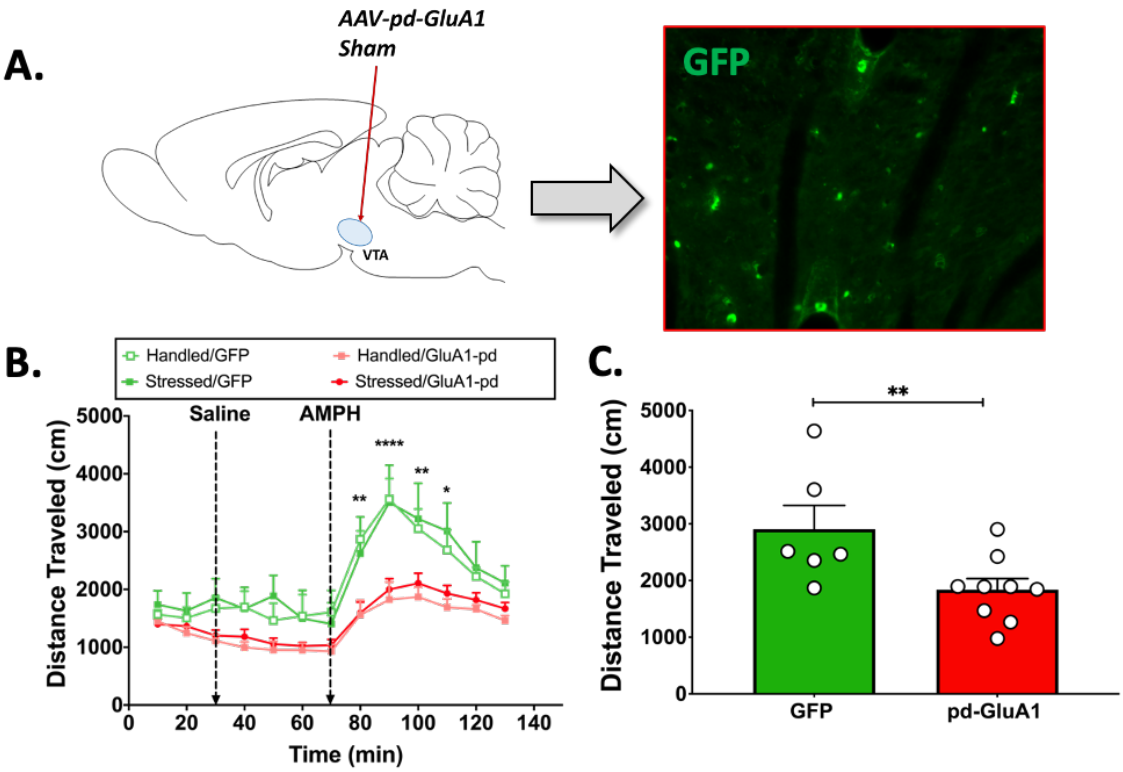

Figure 3.6. Functional inactivation of GluA1 in VTA dopamine neurons prevents stress-induced AMPH sensitization. (A) Schematic that depicts the viral infusion site (left), and representative fluorescent GFP labeling (right) in rats with viral infusions. (B) GFP-Stressed rats traveled a significantly greater distance at 80, 90, 100, and 110 min compared to GluA1-pd-Stressed rats (****p<0.0001; **p<0.005; *p<0.05). (C) On average, stressed-GFP rats traveled significantly more following AMPH administration than did pd-GluA1-stressed rats (**p<0.005).

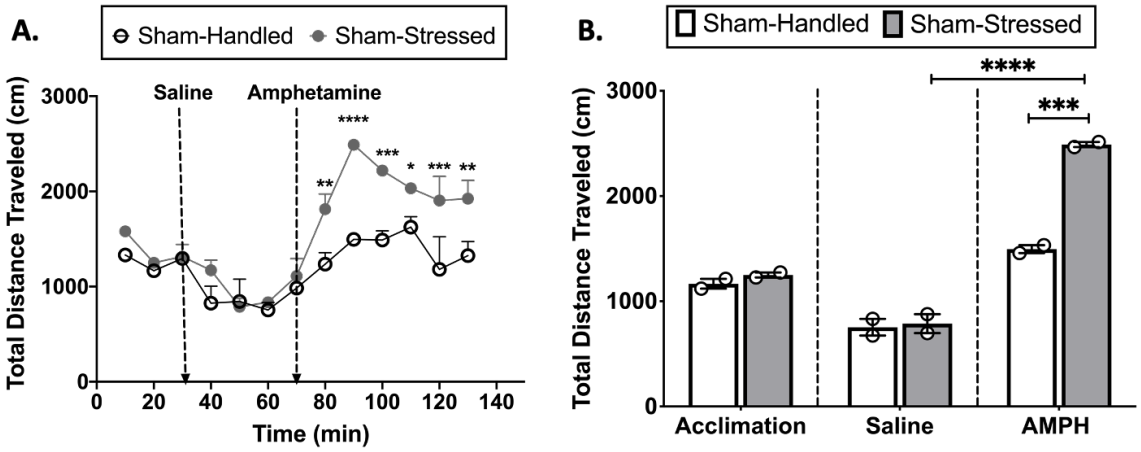

Figure 3.7. Verification of the effects of social stress on cross-sensitization effects in female rats in experiment 2: intermittent social defeat stress increases locomotor effects of AMPH compared to handled animals. To verify that the attenuation of stress-induced locomotion occurred as a result of viral construct and ensure we had differentiation of locomotion in stressed and handled animals, we performed the AMPH challenge in stressed and handled SHAM rats. (A) Locomotor activity over time before and after saline, and following AMPH administration (0.5 mg/kg, i.p.) in rats cycling in proestrus/estrus in experiment 2, without AAV-pd-GluA1 infusions. Rats subjected to social stress moved significantly more than handled rats at 80-130 min compared to handled rats (****$p<0.0001$; ***$p<0.0005$; **$p<0.005$; *$p<0.05$). (B) On average, sham-stressed rats moved significantly more after AMPH administration compared to sham-handled rats. There was no difference in locomotor activity between baseline (acclimation) and after saline injections, but stressed female rats traveled significantly more than handled rats in response to the low dose AMPH challenge, which was significantly higher than distance traveled after saline (****$p<0.0001$; ***$p<0.0005$).

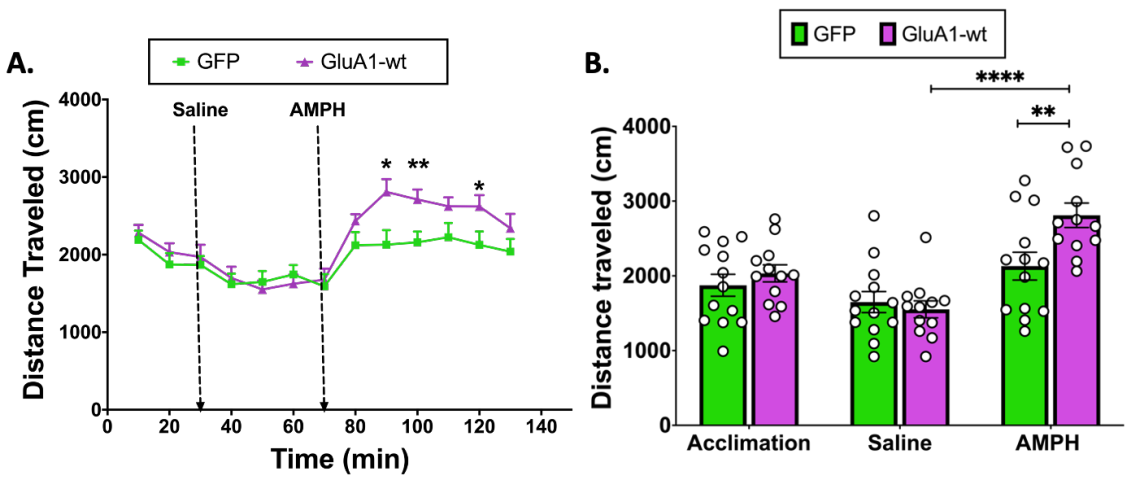

Figure 3.8. Functional overexpression of GluA1 by bilateral AAV-wt-GluA1 infusions in VTA dopamine neurons mimics the effects of stress on locomotor activity. (A) Locomotor activity over time before and after saline, and following AMPH administration (0.5 mg/kg, i.p.) in rats cycling in pro/estrus with or without AAV-wt-GluA1 expression. Rats expressing AAV-wt-GluA1 in VTA DA neurons traveled a significantly greater distance at 90, 100, and 120 min compared to AAV-GFP-expressing rats **p<0.005; *p<0.05). (B) On average, stressed-GFP rats traveled significantly more following AMPH administration than did pd-GluA1-stressed rats (**p<0.005). (B) There was no difference in locomotor activity between baseline (acclimation) and after saline injections, but female rats expressing AAV-wt-GluA1 traveled significantly more than control rats in response to the low dose AMPH challenge, which was significantly higher than distance traveled after saline (****p<0.0001; **p<0.005).

Chapter 4: ELUCIDATING THE NEURONAL CIRCUITRY UNDERLYING SOCIAL STRESS-INDUCED PSUCHOSTIMULANT CROSS-SENSITIZATION USING BIDIRECTIONAL WIRELESS OPTOGENETICS

ABSTRACT

Drug addiction is a significant concern in the United States, accounting for over 70,000 deaths annually. Stress is a factor that leads to addiction-related behaviors, including addiction vulnerability and behavioral sensitization to drugs of abuse. Our recent data show that glutamate AMPA receptor (AMPAR) GluA1 subunits in VTA dopamine neurons play a critical role in the stress-induced amphetamine (AMPH) sensitization response. In addition, we found that intermittent social defeat stress preferentially activates infralimbic (IL) cortical neurons that project to VTA dopamine neurons. However, the precise neural pathway that drives social stress-induced psychostimulant sensitization remains unknown. The present study utilized wireless optogenetics to determine the causal role of the IL-VTA pathway in the induction of this stress-induced sensitized response. AAVretro-ChR2 viral constructs, in conjunction with 473-nm pulsatile light stimulation of IL-VTA neurons, preferentially activated VTA dopamine (DA) neurons, as demonstrated by Fos/tyrosine hydroxylase (TH) double-labeling in the VTA of experimental rats. Furthermore, optogenetic inactivation of IL-VTA neurons through the utilization of AAVretro-JAWS, a novel inhibitory cruxhalorhodopsin, in the VTA in conjunction with 630-nm continuous light stimulation of the IL, prevented stress-induced cross-sensitization, or augmented locomotor response to low dose AMPH challenge (1.0 mg/kg, i.p.). In addition, this optogenetic inactivation of the IL-VTA pathway prevented mesolimbic signaling to the NAc, as demonstrated by higher Fos labeling in the NAc of control rats, rather than AAVretro-JAWS-expressing rats. This suggests that the IL-VTA pathway is crucial in mesolimbic signaling which drives stress-induced AMPH sensitization of rats. These novel findings could shed some light on potential pharmacotherapeutics in the treatment of stress-induced substance abuse susceptibility.

Introduction

With annual health-, productivity-, and crime-related costs of approximately $740 billion related to substance abuse, drug addiction represents a significant and persistent health concern in the United States (NIDA, 2014). Some observable characteristics of addiction that are frequently studied include drug sensitization, drug self-administration, and drug-seeking behavior. Sensitization induces augmented brain circuitry sensitivity to addictive drugs (Robinson & Berridge, 2001), and is believed to be an important characteristic of addiction. Converging evidence from human and animal models demonstrates that the effects of drug sensitization induce long-lasting neurochemical changes in the mesocorticolimbic pathway of the brain, which is comprised of dopaminergic neurons that originate in the ventral tegmental area (Boileau *et al.*, 2006; Castner & Williams, 2007; Vezina & Leyton, 2009).

Additionally, such evidence suggests that stress is a significant factor leading to addiction-related behaviors. Humans are more vulnerable to drug addiction, particularly psychostimulants, after enduring stressful life events (Sinha, 2008). Similarly, it has been shown in animal models that various types of stress can increase sensitivity and vulnerability to addictive drugs (Robinson *et al.*, 1985; Piazza *et al.*, 1990; Miczek *et al.*, 2004; Nikulina *et al.*, 2004; de Jong *et al.*, 2005). Drug sensitization is a phenomenon that is clearly observed and rapidly measured, has long-term effects, and does not need to be trained/taught, making it an ethologically relevant measure of addiction. For example, social stress has been shown to increase sensitivity to psychostimulants, and induce enduring cross-sensitization, with significant effects several months after the last episode of social stress (Covington & Miczek, 2001; Nikulina *et al.*, 2004).

Social defeat stress is a salient and unpredictable stressor where the defeated animal has no control during the conflict situation. In particular, intermittent social defeat stress in rats replicates the human response to stress and the resulting changes in social hierarchy, as rats do not habituate to recurrent episodes of social stress (Amat *et al.*, 2005). Similar to the persistence of sensitization which occurs after repeated drug exposure (Paulson *et al.*, 1991), intermittent

social defeat stress induces long-lasting changes in the mesocorticolimbic dopamine system, and enhances psychostimulant self-administration (Covington & Miczek, 2001; Nikulina *et al.*, 2004; Covington *et al.*, 2005). Additionally, social defeat stress increases VTA dopamine neuron firing rate and bursting patterns (Razzoli *et al.*, 2011), and induces heightened α-amino-3-hydroxy-5-methyl-4-isoxazoleproionic acid (AMPA) receptor subunit, GluA1, expression in the VTA of rats (Covington et al., 2008), which we recently found occurs predominantly in dopaminergic (DAergic) neurons, and is involved in the induction of social stress-induced amphetamine (AMPH) sensitization (Rudolph et al., 2020). Given that recent data show alterations in glutamatergic signaling (Wang et al., 2015), and that intermittent social defeat stress induces persistent ΔFosB expression selectively in IL neurons that project to the VTA, (Nikulina *et al.*, 2012), IL projection neurons could be the source of VTA glutamate whose function is selectively altered following intermittent social stress.

To address this question, we used wireless optogenetics to selectively inactivate the IL-VTA pathway in freely behaving rats that were subjected to intermittent social defeat stress or handling in order to identify whether this pathway drives social stress-induced AMPH cross-sensitization. To first identify whether we could specifically stimulate IL neurons that project to VTA dopamine (DA) neurons using optogenetics, we performed intra-VTA infusions of a retrograde-transporting AAV viral construct that expresses channelrhodopsin (ChR2) in male Sprague-Dawley rats. We then used 473-nm pulsatile LED light stimulation to stimulate this pathway, and then we analyzed Fos expression in VTA DA neurons. To investigate whether the IL-VTA pathway is *necessary* to drive stress-induced AMPH sensitization, we performed bilateral intra-VTA infusions of a retrograde-transporting AAV viral construct that expresses the inhibitory cruxhalorhodopsin, JAWS. We used 630-nm continuous light stimulation of IL-VTA neurons to inactivate this pathway in freely-behaving rats to see if this prevents stress-induced AMPH sensitization.

2. MATERIALS AND METHODS

2.1. Subjects

Experimental subjects were male Sprague Dawley homozygous tyrosine hydroxylase (TH)-Cre rats (Sage Laboraties, MO) weighing 200-250 g at the start of experimentation. Two breeding pairs of TH-Cre Sprague Dawley rats were ordered from SAGE laboratory, where they were verified to be homozygous and to have no observed ectopic expression of cre (Sage Laboratories, MO). All experimental animals were bred onsite in the University of Arizona College of Medicine-Phoenix animal facility, and were maintained under a reverse light/dark cycle (12h:12h, lights on at 9:00 am), with *ad libitum* access to food (Purina Rodent Diet, Brentwood, MO) and water. Three days prior to the first social stress exposure, subjects were individually housed in standard plastic cages (25 x 50 x 20 cm^3). Male Long-Evans rats (weighing 550-700 g) termed “residents”, were pair-housed with female Long-Evans rats in larger plastic cages (37 x 50 x 20 cm^3). Residents were screened for aggressive behavior as described previously (Nikulina et al., 2012), and were used to induce social defeat stress in the experimental “intruder” TH-Cre rats as described below. All experimental procedures were approved by the University of Arizona Institutional Animal Care and Use Committees. All studies were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011), and efforts were made to minimize pain and suffering and reduce the number of animals used.

2.2. Viral vectors

Rats that were assigned to control viral groups received bilateral intra-VTA infusions of retrograde-transporting adeno-associated viral (AAVretro) constructs that express green fluorescent protein (GFP) (AAVretro-hsyn-GFP; AAVretro-GFP; Addgene viral prep# 50465-AAVrg). Rats that were assigned to ChR2-mediated optogenetic activation groups received intra-VTA infusions of a retrograde-transporting AAV that expresses ChR2 (AAVretro-Syn-ChR2(H134R)-GFP; AAVretro-ChR2; Addgene viral prep# 58880-AAVrg), and rats that were assigned to Jaws-mediated optogenetic inhibition groups received intra-VTA infusions of a retrograde-transporting AAV that expresses Jaws (AAVretro-hsyn-Jaws-KGC-GFP-ER2; AAVretro-JAWS; Addgene viral prep# 65014-AAVrg). We specifically chose to use AAVretro-

JAWS, as it is a relatively novel cruxhalorhodopsin that is commercially available to transport retrogradely and is capable of powerful optical hyperpolarization by utilizing red light stimulation (Chuong et al., 2014).

2.3. Bilateral intracranial viral infusions and optic fiber implants

After random group assignment, rats were anesthetized using isofluorane and positioned in a stereotaxic frame (Leica Angle Two; Richmond, IL). Rats underwent two surgeries, approximately 6-7 weeks apart. First, the respective viral construct was bilaterally infused (1.0 µl per side) into the VTA (AP -5.0 to -5.3, ML ±2.1, DV -8.2, tilt 10°; Paxinos and Watson, 2007) at a constant flow rate of 0.1 µl/min (Hamilton; Model 7105 KH; 24-gauge tip; Reno, NV). After infusion, the needle remained at the infusion site for 5 min to prevent retrace of the virus. Rats recovered in their cages for 4-5 weeks to allow for optimal viral expression prior to the start of social stress or handling procedures. Second, rats were implanted with unilateral (TeleL-B-500-4.8) or bilateral (TeleLCD-R-4.8-500-1.7) LED optic fibers (500-µm core, 0.37 NA; Teleopto, Nagoya, Japan) just above the site of interest in the IL (AP +3.2 mm, ML +0.8 mm, DV -4.8 mm, tilt 10°; Paxinos and Watson, 2007) of rats in the optogenetic activation or inhibition experiments, respectively. Implants were secured to the skull with dental acrylic (Ortho-Jet BCA, B1320, Lang Dental, Wheeling, IL, USA). Viral infusion site and optic fiber implant localization was performed using fluorescent immunohistochemistry (described below) after each experiment to ensure accurate viral expression in medial/anterior portions of the VTA, which are known to have a high density of DA neurons (Morales & Margoles, 2017), as well as specific implant localization in the IL.

2.4. Experimental design

We conducted two separate experiments that were designed to elucidate the neural pathway that drives social stress-induced psychostimulant sensitivity in rats (Fig. 4.1).

Experiment 1: optogenetic activation of the IL-VTA pathway. In order to see whether we could use optogenetics to preferentially stimulate IL neurons that project to VTA DA neurons, rats were randomly assigned to one of two groups based on viral manipulation (AAVretro-ChR2 [*n=3*] vs AAVretro-GFP [*n=3*). These subjects were subjected to pulsatile light stimulation (pulse width, 15

ms; frequency, 10 Hz; 473-nm LED). Rats were euthanized 90 min after optogenetic stimulation, and Fos/Tyrosine Hydroxylase (TH) double-labeling in the VTA was assessed (Fig. 1a, b).

Experiment 2: Effect of optogenetic inhibition of IL-VTA neurons on behavioral response to AMPH in stressed and handled animals. Rats were randomly assigned to one of four groups based on two experimental factors: virus (AAVretro-GFP vs AAVretro-JAWS) or behavioral treatment (handling vs stress). The groups were as follows: AAVretro-GFP-handled (*n=6*), AAVretro-GFP-stressed (*n=5*), AAVretro-JAWS-handled (*n=6*); AAVretro-JAWS-stressed (*n=5*). These rats were subjected to AMPH challenge paired with continuous light stimulation (632-nm LED) approximately 10 days after the last instance of social defeat stress/handling, which is the length of time shown to produce long-term behavioral effects of intermittent social defeat stress in rats (Miczek et al., 2011; Covington and Miczek, 2001; Nikulina et al., 2004). Animals were perfused 90 min after the start of light stimulation and Fos expression was assessed in the VTA and NAc (Fig. 1a, c).

2.5. Intermittent social defeat stress and handling procedure

Social stress was induced by a brief confrontation between an aggressive resident rat and an experimental intruder rat, as previously described (Nikulina et al., 2012; Rudolph et al., 2020). This defeat procedure was performed in a sound-attenuated room to prevent ultrasonic stimuli from affecting unstressed subjects. After removing the female from the resident's home cage, an experimental intruder rat was placed inside the resident's home cage for 5 min under a small metal protective cage (15 x 25 x 15 cm^3). The protective cage was then removed, allowing the resident to attack the experimental rat until it displayed a submissive supine posture for at least 6 s, or for 5 min, whichever came first. The experimental rat was then placed back into the protective cage for an additional 20 min before returning to its home cage. The social stress procedure was performed every third day for 10 days, producing intermittent exposure to social defeat stress. On the same days as social stress, rats in the control groups were handled for 2-3 min and returned to their home cages.

2.6. Optogenetic stimulation and AMPH challenge

For both experiments, for three days prior to light stimulation, rats were habituated with dummy Teleopto receivers (2 g, TeleDummy, Bio Research Center) attached to the unilateral or bilateral LED cannula unit for 30min.

Experiment 1: optogenetic activation of the IL-VTA pathway in ChR2-expressing rats using 473-nm pulsatile light stimulation

First, to test the ability to preferentially stimulate neurons which project from the IL to VTA DA neurons, rats that expressed ChR2 or the control GFP viral construct in cell bodies of the IL were exposed to pulsatile 473-nm light stimulation. For these experiments, rats were allowed to recover from optic fiber implants for 7-8 days before optogenetic activation. On the day of light stimulation, an infrared light-driven wireless LED unit Teleopto receiver (2 g, TeleR-2-P, Bio Research Center) was attached to the unilateral LED cannula unit in the home cage, and rats were placed in the behavior room. Rats first acclimated to this wireless receiver for 20 min, after which rats received pulsatile light stimulation (pulse width, 15 ms; frequency, 10 Hz; 473-nm LED) for 20 min (1 min on and 1 min off for 10 cycles) to stimulate IL-VTA neurons. These stimulation settings are based on firing patterns of IL glutamatergic neurons and optogenetic stimulation of IL PFC neurons that reproduce these firing patterns (Fuchikami et al., 2015). The delivery of these light pulses was controlled by a programmable stimulator (STOmk-2, Teleopto, Nagoya, Japan). The optical intensity was measured prior to each experiment to ensure consistent output of ~5 mW measured at the tip of an optic fiber.

Experiment 2: Effect of optogenetic inhibition of IL-VTA neurons on behavioral response to AMPH in stressed and handled animals.

To identify whether the IL-VTA pathway is necessary to drive stress-induced AMPH sensitivity in male rats, 632-nm pulsatile light inhibition was paired with a low dose of AMPH, administered as previously described (Nikulina et al., 2012). On the day of light stimulation, an infrared light-driven wireless LED unit Teleopto receiver (2 g, TeleR-2-C, Bio Research Center) was attached to the bilateral LED cannula unit (implanted in the IL). Rats were injected with vehicle (0.9% sterile saline; 1.0 ml/kg, i.p. daily) on several days prior to the AMPH challenge to acclimate them to i.p.

injections. On the day of the AMPH challenge/light stimulation, rats were moved into the procedure room, where locomotor activity measured as total distance traveled (in cm) was recorded in square open field apparatus during sequential 10 min bins using Ethovision. To control for potential variability in locomotion due to the wireless receiver, baseline locomotor activity was first recorded for 20 min, after which a 0.9% saline injection (1.0 ml/kg, i.p.) was administered and locomotor activity was recorded for 30 min. Finally, rats received an injection of D-AMPH sulfate (1.0 ml/kg in saline vehicle, i.p.); after 10 min, an infrared light-driven remote controller was used to turn on the bilateral 630-nm LED optic fibers (20 min, continuous). These stimulation settings were selected based on paramaters known to induce optogenetic inhibition of rat prefrontal neurons in a similar experimental setup (Calu et al., 2013). Locomotor activity was recorded for 60 min following AMPH administration (30 min following the termination of light stimulation). The optical intensity was measured prior to each experiment to ensure consistent output of ~5 mW measured at the tip of an optic fiber.

2.7. Tissue harvesting for immunohistochemistry

Within 90 min after the start of optogenetic stimulation, all rats were deeply anesthetized with sodium pentobarbital (100 mg/kg, i.p.; Euthasol, Virbac Co., St. Louis, MO) and perfused transcardially with 4% paraformaldehyde, like described previously (Fanous et al., 2011). Brains were extracted, post-fixed for 90 min at 4°C, and placed into graded sucrose solutions. Frozen brain tissue was sectioned on a cryostat (20 μm) and serial IL (AP = + 3.0 to + 3.5, Paxinos and Watson, 2007), NAc (AP = + 1.0 to +1.5, Paxinos and Watson, 2007), and VTA sections (AP = -5.0 to -5.6, Paxinos and Watson, 2007) were mounted onto slides. Adjacent IL, NAc, and VTA slides from each brain were processed for either Fos, Fos/TH double-labeling, or fluorescent localization of GFP expression.

2.8. Immunohistochemistry

To determine the cellular localization of Fos expression in the IL, NAc, and VTA, we performed fluorescent double-labeling of Fos and TH in the VTA, and fluorescent single labeling of Fos in the IL and NAc. Sections were first washed in 0.05M potassium phosphate buffered-saline (KPBS), then blocked for 1 h in 10% normal goat serum (NGS) and 0.4% Triton X-100 in 0.05M

KPBS. *IL and NAc tissue*: In single-labeled tissue, sections were incubated in rabbit anti-cFos (2250, 1:2000 dilution; Cell Signaling; Beverly, MA) at 4°C for 48 h. Alexa Fluor 488 Goat Anti-Rabbit (1:1000 dilution, Vector Laboratories, CA) was then applied for 2.5 h. After washing with 0.05M KPBS, coverslips were applied with ProLong Diamond Antifade Mountant (Invitrogen, San Diego, CA). *VTA tissue*: double-labeled sections were incubated simultaneously in rabbit anti-cFos (2250, 1:2000 dilution; Cell Signaling; Beverly, MA) and mouse anti-TH (SC-7837, 1:500 dilution; Santa Cruz Biotechnology; Santa Cruz, CA) at 4°C for 48 h. Alexa Fluor 488 Goat Anti-Rabbit (1:1000 dilution, Vector Laboratories, CA) and Alexa Fluor 647 Goat Anti-Mouse (1:1000 dilution; Invitrogen; San Diego, CA) were applied simultaneously for 2.5 h. After washing with 0.05M KPBS solution, coverslips were then applied with ProLong Diamond Antifade Mountant (Invitrogen, San Diego, CA).

2.9. Modified stereological quantification

Tissue sections were imaged using a Zeiss Axioscope with a 20x objective lens, and were digitalized using a color digital camera. The number of Fos/TH double-labeled neurons was quantified using ImageJ software (NIH), and the analysis was conducted using a modified stereology counting procedure as described previously (Fanous et al., 2011a; Nikulina et al., 2012). A grid of 30 squares (0.0075 mm^2) was superimposed on 2-3 adjacent VTA sections bilaterally from each subject. Double-labeled neurons were counted in 15 grid random squares such that labeled cells intersecting the bottom or right lines of each square were included, while cells intersecting the top or left lines of the square were excluded from the analysis. Double-labeled cell density (in mm^2) was calculated by averaging the bilateral counts across sections, then dividing the total number of counted cells by the total area that was assessed (0.111 mm^2).

To count the number of Fos-labeled cells in the NAc shell after optogenetic inhibition, a square (4 mm^2) was superimposed on 2-3 adjacent NAc sections bilaterally from each subject using ImageJ software (NIH). All Fos-labeled cells were included, except those that intersected the edges of the superimposed square.

2.10. Statistical analyses

Across all experiments, the results are expressed as mean ± standard error (SEM) and a p value ≤ 0.05 was considered significant. GraphPad Prism version 8 (GraphPad Prism; La Jolla, CA) was used to perform all statistical analyses, and Tukey's HSD was the preferred post hoc test across all experiments, except in the AMPH challenge, where we utilized the more conservative Fisher's LSD test. In experiment 1, a paired two-tailed t-test was used to analyze Fos/TH double-labeled cell counts in rats expressing the control GFP viral construct or the experimental ChR2 viral construct after optogenetic stimulation. In experiment 2, a two-way ANOVA with multiple comparisons was used to analyze locomotor activity and immunohistochemical labeling density (between-subjects factors: viral vector (AAVretro-GFP or AAVretro-JAWS) and behavioral treatment (handling or stress)). In both experiments, data were excluded in the case of incorrect viral infusion sites (n=3), medical-related issue at the time of surgery or social defeat (n=3), or loss of data due to optogenetics technical error (n=4).

3. RESULTS

3.1. Experiment 1

Optogenetic excitation of the IL-VTA pathway induces cellular activation of VTA DA neurons.

To evaluate DA neuron activation in the VTA following optogenetic stimulation of the IL, Fos/TH double-label fluorescent immunohistochemistry was performed in the rostral VTA (Fig. 4.2A). A paired t-test was performed to compare the mean number of Fos-expressing DA neurons in in control rats (expressing AAVretro-GFP) and experimental rats (expressing AAVretro-ChR2) following 473-nm light stimulation. There was a trend toward more Fos/TH double-labeled cells in the rostral-medial VTA in GFP rats compared to ChR2 rats (n=6).

3.2. Experiment 2

Optogenetic inhibition of the IL-VTA pathway decreases social stress-induced AMPH sensitization effects

AAVretro-JAWS/stressed rats exhibited significantly less locomotor activity during AMPH challenge after 20 min of 632-nm light stimulation than did AAVretro-GFP/stressed rats (p=0.0358). Two-way ANOVA demonstrated significant main effects of experimental group (n=18,

$F_{3,14}$=6.700, p=0.0049) and AMPH administration ($F_{2.807,39.30}$=30.76, p<0.0001), and there was an interaction between the two factors ($F_{30,140}$=2.726, p<0.0001; Fig. 4.3A). Specifically, post hoc analysis revealed that AAVretro-GFP/stressed rats displayed greater locomotor activity than did AAVretro-JAWS/stressed rats at 20 (p=0.0358), 30 (p=0.0102), 40 (p=0.0013), and 50 (p=0.0002) min after the start of red light stimulation, but there was no difference at any other timepoints (p>0.05). Additionally, a two-way ANOVA was conducted to compare the average distance traveled during acclimation, following saline treatment, and after AMPH challenge (paired with light stimulation) in the different experimental groups. Two-way ANOVA revealed a significant main effect of experimental group ($F_{3,42}$=6.886, p=0.0007) as well as experimental timepoint ($F_{2,42}$=31.50, p=0.0007), but no interaction between the two factors ($F_{6,42}$=0.8457, p=0.5422; Fig. 4.3B). Furthermore, a two-way ANOVA was used to compare locomotor activity between AAVretro-JAWS/stressed rats and AAVretro-GFP/stressed rats at the start and end of 632-nm light stimulation paired with AMPH administration. Two-way ANOVA revealed a significant difference at the start (p=0.0203) and at the end (p=0.0020) of stimulation, revealing a significant effectiveness of optogenetic inhibition of the IL-VTA pathway in blocking stress-induced sensitization to AMPH (Fig. 4.3C).

Inhibition of IL-VTA projection neurons decreases Fos expression in the VTA and NAc shell

To identify whether inhibition of the IL-VTA pathway blocks mesolimbic signaling to the NAc, fluorescent Fos immunohistochemistry was performed in the NAc shell to compare mean number of Fos-labeled cells in rats subjected to optogenetic light stimulation compared to control rats (Fig. 4.4). A two-way ANOVA revealed a significant main effect of viral vector (JAWS vs GFP) (n=11; $F_{1,7}$=20.5, p=0.0027), but no effect of stress group ($F_{1,7}$=0.77, p=0.4091) or interaction between the two factors ($F_{1,7}$=0.00, p=0.9789; Fig. 4). A Tukey's multiple comparisons test revealed that significantly more Fos labeling was observed in the NAc shell in AAVretro-GFP/stressed rats compared to AAVretro-JAWS/stressed rats (p=0.030), and AAVretro-GFP/handled rats trended to have higher Fos labeling in the NAc shell than AAVretro-JAWS/handled rats.

4. DISCUSSION

In this study, we assessed the causal role of the IL-VTA neural pathway in social stress-induced psychostimulant sensitivity. The present results demonstrate that the inhibition of IL neurons that project to the VTA prevents social stress-induced AMPH cross-sensitization and blocks mesolimbic signaling to the NAc. These results indicate that the IL-VTA pathway plays an essential role in the induction of social stress-induced psychostimulant sensitivity, suggesting that this pathway could be a potential target in preventing stress-induced drug addiction vulnerability.

The medial prefrontal cortex (mPFC) is a major regulator of the DA system and plays a role in mediating goal-directed behaviors and impulse control. Two predominant subdivisions of the mPFC, the infralimbic (IL) and the prelimbic (PL) cortices, mainly consist of glutamatergic pyramidal neurons and have distinct projections to the VTA (Goldstein & Volkow, 2011; Sesack and Carr, 2002; Gabbott et al., 2005), along with other regions of the mesolimbic DA system, including the basolateral amygdala (BLA) and NAc. In rodents, the administration of psychostimulants increases glutamate transmission in portions of the mesocorticolimbic pathway involved in behavioral sensitization to psychostimulants, including the NAc and VTA (Xue et al., 1996; Reid et al., 1997; Del Arco et al., 1998; Wolf & Xue, 1999). This glutamate input, which mainly originates from prefrontal cortical regions, modulates VTA DA neurons. A majority of studies on cortical control over addiction have focused on the PL, largely because a homologous region in the human PFC (Uylings et al., 2003; Farovik et al., 2008) regulates decision making and inhibitory response control (Gregoire et al., 2012; Hare et al., 2009; Balleine & O'Doherty, 2009). In rodents, the PL has been shown to play a role in modulating cocaine-seeking behavior (Chen et al., 2013; Limpens et al., 2015), and the IL has been shown to play a role in the development of habitual reward seeking and extinction-related behavior (Barker et al., 2014), but little is understood about the role of the IL-VTA pathway in stress-induced addiction susceptibility.

Optogenetic stimulation of the mPFC has been shown to increase extracellular glutamate concentration in the VTA (You et al., 2007), as well as activate both nondopaminergic and dopaminergic neurons, exhibited by increased burst firing of VTA DA neurons followed by stimulation of the mPFC (Tong et al., 1996; Gariano & Groves, 1988; Moorman & Aston-Jones,

2010). In accordance with such results, we observed Fos expression in VTA DA neurons, exhibited by Fos/TH double-labeling, after optogenetic excitation of the IL-VTA pathway (Fig. 4.2). This was essentially a confirmation that we were able to use a novel wireless optogenetics technique to induce preferential neuronal activation of glutamatergic IL neurons which synapse onto VTA DA neurons.

Glutamate transmission onto VTA DA neurons undergoes synaptic plasticity that plays a critical role in establishing addiction-related behaviors, including drug sensitization (Kalivas et al., 2008). Glutamate then binds to metabotropic glutamate receptors (mGluRs) or ionic receptors such as AMPARs or N-methyl-D-aspartate (NMDA) receptors. AMPARs are ionic transmembrane receptors that contain four subunits, GluA1-4, which modulate receptor trafficking and channel functions (Carlezon & Nestler, 2002; Straub & Tomita, 2012; Wolf & Tseng, 2012). GluA1-homomeric AMPARs (GluA2-lacking) are Ca^{2+}-permeable, so they allow Ca^{2+} to pass through the channel and amplify intracellular Ca^{2+}-dependent signaling pathways (Straub & Tomita, 2012; Wolf & Tseng, 2012). Contrarily, all NMDA receptors, which are comprised of an NR1 subunit and multiple NR2 subunits, are Ca^{2+}-permeable; however, NMDA receptors require membrane depolarization to open with high probability due to the presence of a voltage-gated Mg^{2+} block (Derkach et al., 2007).

Repeated stress has been exhibited to increase neural activity of VTA DA neurons that project to the NAc (Tidey & Miczek, 1997), as well as enhance glutamatergic synaptic plasticity through NMDA receptors (NMDARs) in the VTA (Stelly et al., 2016). Our previous studies showed that GluA1, preferentially in VTA DA neurons, plays a critical role in the induction of stress-induced AMPH cross-sensitization (Rudolph et al., 2020); in addition, converging evidence has shown that GluA1 in VTA DA neurons, which receives glutamate input from the IL, is a trigger for drug-induced sensitization (Paluson et al., 1991; Yap & Miczek, 2007). Furthermore, we previously found that intermittent social defeat stress induces persistent deltaFosB expression selectively in IL neurons that project to the VTA, but not in other VTA-projecting regions, including the PL cortex (Nikulina et al., 2012), telling us that this pathway is activated as a result of intermittent social defeat stress. In accordance with such results, our present data shows that

inhibition of IL neurons that project to the VTA, presumably DA neurons, blocks stress-induced AMPH sensitization. There are a variety of mediators of stress-induced plasticity in the VTA. Importantly, corticotropin releasing hormone (CRH) modulates the autonomic and neuroendocrine response to stress, and increases the excitability of VTA DA neurons upon stress exposure (Douma & de Kloet, 2020; Holly et al., 2016; Korotkova et al., 2006; Leonard et al., 2017). In addition, stress induces glucocorticoid release, which acts on mesolimbic DA neurons to exacerbate the locomotor effects of addictive drugs (Miczek et al. 2008; Kalivas et al., 2009). It is likely this, along with glutamatergic potentiation of VTA DA neurons, which drives stress-induced AMPH sensitization.

When we shined 632nm light on JAWS-expressing neurons in the IL which project to VTA neurons, we found a decrease in Fos expression in the NAc shell. This tells us that inhibition of the IL-VTA pathway prevents mesolimbic signaling in the NAc. While some evidence suggests that mPFC afferents to the VTA synapse onto mPFC-projecting, rather than NAc-projecting DA neurons (Carr & Sesack, 2000), and that mPFC directly synapse onto VTA GABA neurons, which indirectly innervate DA neurons (Tong et al., 1996), there is a lack of afferent-specific research performed to corroborate those findings. In addition, DA neurons in the VTA may co-release glutamate (Stuber et al., 2010; Zhang et al., 2015) or GABA (Tritsch et al., 2012; Stamatakis et al., 2013), so it is possible that some of the NAc-projecting DA neurons may have co-released GABA, which would influence DA transmission.

In addition, IL glutamatergic projections to the NAc play a role in modulating cocaine seeking behavior in rats (Ewald et al., 2019; Ma et al., 2014). It is possible that some of the results we observed could have been due to nonspecific light stimulation of the IL-NAc pathway rather than the IL-VTA pathway. However, the viral construct we used was a retrograde-transporting AAV, which was injected into the VTA. This means that it should travel from the terminals in the VTA to cell bodies which have afferent projections to the VTA, including the NAc. To ensure that the decreased locomotion we observed was due to IL-VTA inhibition rather than IL-NAc inhibition, we examined levels of fluorescent expression of GFP in the NAc in experimental animals and saw minimal GFP expression, indicating that we mainly preferentially

stimulated the IL-VTA pathway, but it is important to consider potential effects of nonspecific light emission. Taken together, GluA1 in VTA DA neurons plays a critical role in the induction of stress-induced AMPH cross-sensitization (Rudolph et al., 2020), and our present results showed that optogenetic inhibition of IL-VTA glutamatergic neurons blocked stress-induced AMPH sensitivity. Therefore, we suggest that intermittent social stress increases glutamate release from pyramidal glutamatergic neurons in the IL that synapse onto GluA1-homomeric AMPARs on VTA DA neurons. It is this stress-induced plasticity onto VTA DA neurons that drives the mesolimbic DA signaling and resulting behavioral effects of stress-induced AMPH sensitization.

Very little work has been conducted on the elucidation of neural pathways that drive stress-induced drug addiction vulnerability, specifically on the effects of psychostimulant sensitization. Many studies have focused on prelimbic projections to regions of the mesolimbic pathway; as a control, it would be helpful to replicate these experiments in the PL-VTA pathway to see if we observe similar or converse effects. In conclusion, our experiments utilize wireless optogenetics to present the novel finding that the IL-VTA pathway is necessary for the induction of social stress-induced sensitization to psychostimulants in rats, thereby providing a potential neural target for intervention for drug abuse sus

FIGURES

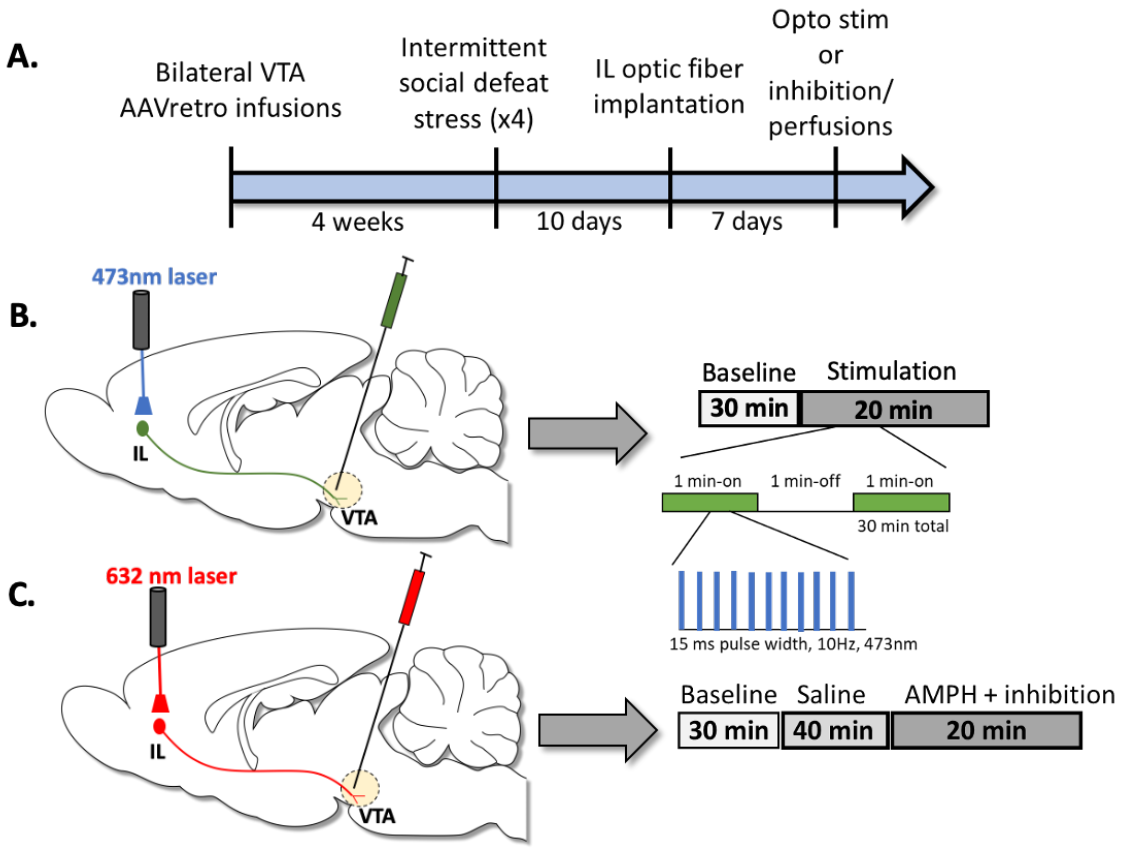

Figure 4.1. Experimental timeline and schematic of optogenetic stimulation parameters. (A) Timeline of surgical and behavioral procedures in both optogenetics experiments. (B) Experiment 1: Effect of 473-nm light stimulation of the IL-VTA pathway in ChR2-expressing rats. AAVretro-ChR2 or AAVretro-GFP was infused into the VTA and optic fibers containing 473-nm LEDs were implanted in the IL. Animals in both viral groups received 473-nm light stimulation. (C) Experiment 2: Effect of 630-nm light inhibition of the IL-VTA pathway in JAWS-expressing rats. AAVretro-JAWS or AAVretro-GFP was bilaterally infused into the VTA and bilateral optic fibers containing 630-nm LEDs were implanted in the IL. Animals were exposed to intermittent social defeat stress or handling, and all animals received 630-nm continuous light stimulation, paired with low-dose AMPH (1.0 mg/kg, i.p.) administration.

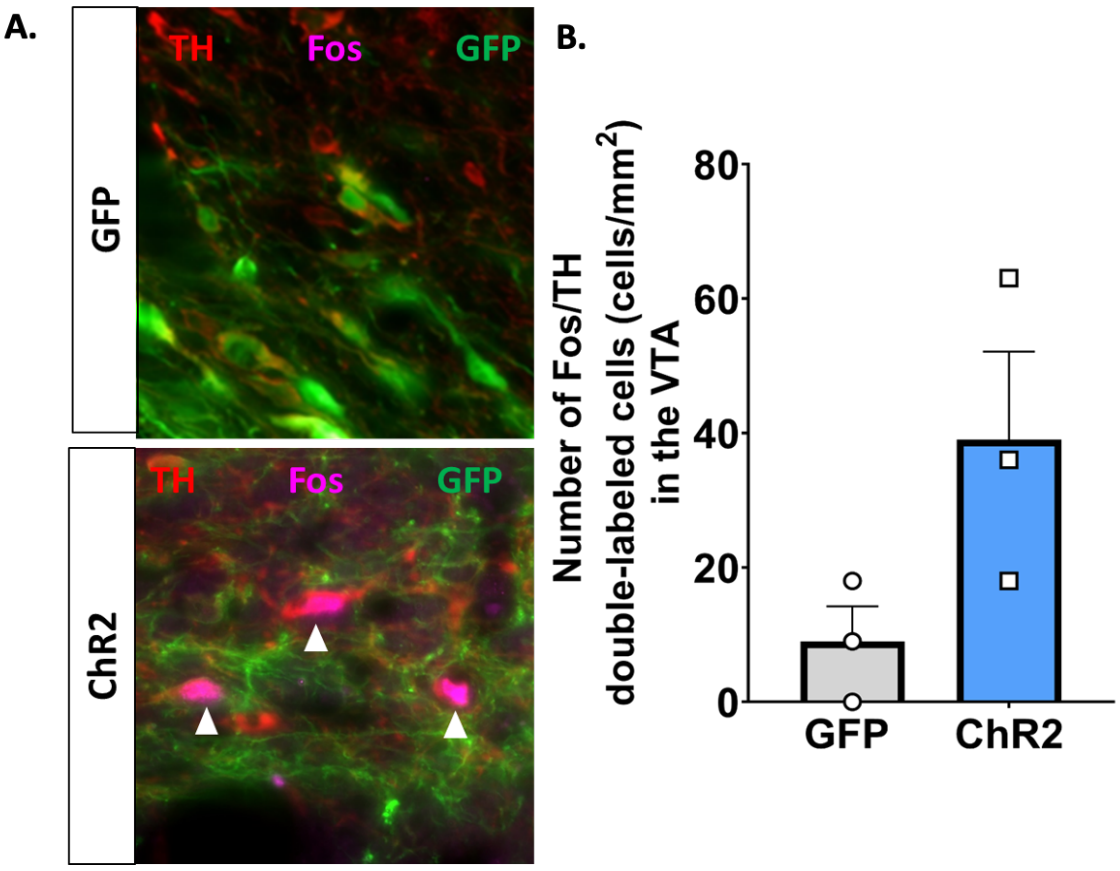

Figure 4.2. 473-nm LED light stimulation of lL-VTA pathway induces cellular activation of VTA DA neurons, exhibited by increased Fos/TH labeling. (A) Representative images of Fos/TH double labeling (triangle arrows) at 40x original magnification. (B) Rats that were infused with AAVretro-ChR2 and exposed to 473-nm light stimulation trended to express more Fos in VTA TH+ neurons than those with AAVretro-GFP, exposed to the same light stimulation (n=3 per group).

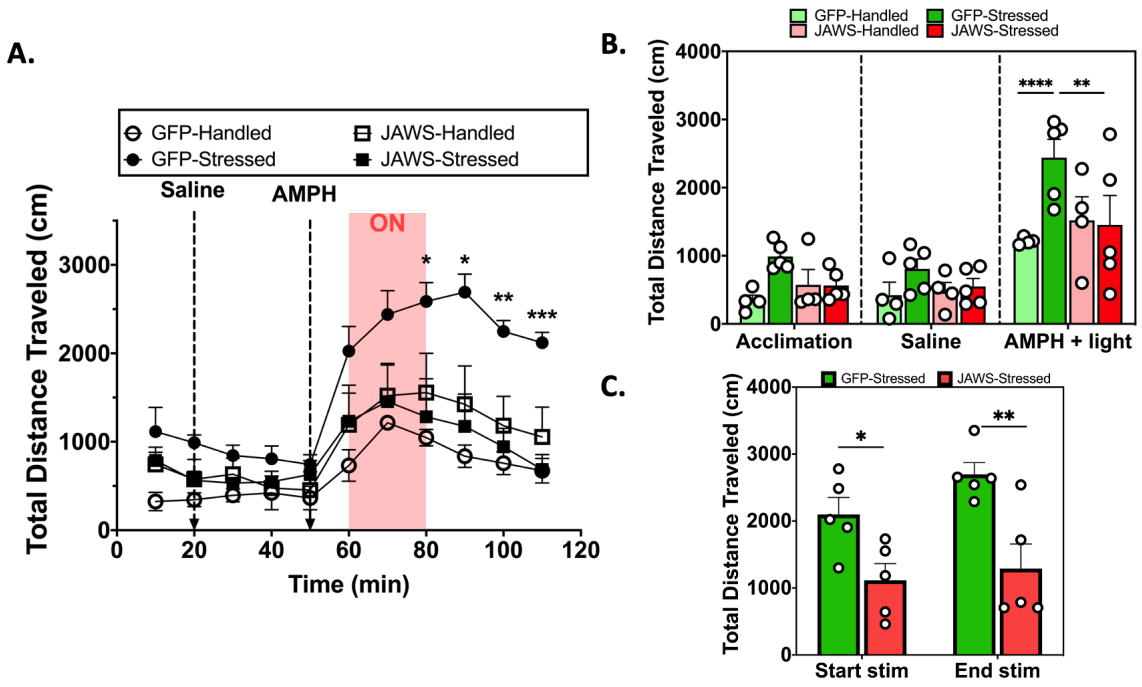

Figure 4.3. Optogenetic inhibition of the IL-VTA pathway decreases stress-induced AMPH sensitization effects. (A) Locomotor activity (total distance traveled in cm) over time before and after saline, and following AMPH administration (1.0 mg/kg, i.p.) paired with 630-nm light stimulation. GFP-stressed rats traveled a significantly greater distance at 80, 90, 100, and 110 min compared to JAWS-stressed rats. Importantly, GFP-stressed rats moved significantly more than JAWS-stressed rats 20 min following the start of 630-nm light stimulation (***p<0.001, **p<0.005, *p<0.05). (B) There was no difference in average locomotor activity between groups during the acclimation period or after saline injections, but GFP-stressed rats moved significantly more than GFP-handled rats and between JAWS-stressed rats after AMPH challenge paired with 630-nm continuous light stimulation (****p<0.0005; **p<0.005). (C) GFP-stressed rats moved significantly more than JAWS-stressed rats at the start and end of 630-nm light stimulation, which was paired with AMPH administration (**p<0.005; *p<0.05).

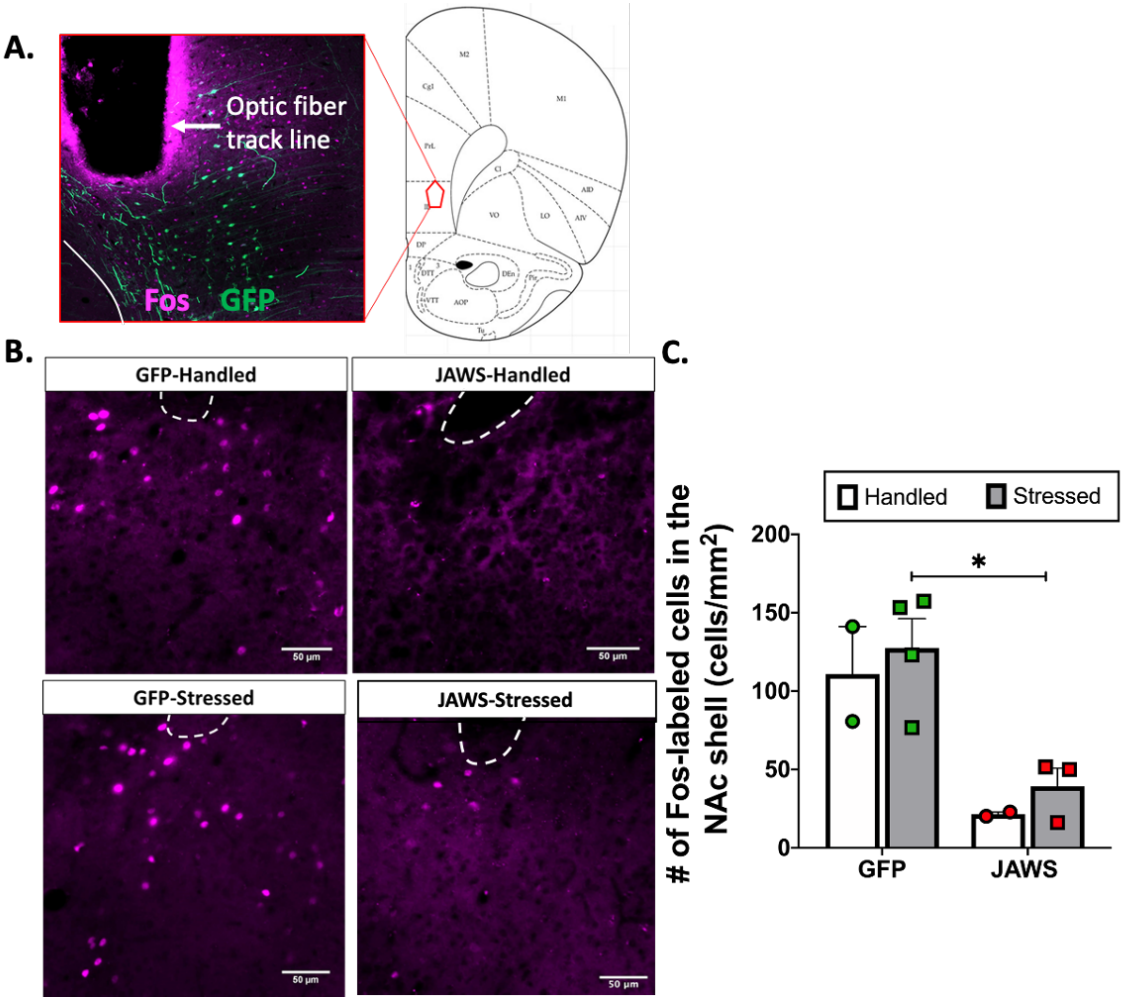

Figure 4.4. 630-nm light inhibition of IL-VTA projection neurons decreases Fos expression in the NAc shell. (A) Left: representative fluorescent image of optic fiber implant site (5x objective magnification); Right: Schematic depicting optic fiber implant location in the IL at +3.2mm from bregma. (B) Representative fluorescent images show higher Fos labeling in the NAc shell in rats expressing AAVretro-GFP following 630-nm light stimulation, compared to those expressing AAVretro-JAWS (scale bar = 50 µm). (C) Optogenetic inhibition of IL-VTA neurons decreased Fos labeling in the NAc shell in experimental animals expressing AAVretro-JAWS compared to control rats expressing AAVretro-GFP.

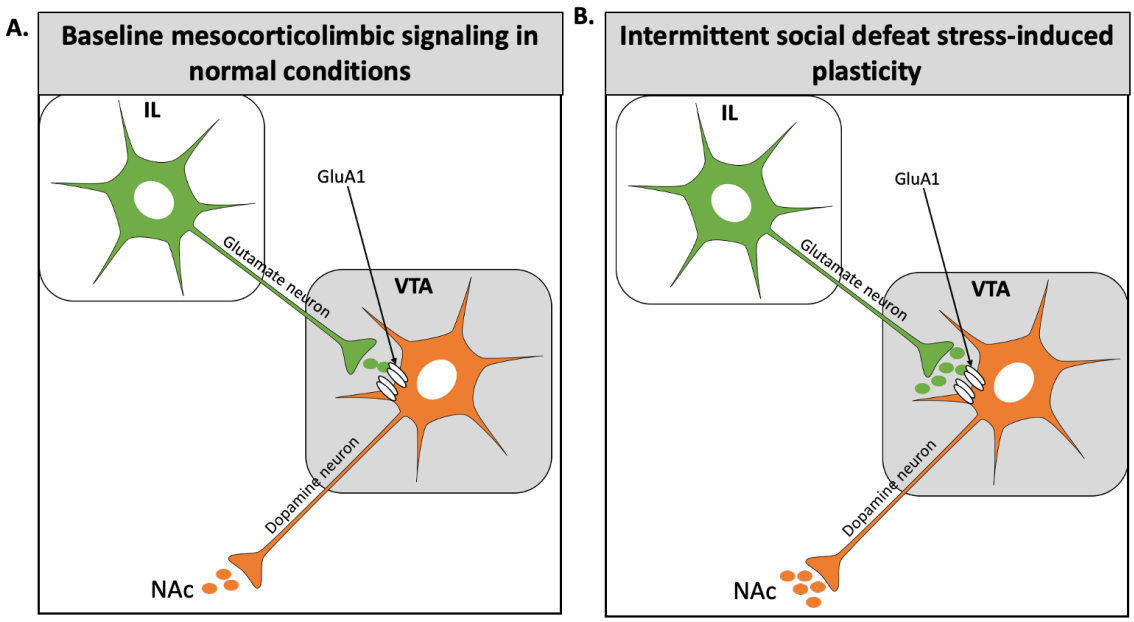

Figure 4.5. Simplified schematic of a proposed mechanism for IL-VTA control of stress-induced AMPH sensitization. (A) In normal conditions, IL glutamatergic neurons (green) synapse onto dopaminergic (and nondopaminergic) neurons in the VTA (orange), which have mesolimbic projections to the NAc to produce downstream effects; (B) Intermittent social defeat stress increases glutamate signaling from the IL to the VTA. We propose that intermittent social stress augments expression of GluA1-homomeric AMPARs, so glutamate released from IL neurons binds to these GluA1 AMPARs. This enhanced potentiation of glutamatergic neurons and resulting plasticity promotes augmented DA release in the NAc, which increases the effects of stress-induced psychostimulant sensitization in rats.

Chapter 5: GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

Summary of Major Results

The aim of the experiments discussed in the contents of this document was to investigate whether GluA1 homomeric AMPARs in VTA DA neurons plays a critical role in the induction of stress-induced vulnerability to psychomotor stimulant drugs. To do so, we used the social defeat stress model, which has high face validity in replicating interpersonal conflict and peer pressure in humans. In chapter 2, we examined whether GluA1 is necessary and sufficient to drive the behavioral response to intermittent social stress using bilateral AAV-mediated manipulation of GluA1 AMPARs in VTA DA neurons. In chapter 3, we utilized fluorescent immunohistochemistry and viral-mediated gene transfer to examine the role of ovarian hormones in sex-dependent differences in the behavioral and neurochemical response to stress, and mesolimbic mechanisms that may drive this differential response. In chapter 4, we utilized wireless optogenetics to further elucidate the neural pathway that drives stress-induced psychostimulant sensitivity.

In chapter 2, we first found that GluA1 expression is heightened in VTA DA neuros following intermittent social defeat stress, which is in accordance with our previous data that utilized western blot analysis to show higher GluA1 expression in the VTA following social stress, which occurs concomitantly with stress-induced AMPH sensitization (Wang et al., 2014). Next, GluA1 AMPARs in VTA DA neurons were functionally inactivated using viral-mediated gene transfer in a dominant-negative fashion to replace wildtype GluA1 AMPARs with pore-dead variants. This was performed several weeks prior to intermittent social defeat stress/handling procedures, and subjects were tested for stress-induced social avoidance behavior and AMPH cross-sensitization. Functional inactivation of GluA1 in VTA DA neurons prevented social stress-induced sensitization to AMPH without impacting social avoidance behavior; however, it had no effect on social avoidance behavior. These differential responses suggest that there is a different mechanism that drives stress-induced social avoidance behavior in rats, including BDNF-TrkB signaling and mu-opioid receptor signaling (Wang et al., 2013; Wang et al., 2014; Johnston et al., 2015). To determine whether GluA1 in VTA DA neurons is sufficient to mimic the effects of stress

on AMPH sensitization, we utilized an AAV construct to functionally overexpress GluA1 wildtype in VTA DA neurons several weeks prior to handling rats. Overexpression of wildtype GluA1 in VTA DA neurons induced augmented response to low-dose AMPH, even in the absence of social stress, indicating it was *sufficient* to mimic the effects of stress on AMPH sensitization. Those aforementioned results supported our hypothesis that GluA1 in VTA DA neurons plays a critical role in the induction of stress-induced sensitization to psychostimulants.

In chapter 3, we first found that females are more sensitive to stress-induced sensitization effects, and these effects are largely driven by differences in ovarian hormones during varying stages of the rat estrous cycle. Females cycling in pro/estrus, during the high estradiol stage of the estrous cycle, traveled significantly more in response to low-dose AMPH challenge than did females cycling in met/diestrus. In addition, we found that GluA1 expression in VTA DA neurons was significantly higher in rats subjected to intermittent social defeat stress compared to handling, but there was no effect of ovarian hormone on GluA1 expression. Contrarily, TrkB expression in VTA DA neurons was largely influenced by ovarian hormones, with higher TrkB expression in rats cycling in pro/estrus compared to met/diestrus, but there was no difference in TrkB/TH double-labeling as a result of intermittent social defeat stress. This finding is in accordance with previous research that showed differential TrkB expression in the hippocampus during different stages of the estrus cycle, with highest expression during pro/estrus compared to met/diestrus (Spencer et al., 2008). To identify whether GluA1 signaling plays a necessary and sufficient role in the induction of stress-induced AMPH sensitization in females as it does in males, we bilaterally manipulated GluA1 in VTA DA neurons. Like we found in males, we found that GluA1 inactivation in VTA DA neurons prevented cross-sensitization effects, whereas GluA1 overexpression in VTA DA neurons augmented the response to AMPH, even in the absence of social stress. These findings suggest that BDNF/TrkB signaling may influence GluA1 expression in the VTA through intracellular signaling pathways, especially since intra-NAc infusions of BDNF rapidly increases GluA1 surface expression in male rats (Li & Wolf, 2011). This

could explain why there is a differential expression of GluA1 in VTA DA neurons and TrkB in VTA DA neurons between male and female rats and during different periods of the estrus cycle.

In chapter 4, wireless optogenetics was used to inactivate the IL-VTA pathway in male rats to examine whether it is this pathway that drives stress-induced AMPH sensitization. Our previous studies showed intermittent social defeat stress induces persistent deltaFosB expression selectively in IL neurons that project to the VTA, but not in other VTA-projecting regions, including the PL cortex (Nikulina et al., 2012), telling us that this pathway is activated as a result of intermittent social defeat stress. For this reason, we utilized continuous red-light inhibition of IL neurons that project to the VTA by using a novel red-light shifted cruxhalorhodopsin, JAWS. We paired red light inhibition with low-dose AMPH challenge following intermittent social defeat stress and found that inhibition of the IL-VTA pathway prevented stress-induced AMPH sensitization. In addition, fluorescent immunohistochemical analyses were performed to examine mesolimbic cellular activity as a result of this IL-VTA optogenetic inhibition. We found that optogenetic inhibition of the IL-VTA significantly reduced Fos labeling in the NAc compared to GFP-expressing rats following AMPH administration, indicating a significant reduction in VTA-NAc mesolimbic signaling. Taken together, GluA1 in VTA DA neurons plays a critical role in the induction of stress-induced AMPH cross-sensitization (Rudolph et al., 2020), and our present results showed that optogenetic inhibition of IL-VTA glutamatergic neurons blocked stress-induced AMPH sensitivity. Therefore, we suggest that intermittent social stress increases glutamate release from pyramidal glutamatergic neurons in the IL that synapse onto GluA1-homomeric AMPARs on VTA DA neurons. It is this stress-induced plasticity onto VTA DA neurons that drives the mesolimbic DA signaling and resulting behavioral effects of stress-induced AMPH sensitization.

Mesolimbic mechanisms underlying sex-dependent differences in stress-induced sensitization response: a potential interaction between GluA1 AMPARs and BDNF-TrkB signaling

Initially, BDNF was characterized as a nerve growth factor that enhances survival and activity of dopaminergic neurons (Hyman et al., 1991). BDNF binds to the TrkB receptor with high

affinity as the primary method of signal transduction; both BDNF and TrkB mRNA are densely expressed in VTA DA neuron cell bodies and terminals (Ip et al., 1992). Given the role of BDNF in dopaminergic neuron activity and survival (Alter et al., 1992; Shen et al., 1994; Siuciak et al., 1996), persistently enhanced VTA BDNF signaling leads to augmented DA transmission in the mesolimbic circuit, and further potentiates stress effects on DA transmission in the mesolimbic circuit. It has been shown that BDNF in the VTA potentiates stress-induced AMPH sensitization, implying that VTA BDNF signaling acts as a risk factor in stress-induced drug abuse susceptibility. BDNF can also regulate the assembly of GluA1-homomeric AMPARs on the cell membrane (Fortin et al., 2012), which facilitates LTP and increases membrane conductance. Since BDNF-TrkB signaling potentiates GluA1 signaling, which also plays a critical role in the induction of intermittent social stress-induced sensitization to AMPH, we believe these mechanisms work together to induced the heightened psychomotor response, especially in females.

In addition, we found that GluA1 in VTA DA neurons also plays a critical role in the induction of stress-induced sensitization to AMPH in female rats; we found higher GluA1 expression in VTA DA neurons in stressed rats compared to handled, but this was independent of estrus stage. In addition, GluA1 inactivation in VTA DA neurons prevented cross-sensitization effects, By contrast, we found that TrkB is expressed more in VTA DA neurons during proestrus/estrus compared to metestrus/diestrus but is independent of stress in female rats, which is different than in male rats. This is an accordance with previous studies that show that DA signaling is enhanced during proestrus, which drives behavioral aspects of addiction, including CPP (Calipari et al., 2017). In addition, previous studies show that TrkB expression in the hippocampus fluctuates with estrus stage, and is highest when females are cycling in proestrus (Spencer et al., 2008). We propose the following cellular mechanism for the social stress-induced psychostimulant sensitization response in males and females: (1) Stress induces GluA1 AMPAR-mediated plasticity on VTA DA neurons. Glutamate is released from IL-originating neurons that synapse onto these GluA1-homomeric AMPARs; (2) GluA1 AMPARs are Ca^{2+}-permeable, and

increases channel conductance, leading to action potentials that drive enhanced DA release in the NAc; (3) this enhanced DA signaling drives the stress-induced psychomotor sensitization response; (4) in females, estradiol potentiates BDNF-TrkB signaling, which also potentiates further insertion of GluA1-homomeric AMPARs into the cell membrane; (5) the stress- and BDNF-TrkB-mediated plasticity in VTA DA neurons augments DA signaling in the NAc and drives the heightened sensitization response in females (Fig. 5.1).

Methodological Considerations and Future Studies

Although bilateral manipulation of GluA1 AMPARs in VTA DA neurons affected social stress-induced sensitization to AMPH, there are several methodological considerations that must be noted. First, the VTA is extremely heterogeneous in nature, with 60-65% of the cells being dopaminergic, approximately 30-35% GABAergic, and the remaining cells being glutamatergic, or co-expressing multiple neurotransmitters. With that in mind, our present studies specifically examined the effects of manipulating GluA1 in VTA dopaminergic neurons but failed to examine potential effects in other cell types. For this reason, future studies should be conducted in which GluA1 AMPARs are manipulated in GAD-Cre rats to elucidate whether the observed effects are specific to DA neurons. In addition, for the optogenetics experiments, we utilized a retrograde-transporting AAV to express JAWS in the VTA, which was transported to afferent cell bodies, including those in the IL. Optic fibers were implanted in the IL so we were able to use red light to inhibit neurons that project from the IL to the VTA. In this case, it would have been beneficial to perform additional control experiments, such as experiments in which optic fibers are implanted in the PL or in the Anterior cingulate. Many studies about addiction have been conducted involving the PL rather than the IL, and in rodents, the PL has been shown to play a role in modulating cocaine-seeking behavior (Chen et al., 2013; Limpens et al., 2015), so it would be useful to rule out any contributions from the PL to VTA.

In our second experiment in which we studied ovarian cycle-dependent differences in the effects of stress on AMPH sensitization, we utilized viral-mediated gene transfer to bidirectionally manipulate GluA1 in VTA DA neurons, and utilized fluorescent immunohistochemistry to identify

potential cellular mechanisms underlying enhanced sensitivity during proestrus/estrus. While our techniques were innovative and relatively conclusive, there are still some gaps in knowledge and unknowns that should be investigated. First, we should better characterize cell types where GluA1 expression occurs following intermittent social defeat stress. To do that, fluorescent immunohistochemistry should be used with antibodies against GAD65/67 and/or VGlut2 to characterize expression in GABA and glutamate cells, as well as TH cells. In addition, we should replicate our viral manipulation experiments using GAD-cre rats to examine whether the effects of GluA1 on stress-induced sensitization effects is specific to DA neurons. Furthermore, to better understand the role of BDNF-TrkB signaling in GluA1 signaling, as well as behavioral effects of stress on AMPH sensitization, it would be ideal to use a viral cre-dependent viral construct to preferentially manipulate TrkB in VTA DA neurons. In addition, additional fluorescent immunohistochemistry should be performed to examine whether GluA1 inactivation affects TrkB or BDNF expression in the VTA, and if TrkB knockdown in the VTA prevents GluA1 expression in the VTA. This would give us a more conclusive explanation about the mechanism in which sex-dependent differences in stress-induced sensitization occurs.

Concluding Remarks

The studies described in chapters 2-4 show that intermittent social stress increases GluA1 expression in VTA DA neurons in male and female rat, which is critical for the induction of stress-induced AMPH sensitization. However, knockdown of GluA1 in VTA DA neurons does not play a role in stress-induced social avoidance behavior, suggesting that a different mechanism governs this response, such as the previously studied mu-opioid receptor signaling in the VTA (Johnston et al., 2015). We also showed that female rats are more sensitive to the effects of stress on AMPH sensitization during proestrus/estrus, when estradiol levels are highest, and that mesolimbic BDNF/TrkB signaling likely plays a role in the ovarian hormone-governed response; GluA1 AMPAR signaling, however, is not affected by sex hormones, but it does play a role in the sensitization response to social defeat stress. This leads us to suggest that BDNF-TrkB signaling and GluA1 AMPAR signaling in VTA DA neurons essentially work together to drive this estrus

cycle-dependent enhanced sensitization response. Finally, we used red-light inhibition of the IL-VTA pathway during a low-dose AMPH challenge to show that this neural pathway plays a critical role in the induction of stress-induced heightened psychomotor response to AMPH in males. While we found critical information on the role of GluA1 in stress-induced AMPH sensitization, and we elucidated the neural pathway that drives this response, further studies should be conducted using GAD-Cre rats to further elucidate the cell types affected by intermittent social stress-induced plasticity that drives the sensitization response. Taken together, these studies suggest that IL glutamate signaling onto GluA1 AMPAR in VTA DA neurons underlie susceptibility to psychomotor stimulants, and that interruption of this signaling may be a potential pharmacotherapeutic intervention for stress-induced neuropsychiatric disorders and substance abuse susceptibility.

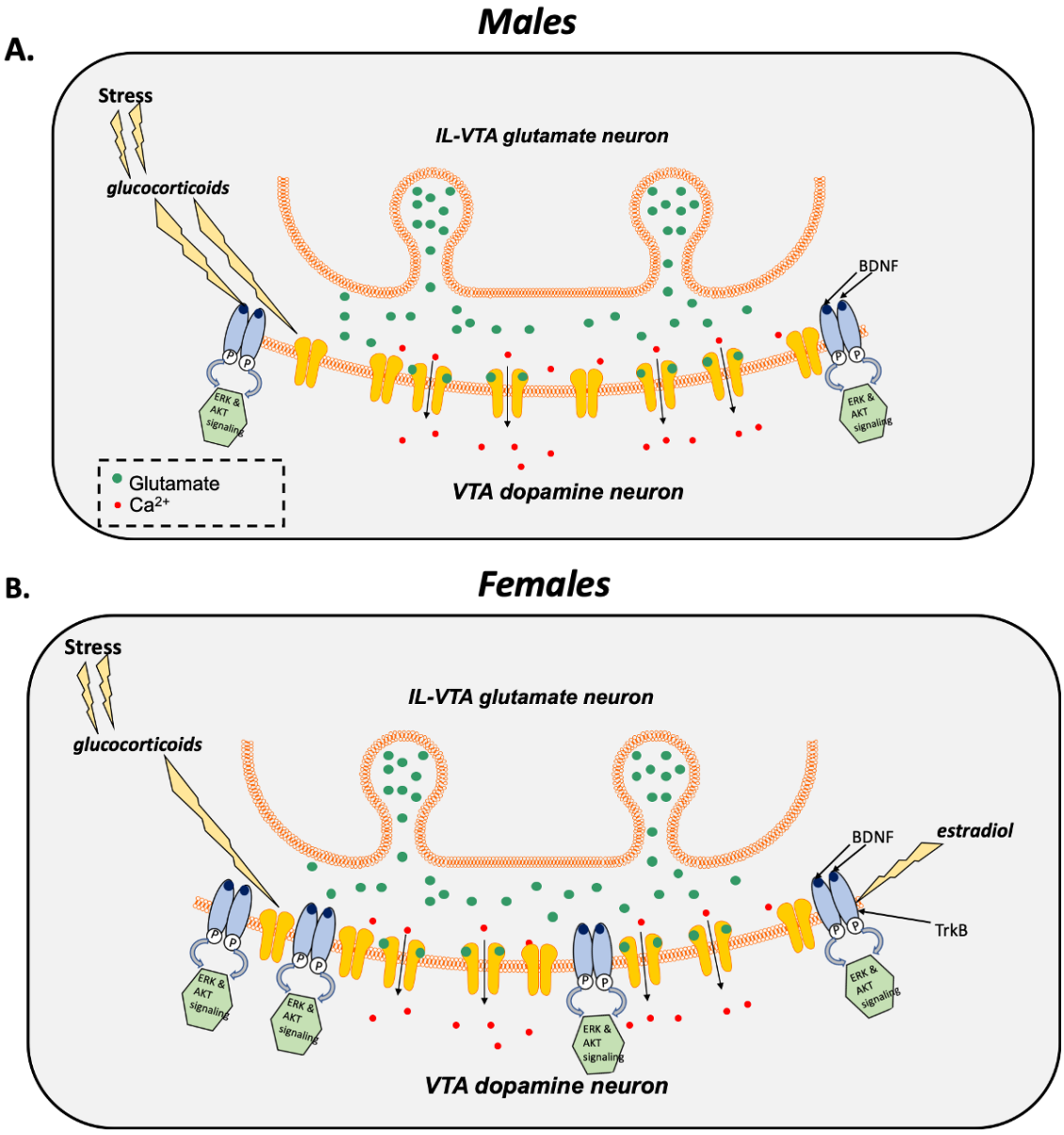

Figure 5.1. Social stress-induced changes in the mesolimbic pathway that leads to sex-related differences in psychomotor response to AMPH. Simplified schematic showing a summary of the aforementioned mesolimbic mechanisms underlying stress-induced AMPH sensitization. In both males and females, stress induces glucocorticoid release, which amplifies the effects of IL-VTA glutamatergic signaling. (A) In male rats, intermittent social defeat stress increases Ca^{2+}-permeable GluA1 expression in VTA DA neurons. In addition, intermittent social defeat stress induces augmented BDNF/TrkB signaling in the VTA, which activates ERK and AKT signaling cascades, which plays a role in DA neuron plasticity, mRNA transcription, as well as the potentiation of further GluA1-homomeric AMPAR formation in the membrane. This ultimately

results in more DA release in the NAc and the resulting psychomotor sensitization to psychostimulants. (B) In females, intermittent social defeat stress also increases Ca^{2+}-permeable GluA1 expression in VTA DA neurons. We suggest that estradiol (which is highest during proestrus) plays a modulatory role in TrkB expression, resulting in significantly higher TrkB expression in VTA DA neurons during proestrus/estrus compared to metestrus/diestrus. The binding of BDNF to TrkB receptors activates intracellular ERK and AKT signaling cascades, which ultimately results in further potentiation of GluA1-homomeric AMPAR formation in the membrane. The combined effects of glutamate signaling on VTA GluA1-homomeric AMPARs and BDNF-TrkB signaling results in more DA release in the NAc and the amplified response to stress-induced AMPH sensitization in females during proestrus/estrus compared to metestrus/diestrus.

REFERENCES

Adamantidis, A.R., Tsai, H.C., Boutrel, B., Zhang, F., Stuber, G.D., Budygin, E.A., Tourino, C., Bonci, A., Deisseroth, K., de Lecea, L., 2011. Optogenetic interrogation of dopaminergic modulation of the multiple phases of reward-seeking behavior. *J. Neurosci*., 31, 10829-10835.

Airan, R.D., Thompson, K.R., Fenmo, L.E., Bernstein, H., Deisseroth, K., 2009. Temporally precise *in vivo* control of intracellular signalling. *Nature*, 458, 1025-1029.

Anderson, S.M., Famous, K.R., Sadri-Vakili, G., Kumaresan, V., Schmidt, H.D., Bass, C.E., Terwilliger, E.F., Cha, J.J., & Pierce, R.C., 2008. CaMKII: a biochemical bridge linking accumbens dopamine and glutamate systems in cocaine seeking. Nat Neurosci. 11, 344-353.

Anker J.J., Carroll M.E., 2011. Females are more vulnerable to drug abuse than males: evidence from preclinical studies and the role of ovarian hormones. *Curr Top Behav Neurosci.* 8, 73-96.

Appleby V.J., Correa, S.A.L., Duckworth, J.K., Nash, J.E., Noel, J., Fitzjohn, S.M., Collingridge, G.L., Molnar, E., 2010. LTP in hippocampal neurons is associated with a CaMKII-mediated increase in GluA1 surface expression. J Neurochem. 116, 530-543.

Argilli, E., Sibley, D.R., Malenka, R.C., England, P.M., Bonci, A. (2008) Mechanism and time course of cocaine-induced long-term potentiation in the ventral tegmental area. *J. Neurosci.*, **28**, 9092-9100.

Bachtell, R.K., Choi, K.H., Simmons, D.L., Falcon, E., Monteggia, L.M., Neve, R.L., & Self, D.W., 2008. Role of GluR1 expression in nucleus accumbens neurons in cocaine sensitization and cocaine-seeking behavior. Eur J Neurosci. 28, 9092-9100.

Bale, T.L. & Vale, W.W., 2004. CRF and CRF receptors: role in stress response and other behaviors. *Ann. Rev. Pharmacol. Toxic*., 44, 525-557.

Balleine, B.W. & O'Doherty, Human, J.P. (2009), Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action, *Neuropsychopharmacol*., 35, 48-69.

Bamann, C., Kirsch, T., Nagel, G., Bamberg, E., 2008. Spectral charecterizations of the photocycle of channelrhodopsin-2 and its implication for channel function. *J. Mol. Biol.,* 375 (3), 686-694.

Banke, T.G., Greenwood, J.R., Christensen, J.K., Liljefors, T., Traynelis, S.F., Schousboe, A., Pickering, D.S. 2001, Identification of amino acid residues in GluR1 responsible for ligand binding and desensitization. *J. Neurosci*., 21(9), 3052-3062.

Barker, J.M., Taylor, J.R., Chandler, L.J. (2014), A unifying model of the role of the infralimbic cortex in extinction and habits, *Learn. Mem*., 21, 441-448.

Bartlett, E., Hallin, A., Chapman, B., and Angrist, B., 1997. Selective sensitization to the psychosis-inducing effects of cocaine: a possible marker for addiction relapse vulnerability? *Neuropsychopharmacology*, 16, 77-82.

Bass, C.E. Grinevich, V.P., Vance, Z.B., Sullivan, R.P., Bonin, K.D., Budygin, E.A., 2010. Optogenetic control of striatal dopamine release in rats. *J. Neurochem*., 114, 1344-1352.

Baxter, J.D. & Rousseau, G.G. 1979, Glucocorticoid hormone action. Berlin, New York: Springer-Verlag.

Becker, J.B., Cha, J. 1989, Estrous cycle-dependent variation in amphetamine-induced behaviors and striatal dopamine release assessed with microdialysis, *Behav. Brain Res*., 25(2), 117-125.

Becker, J.B. & Hu, M. 2008, Sex differences in drug abuse. *Front. Neuroend*., 29(1), 36-47.

Becker, J.B. & Koob, G.F. 2016, Sex differences in animal models: focus on addiction, *Pharm. Rev.*, 68(2), 242-263.

Becker, J.B., Perry, A.N., Westenbroek, C. 2012, Sex differences in the neural mechanisms mediating addiction: a new synthesis and hypothesis, *Biol. Sex Diff*., 3, 14, http://www.bsd-journal.com/content/3/1/14

Bellone, C., Luscher, C. 2005, mGluRs induce a long-term depression in the ventral tegmental area that involves a switch of the subunit composition of AMPA receptors, *Eur. J. Neurosci*., 21(5), 1280-1288.

Bellone, C., Luscher, C. 2006, Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. *Nat. Neurosci*., 9, 636-641.

Berhow, M.T., Hiroi, N., Nestler, E.J. 1996. Regulation of ERK (extracellular signal regulated kinase), part of the neurotrophin signal transduction cascade, in the rat mesolimbic dopamine system by chronic exposure to morphine or cocaine*. J. Neurosci.,* 16(15), 4707-5715.

Berthet, A.M., Margolis, E.B., Zhang, J., Hsieh, I., Zhang, J., Hnasko, T.S., Ahmad, J., Edwards, R.H., Sesaki, H., Huang, E.J., Nakamura, K. 2014, Loss of mitochondrial fission depletes axonal mitochondria in midbrain dopamine neurons. *J. Neurosci*., 34(43), 14304-14317.

Berton, O., McClung, C.A., Dileone, R.J., Krishnan, V., Renthal, W., Russo, S.J., Graham, D., Tsankova, N.M., Bolanos, C.A., Rios, M., Monteggia, L.M., Self, D.W. & Nestler, E.J., 2006. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science. 311, 864-868.

Bjorklund, A. & Dunnett, S.B. 2007, Dopamine neuron systems in the brain: an update. *Trends Neurosci*., 30(5), 194-202.

Blanchard, R.J., McKittrick, C.R., Blanchard, D.C., 2001, Animal models of social stress: effects on behafvior and brain neurochemical systems. *Physiol. Behav.*, 73, 261-271.

Blanchard, C., Griebel, G., Blanchard, R.J. 2003, Conditioning and residual emotionality effects of predator stimuli: some reflections on stress and emotion. P*rog. Neuro-Psychopharm. Bio. Psych*., 27(8), 1177-1185.

Bobzean S.A., DeNobrega A.K., Perrotti L.I., 2014. Sex differences in the neurobiology of drug addiction. *Experimental Neurology* 259, 64-74.

Bock, R., Shin, J.H., Kaplan, A.R., Dobi, A., Markey, E., Kramer, P.F., Alvarez, V.A. 2013, Strengthening the accumbal indirect pathway promotes resilience to compulsive cocaine use. *Nat. Neurosci*. 16, 632-638.

Bolla, K., Ernst, M., Kiehl, K., Mouratidis, M., Eldreth, D., Contoreggi, C., Matochik, J., Kurian, V., Cadet, J., Kimes, A., Funderburk, F., London, E. 2004, Prefrontal cortical dysfunction in abstinent cocaine abusers. *J. Neuropsych. and Clin. Neurosci.*, 16(4), 456-464.

Bowie, D. 2008. Ionotropic glutamate receptors and CNS disorders. C*NS Neurol. Disord.: Drug Targets*, 7(2), 129-143.

Boyden, E.S., Zhang, F., Bamberg, E., Nagel, G., Deisseroth, K., 2005. Millisecond-timescale, genetically targeted optical control of neural activity. *Nature Neurosci.*, 8, 1263-1268.

Boyson, C.O., Miguel, T.T., Quadros, I.M., DeBold, J.F., Miczek, K.A. 2011, Prevention of social stress-escalated cocaine self-administration by CRF-R1 antagonist in the rat VTA, *Psychopharmacology*, 218, 257-269.

Brady, K.T., Myrick, H., McElroy, S., 1998. The relationship between substance use disorders, impulse control disorders, and pathological aggression. *American J. Add.*, 7, 221-230.

Brady, K.T., Randall, C.L. 1999, Gender differences in substance use disorders. *Psych. Clin. N. Am.*, 22, 241-252.

Brewer, D.D., Catalano, R.F., Haggerty, K., Gainey, R.R., Fleming, C.B. 1998, A meta-analysis of predictors of continued drug use during and after treatment for opiate addiction. *Addiction*, 93, 73-92.

Britt, J.P., McDevitt, R.A., Bonci, A. 2012, Use of channelrhodopsin for activation of CNS neurons. *Current Prot. in Neurosci.*, 58(1), 2.16.1-2.16.19

Brodish, A. & Redgate, E.S. 1973, Brain-pituitary-adrenal interrelationships. Basel, New York,: S. Karger.

Brown, J.M., Hanson, G.R., Fleckenstein, A.E. 2001, Cocaine-induced increases in vesicular dopamine uptake: role of dopamine receptors. *Neuropharmacology*, 298(3), 1150-1153.

Brown, M.R., Koob, G.F., Rivier, C. 1990, Stress: neurobiology and neuroendocrinology. New York: Dekker.

Cador, M., Bjijou, Y., Cailhol, S., Stinus, L. 1999, D-amphetamine-induced behavioral sensitization: implication of a glutamatergic medial prefrontal cortex-ventral tegmental area innervation. *Neuroscience*, 94(3), 705-721.

Calipari, E.S., Juarez, B., Morel, C., Walker, D.M., Cahill, M.E., Ribeiro, E., Roman-Ortiz, C., Ramakrishnan, C., Deisseroth, K., Han, M., Nestler, E.J. 2017, Dopeminergic dynamics underlying sex-specific cocaine reward, *Nat. Comm*, 8, 13877, https://doi.org/10.1038/ncomms13877

Calu, D.J., Kawa, A.B., Marchant, N.J., Navarre, B.M., Henderson, M.J., Chen B., Yau, H., Bossert, J.M., Schoenbaum, G., Deisseroth, K., Harvey, B.K., Hope, B.T., Shaham, Y. (2013), Optogenetic inhibition of dorsal medial prefrontal cortex attenuates stress-induced reinstatement of palatable food seeking in female rats, *J. Neurosci.*, 33, 214-226.

Cao J., Covington H.E., 3rd, Friedman, A.K., Wilkinson, M.B., Walsh, J.J., Cooper, D.C., Nestler, E.J., Han, M., 2010. Mesolimbic dopamine neurons in the brain reward cicuit mediate susceptibility to social defeat and antidepressant action. *J Neurosci.* 30(49), 16453-16458.

Carlezon Jr., W.A., Boundy, V.A., Haile, C.N., Lane, S.B., Kalb, R.G., Neve, R.L., Nestler, E.J. 1997, Sensitization to morphine induced by viral-mediated gene transfer, *Science*, 277, 812-815.

Carlezon Jr., W.A., Nestler, E.J., 2002. Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? *Trends Neurosci*. 25(12), 610-615.

Carlezon Jr., W.A., Neve, R.L., 2003. Viral-mediated gene transfer to study the behavioral correlates of CREB function in the nucleus accumbens of rats. In: Wang J.Q. (eds) Drugs of Abuse. Methods in Molecular Medicine ™, vol 79. Humana Press.

Carlson, J.N., Herrick, K.F., Baird, J.L., Glick, S.D. 1987, Selective enhancement of dopamine utilization in the rat prefrontal cortex by food deprivation. *Brain Res*., 400(1), 200-203.

Carr, D.B. & Sesack, S.R. 2000, Projections form the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons, *J. Neurosci.*, 20(10), 3864-3873.

Carroll, M.E., Lynch, W.J., Roth, M.E., Morgan, A.D., Cosgrove, K.P. 2004, Sex and estrogen influence drug abuse, T*rends in Pharmacol. Sci*., 25(5), 273-279.

Castner, S.A. & Williams, G.V. 2007, From vice to virtue: insights from sensitization in the nonhuman primate, *Prog Neuro-Psychopharm. Biol. Psych*., 31(8), 1572-1592.

Chen, K. & Kandel, D. 2002, Relationship between extent of cocaine use and dependence among adolescents and adults in the United States, *Drug Alcoh. Dep*., 68(1), 65-85.

Chen, B.T., Yau, H., Hatch, C., Kusumoto-Yoshida, I., Cho, S.L., Woodward Hopf, F., Bonci, A. (2013), Rescuing cocaine-induced prefrontal cortex hypoactivity prevents compulsive cocaine seeking, *Nature*, 496, 359-362.

Choi K.H., Edwards, S., Graham, D.L., Larson, E.B., Whisler, K.N., Simmons, D., Friedman, A.K., Walsh, J.J., Rahman, Z., Monteggia, L.M., Eisch, A.J., Neve, R.L., Nestler, E.J., Han, M., Self., D.W. (2011) Reinforcement-related regulation of AMPA glutamate receptor subunits in the ventral tegmental area enhjances motivation for cocaine. J *Neurosci.,* **31(21)**, 7927-7937.

Chung, L. 2015, A brief introduction to the transduction of neural activity into fos signal, *Dev. Reprod.*, 19(2), 61-67.

Chuong, A.S., Miri, M.L., Busskamp, V., Matthews, G.A.C., Acker, L.C., Sorensen, A.T., Young, A., Klapoetke, N.C., Henninger, M.A., Kodandaramaiah, S.B., Ogawa, M., Ramanlal, S.B., Bandler, R.C., Allen, B.D., Forest, C.R., Chow, B.Y., Han, X., Lin, Y., Tye, K.M., Roska, B., Cardin, J.A., Boyden, E.S. 2014, Noninvasive optical inhibition with a red-shifted microbial rhodopsin, *Nat. Neurosci*., 17, 1123-1129.

Churchill, L., Swanson, C.J., Urbina, M., Kalivas, P.W., 1999. Repeated cocaine alters glutamate receptor subunit levels in the nucleus accumbens and venral tegmental area of rats that develop behavioral sensitization. *J Neurochem*. 72, 157-165.

Clure, C., Brady, K.T., Saladin, M.E., Johnson, D., Waid, R., Rittenbury, M. 1999, Attention-deficit/hyperactivity disorder and substance use: Symptom pattern and drug choice. *Am. J. Drug & Alcohol Abuse*, 25, 441.

Conner, J.M., Lauterborn, J.C., Yan, Q., Gall, C.M., Varon, S. 1997, Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: evidence for anterograde axonal transport, *J. Neurosci*., 17(7), 2295-2313.

Covington, H.E., 3rd, Kikusui, T., Goodhue, J., Nikulina, E.M., Hammer, R.P., & Miczek, K.A., 2005. Brief social defeat stress: long lasting effects on cocaine taking during a binge and zif268mRNA expression in the amygdala and prefrontal cortex. *Neuropsychopharmacology*. 30, 310-321.

Covington, H.E., 3rd. & Miczek, KA., 2001. Repeated social-defeat stress, cocaine or morphine effects on behavioral sensitization and intravenous cocaine self-administration "binges". *Psychopharmacology (Berl)*. 158, 388-398.

Covington, H.E., 3rd, Tropea, T.F., Rajadhyaksha, A.M., Kosofsky, B.E., & Miczek, K.A., 2008. NMDA receptors in the rat VTA: a critical site for social stress to intensify cocaine taking. *Psychopharmacology (Berl*). 197, 203-216.

Craddock, C.G. 1978, Corticosteroid-induced lymphopenia, immunosuppression, and body defense. *Ann Intern. Med*, 88(4), 564-566.

Curran, T.A., Miller, D., Zokas, L., Verma, I.M. 1984, Viral and cellular fos proteins: a comparative analysis, *Cell*, 36(2), 259-268.

Del Arco, A., Gonzalez-Mora, J.L., Armas, V.R., & Mora. F., 1999. AMPH increases the extracellular concentration of glutamate in striatum of the awake rat: involvement of high affinity transporter mechanisms. *Neuropharmacology*. 38, 943-954.

Derkach, V., Barria, A., Soderling, T.R., 1999, Ca^{2+}/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazoleproprionate type glutamate receptors. *Proc Natl Acad Sci USA*. 96, 3269-3274.

Derkach, V.A., Oh, M.C., Guire, E.S., Soderling, T.R. 2007, Regulatory mechanisms of AMPA receptors in synaptic plasticity. *Nat. Neurosci. Rev*., 8, 101-113.

Deisseroth, K., 2011. Optogenetics, *Nat. Methods*, 8, 26-29.

Deisseroth, K., Feng, G., Majewska, A.K., Miesenbock, G., Ting, A., Schnitzer, M.J., 2006. Next-generation optical technologies for illuminating genetically targeted brain circuits. *J. Neurosci*. 26, 10380-10386.

Deroche, V., Marinelli, M., Maccari, S., Le Moal, M., Simon, H., Piazza, P.V. 1995, Stress-induced sensitization and glucocorticoids. I. Sensitization of dopamine-dependent locomotor effects of amphetamine and morphine depends on stress-induced corticosterone secretion. *J. Neurosci.*, 15(11), 7181-7188.

Deutch, A.Y., Clark, W.A., Roth, R.H. 1990 Prefrontal cortical dopamine depletion enhances the responsiveness of mesolimbic dopamine neurons to stress. *Brain Res*., 521(1-2), 311-315.

Deutch, A.Y., Tam, S.Y., Roth, R.H. 1985, Footshock and conditioned stress increase 3,4-dihydroxypenylacetic acid (DOPAC) in the ventral tegmental area but not substantia nigra. *Brain Res*., 333(1), 143-146.

Di Chiara, G., 1995. The role of dopamine in drug abuse viewed from the perspective of its role in motivation., *Drug and Alc. Dep*., 39, 155.

Di Chiara, G. & Imperato, A., 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc Natl Acad Sci USA. 85, 5274-5278.

Dingledine, R., Borges, K., Bowie, D., Traynelis, S.F. (1999) The glutamate receptor ion channels. *Pharmacol. Rev*, 51, 7-61.

Douma, E.H., de Kloet, E.R. (2020), Stress-induced plasticity and functioning of ventral tegmental area dopamine neurons, *Neurosci. Biobehav. Rev*., 108, 48-77.

Dunn, A.J. 1988, Stress-related activation of cerebral dopaminergic systems. *Ann. N.Y. Acad. Sci.,* 537, 188-205.

Edwards, N.J., Tejeda, H.A., Pignatelli, M., Zhang, S., McDevitt, R.A., Wu., J., Bass, C.E., Bettler, B., Morales, M., Bonci, A. (2017) Circuit specificity in the inhibitory architecture of the VTA regulates cocaine-iduced behavior. *Nat. Neuroscience*, **20**, 438-448.

Emmett-Oglesby, 1995. Sensitization and tolerance to the motivational and subjective effects of psychostimulants. In: the Neurobiology of Cocaine: Cellular and Molecular Mechanisms, pp. 31-47. Ed. R.T. Hammer. CRC Press: Boca Raton.

Ersche, K.D., Fletcher, P.C., Lewis, S.J., Clar, L., Stocks-Gee, G., London, M., Shakian, B.J. 2005, Abnormal frontal activations related to decision-making in current and former amphetamine and opiate dependent individuals. *Psychopharmacology (Berl)*, 180(4), 612-623.

Evans, S.M. 2007, The role of estradiol and progesterone in modulating the subjective effects of stimulants in humans. *Exp. and Clin. Psychopharm.*, 15(5), 418-426.

Evans S.M., Haney M., Foltin R.W., 2002. The effects of smoked cocaine durin the follicular and luteal phases of the menstrual cycle in women. *Psychopharmacology* 159, 397-406.

Evans S.M., Foltin R.W., 2006. Exogenous progesterone attenuates the subjective effects of smoked cocaine in women, but not in men. *Neuropsychopharmacology* 31, 659-674.

Evans, S.M. & Foltin, R.W. 2010, Does the response to cocaine differ as a function of sex or hormonal status in human and non-human primates? H*orm. Behav.*, 58(1), 13-21.

Ewald, V.A., De Corte, B.J., Gupta, S.C., Lillis, K.V., Narayanan, N.S., Wemmi, J.A., LaLumiere, R.T. (2019), Attenuation of cocaine seeking in rats via enhancement of infralimbic cortical activity using stable step-function opsins, *Psychopharmacology*, 236, 479-490.

Fanous, S., Hammer, R.P., Jr., Nikulina, E.M. 2010, Short- and long-term effects of intermittent social defeat stress on brain-derived neurotrophic factor expression in mesocorticolimbic brain regions. *Neuroscience*, 167, 598-607.

Fanous, S., Terwilliger, E.F., Hammer, R.P. & Nikulina, E.M., 2011. Viral depletion of VTA BDNF in rats modulates social behavior, consequences of intermittent social defeat stress, and long-term weight regulation. *Neurosci Lett*. 502, 192-196.

Farovik, A., Dupont, L.M., Arce, M., and Eichenbaum, H. (2008), Medial prefrontal cortex supports recollection, but not familiarity in the rat. *J. Neurosci*., 28, 13428-13434.

Feder, H.H. 1981, Estrous Cyclicity in Mammals. In: Adler N.T. (eds) Neuroendocrinology of Reproduction. Springer, Boston, MA.

Ferrario, C.R., Loweth, J.A., Milovanovic, M., Ford, K.A., Galinanes, G.L., Heng, L., Tseng, K.Y., Wolf, M.E. 2011, Alterations in AMPA receptor subunits and TARPs in the rat nucleus accumbens related to the formation of Ca^{2+}-permeable AMPA receptors during the incubation of cocaine craving. *Neuropharmacology,* 61(7), 1141-1151.

Festa, E.D. & Quinones-Jenab, V. 2004, Gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine. *Hormones and Behav.*, 46(5), 509-519.

Fields, H.L., Hjelmstad, G.O., Margolis, E.B., Nicola, S.M. 2007, Ventral tegmental area neurons in learned appetitive behavior and positive reinforcement. *Annu Rev Neurosci,* 30, 289-316.

Fiorillo, C.D. & Williams, J.T. 1998, Glutamate mediates an inhibitory postsynaptic potential in dopamine neurons. *Nature*, 394, 78-82.

Fitzgerald, L.W., Ortiz, J., Hamedani, A.G., Nestler, E.J., 1996. 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. *J Neurosci*. 16, 274-282.

Fleckenstein, A.E., Volz, T.J., Riddle, E.L., Gibb, J.W., Hanson, G.R., 2007. New insights into the mechanism of action of amphetamines. Annu Rev Pharmacol Toxicol. 47, 681-698.

Freeman, M.E. 1988, The ovarian cycle of the rat. In: E. Knobil & J. Neil (eds.), Physiology of reproduction. Raven Press Ltd., New York, p. 1893-1928.

Freeman, M.E. 1994, The neuroendocrine control of the ovarian cycle of the rat. The Physiology of Reproduction, E Knobil, JD Neill. Raven Press, New York, 613-658.

Fuchikami, M., Thomas, A., Liu, R., Wohleb, E.S., Land, B.B., DiLeone, R.J., Aghajanian, G.K., Duman, R.S. (2015), Optogenetic stimulation of infralimbic PFC reproduces ketamine's rapid and sustained antidepressant actions, *Proc. Nat. Acad. USA*., 112, 8106-8111.

Gabbott, P.L.A., Warner, T.A., Jays, P.R.L., Salway, P., Busby, S.J. (2005), Prefrontal cortex in the rat: projections to subcortical autonomic, motor, and limbic centers, *J. Comp. Neurology*, 492, 145-177.

Gall, C.M., Gold, S.J., Isackson, P.J., Seroogy, K.B. 1992, Brain-derived neurotrophic factor and neurotrophin-3 mRNAs are expressed in ventral midbrain regions containing dopaminergic neurons. *Mol. Cell. Neurosci*., 3(1), 56-63.

Gallop, R.J., Crits-Christoph, P., Ten Have, T.R., Barber, J.P., Frank, A., Griffin, M.L., Thase, M.E. 2007, Differential transitions between cocaine use and abstinence for men and women. J. *Cons. Clin. Psych*., 75(1), 95-103.

Gariano, R.F., Groves, P.M. (1988), Burst firing induced in midbrain dopamine neurons by stimulation of the medial prefrontal and anterior cingulate cortices. *Brain Res*., 462, 194-198.

Gerdeman, G.L., Partridge, J.G., Lupica, C.R., Lovinger, D.M. 2003, It could be habit forming; drugs of abuse and striatal synaptic plasticity. *Trends in Neurosci*., 26(4), 184-192.

Goldstein, R.Z., Alia-Klein, N., Tomasi, D., Carrillo, J.H., Maloney, T., Woicik, P.A., Wang, R., Telang, F., Volkow, N.D. 2009, Anterior cingulate cortex hypoactivations to an emotionally salient task in cocaine addiction. *Proc. Nat. Acad. Sci. U.S.A.*, 106(23), 9453-9458.

Goldstein, R.Z., Volkow, N.D. (2011), Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. *Nat. Rev. Neurosci*., 12, 652-669.

Graziane, N.M., Polter, A.M., Briand, L.A., Pierce, R.C., & Kauer, J.A., 2013. Kappa opioid receptors regulate stress-induced cocaine seeking and synaptic plasticity. *Neuron*. 77, 942-954.

Greenberg, M.E., Xu, B., Lu, B., Hempstead, B.L. 2009, New insights in the biology of BDNF synthesis and release: implications in CNS function, *J. Neurosci.,* 29(41), 12764-12767.

Gregoire, S., Rivalan, M., Le Moine, C., and Dellu-Hagedorn, F. (2012), The synergy of working memory and inhibitory control: behavioral pharmacological and neural functional evidences. *Neurobiol. Learn. Mem*., 97, 202-212.

Gresch, P.J., Sved, A.F., Zigmond, M.J., Finlay, J.M. 1994, Stress-induced sensitization of dopamine and norepinephrine efflux in medial prefrontal cortex of the rat. *J. Neurochem*., 63(2), 575-583.

Grimm, J.W., Lu, L., Hayashi, T., Hope, B.T., Su, T., Shaham, Y. 2003, Time-dependent increases in brain-derived neurotrophic factor protein levels within the mesolimbic dopamine system after withdrawal from cocaine: implications for incubation of cocaine craving, J*. Neurosci.*, 23(3), 742-747.

Grueter, B.A., McElligott, Z.A., Winder, D.G. 2007, Group I mGluRs and Long-Term Depression: potential roles in addiction? *Mol. Neurobiol*., 36, 232-244.

Guillin, O., Diaz, J., Carroll, P., Griffon, N., Schwartz, J., Sokoloff, P. 2001, BDNF controls dopamine D_3 receptor expression and triggers behavioural sensitization, *Nature*, 411, 86-89.

Gulaboski, R., Cordeiro, M.N.D.S., Milhazes, N., Garrido, J., Borges, F., Jorge, M., Pereira, C.M., Bogeski, I., Morales, A.H., Naumoski, B., Silva, A.F., 2007. Evaluation of the lipophilic properties of opioids, amphetamine-like drugs, and metabolites through electrochemical studies at the interface between two immiscible solutions. *Analytical Biochemistry*. 361(2), 236-243.

Guzman, D., Carreira, M.B., Friedman, A.K., Adachi, M., Neve, R.L., Monteggia, L.M., Han, M., Cowan, C.W., Self, D.W., 2018. Inactivation of NMDA receptors in the ventral tegmental area during cocaine self-administration prevents GluA1 upregulation but with paradoxical increases in cocaine-seeking behavior. *J Neurosci*. 38(3), 575-585.

Haller, J., Fuchs, E., Halasz, J., Makara, G.B. 1999, Defeat is a major stressor in males while social instability is stressful mainly in females: towards the development of a social stress model in female rats. *Brain Res Bul*l, 50, 33-39.

Hayashi, Y., Shi, S.H., Esteban, J.A., Piccini, A., Poncer, J.C., Malinow, R., 2000. Driving AMPA receptors into synapses by LTP and CaMKII: requirement for GluA1 and PDZ domain interaction. *Science*. 287, 2262-2267.

Hare, T.A., Camerer C.F., and Rangel, A. (2009), Self-control in decision-making involves modulation of the vmPFC valuations. *Science*, 324, 646-648.

Hegemann, P., and Moglich, A., 2011. Channelrhodopsin engineering and exploration of new optogenetic tools. *Nature Methods*. 8, 39-42.

Heimer, L., Zahm, D.S., Churchill, L., Kalivas, P.W., Wohltmann, C. 1991, Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience*, 41(1), 89-125.

Henry, D.J., Margaret, A.G., White, F.J. 1989. Electrophysiological effects of cocaine in the mesoaccumbens dopamine system: repeated administration. *J. Pharmacol. Exp. Ther*., 251, 833-839.

Hernandez-Avila, C.A., Rounsaville, B.J., Kranzler, H.R. 2004, *Drug Alcoh. Dep*., 74(3), 265-272.

Holly, E.N., Boyson, C.O., Montagud-Romero, S., Stein, D.J., Gobrogge, K.L., DeBold, J.F., Miczek, K.A. (2016), Episodic social stress-escalated cocaine self-administration: role of phasic and tonic corticotropin releasing factor in the anterior and posterior ventral tegmental area. *J. Neurosci*., 36, 4093-4105.

Holly E.N., Shimamoto A., DeBold J.F., Miczek K.A., 2012. Sex differences in behavioral and neural cross-sensitization and escalated cocaine taking as a result of episodic social defeat stress in rats. *Psychopharmacology (Berl)* 224. 179-188.

Hu, M. & Becker, J.B. 2003, Effects of sex and estrogen on behavioral sensitization to cocaine in rats, *J. Neurosci*., 23(2), 693-699.

Ignjatova, L., Raleva, M. 2009, Gender difference in the treatment outcome of patients served in the mixed-gender program. *Bratisl. Lek. Listy*, 110, 285-289.

Ikemoto, S. 2007, Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Res. Rev*., 56(1), 27-78.

Imperato, A., Angelucci, L., Casolini, P., Zocchi, A., Puglisi-Allegra, S. 1992, Repeated stressful experiences differently affect limbic dopamine release during and following stress. *Brain Res*., 577(2), 194-199.

Imperato, A., Puglisi-Allegra, S., Casolini, P., Zocchi, A., Angelucci, L., 1989. Stress-induced enhancement of dopamine and acetylcholine release in limbic structures: role of corticosterone. *Eur J Pharmacology*. 165, 337-338.

Jacobs E., D'Esposito M., 2011. Estrogen shapes dopamine-dependent cognitive processes: implications for women's health. *J Neurosci*. 31, 5286-5293.

Jennings, J.H. Sparta, D.R., Stamatakis, A.M., Ung, R.L., Pleil, K.E., Kash, T.L. Stuber, G.D. 2013, Distinct extended amygdala circuits for divergent motivational states. *Nature*, 496, 224-228.

Johnson, S.W. & North, R.A., 1992. Two types of neurons in the rat ventral tegmental area and their synaptic inputs. *J Physiol*. 450, 455-468.

Johnston, C.E., Herschel, D.J., Lasek, A.W., Hammer Jr., R.P., Nikulina, E.M., 2015. Knockdown of ventral temgmental area mu-opiod receptrs in rats prevents effects of social defeat stress: implications for amphetamine cross-sensitization, social avoidance, weight regulation and expression of brain-derived neurotrophic factor. *Neuropharm*. 89, 325-334.

Jones, S.R., Joseph, J.D. Barak, L.S., Caron, M.G., and Wightman, R.M., 1999. Dopamine neuronal transport kinetics and effects of amphetamine [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]. *J Neurochem*, 73(6), 2406-2414.

Jongen-Relo, A.L., Voorn, P., Gfoenewegen, H.J. 1994, Immunohistochemical characterization of the shell and core territories of the nucleus accumbens in the rat. *Eur. J. Neurosci*., 6(8), 1255-1264.

Joyce, E.M. & Iverson, S.D. 1979, The effect of morphine applied locally to mesencephalic dopamine cell bodies on spontaneous motor activity in the rat. *Neurosci Lett,* 14, 207-212.

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

Kalivas, P.W., 2009. The gluatamate homeostasis hypothesis of addiction. *Nat Rev Neurosci*. 10**,** 561-572.

Kalivas, P.W., Duffy, P., Barrow, J., 1989. Regulationof the mesocorticolimbic dopamine system by glutamic acid receptor subtypes. *Pharmacol Exp Ther*, 251(1), 378-387.

Kalivas, P.W. & Weber, B., 1988. Amphetmaine injection into the ventral mesencephalon sensitizes rats to peripheral amphetamine and cocaine. *J. Pharm. and Exp. Ther.*, 245(3), 1095-1102.

Kamata, K., Rebec, G.V. 1984. Long-term amphetamine treatment attenuates or reverses the depression of neuronal activity produced by dopamine agonists in the ventral tegmental area. *Life Sciences*, 34(24), 2419-2427.

Kelso, S.R., Nelson, T.E., and Leonard, J.P., 1992. Protein kinase C-mediated enhancement of NMDA currents by metabotropic glutamate receptors in Xenopus oocytes. *J. Physiol.*, 449, 705-718.

Kim, K.M., Baratta, M.V., Yang, A., Lee, D., Boyden, E.S., Fiorillo, C.D. 2012, Optogenetic mimicry of the transient activation of dopamine neurons by natural reward is sufficient for operant reinforcement. *PLoS One*, 7(4): e33612.

Kim, J., & Vezina, P. 1998, Metabotropic glutamate receptors are necessary for sensitization by amphetamine, *Neuroreport*, 9(3), 403-406.

Koppel, B.S., Samkoff, L., Daras, M., 1996. Relation of cocaine use to seizures and epilepsy, *Epilepsia*, 37(9), 875-878.

Kreek, M.J., Nielsen, D.A., Butelman, E.R., LaForge, K.S. 2005, Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. *Nat. Neurosci*., 8, 1450-1457.

Krishnan, V., Han, M.H., Graham, D.L., Berton, O., Renthal, W., Russo, S.J., Laplant, Q., Graham, A., Lutter, M., Lagace, D.C., Ghose, S., Reister, R., Tannous, P., Green, T.A., Neve, R.L., Chakravarty, S., Kumar, A., Eisch, A.J., Self, D.W., Lee, F.S., Tamminga, C.A., Cooper, D.C., Gershenfeld, H.K., Nestler, E.J., 2007, Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell*, 131, 391-404.

Komatsu, H., Ohara, A., Sasaki, K., Abe, H., Hattori, H., Hall, F.S., Uhl, G.R. & Sora, I., 2011. Decreased response to social defeat stress in μ-opioid-receptor knockout mice. Pharmacol *Biochem Behav*. 99, 676-682.

Korotkova, T.M., Brown, R.e>, Sergeeva, O.A., Ponomarenko, A.A., Haas, H.L. (2006), Effects of arousal- and feeding-related neuropeptides on dopaminergic and GABAergic neurons in the ventral tegmental area of the rat. *Eur. J. Neurosci*., 23, 2677-2685.

Kosten, T.R., Rousaville, B.J., Kleber, H.D. 1985, Ethnic and gender differences among opiate addicts. *Int. J. Addictions*, 20, 1143-1162.

Kourrich, S., Rothwell, P.E., Klug, J.R., Thomas, M.J., 2007. Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. J Neurosci. 27(30), 7921-7928.

Kravitz, A.V., Tye, L.D., Kreitzer, A.C., 2012. Distinct roles for direct and indirect pathway striatal neurons in reinfocement. *Nat. Neurosci.*, 15, 123-130.

Krishnan, V., Han, M., Graham, D.L., Berton, O., Renthal, w., Russo, S.J., LaPlant Q., Graham, A., Lutter, M., Lagace, D.C., Ghose, S., Reister, R., Tannous, P., Green, T.A., Neve, R.L., Chakravarty, S., Kumar, A., Eisch, A.J., Self, D.W., Lee, F.S., Tamminga, C.A., Cooper, D.C., Gershenfeld, H.K., Nestler, E.J., 2007. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. *Cell.* 131, 391-404.

Kritis, A.A., Stamoula, E.G., Paniskaki, K.A., Vavilis, T.D., 2015. Researching glutamate-induced cytotoxicity in different cell lines: a comparative/collective analysis/study. *Front. Cell. Neurosci.*, https://doi.org/10.3389/fncel.2015.00091

Kreek, M.J., Nielsen, D.A., Butelman, E.R., LaForge, K.S. 2005, Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction., *Nat. Neurosci.*, 8, 1450-1457.

Kudo, T., Uchigashima, M., Miyazaki, T., Konno, K., Yamasaki, M., Yanagawa, Y., Minami, M., Watanabe, M. 2012, Three types of neurochemical projection from the bed nucleus of the stria terminals to the ventral tegmental area in adult mice. *J. Neurosci*, 32(50), 18035-18046.

Laflamme, N., Nappi, R.E., Drolet, G., Labrie, C., Rivest, S. 1999, Expression and neuropeptidergic characterization of estrogen receptors (ERa and ERb) throughout the rat brain: anatomical evidence of distinct roles of each subtype, *J. Neurobiol.*, 36(3), 357-378.

Lammel, S., Hetzel, A., Hackel, O., Jones, I., Liss, B., Roeper, J. 2008, Unique properties of mesoprefrontal neurons within a dual mesocorticolimbic dopamine system. *Neuron,* 57(5), 760-773.

Lammel, S., Lim, B.K., Ran, C., Huang, K.W., Betley, M.J., Tye, K.M., Deisseroth, K., Malenka, R.C. (2012) Input-specific control of reward and aversion in the ventral tegmental area. *Nature*, **491**, 212-217.

Larson, E.B., Anker, J.J., Gliddon, L.A., Fons, K.S., Carroll, M.E. 2007, Effects of estrogen and progesterone on the escalation of cocaine self-administration in female rats during extended access, *Exp. and Clin. Psychopharm.*, 15(5), 461-471.

Leonard, M.Z., DeBold, J.F., Miczek, K.A. (2017), Escalated cocaine “binges” in rats: enduring effects of social defeat stress or intra-VTA CRF. *Psychopharmacology (Berl.)*, 234, 2823-2836.

Lessmann, V., Gottmann, K., Malcangio, M. 2003, Neurotrophin secretion: current facts and future prospects. *Prog. Neurobiology*, 69(5), 341-374.

Li, X. & Wolf, M.E. 2011, Brain-derived neurotrophic factor rapidly increases AMPA receptor surface expression in rat nucleus accumbens, *Eur. J. Neurosci*., 34(2), 190-198.

Li, Y., Vartanian, A.J., White, F.J., Xue, C.J., & Wolf, M.E., 1997. Effects of the AMPA receptor antagonist NBQX on the development and expression of behavioral sensitization to cocaine and AMPH. *Psychopharamacology (Berl)*. 134, 266-276.

Limpens, J.H.W., Damsteegt, R., Broekhoven, M.H., Voorn, P., Vanderschuren, L.J.M.J. (2015), Pharmacological inactivation of the prelimbic cortex emulates compulsive reward seeking in rats. *Brain Res*., 1628, 210-218.

Liu, X., Liu, Y., Zhong, P., Wilkinson, B., Qi, J., Olsen, C.M., Bayer, K.U., Liu, Q., 2014. CaMKII activity in the ventral tegmental area gates cocaine-induced synaptic plasticity in the nucleus accumbens. *Neuropsychopharm*. 39, 989-999.

Long J. A., and Evans H. M., 1922. Oestrus cycle in the rat and its associated phenomena. *Mem. Univ. Calif.* 6, 1–146.

Loweth, J.A., Scheyer, A.F., Milovanovic, M., LaCrosse, A.L., Flores-Barrera, E., Werner, C.T., Li, X., Ford, K.A., Le, T., Olive, M.F., Szumlinski, K.K., Tseng, K.Y., Wolf, M.E. 2014, *Nature Neurosci*., 17, 73-80.

Loweth J.A., Tseng, K.Y., Wolf, M.E., 2013. Using metabotropic glutamate receptors to modulate cocaine's synaptic and behavioral effects: mGluR1 finds a niche. *Curr Opin Neurobiol*. 23, 500-506.

Lu, B., Pang, P.T., Woo, N.H. 2005, The yin and yang of neurotrophin action. *Nat. Rev. Neurosci.*, 6, 603-614.

Lu, L., Shepard, J.D., Hall, F.S., Shaham, Y. 2003, Effect of environmental stressors on opiate and psychostimulant reinforcement, reinstatement and discrimination in rats: a revew. *Neurosci. Biobehav. Rev.*, 27(5), 457-491.

Luscher, C. & Huber, K.M. 2010, Group I mGluR-dependent synaptic long-term depression: mechanisms and implications for circuitry and disease. *Neuron*, 65(4), 445-459.

Luscher, C. & Malenka, R.C., 2011. Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. *Neuron*. 69, 650-663.

Lynch, W.J., Arizzi, M.N., Carroll, M.E. 2000, Effects of sex and the estrous cycle on regulation of intravenously self-administered cocaine in rats, *Psychopharmacology*, 152, 132-139.

Lynch, W.J. & Carroll, M.E. 1999, Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats, *Psychopharmacology*, 144, 77-82.

Lynch, W.J., Roth, M.E., Mickelberg, J.L., Carroll, M.E. 2001, Role of estrogen in the acquisition of intravenously self-administered cocaine in female rats. *Pharm. Biochem. Behav.*, 68(4), 641-646.

Lynch, W.J., Roth, M.E., Carroll, M.E. 2002, Biological basis of sex differences in drug abuse: preclinical and clinical studies, *Psychopharmacology*, 164, 121-137.

Lynch, W.J. & Taylor, 2004. Sex differences in the behavioral effects of 24-h/day access to cocaine under a discrete trial procedure, *Neuropsychopharmacology*, 29, 943-951.

Ma, Y., Lee, B.R., Wang, X., Guo, C., Liu, L., Cui, R., Lan, Y., Balcita-Pedicino, J.J., Wolf, M.E., Sesack, S.R., Shaham, Y., Schluter, O.M., Huang, Y.H., Dong, Y. 2014, Bidirectional modulation of incubation of cocaine craving by silent synapse-based remodeling of prefrontal cortex to accumbens projections, *Neuron*, 83(6), 1453-1467.

Malenka, R.C., Nicoll, R.A. 1999, Long-term potentiation--a decade of progress? *Science*, 285, 1870-1874.

Mameli, M., Balland, B., Lujan, R., Luscher, C. 2007, Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area. *Science*, 27, 530-533.

Mameli, M., Halbout, B., Creton, C., Engblom, D., Rodriguez, J., 2009. Cocaine-evoked synaptic plasticity: persistence in the VTA triggers adaptations in the NAc. *Nat Neurosc*i. **12**, 1036-1042.

Mandl, A.M. 1951, The phases of the oestrous cycle in the adult white rat. *J. Exp. Biol.*, 28, 576-584.

Marcondes, F.K., Bianchi, F.J., Tanno, A.P., 2002. Determination of the estrous cycle phases of rats: some helpful considerations. *Braz. J. Biol.* 63, 609-614.

Marcondes, F.K., Miguel, K.J., Melo, L.L., Spadari-Bratfisch, R.C. 2001, Estrous cycle influences the response of female rats in the elevated plus-maze test. *Phys. and Behav*., 74(4-5), 435-440.

Margolis, E.B., Lock, H., Hjelmstad, G.O., Fields, H.L. 2006, The ventral tegmental area revisited: is there an electrophysiological marker for dopaminergic neurons? *J Physiol,* 577(3), 907-924.

Margolis, E.B., Toy, B., Himmels, P., Morales, M., Fields, H.L. 2012. Identification of rat ventral tegmental area GABAergic neurons. PLoS ONE. 7(7): e42365. doi:10.1371/journal.pone.0042365

McCutcheon, J.E., Wang, X., Tseng, K.Y., Wolf, M.E., & Marinelli, M., 2011. Calcium-permeable AMPA receptors are present in nucleus accumbens synapses after prolonged withdrawal from cocaine self-administration but not experimenter-administered cocaine. J Neurosci. 31(15), 5737-5743.

Meerlo, P., Overkamp, G.J., Daan, S., Van Den Hoofdakker, R.H., Koolhaas, J.M. 1996, Changes in behaviour and body weight following a singloe or double social defeat in rats. *Stress*, 1, 21-32.

Mehta, A.K., Halder, S., Khanna, N., Tandon, O.P., Singh, U.R., Sharma, K.K. 2012. Role of NMDA and opioid receptors in neuropathic pain induced by chronic constriction injury of sciatic nerve in rats. J. Basic and Clin. Physiol. Pharmacol., 23, 49-55.

Menetrey, D., Gannon, A., Levine, J.D., Basbaum, A.I. 1989. Expression of c-fos protein in interneurons and projection neurons of the rat spinal cord in response to noxious somatic, articular, and visceral stimulation. *J. Comp. Neurol*., 285, 177-195.

Miczek K.A., Yap J.J., Covington H.E., 3rd, 2008. Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake, Pharmacol Ther. 120,102-128.

Moorman, D.E., Aston-Jones, G. (2010), Orexin/hypocretin modulates response of ventral tegmental dopamine neurons to prefrontal activation: Diurnal influences. *J. Neurosci*., 30, 15585-15599.

Morales, M., Margolis, E.B., 2017. Ventral tegmental area: cellular heterogeneity, connectivity and behaviour. Nat Rev neurosc. 18, 73-85.

Morales, M. & Pickel, V.M., 2011. Insights to drug addiction derived from ultrastructural views of the mesocorticolimbic system. *ANNALS of the New York Academy of Sciences*., 1248, 71-88.

Morales, M. & Root, D.H. 2014, Glutamate neurons within the midbrain dopamine regions, *Neuroscience*, 282(12), 60-68.

Morgan, J.I. & Curran, T., 1986. Role of ion flux in the control of c-fos expression. *Nature*, 322, 552-555.

Morgan, P.F., Nakajima, T., Linnoila, M., 1988. Psychological induction of brain c-fos mRNA. *Abs. Soc. Neurosci.*, 1:289.

Morisette, M., Le Saux, M., D'Astous, M., Jourdain, S., Al Sweidi, S., Morin, N., Estrada-Camarena, E., Mendez, P., Garcia-Segura, L.M., Di Paola, T. 20087, Contribution of estrogen receptors alpha and beta to the effects of estradiol in the brain. J*. Steroid Biochem. and Mol. Biol.*, 108(3-5), 327-338.

Mowla, S.J., Pareek, S., Farhadi, H.F., Petrecca, K., Fawcett, J.P., Seidah, N.G., Morris, S.J., Sossin, W.S., Murphy, R.A. 1999, Differential sorting of nerve growth factor and brain-derived neurotrophic factor in hippocampal neurons. *J. Neurosci.*, 19(6), 2069-2080.

Mugnaini, E., Berrebi, A.S., Morgan, J.I., Curran, T. 1989, Fos-like immunoreactivity induced by seizure in mice is specifically associated with euchromatin in neurons. Eur. J. Neurosci., 1, 46-52.

Murase, S., Grenhoff J., Chouvet, G., Gonon, F.G., Svensson, T.H. (1993), Prefrontal cortex regulates burst firing and transmitter release in rat mesolimbic dopamine neurons studied in vivo. *Neuroscience Letters*, 157, 53-56.

Nair-Roberts, R.G., Chatelain-Badie, S.D., Benson, E., White-Cooper, H., Bolam, J.P., Ungless, M.A. 2008, Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, subtantia nigra and retrorubral field in the rat. *Neuroscience*, 152(4), 1024-1031.

Nayerossadat, N., Maedeh, T., Ali, P.A., 2012. Viral and nonviral delivery systems for gene delivery

Nestler, E.J. 2001, Molecular basis of long-term plasticity underlying addiction, *Nat. Rev. Neurosci.*, 2, 119-128.

Nestler, E.J. 2008, Transcriptional mechanisms of addiction: role of DeltaFosB. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.*, 363, 3245-3255.

Nestler, E.J., 2014. Epigenetic mechanisms of drug addiction. *Neuropharmacology*, 76, 259-268.

Nequin, L.G., Alvarez, J., Schwartz, N.B. 1979, Measurement of serum steroid and gonadotropin levels and uterine and ovarian variables throughout 4 day and 5 day estrous cycles in the rat. *Biol. Reprod*., 20, 659-670.

NIDA, 2014. Drug facts: understanding drug abuse and addiction., p 1-4, Washington D.C.: National Institutes of Health, National Institute on Drug Abuse.

Niehaus, J., Murali, M., & Kauer, J., 2010. Drugs of abuse and stress impair LTP at inhibitory synapses in the ventral tegmental area. Eur J Neurosci. 32, 108-117.

Nikulina, E.M., Arrilaga-Romany, I., Miczek, K.A., Hammer, R.P., Jr. 2008, Long-lasting alteration in mesocorticolimbic structures after repeated social defeat stress in rats: time course of mu-opioid receptor mRNA and fosB/delta-fosB immunoreactivity. *E. J. Neurosci,* 27(9), 2272-2284.

Nikulina, E.M., Covington, H.E., 3rd, Ganschow, L., Hammer Jr., R.P., & Miczek, K.A., 2004. Long-term behavioral and neuronal cross-sensitization to AMPH induced by repeated brief social defeat stress: Fos in the ventral tegmental area and amygdala. Neuroscience. 123, 857-865.

Nikulina, E.M., Jonston, C.E., Wang, J., Hammer, R.P. Jr. 2014, Neurotrophins in the ventral tegmental area: role in social stress, mood disorders and drug abuse. *Neuroscience*, 282, 122-138.

Nikulina, E.M., Lacagnina, M.J., Fanous, S., Wang, J., & Hammer Jr., R.P., 2012. Intermittent social defeat stress enhances mesocorticolimbic ΔFosB/BDNF co-expression and persistently activates corticotegmental neurons: implication for vulnerability to psychostimulants. Neuroscience. 212, 38-48.

Oades, R.D. & Halliday, G.M. 1987, Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. *Brain Res. Rev*., 12(2), 117-165.

Overton, P.G., Tong, Z.Y., Brain, P.F., Clark, D. (1996), Preferential occupation of mineralocorticoid receptors by corticosterone enhances glutamate-induced burst firing in rat midbrain dopaminergic neurons, *Brain Research*, 737, 146-154.

Pacchioni, A.M., Gioino, G., Assis, A., Cancela, L.M. 2002, A single exposure to restraint stress induces behavioral and neurochemical sensitization to stimulating effects of amphetamine. *Ann. N.Y. Acad. Sci.*, 965, 233-246.

Park, H. & Poo, H. 2013, Neurotrophin regulation of neural circuit development and function. *Nat. Rev. Neurosci*., 14, 7-23.

Paxinos, G. & Watson, C., 2007. *The rat brain in stereotaxic corrdinates*, New York, New York.

Perry, R.I., Krmpotich, T., Thompson, L.L., Mikulich-Gilbertson, S.K., Banich, M.T., Tanabe, J. 2013, Sex modulates approach systems and impulsivity in substance dependence. Drug Alcohol. Depend., 133, 222-227.

Piazza, P.V., Deminiere, J.M., le Moal, M., Simon, H. 1990, Stress- and pharmacologically-induced behavioral sensitization increases vulnerability to acquisition of amphetamine self-administration. *Brain Res.*, 514(1), 22-26.

Pierce, R.C., Kumaresan, V. 2006, The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neurosci. & Biobehav. Rev.*, 30(2), 215-238.

Ping, A., Xi, J., Prasad, B.M., Wang, M.H., & Kruzich, P.J., 2008. Contributions of nucleus accumbens core and shell GluR1 containing AMPA receptors in AMPA- and cocaine-primed reinstatement of cocaine-seeking behavior. Brain Res. 1215, 173-182.

Pu, L., Liu, Q.S., Poo, M.M. (2006) BDNF-dependent synaptic sensitization in midbrain dopamine neurons after cocaine withdrawal., Nat Neurosci. 9, 605-607.

Pulliam, J.V., Dawaghreh, A.M., Alema-Mensah, E., Plotsky, P.M. 2010, Social defeat stress produces prolonged alterations in acoustic startle and body weight gain in male Long Evans rats. *J. Psych. Res.*, 44, 1405-1416.

Quinones-Jenab V., Jenab S., 2010. Progesterone attenuates cocaine-induced responses. *Horm Behav.* 58, 22-32.

Reed B.G., Carr B.R., 2015. The normal menstrual cycle and the control of ovulation. In: De Groot L.J., Chrousos G., Dungan K., *et al.,* eds. *Endotext [Internet]*. MDText.com, Inc. Available online at: https://www.ncbi.nlm.nih.gov/books/ NBK279054

Reid, M.S., Hsu Jr. K., Berger, S.P., 1997, Cocaine and amphetamine preferentially stimulate glutamate release in the limbic system: studies on the involvement of dopamine. Synapse. 27, 95-105.

Reid, M.S. & Thakkar, V., 2009. Valproate treatment and cocaine cue reactivity in cocaine dependent individuals. Drug and Alcohol Dependence. 102, 144-150.

Rivier, C. 1990, Role of interleukins in the stress response. In Stress: neurobiology and neuroendocrinology (eds G. Jasmin and M. Cantin). New York: Dekker.

Roberts, D.C., Koob, G.F., Klonoff, P., Fibiger, H.C. 1980. Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. *Pharmacol. Biochem. Behav*., 12(5), 781-787.

Robinson, T.E. 1984, Behavioral sensitization: characterization of enduring changes in rotational behavior produced by intermittent injections of amphetamine in male and female rats. *Psychopharmacology (Berl)*, 84, 466-475.

Robinson, T.E. & Berridge, K.C. 1993, The neural basis of drug craving: an incentive-sensitization theory of addiction, *Brain Res. Rev*., 18, 247-291.

Robinson, T.E. & Berridge, K.C. 2008. The incentive sensitization theory of addiction: some current issues, *Philosophical Transactions of the Royal Society B*, 363, 3137-3146.

Rodnitzky, R.L. & Keyser, D.L. 1992, Neurologic complications of drugs. Tardive dyskinesias, neuroleptic malignant syndrome, and cocaine-related syndromes. *Psychiatr. Clin. North Am*., 15(2), 491-510.

Roth M.E., Carroll M.E., 2004. Sex differences in the escalation of intravenous cocaine intake following long- or short-access to cocaine self-administration. *Pharmcol. Biochem. Behav.* 78, 199-207.

Roth, R.H., Tam, S.Y., Ida, Y., Yang, J.X., Deutch, A.Y. 1988, Stress and the mesocorticolimbic dopamine systems. *Ann. N.Y. Acad. Sci*., 537, 138-147.

Rouge-Pont, F., Marinelli, M., Le Moal, M., Simon, H., Piazza, P.V. 1995, Stress-induced sensitization and glucocorticoids. II. Sensitization of the increase in extracelluar dopamine induced by cocaine depends on stress-induced corticosterone secretion. *J. Neurosci.*, 15(11), 7189-7195.

Rudolph, M.L., Neve, R., Hammer, R.P. Jr., Nikulina, E.N., 2020. Enhanced psychostimulant response, but not social avoidnace, depends on GluA1 AMPA receptors in VTA dopamine neurons following intermittent social defeat stress in rats. *Eur. J. Neurosci.*, DOI: 10.1111/ejn.14884

Russo, S.J., Festa, E.D., Fabian, S.J., Gazi, F.M., Kraish, M., Jenab, S., Quinones-Jenab, V. 2003, Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats., *Neuroscience.*, 120, 523-533.

SAMHSA. 2014, Results from the 2013 National Survey on Drug Use and Health: summary of national findings, p 1-170 Rockville, MD, USA: Substance Abuse and Mental Health Services Administration.

Satel, S.L., Price, L.H., Palumbo, J.M., McDougle, C.J., Krystal, J.H., Gawin, F., Charney, D.S., Henninger, G.R., Kleber, H.D., 1991. Clinical phenomenology and neurobiology of cocaine abstinence: a prospective inpatient study, *Am. J. Psych*., 148, 1712-1716.

Sato, M., Chen, C.C., Akiyama, K., Otsuki, S., 1983. Acute exacerbation of paranoid psychotic state after long-term abstinence in patiens with previous metamphetamine psychosis. *Biol. Psychiat*., 18, 429-440.

Sawchenko, P.E., Arias, C.A., Mortrud, M.T. 1993, Local tetrodotoxin blocks chronic stress effects on corticotropin-releasing factor and vasopressin messenger ribonucleic acids in hypophysiotropic neurons. *J. Neuroend*., 5(4), 341-348.

Schlauch, R.C., Breiner, M.J., Stasiewicz, P.R., Christensen, R.L., Lang, A.R. 2013, Women inmate substance abusers' reactivity to visual alcohol, cigarette, marijuana, and crack-cocaine cues: approach and avoidance as separate dimensions of reactivity. J. *Psychopathol. Behav. Assess*. 35, 45-56.

Schmitz, Y., Lee, C.J., Schmauss, C., Gonon, F., and Sulzer, D. 2001, Amphetamine distorts stimulation-dependent dopamine overflow: effects on D2 autoreceptors, transporters, and synaptic vesicle stores. *J. Neurosci*., 21, 5916-5924.

Schobert, B., Lanyi, J.K. 1982. Halorhodopsin is a light-driven chloride pump. *J. Biol. Chem*., 257 (17), 10306-10313.

Schwartz, N.B. 1964, Acute effects of ovariectomy on pituitary LH, uterine weight, and vaginal cornification. *Am. J. Physiol.*, 107, 1251-1259.

Schultz, K.N., von Esenwein, S.A., Hu, M., Bennett, A.L., Kennedy, R.T., Musatov, S., Toran-Allerand, C.D., Kaplitt, M.G., Young, L.J., Becker, J.B. 2009, Viral vector-mediated overexpression of estrogen receptor-a in striatum enhances the estradiol-induced motor activity in female rats and estradiol-modulated GABA release, J. Neurosci., 29(6), 1897-1903.

Segarra A.C., Agosto-Rivera J.L., Febo M., Lugo-Escobar N., Menéndez-Delmestre R., Puig-Ramos A., Torres-Diaz Y.M., 2010. Estradiol: a key biological substrate mediating the response to cocaine in female rats. *Horm Behav.* 58, 33-43.

Seidah, N.G., Benjannet, S., Pareek, S., Chretien, M., Murphy, R.A. 1996, Cellular processing of the neurotrophin precursors of NT3 and BDNF by the mammalian proprotein convertases. FEBS Press, https://doi.org/10.1016/0014-5793(95)01520-5

Seroogy, K.B., Lundgren, K.H., Tran, T.M.D., Guthrie, K.M., Isackson, P.J., Gall, C.M. 1994, Dopaminergic neurons in rat ventral midbrain express brain-derived neurotrophic factor and neurotophin-3 mRNAs. *J. Comp. Neuro*., 342(3), 321-334.

Sesack, S.R. & Pickel, V.M., 1992. Prefrontral cortical efferents in the rat synapse on unlabeled neuronal targets of catecholamine terminals in the nucleus accumbens septi on dopamine neurons in the rat ventral tegmental area. J Comp Neurol. 320, 145-160.

Shinamoto A., DeBold J.F., Holly E.N., Miczek K.A., 2011. Blunted accumbal dopamine response to cocaine following chronic social stress in female rats: exploring a link between depression and drug abuse. *Psychopharmacology* 218, 271-279.

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

Sinha, R., 2008. Chronic stress, drug use, and vulnerability to addiction. Ann N Y Acad Sci. 1141, 105-130.

Sinha, R., 2011. New findings on biological factors predicting addiction relapse vulnerability. *Curr Psychiatry Rep*. 13, 398-405.

Sofuoglu M., Dudish-Poulsen S., Nelson D., Pentel P.R., Hatsukami D.K., 1999. Sex and menstrual cycle differences in the subjective effects from smoked cocaine in humans. *Exp. Clin. Psychopharmacol.* 7, 274-283.

Sparta, D.R., Jennings, J.H., Ung, R.L., Stuber, G.D., 2013. Optogenetic strategies to investigate neural circuitry engaged by stress. *Behav. Brain Res*. 255, 19-25.

Spencer, J.L., Waters, E.M., Milner, T.A., McEwen, B.S. 2008, Estrous cycle regulates activation of hippocampal Akt, LIM kinase, and neurotrophin receptors in C57BL/6 mice. *Neuroscience*, 155(4), 1106-1119.

Spornitz, U.M., Socin, C.D., Dravid, A.A., 1999, Estrous stage determination in rats by means of scanning electron microcopic images of uterine surface epithelium., *Anat. Rec*., 254(1), 116-126.

Stamatakis, A.M., Jennings, J.H., Ung, R.L., Blair, G.A., Weinberg, R.J., Neve, R.L., Boyce, F., Mattis, J., Ramakrishnan, C., Deisseroth, K., & Stuber, G.D., 2013. A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward. Neuron. 80, 1039-1053.

Stelly C.E., Pomrenze, M.B., Cook, J.B., Morikawa, H., 2016. Repeated social defeat stress enhances glutamatergic synaptic plasticity in the VTA and cocaine place conditioning. eLife. DOI: 10.7554/eLife. 15448.

Stewart, J. & Vezina, P. 1989, Microinjections of Sch-23390 into the ventral tegmental area and substantia nigra pars reticulata attenuate the development of sensitization to the locomotor activating effects of systemic amphetamine. *Brain Res*, 495(2), 401-406.

Straub, C., Tomita, S., 2012. The regulation of glutamate receptor trafficking and function by TARPs and other transmembrane auxiliary subunits. Curr Opin Neurobiol. 22(3), 488-495.

Strakowski, S.M., Sax, K.W., Setters, M.J., Keck, P., Jr., 1996. Enhanced response to repeated d-amphetamine challenge: evidence for behavioral sensitization in humans. *Biol. Psychiatry*, 40, 872-880.

Stuber, G.D., Hnasko, T.S., Britt, J.P., Edwards, R.H., & Bonci, A., 2010. Dopaminergic terminals in the nucleus accumbens but not the dorsal striatum corelease glutamate. J. Neurosci.. 30, 8229-8233.

Sulzer, D., Chen, T.K., Lau, Y.Y., Kristensen, H., Rayport, S., and Ewing, A., 1995. Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *J. Neurosci*., 15, 4102-4108.

Sulzer, D., Sonders, M.S., Poulsen, N.W., and Galli, A., 2005. Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol*, 75, 406-433.

Swanson, L.W., 1982. The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. Brain Res Bull. 9, 321-353.

Svensson, T.H., Tung, C.S. (1996), Local cooling of pre-frontal cortex induces pacemaker-like firing of dopamine neurons in rat ventral tegmental area in vivo. A*cta Physiologica Scandinavica*, 136, 135-136.

Taber, M.T. & Fibiger, H.C., 1995. Electrical stimulation of the prefrontal cortex increases dopamine release in the nucleus accumbens of the rat: modulation by metabotropic glutamate receptors. J Neurosci. 15, 3896-3904.

Takahashi A., Chung J.R., Zhang S., Zhang H., Grossman Y., Aleyasin H., Flanigan M.E., Pfau M.L, Menard C., Dumitriu D., Hodes G.E., McEwen B.S., Nestler E.J, Han M., Russo S.J., 2017. Establishment of a repeated social defeat stress model in female mice. *Sci Rep* 7, 12838.

Teng, K.K., Felice, S., Kim, T., Hempstead, B.L. 2010, Understanding proneurotrophin actions: recent advances and challenges. *Dev. Neurobiology*, 70(5), 350-359.

Terner, J.M. & de Wit, H. 2006, Menstrual cycle phase and responses to drugs of abuse in humans, *Drug and Alcoh. Dep*., 84(1), 1-13.

Thierry, A.M., Tassin, J.P., Blanc, G., Glowinski, J. 1976, Selective activation of mesocortical DA system by stress. *Nature*, 263(5574), 242-244.

Tidey J.W., Miczek, K.A., 1996. Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. Brain Res. 721, 140-149.

Tidey, J.W., Miczek, K.A. 1997, Acquisition of cocaine self-administration after social stress: role of accumbens dopamine. *Psychopharmacology (Berl)*, 130(3), 203-212.

Tong, Z.Y., Overton, P.G., Clark, D. 1996, Stimulation of the prefrontal cortex in the rat induces patterns of activity in midbrain dopaminergic neurons which resemble natural burst events. *Synapse*, 22, 195-208.

Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., Hansen, K.B., Yuan, H., Myers, S.J., Dingledine, R. 2010, Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol. Rev*., 62, 405-496.

Tritsch, N.X., Ding, J.B., & Sabatini, B.L., 2012. Dopaminergic neurons inhibit striatal output through non-canonical release of GABA. *Nature*. 490, 262-266.

Tye, K.M., Mirzabekov, J.J., Warden, M.R., Ferenczi, E.A., Tsai, H.C., Finkelstein, J., Kim, S.Y., Adhikari, A., Thompson, K.R., Andalman, A.S., Gunaydin, L.A., Witten, I.B., & Deisseroth, K., 2013. Dopamine neurons modulate neural encoding and expression of depression-related behaviour. Nature. 493, 537-541.

Ugolini, A., Corsi, M., and Bordi, F., 1997. Potentiation of NMDA and AMPA responses by group I mGluR in spinal cord motoneurons. *Neuropharmacology*, 36, 1047-1055.

Uhl, G.R. & Grow, R.W., 2004. The burden of complex genetics in brain disorders. *Arch. Gen. Psychiatry*, 61(3), 223-229.

Ungless, M.A., Singh, V., Crowder, T.L., Yaka, R., Ron, D., Bonci, A. Corticotropin-releasing factor requires CRF binding protein to potentiate NMDA receptors via CRF receptor 2 in dopamine neurons, *Neurons*, 39(3), 401-407.

Ungless, M.A., Whistler, J.L., Malenka, R.C. & Bonci, A., 2001. A single cocaine exposure in vivo induces long-term potentiation in dopamine neurons. Nature. 411, 583-587.

UNODC. 2014, World Drug Report, p 1-197: United Nations Office on Drugs and Crime.

Uylings, H.B.M., Groenewegen, J.J., and Kolb, B. (2003), Do rats have a prefrontal cortex? *Behav. Brain Res*., 146, 3-17.

Valenti, O., Gill, K.M., Grace, A.A., 2012. Different stressors produce excitation or inhibition of mesolimbic dopamine neuron activity: response alteration by stress pre-exposure. Eur J Neurosci. 35, 1212-1321.

Vanderschuren, L.J. & Kalivas, P.W., 2000. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl). 151, 99-120.

Vanderschuren, L.J. & Pierce, R.C. 2010, Sensitization processes in drug addiction. *Behav. Neurosci. Drug Addict.*, 179-195.

Venzala, E., Garcia-Garcia, A.L., Elizalde, N., Delagrange, P., Tordera, R.M. 2012, Chronic social defeat stress model: behavioral features, antidepressant action, and interaction with biological risk factors. *Psychopharmacology*, 224, 313-325.

Vezina, P. 2004, Sensitization of midbrain dopamine neuron reactivity and the self-administration of psychomotor stimulant drugs. *Neurosci. and Biobehav. Rev*., 27(8), 827-839.

Vezina, P. & Leyton, M. 2009, Conditioned cues and the expression of stimulant sensitization in animals and humans, *Neuropharmacology*, 56, 160-168.

Vezina, P. & Stewart, J. 1989, The effect of dopamine receptor blockade on the development of sensitization to the locomotor activating effects of amphetamine and morphine. *Brain Res*, 499(1), 108-120.

Walker, Q.D., Cabassa, J., Kaplan, K.A., Shih-Tzung, L., Haroon, J., Spohr, H.A., Kuhn, C.M. 2001, Sex differences in cocaine-stimulated motor behavior: disparate effects of gonadectomy, *Neuropsychopharmacology*, 25, 118-130.

Wanat, M.J., Hopf, F.W., Stuber, G.D., Phillips, P.E.M., Bonci, A. 2008, Corticotropin-releasing factor increases mouse ventral tegmental area dopamine neuron firing through a protein kinase C-dependent enhancement of I_h, *J. Physiol*., 586(8), 2157-2170.

Wang, J., Bina, R.W., Wingard, J.C., Terwilliger, E.F., Hammer Jr., R.P. & Nikulina, E.M., 2014. Knockdown of tropomyosin-related kinase B receptor expression in the nucleus accumbens shell prevents intermittent defeat stress-induced cross-sensitization to amphetamine in rats. Eur J Neurosci. 39, 1009-1017.

Wang, J., Fanous, S., Terwilliger, E.F., Bass, C.E., Hammer Jr., R.P. & Nikulina, E.M., 2013. BDNF overexpression in the ventral tegmental area prolongs social defeat stress-induced cross-sensitization to amphetamine and increases ΔFosB expression in mesocorticolimbic region of rats. Neuropsychopharmacology. 38, 2286-2296.

White, F.J., Wang, R.Y. 1984, A10 dopamine neurons: role of autoreceptors in determining firing rate and sensitivity to dopamine agonists. *Life Sciences*, 34)12), 1161-1170.

White T.L., Justice A.J.H., de Wit H., 2002. *Differential subjective effects of D-amphetamine by gender, hormone levels and menstrual cycle phase*. *Pharmacol Biochem Behav* 73: 729–741.

Witten, I.B., Steinberg, E.E., Lee, S.Y., Davidson, T.J., Zalocusky, K.A., Bordsky, M., Yizhar, O., Cho, S.L., Gong, S., Ramakrishnan, C., Stuber, G.D., Tye, K.M., Janak, P.H., Deisseroth, K. 2011, *Neuron,* 72(5), 721-733.

Wolf, M.E., 2010. Regulation of AMPA receptor trafficking in the nucleus accumbens by domaine and cocaine. Neurotox Res. 18, 393-409.

Wolf, M.E. & Tseng, K.Y., 2012. Calcium-permeable AMPA receptors in the VTA and nucleus accumbens after cocaine exposure: when, how, and why? Front Mol Neurosci. 5, 72.

Wolf, M.E., White, F.J., Nassar, R., Brooderson, R.J., Khansa, M.R. 1993, Differential development of autoreceptor subsensitivity and enhanced dopamine release during amphetamine sensitization. *J. Pharmacol. Exper. Therap*., 264, 249-255.

Wolf, M.E. & Xue, C.J., 1999. AMPH-induced glutamate efflux in the rat ventral tegmental area is prevented by MK-801, SCH 23390, and ibotenic acid lesions of the prefrontal cortex. *J Neurochem.* 73, 1529-1538.

Xue, C.J., Ng, J.P., Li, Y., & Wolf, M.E., 1996. Acute and repeated systemic AMPH administration: effects on extracelular glutamate, aspartate, and serine levels in rat ventral tegmental area and nucleus accumbens. *J Neurochem*. 67, 352-363.

Yamaguchi, T., Sheen, W., Morales, M. 2007, Glutamatergic neurons are present in the rat ventral tegmental area. *Eur. J. Neurosci*., 25(1), 106-118.

Yap, J.J., Covington, H.E. III, Gale, M.C., Datta, R., Miczek, K.A. 2005, Behavioral sensitization due to social defeat stress in mice: antagonism at mGluR5 and NMDA receptors, *Psychopharmacology*, 179, 230-239.

Yizhar, O., Fenno, L.E., Davidson, T.J., Mogri, M., Deisseroth, K., 2011. Optogenetics in neural systems. *Neuron*. 71, 9-34.

Young, W.C., Boiling, J.L., Blandau, R. 1941, The vaginal smear picture, sexual receptivity and time of ovulation in the albino rat. *Anat. Rec*., 80, 37-45.

You, Z.B., Wang, B., Zitzman, D., Azari, S., Wise, R.A. (2007), A role for conditioned ventral tegmental glutamate release in cocaine seeking. *J. Neurosci*., 27, 10546-10555.

Zahm, D.S. 2000, An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. *Neuroscience and Biobehavioral Reviews*, 24(1), 85-105.

Zakharova, E., Wade, D., Izenwasser, S., 2009, Sensitivity to cocaine conditioned reward depends on sex and age. *Pharm. Biochem. Behav*., 92, 131-134.

Zhang, S., Qi, J., Li, X., Wang, H., Britt, J.P., Hoffman, A.F., Bonci, A., Lupica, C.R., & Morales, M., 2015. Dopaminergic and glutamatergic microdomains in a subset of rodent mesoaccumbens axons. *Nat Neurosci.* 18, 386-392.

Zhang, X.F., Hu, X.T., White, F.J., & Wolf, M.E., 1997. Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or AMPH is transient and selectively involves AMPA receptors. *J Pharmacol Exp Ther*. 281, 699-706.

Zhang, F., Vierock, J., Yizhar, O., Fenno, L.E., Tsunoda, S., Kianianmomeni, A., Prigge, M., Berndt, A., Cushman, J., Polle, J., Magnuson, J., Hegemann, P., Deisseroth, K. 2011, The microbial opsin family of optogenetic tools, *Cell,* 147(7), 1446-1457.

Zhen, X., Goswami, S., Abdali, S.A., Frankfurt, M., Friedman. 2007, Estrogen-modulated frontal cortical CaMKII activity and behavioral supersensitization induced by prolonged cocaine treatment in female rats, *Psychopharmacology*, 191, 323-331.