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Cocaine Diminishes Consolidation of Cued Fear Expression in Female Rats Through Interactions With Dopamine D2 Receptors

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Abstract

In addition to cocaine's addictive properties, cocaine use may lead to heightened risk-taking behaviors in individuals despite potentially aversive consequences. One possible reason for this may be cocaine's disruptive effect on aversive memory formation. The present study investigated the effects of cocaine on fear memory formation using a cued fear conditioning paradigm in female Sprague Dawley rats. On day 1, animals received tone-shock pairings and on day 2 (24 hours later) were returned to the fear chamber and tested for recall of fear memory. Fear was measured as percent time the animal spent freezing during the tone presentation. In Experiment 1 (n = 48), cocaine (15mg/kg; i.p.) was administered prior to or immediately after the conditioning trials to assess the effect of cocaine on fear memory acquisition and consolidation. To determine whether cocaine's effects on memory consolidation are mediated by D2 receptors, the D2 receptor antagonist eticlopride (0.1mg/kg; i.p.) was administered concurrently with cocaine. No drugs were administered on test day. Results from Experiment 1 revealed that pre-training cocaine diminishes fear acquisition and that post-conditioning cocaine resulted in diminished fear expression during fear test. Concurrent D2 antagonism attenuated the impairing effect of cocaine on fear memory consolidation, with animals showing increased freezing relative to animals receiving cocaine alone. In Experiment 2 (n = 15), animals received direct infusions of eticlopride (0.05 μl/min) into the ventral hippocampus (VH), a structure known to be involved in cued fear conditioning and a target region of ventral tegmental area, substantia nigra, and locus coeruleus dopaminergic neurons. Intra-VH eticlopride or saline was directly infused into the VH immediately after conditioning concurrent to cocaine administration. Results from Experiment 2 suggest that the antagonism of VH D2 receptors may disrupt the impairing effects of cocaine on fear memory consolidation, suggesting the VH as a potential region mediating this effect. The present study provides evidence that acute cocaine administration impairs aversive memory formation and establishes a potential circuit through which cocaine induces its detrimental effects on fear memory consolidation. Moreover, these results provide insight into why cocaine users might engage in impulsive and risk-taking behavior that could lead to fatal consequences.

Key words: cocaine, eticlopride, fear memory, consolidation, D2 receptors, ventral hippocampus

Chapter 1: Literature Review

Addiction is a recurring condition that affects thousands every year. In 2021, the CDC estimated over 107,500 deaths by overdose, which was a 15% increase from 2020. Since 2015, the number of psychostimulant drug-related deaths has steadily increased, with over 30,000 deaths reported in 2021 [1]. Psychostimulant drugs, like cocaine, have highly addictive potential due to the euphoric feeling they induce, and in 2021 the death toll for cocaine-related overdose surpassed 24,000 [1]. Over the past years, cocaine use has increased dramatically, with 4.8 million people over the age of 12 reporting using cocaine in the past 12 months as of 2021 [2]. Additionally, in 2021 it was reported that approximately 1.4 million people aged 12 or older were diagnosed with cocaine use disorder [2]. Cocaine's highly addictive properties make it one of the most prevalent threats to health and addiction in the United States.

Cocaine use increases the amount of dopamine in the brain by interfering and blocking dopamine transporters [3]. The excess concentration of dopamine in the synapse reinforces the brain's reward circuitry and promotes increased drug-seeking behavior [4]. The strong feelings of reward, euphoria, and pleasure can lead to dangerous, and sometimes lethal, withdrawal symptoms, such as paranoia, anxiety, insomnia, fatigue, and constricted blood vessels [4]. Cocaine use can have further detrimental effects on an individual's wellbeing, not only physical but also psychological. It can cause individuals to isolate themselves and engage in maladaptive behaviors repeatedly, such as using infected needles, increasing the risk of contracting diseases like HIV [4]. It has become evident that cocaine abuse can lead to individuals partaking in threatening situations, disregarding the danger that these pose and leading to potentially fatal consequences.

Drug use often increases impulsive behaviors and impairs inhibition and decision-making, resulting in an increased likelihood of engaging in potentially risky behaviors, such as deviant or risky sexual behavior [5]. Cocaine use has been associated with increased risk taking behavior, and previous neuroimaging studies have shown altered activity in the reward and decision-making circuitry of users, offering a potential explanation as to why cocaine users engage in heightened risk-taking [6]. Additionally, research has found that crack cocaine users present greater levels of impulsivity and risk-taking propensity when compared to other illicit drug users, like heroin users [7]. However, it remains unclear what drives this increase in risky and impulsive behavior. It is a possibility that individuals with cocaine use disorder are more sensitive to losses, which leads to increased risk-taking behavior and further drug use despite potential aversive outcomes [6]. More research is needed to understand why there is an association between cocaine use and increased risk-taking behavior and the potential consequences this may have.

Drug-induced impairments could also influence further drug use through engagement of reward reinforcement systems [8]. One possible reason for cocaine user's engagement in risky and dangerous behavior is cocaine's negative effect on memory formation, particularly aversive memories [9]. Previous research has established that there are impulse-related cognitive dysfunctions in individuals with cocaine use disorder [10]. Impulsivity, often referred to as risk-taking behavior [11], is defined as a poorly conceived action that leads to a response without reflection or conscious judgment [12]. Studies have found that impulse-reward behavior is highly affected by cocaine, resulting in impaired decision making [10]. Multiple clinical literature has demonstrated that cocaine use disorder is associated with increased action without conscious thought as well as increased sensation seeking [13]. While limited research has focused on the

role that cocaine plays in the acquisition and consolidation of fear memories, it is possible that cocaine use may lead to continued risk-taking behavior because it impairs formation of memory related to aversive consequences. While learning and memory has been widely studied in neuroscience, not enough research has been conducted on how cocaine use interferes with memory processes, particularly aversive memory consolidation.

Learning and memory are closely related processes. Learning refers to the acquisition of knowledge over time, while memory refers to the expression of acquired knowledge. Diverse theories of learning explain how mammals acquire knowledge. Pavlov's classical conditioning theory of learning attempts to explain how mammals learn to associate a specific stimulus with a response. Classical conditioning is the process through which a conditioned response (CR) is obtained by pairing a neutral stimulus (NS) with an unconditioned stimulus (US), which usually elicits an unconditioned response (UR) [14]. The subject is presented with the neutral stimulus enough times for them to make an association between the now conditioned stimulus (CS) and the unconditioned response, leading to a conditioned response. Pavlov's theory of classical conditioning can be applied to aversive memory formation. Pavlovian fear conditioning is used to study the acquisition and consolidation of fearful memories and can be implemented utilizing a contextual or cued fear conditioning paradigm [15].

Pavlovian cued fear conditioning is a paradigm used to measure aversive memories and understand the neurobiological mechanisms underlying fear learning and memory. In such a paradigm, a rodent is placed in a conditioning chamber in which they are presented with an auditory cue, often a tone, followed by a mild footshock. Following classical conditioning learning theory, the auditory cue represents the neutral stimulus, as it elicits no response when presented on its own at the beginning of the paradigm. The shock represents the unconditioned

stimulus, leading to an unconditioned response that is measured in rodents as freezing behavior. Over time, the rodent will learn to associate the auditory cue with the footshock, which will now elicit the conditioned response of freezing behavior even without the presence of the unconditioned stimulus. During fear conditioning, the animal will show increased fear memory acquisition, indicating that the rodent is learning to make the association between the CS and the US [16]. Acquisition of fear memory occurs when the animal learns to make an association between a context (auditory cue) and a consequence (footshock) [17]. Pavlovian cued fear conditioning can be helpful in assessing whether a fear memory has been acquired and consolidated. During the conditioning trials, a clear acquisition curve that depicts the animal's learning process should be observed. Fear memory consolidation is then tested with the fear test, which usually takes place 24 hours after the conditioning trials to test for recall of fear memory.

Aversive learning is a complex process that involves the activation of several brain structures. Evidence suggests that the amygdala is involved in the acquisition, consolidation, and expression of fear memories [18]. Pharmacological and neurophysiological studies identify the amygdala as the key neural structure subserving fear conditioning [19][20]. The amygdala has been implicated as an emotive brain center in the mammalian brain which receives sensory inputs from multiple brain regions, such as the thalamus, neocortex, and the hippocampus [18]. The amygdala sends projections to diverse autonomic and somatomotor structures that seem to mediate fear responses, such as the bed nucleus of stria terminalis to activate stress hormones and the periaqueductal gray (PAG) matter to elicit freezing behavior [18]. It is believed that CS-US association takes place in the basal and lateral nuclei of the amygdala (BLA), where sensory information first enters the amygdala [18]. This information then enters the central nucleus of the amygdala (CeA), which sends projections to multiple autonomic and somatomotor

centers that mediate fear responses [18]. Previous research has also implicated projections from the acoustic thalamus to the amygdala in the classical conditioning of emotional responses when an auditory stimulus is involved [21][22].

The hippocampus is a brain region known as the memory center of the brain due to its heavy involvement in memory formation. Several memory mechanisms within the hippocampus are crucial in the neural circuitry of fear conditioning [23]. The ventral hippocampus (VH) has specifically been associated in memory formation that involves stress, emotion, and affect [24]. Additionally, research has shown that the VH is reciprocally connected with other limbic structures mediating aversive memory formation, such as the amygdala [25]. The ventral hippocampus is known to be an efferent target of the BLA and previous research suggests that BLA to VH projections are involved in the consolidation of footshock memory in rats [25] and that post-training inactivation of the VH impairs auditory fear conditioning [26][27]. Efferent connectivity suggests that the VH modulates emotional behaviors through projections with the amygdala [28]. Neurons in the VH are heavily involved in the acquisition of cued fear conditioning and the later expression of auditory fear [26] and both pre- and post-training lesions to the VH impair the acquisition and expression of auditory fear conditioning [29]. It appears that the ventral region of the hippocampus is a crucial structure involved in the formation of aversive memories due to its bidirectional connections with the basal amygdala [30].

The ventral hippocampus is crucial for emotional processing [31] due to its connections to the amygdala and the hypothalamic-pituitary-adrenal axis [32]. Functionally, the dorsal and ventral regions of the hippocampus mediate distinct behaviors and processes [32]. While the dorsal hippocampus is associated with spatial memory performance, the ventral hippocampus has been closely associated with anxiety-like behaviors [32]. Lesions to the VH have shown to

reduce anxiety on a variety of behavioral tests, including the elevated plus maze [33]. Furthermore, VH lesions significantly attenuate conditioned defensive behavior after administration of a footshock and result in a reduction in freezing behavior following delivery of a footshock in an operant chamber [34][35]. This suggests that the VH plays an important role in fear and anxiety-like behavior and memory.

Memory formation is a complex process that involves several distinct phases. The formation of long-term memory (LTM) involves the conversion of a short-term memory (STM) into a stable trace through consolidation [36]. Consolidation of memory occurs when the newly learned association and acquired knowledge is moved into long-term memory [17]. The consolidation theory states that STM evolves into LTM through protein synthesis that makes changes in synaptic efficacy and functioning [37]. Immediately after learning, during memory consolidation, a memory remains susceptible to disruption and subject to interference and is later stabilized [38][39]. Memory consolidation involves a cascade of cellular events that change synaptic efficacy [39].

The underlying molecular and cellular mechanisms of memory consolidation are often explained through long-term potentiation (LTP) as an experimental model. Previous research has established the NMDA receptor as a crucial molecular structure for the induction of LTP, and the Ca2+/calmodulin-dependent protein kinase II (CaMKII) downstream signaling pathway is thought to be a key mediator in regulating early LTP [40]. Late LTP includes long-lasting synaptic changes, including changes in the structure and content of dendritic spines [41], and is essential for memory storage [42]. Late LTP is dependent on cAMP response element-binding protein (CREB) phosphorylation [43] and brain-derived neurotrophic factor (BDNF) synthesis [44], which is activated via a calcium-induced protein synthesis chain. While the role of

glutamate receptors and calcium signaling has been well established in long-term memory formation, the role of other neurotransmitters, like dopamine (DA), is still being studied. However, research has found that DA seems to be involved in synaptic plasticity within the CA1 region of the hippocampus, and the CA1 region of the ventral hippocampus has been found to be crucial for the formation of fear memories [45][46]. Due to the potential involvement of DA in the formation of memories, it is likely that psychostimulant drugs that act on the dopaminergic signaling system, such as cocaine, could disrupt the memory consolidation processes.

Cocaine's mechanism of action is through the dopaminergic signaling pathway in the limbic system, which contains a high concentration of dopamine-responsive cells [47]. Cocaine binds and blocks dopamine transporters (DAT), thus inhibiting the reuptake of DA and increasing transmission in the synapse [48]. Increased transmission in the synapse results in enhanced and prolonged sympathetic and euphoric effects [48]. Within the limbic system, the nucleus accumbens (NAc) seems to be closely associated with the euphoric feelings produced by cocaine consumption [47]. The NAc receives robust inputs from the ventral tegmental area (VTA), which is composed of approximately 60% of dopaminergic neurons [49]. Despite the VTA-NAc projections being most closely associated with the underlying mechanism of cocaine, other projections from the VTA to varying brain structures, such as the ventral hippocampus and the amygdala, might also be targeted by cocaine.

Dopaminergic neurons originating in the VTA and locus coeruleus innervate the VH [50] and are hypothesized to regulate memory storage [50][52]. It is also hypothesized that encountering a novel stimulus increases dopamine levels in the hippocampus, which induces long-term potentiation and storage of long-term memories [52]. Previous research has shown that increased release of dopamine into the rat ventral hippocampus mediates recognition memory

[52]. Due to the VH's involvement in the formation of fear memories and its high concentration of dopaminergic terminals, it is likely that cocaine might be enacting its effects directly on dopaminergic VH synapses to affect fear learning. The ventral hippocampus is also known to contain a robust concentration of dopaminergic D2 receptors [53]. Evidence has found that VTA stimulation evokes binding of D2 receptors in the hippocampus and mediates working memory [54]. Very little is known about the specific distribution of D2 receptors within the dorso-ventral axis of the hippocampus and how this contributes to spatial and affective memory. Past immunohistochemical studies have assessed D2 protein expression in distinctive sublayers of the hippocampus along the dorso-ventral axis [53]. There are significantly higher levels of D2 protein expression in the ventral hippocampus with regard to the dentate gyrus, CA1, CA2, and C3 region [53]. Given the dense GABAergic interneuron and glutamatergic neuron expression in the CA1 region of the hippocampus [55], it is plausible that dopaminergic innervations target D2 receptors in these neurons.

The VTA consists of dopamine, GABA, and glutamate-releasing neuron populations [56][57] with dopaminergic neurons being the most abundant [58]. Research has shown that VTA projections to the hippocampus are approximately 15-18% dopaminergic, and the hippocampal formation primarily receives DA input from dopamine neurons in the ventral half of the VTA [59]. Dopamine modulates LTP in the hippocampus and is involved in the mediation of recognition learning and memory through interactions with D2 receptors [60]. However, research has found that VTA neurons can co-release neurotransmitters into its target regions, with inputs from the VTA into the hippocampal dentate gyrus co-releasing dopamine, GABA, and glutamate, which could also influence memory processes [61]. While dopamine projections

from the VTA to the hippocampus have been established, more research is needed to fully characterize a mesohippocampal dopamine pathway and its interactions with memory formation.

In order to investigate cocaine's effect in fear memory consolidation, the present thesis proposes a study in which a Pavlovian cued fear conditioning paradigm is used to measure fear memory. Study 1A will assess both the effects of cocaine on fear memory acquisition and fear memory consolidation by administering cocaine prior to fear conditioning and immediately after fear conditioning. Subsequently, to determine the role of dopaminergic D2 receptors, in study 1B, D2 receptor antagonist eticlopride will be concurrently administered with cocaine. Lastly, to establish the role of the ventral hippocampus on fear memory consolidation, study 2 will target this brain region through bilateral cannulation. Based on previous research supporting that the ventral hippocampus is involved in fear memory formation [25][26][27] and that the VH contains highly concentrated dopaminergic terminals [52], I hypothesized that cocaine will impair the consolidation of cued fear memory through interactions with dopaminergic D2 receptors in the ventral hippocampus.

Chapter 2: Assessing cocaine's effect on fear memory through interactions with dopamine D2 receptors in the ventral hippocampus

Cocaine use has been long associated with cognitive decline and impairments in memory processes, such as working memory [62][10]. Research suggests that, when taken at high doses, cocaine attenuates neurogenesis in the hippocampus and consequently impairs working memory [63]. Chronic cocaine abuse has deleterious effects on learning and memory storage, and past research has established a pattern of cognitive decline and significant impairment in short-term memory in cocaine users [64][65]. However, there remains a high level of uncertainty regarding cocaine's effect on memory performance. Previous studies have found that while cocaine administration affects long-term potentiation, it does not notably affect performance in a Morris water maze paradigm [66]. Contrastingly, other studies have found that cocaine can facilitate spatial memory performance in a highly demanding water maze task [67][68]. Past research has established that cocaine administration, at high doses, disrupts aversive memory [9] while other studies have found that it enhances fear memory [69]. The conflicting evidence regarding cocaine's effects on memory reveal that relatively few studies have examined the effects of acute cocaine administration on learning and memory behavioral paradigms in animal models.

Due to cocaine's known anxiogenic effects [70], it seems possible that acute cocaine administration could potentiate fear conditioning. It has been hypothesized that increased anxiety and arousal could enhance the learning of an anxiety-inducing stimulus [9]. Alternatively, it is also possible that cocaine impairs fear conditioning due to its disruptive effects in the amygdala and hippocampus, as well as other fear and attention-related brain structures like the prefrontal cortex [9]. Previous studies have demonstrated that pre-conditioning cocaine administration at low doses enhances fear conditioning while high-to-moderate doses significantly impair cued

fear conditioning [9]. The effects of cocaine on fear memory formation are complex and seem dose-dependent. More research has to be done to determine a clear relationship between acute cocaine administration and fear memory processes, such as acquisition and consolidation. The present study aimed to assess the effects of a moderate-to-high cocaine dose on both fear memory acquisition and consolidation using a Pavlovian cued fear conditioning paradigm.

In order to establish what cocaine's effect on fear memory is, the present study focused on administering cocaine both prior and immediately after fear conditioning. To confirm past research showing that a moderate-to-high dose of cocaine administered prior to cued fear conditioning impairs fear expression [9], measured as freezing behavior, in Experiment 1A cocaine (15mg/kg; i.p.) was administered 15 minutes prior to fear conditioning. It is important to consider that cocaine, being a stimulant, induces a hyper locomotive effect [71]. Based on the increased locomotion observed after cocaine administration, it is possible that animals receiving cocaine pre-conditioning will not display freezing behavior due to increased locomotor activity. To account for this effect, cocaine will be administered immediately after the conditioning trial in Experiment 1B. Previous studies assessing the effects of cocaine on fear memory formation have not focused specifically on the consolidation process. Cocaine administration immediately after conditioning will allow for the observation and assessment of cocaine's effects on fear memory consolidation.

The underlying mechanism through which cocaine might be impairing fear memory acquisition and consolidation is not fully understood. Previous research has shown that dopaminergic signaling in the hippocampus is associated with memory formation [72]. In particular, it seems that dopamine receptor stimulation in the hippocampal formation plays a role in the encoding and consolidation phases [72]. In addition to dopamine's role in memory

formation, past research has shown that dopamine signaling is closely associated with modulating mechanisms of fear and anxiety [73]. Some evidence suggests that the dopaminergic system is heavily involved in the formation of fear memories and the expression of fear conditioning, and that both D1 and D2 receptors are involved in this process [74].

Modulation of D2 receptor signaling seems to regulate the consolidation of fear responses [75] and past studies have shown that administering a D2 receptor agonist impairs a passive avoidance task in mice [76]. While some studies suggest that D2 receptors may be modulating the formation of aversive memories within the basolateral amygdala due to their robust presence [77], it is also possible that D2 receptors in the ventral hippocampus are associated with memory processes. The ventral hippocampus is a brain structure closely associated with the formation of emotional memories and has been implicated in the acquisition of cued fear memory [24][78]. Past studies have established that the ventral hippocampus contains an extensive concentration of D2 receptors and that these receptors have important influences on working memory [52][53]. D2 receptor agonism and antagonism in the ventral hippocampus mediates memory performance [79]. However, not much is known about the role of ventral hippocampal D2 receptors in mediating fear memory formation. Due to the extensive role of dopamine D2 receptors in memory processes, it is possible that cocaine's effects on fear memory consolidation are mediated by D2 receptor signaling.

To assess whether D2 receptors are involved in fear memory consolidation, D2 receptor antagonist eticlopride (0.1mg/kg; i.p.) was concurrently administered with cocaine immediately after fear conditioning in Experiment 1B. Furthermore, the present study aimed to uncover where in the brain D2 receptors are mediating cocaine's effect on fear memory consolidation. It was hypothesized that the ventral hippocampal D2 receptors could be involved in mediating fear

memory consolidation due to it being a target structure of dopaminergic projections. The ventral hippocampus receives dopamine innervations from the VTA, the substantia nigra, and the locus coeruleus [80]. Dopamine neurons projecting from the VTA to the ventral portion of the hippocampus are hypothesized to be involved in a functional loop through which dopamine signaling strengthens memory consolidation in the hippocampus [81]. Within this loop, activation of dopaminergic neurons produces an enhancement of long-term potentiation and learning, regulating the entry of information into long-term memory [81].

Past research has shown that encountering a novel stimulus increases dopamine activity in the hippocampus and that increased release of dopamine into the hippocampus is crucial for recognition memory [51]. Additionally, midbrain dopaminergic projections into the hippocampus modulate hippocampus-dependent aversive learning [82]. Furthermore, dopamine concentrations in the hippocampus seem to increase in operant-style and discriminative-style learning tasks [83]. VTA-derived dopamine signaling into the ventral hippocampus seems to mediate contextual fear conditioning [84]. Taking these findings into consideration, we wanted to assess whether dopamine signaling in the hippocampus, specifically through D2 receptors, is involved in mediating fear memory consolidation.

To evaluate the role of D2 receptors in the ventral hippocampus in mediating fear memory consolidation, Experiment 2 consisted of intra-VH infusions of the D2 receptor antagonist eticlopride. In Experiment 2, eticlopride (0.05µl/min) or saline was directly infused into the VH and was paired with a cocaine (15mg/kg) or saline injection immediately after fear conditioning. The present study examines the effect of cocaine administration on fear memory acquisition and consolidation, and aims to establish the role of dopamine D2 receptors in the

ventral hippocampus in mediating cocaine's effect on fear memory consolidation in a Pavolvian cued fear conditioning paradigm.

Methods

Subjects

Adult female Sprague-Dawley rats (Charles River Laboratories; $n = 63$) were used in this study. All animals were housed on a reverse 12:12 light-dark cycle in a temperature and humidity-controlled vivarium. Animals were given rat chow and water access *ad-libitum*.

Apparatus

The Pavlovian cued fear conditioning apparatus consists of a conditioning chamber with an electric grid on the floor of the chamber, a house light, and a tone that is presented at an interval. The conditioning chamber is equipped with a mini camera on the top that is used to record all conditioning and testing sessions. The chamber is controlled by a computer program in which the shock is set to 0.5mA and the tone is set to 80dB. The animals movement is tracked with an activity count motion sensor and the freezing time is recorded with the mini camera.

Drugs

Cocaine hydrochloride (Sigma, St. Louis, MO, USA) was administered. Cocaine was dissolved in saline (0.9% NaCl) and administered via intraperitoneal injection (i.p.) at a dose of 15mg/kg. Vehicle animals received a saline injection.

Dopamine D2 receptor antagonist eticlopride hydrochloride was dissolved in saline (0.9% NaCl) and administered at a dose of 0.1mg/kg (i.p.) or infused at a rate of 0.05µl/min.

Procedure

For this study, Pavlovian cued fear conditioning consisted of a two-day paradigm in which the rodent was conditioned in a fear conditioning chamber the first day and tested for

recall of fear memory and fear expression on the second day. Conditioning day (day 1) consisted of a 19 minute session in which the rodent was exposed to six tone-shock pairings. Each tone lasted for a total of 20 seconds at a volume of 80 dB. On second 19 of the tone, a one-second footshock, with an intensity of 0.5 mA, was presented. The rodent had five minutes of habituation on day 1 of the paradigm before the first tone-shock pairing was presented. After each tone-shock presentation, there was an inter trial interval (ITI) of 2 minutes until the next shock was presented. On day two of the experiment, which took place 24 hours after conditioning, the rodent was placed back in the conditioning chamber for a two minute and 20 second session. The first two minutes were used for habituation. After two minutes, a single tone without a shock was presented to test for recall of fear memory and fear expression. Fear expression was scored as time the rodent spent freezing during the presentation of the tone alone.

All conditioning and testing sessions were recorded with a mini camera attached to the top of the conditioning chamber. Using the camera's recording program, the videos for each session were collected and scored with a stopwatch to calculate the percent time the rodent spent freezing. The scores and rodent's freezing time were then confirmed by an additional blind observer.

In Experiment 1A, the rodent was placed in the conditioning chamber for day one of the paradigm 15 minutes after receiving cocaine (15mg/kg; i.p.) or saline. No drugs were administered 24 hours later for the fear test. Figure 1 depicts the experimental timeline for Experiment 1A. Experiment 1B consisted of a cocaine alone (15mg/kg; i.p.) or a saline alone injection immediately after the conditioning trial on day one, or cocaine or saline injection concurrently administered with D2 receptor antagonist eticlopride (0.1mg/kg; i.p.). Eticlopride

was administered immediately after conditioning and five minutes prior to the cocaine or saline injection. Figure 2 demonstrates the experimental timeline for Experiment 1B.

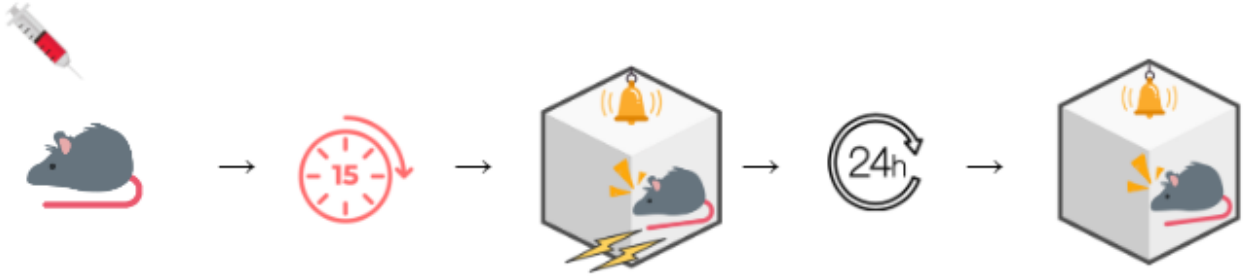

Figure 1. Experimental timeline for experiment 1A. Saline or cocaine (i.p., 15mg/kg) injection is administered prior to fear conditioning.

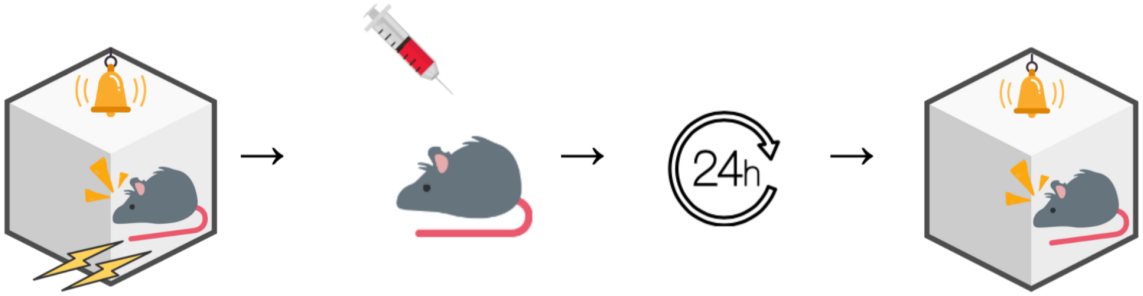

Figure 2. Experimental timeline for experiment 1B. Saline or cocaine (i.p., 15mg/kg) injection alone is administered immediately after fear conditioning, or saline and cocaine concurrently administered with eticlopride (0.1mg/kg, i.p.) immediately after conditioning.

Experiment 2 consisted of eticlopride or saline infusions directly into the ventral hippocampus paired with a cocaine (15mg/kg; i.p.) or saline injection. Prior to conditioning, animals were subjected to stereotaxic surgery and allowed to recover for at least three days. Once recovered, animals were placed in the conditioning chamber and received concurrent administration of eticlopride (0.05µl/min) via intracranial infusion with a cocaine injection. No drugs were administered 24 hours later for the fear test. The experimental timeline for Experiment 2 is shown in Figure 3.

Figure 3. Experimental timeline for experiment 2. Eticlopride (0.05µl/min) or saline infusion directly into the ventral hippocampus immediately after conditioning, paired with a cocaine (i.p., 15mg/kg) or saline injection.

Intracranial Surgeries and Cannula Placement

Stereotaxic surgery was performed in order to target the ventral hippocampus with a bilateral cannulation and the later direct infusion of D2 receptor antagonist eticlopride. Before surgery, animals were anesthetized with isoflurane and given analgesic Rimadyl (0.03ml; i.p.) and antibiotic cefazolin (0.2ml; i.p.). The scalp was then disinfected and incised in order to expose the skull. After exposing the skull, the area was cleaned and two holes, where the cannulas were placed, were drilled with respect to bregma. The cannulas targeted the ventral hippocampus (AP = -5.2mm; ML = +/- 5.2mm; DV = -5mm from dura; Figure 5). After surgery,

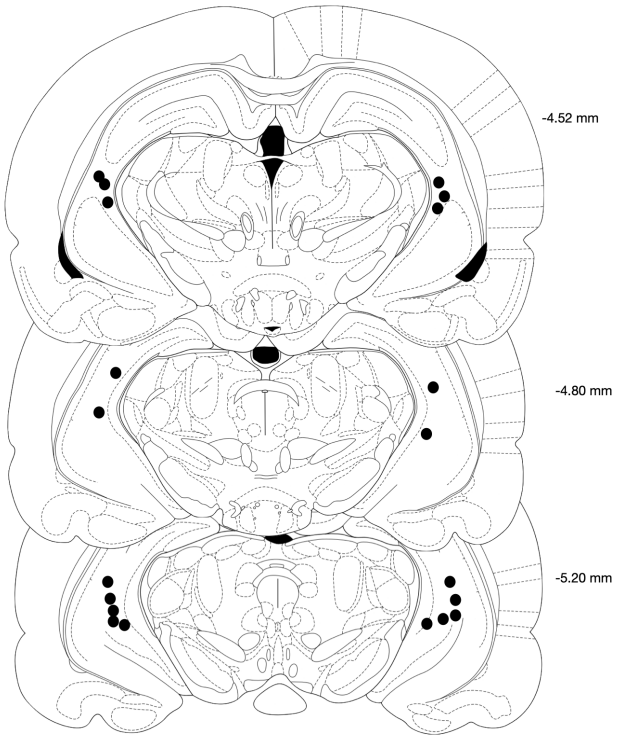

Figure 4. Bilateral cannulation of the ventral hippocampus.

animals will receive at least three days of post-operative care with daily injections of Cefazolin and Rimadyl.

Tissue Collection and Placement Scoring

Once the fear test concluded and data was collected, rats were transcardially perfused. To initiate the perfusion, rats were anesthetized with euthasol. For the perfusion we utilized 150-200 mL of cold 0.9% PBS and 200-300 mL of 10% formalin. Brains were collected and stored in formalin for the following 24 hours. After 24 hours, the brains were transferred to a 20% sucrose azide solution for 48 hours. After 48 hours, the brains were removed from the solution and frozen at -80ºC.

Brains were sliced using a cryostat slicer and mounted onto slides. They were let dry for 48-72 hours before staining with cresyl violet. After staining, slides were cover-slipped. Cannula placement was then scored under a microscope for accuracy (Figure 4).

Results

Study 1: Cocaine impairs acquisition and consolidation of cued fear memories

An unpaired t-test comparing freezing percentage behavior during test between rats receiving cocaine and rats receiving saline revealed that rats that received cocaine administration prior to fear conditioning showed significantly decreased freezing (M = 13.47; SD = 6.80) during fear test when compared to rats that received saline (M = 52.92 ; SD = 13.37), $t(14) = 7.44$, $p <$ .001 (Figure 5B). This demonstrates that cocaine administration pre-conditioning impairs fear memory acquisition, as seen by reduced freezing behavior during the fear test. A two-way repeated measures ANOVA comparing freezing behavior between cocaine-treated and eticlopride-treated rats revealed a significant main effect of cocaine [$F(1,28) = 40.50$, $p < .001$] and eticlopride [$F(1,28) = 59.45$, $p < .001$]. There was a statistically significant interaction

between cocaine and eticlopride [$F(1,28) = 49.76$, $p < .001$] (Figure 6B). A post-hoc Tukey's multiple comparison's test revealed a significant difference between the eticlopride-cocaine-treated rats ($M = 64.53$; $SD = 5.73$) and the cocaine-treated rats ($M = 13.29$; $SD = 7.08$; $p < .001$), a significant difference between the eticlopride-saline-treated rats ($M = 62.14$; $SD = 12.95$) and the cocaine-treated rats ($p < .001$), and a significant difference between the cocaine-treated rats and the saline-treated rats ($M = 59.86$; $SD = 11.61$; $p < .001$). These results indicate that animals receiving cocaine immediately after conditioning froze significantly less than animals receiving saline alone or the D2 receptor antagonist eticlopride.

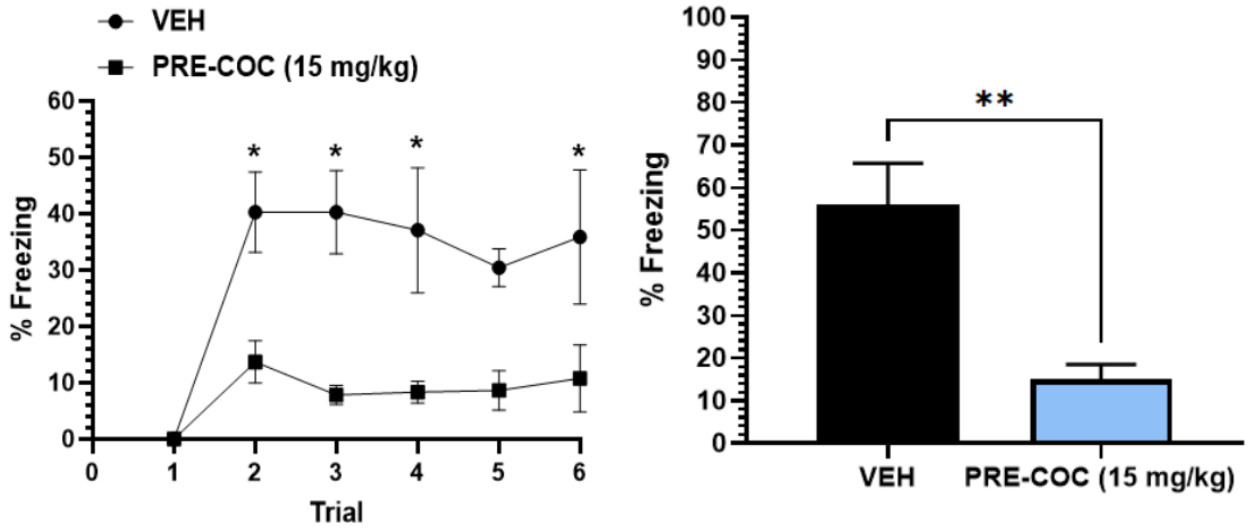

Figure 5. A) Effect of cocaine administration prior to conditioning on percent time spent freezing during the six tone-shock presentations in the conditioning trial. B) Effect of cocaine administration prior to conditioning on percent time spent freezing during fear test. Cocaine-treated rats spent less time freezing during fear test than saline-treated rats.

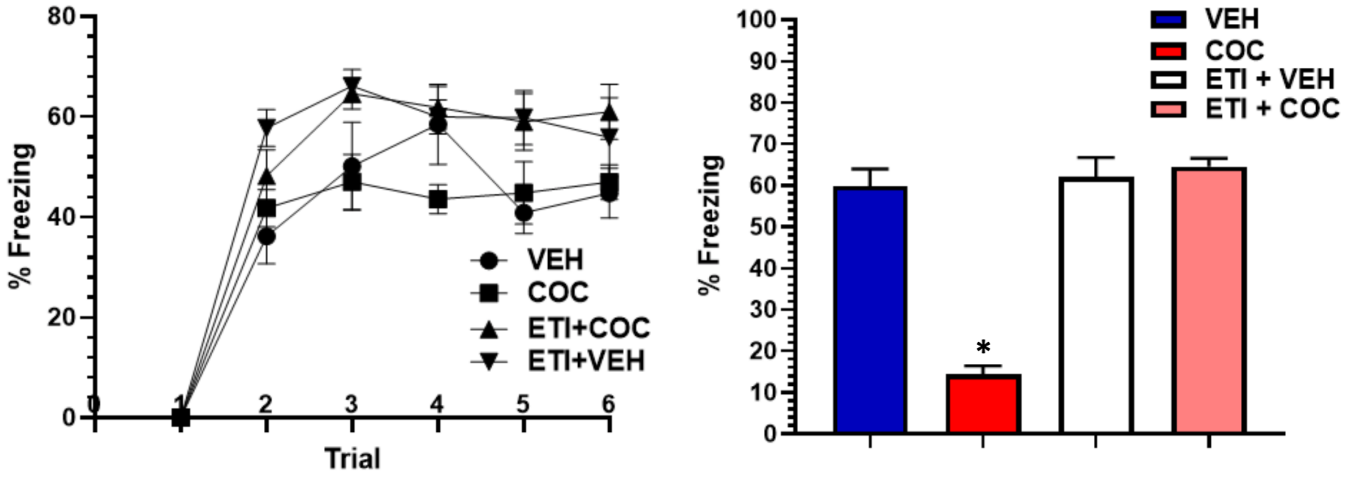

Figure 6. A) Fear learning during the six-tone shock presentations. B) Effect of cocaine administration alone or concurrent with eticlopride immediately after conditioning on percent time spent freezing during fear test. Cocaine-treated rats spent less time freezing during fear test than saline-treated rats. Eticlopride-cocaine-treated rats spent more time freezing during fear test when compared to cocaine-treated rats.

Study 2: Antagonism of D2 receptors in the ventral hippocampus mediates cocaine's impairing effect on fear memory consolidation

When comparing freezing behavior during fear test of animals receiving intra-VH eticlopride infusion with a cocaine injection, a saline infusion with a cocaine injection, or a saline infusion with a saline injection, a one-way ANOVA revealed a significant difference across all groups [$F(2,12) = 10.05$, $p < .01$]. A post hoc Tukey's multiple comparison's test revealed a significant difference between the eticlopride infusion-cocaine injection rats (M = 68.8; SD = 17.66) and the saline infusion-cocaine injection rats (M = 36.29; SD = 20.38; $p < .05$), and a significant difference between the saline infusion-saline injection rats (M = 99.21; SD = 1.16) with the saline infusion-cocaine injection rats ($p < .01$). However, there was no significant

difference between the saline infusion-saline injection and the eticlopride infusion-cocaine injection groups. These results suggest that animals receiving a saline infusion concurrently with a cocaine injection immediately after conditioning froze significantly more during fear test than animals receiving an eticlopride infusion with a cocaine injection. Additionally, these results show that animals treated with saline alone froze at comparable percentages than animals receiving an eticlopride infusion with a cocaine injection.

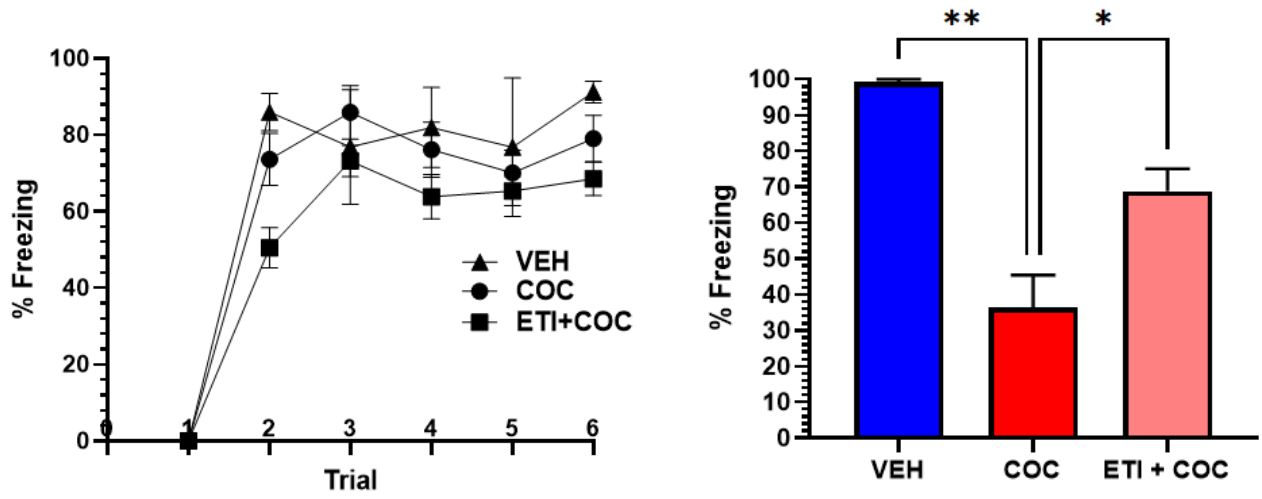

Figure 7. A) Fear learning during the six-tone shock presentations. B) Effect of cocaine administration paired with a saline or eticlopride infusion. Animals administered saline with cocaine froze significantly less than animals treated with eticlopride and cocaine.

Chapter 3: Discussion

The present study assessed the effects of cocaine on fear memory acquisition and consolidation, as well as the role that dopamine D2 receptors have in mediating cocaine's impairing effect on fear memory consolidation. Cocaine administration prior to fear conditioning in a Pavlovian cued fear conditioning paradigm impaired fear memory acquisition, demonstrated by the animals' significantly reduced percent time spent freezing during the six tone-shock presentations. Similarly, cocaine administration immediately after fear conditioning impaired fear memory consolidation, as seen by the animals' decrease in freezing behavior during the fear test. Additionally, peripheral administration of D2 receptor antagonist eticlopride successfully reversed cocaine's impairing effect on fear memory consolidation, suggesting that D2 receptors mediate this effect. To further understand which brain structure is implicated in mediating cocaine's impairing effect on fear memory consolidation, we targeted the ventral hippocampus via stereotaxic surgery. After bilaterally cannulating the VH, a direct infusion of eticlopride was made immediately after fear conditioning but prior to cocaine administration. Results showed that direct infusion of D2 receptor antagonist eticlopride into the ventral hippocampus significantly increased the animals' freezing during the fear test when compared to animals that received a saline infusion with a cocaine injection, thus successfully reversing cocaine's impairing effect on fear memory consolidation. These findings suggest that dopamine D2 receptors in the ventral hippocampus are involved in mediating fear memory consolidation in a Pavlovian cued fear conditioning paradigm.

These findings support previous research showing that the ventral hippocampus is heavily involved in fear memory formation and expression, and that dopamine D2 receptors are involved in mediating memory processes [26][79]. Additionally, this study supports previous

findings that cocaine has detrimental effects on different memory processes, like working memory [10][62]. Specifically, research has shown that acute cocaine administration at high doses disrupts aversive memories [9]. The present study demonstrates that cocaine has impairing effects on both aversive memory acquisition and aversive memory consolidation with only a single exposure to a moderate-to-high dose of cocaine. While previous studies have demonstrated that cocaine administration disrupts hippocampus-dependent fear conditioning [9], few studies have demonstrated the underlying mechanism through which cocaine may be impairing fear memory formation . The present study suggests that cocaine's impairment of fear memory consolidation is mediated by dopaminergic D2 receptors in the ventral hippocampus.

In mammals, the hippocampus is known to be involved in learning and memory processes and is thought to be involved in classical fear conditioning [85]. Previous studies suggest that lesions to the hippocampus impair the expression of conditioned fear, resulting in a disruption in freezing behavior [86]. The ventral hippocampus has been linked to neuronal processes that underlie classical fear conditioning and has long been associated with the formation of memories involving stress, emotion, and affect [85]. The VH also has reciprocal projections with the amygdala, a structure crucial to the formation of aversive memories [87][88]. Behavioral studies have demonstrated that proper function of the VH is necessary for conditioning of aversive memories [85]. Inactivation and lesions to the VH, both prior to and after classical fear conditioning, impair conditioned freezing to acoustic conditioned stimuli, thus suggesting that the VH is crucial for the acquisition, consolidation, and expression of conditioned fear [89][85]. It seems possible that impaired activity of the VH during and immediately after fear conditioning might prevent fear memory formation due to disrupted signaling with amygdala.

The CA1 region of the hippocampus contains both GABAergic interneurons and glutamatergic pyramidal cells [55]. Ventral hippocampal neurons project to distinct regions involved in aversive memory formation, fear expression, and fear renewal, such as the amygdala and the prelimbic region of the medial prefrontal cortex (PL; mPFC) [90]. The PL has been associated with the expression of conditioned fear and past studies have shown that the PL receives extensive glutamatergic projections from the ventral hippocampus [91][92]. Based on previous research establishing hippocampal projections to the PL, it seems possible that D2-expressing pyramidal neurons in the CA1 region of the ventral hippocampus are involved in these projections. Additionally, it has been established that the PL projects to the basolateral amygdala, and that activation of PL-BLA projections increases avoidance [93]. Stimulation of the PL has been shown to result in freezing behavior and increases in BLA firing [94][95]. The VH-PL-BLA circuit appears to be crucial for contextual fear processing [96].

The PL mediates emotional processes, including the formation of fear memories when the anticipation of the threat demands learning of contextual cues [97]. The association of a cue and footshock requires firing of the PL [97]. The hippocampus is a prominent input to the PL and it shares a role in contextual processing [97]. The disruption of PL firing during memory acquisition due to optogenetic silencing of VH-PL projections is detrimental to the retrieval of aversive contextual memory [97]. Functional disruption of either the PL or the hippocampus impairs the acquisition of trace fear conditioning, which requires the association of an auditory stimulus to an aversive footshock [98][99]. Inhibition of PL firing during trace interval impaired learning of the predictive cue-shock relationship [100], suggesting that specific signaling of the PL promotes the learned anticipation to threat.

The CA1 region of the ventral hippocampus is a major glutamatergic afferent to the PL and VH neurons robustly respond to aversive unconditioned stimuli [101][102]. It is possible that the VH mediates aversive memory formation through interactions with the PL and the BLA [103]. Interestingly, research has shown that convergent inputs from the PL and the VH within the BLA are required for modulation of fear [103]. The BLA has also been shown to have strong axonal projections to the PL [103], suggesting that a connective circuit between these regions mediates aversive memory. The BLA also sends long-range projections to the VH [103], which also sends projections back into the BLA [104]. The reciprocal interconnected network between the amygdala, the ventral hippocampus, and the prelimbic cortex seem to mediate the acquisition of conditioned fear responses and behavior. Our results suggest that dopamine signaling within the VH may be involved in this circuit regulating fear memory consolidation and expression.

The involvement of dopamine systems within the hippocampus have received little attention in regards to memory formation. It appears that manipulation of dopaminergic systems affects memory function [53]. Dopamine innervation into the hippocampus arises primarily from the ventral tegmental area, substantia nigra, and locus coeruleus, with the ventral portion of the CA1 region of the hippocampus receiving the highest density of DA innervations [53][80][50][105][52]. Additionally, the VH has been suggested to contain dense expression of dopamine D2 receptors in the CA1 region [52][106]. Previous research has established the role of D2 receptors in the VH in mediating memory [53]. D2 receptors are involved in memory-related neuroplasticity in the hippocampus and the acquisition and consolidation of spatial and associative memories [106]. Evidence suggests that D2 receptors are involved in aversive behaviors through the detection and regulation of dopamine signaling and that these receptors are necessary for the induction and maintenance of LTP at the hippocampal CA3-CA1

synapse [107]. The present study demonstrates that dopamine D2 receptors in the ventral hippocampus are largely involved in the consolidation of aversive memories in a Pavlovian, cued, fear conditioning paradigm.

It is important to address several limitations in this study. First, the D2 receptor antagonist eticlopride also has binding affinity for dopamine D3 receptors [108][109]. It is possible that administration of eticlopride into the VH not only resulted in antagonism of D2 receptors, but also blockade of D3 receptors. However, it must be noted that previous studies utilizing epidepride, a benzamide with high affinity to D2 and D3 receptors, suggest that there is decreased binding of epidepride to D3 receptors in the hippocampus [110][111][112]. Additionally, while D3 receptors are expressed in mesolimbic regions, there is little overlap with D2 receptor expression [113]. Most D3 receptor expression has been localized in the nucleus accumbens, ventral striatum, substantia nigra, the globus pallidus, and the anteroventral nucleus of the thalamus [113]. More research is needed in order to determine the proportion of D2 to D3 receptors in the ventral hippocampus.

Another limitation of the present study is the potential co-occurrence of contextual fear conditioning in the cued fear conditioning paradigm. For the fear test, animals were returned to the same conditioning chamber they were exposed to during the conditioning trials. It is possible that animals associated both the context and the tone with the footshock. However, overall contextual freezing was not scored given that the animal's freezing behavior was only assessed during the presentation of the auditory cue. Moreover, the present study utilized a single dose of cocaine to assess its effects on fear memory acquisition and consolidation, as well as a single dose of eticlopride. It is plausible that higher or lower doses of cocaine may have different

effects on the acquisition and consolidation processes, and that varying doses of eticlopride could result in opposite effects from the one observed.

It is also important to assess the placement of the cannulas during stereotaxic surgery (Figure 4). The targeted AP was -5.2mm from bregma and tissue scoring revealed that some cannula placements were approximately 0.4-0.7mm closer to bregma, potentially missing the ventral portion of the hippocampus. Due to this cannula misplacement, it is possible that the full effects of D2 receptor antagonist eticlopride were not observed in the intra-VH infusions. Furthermore, the extent to which the drug spread in the brain from the point of infusion was not accounted for and it is possible that diffusion of eticlopride targeted neighboring D2 receptor-expressing brain structures.

The present study demonstrated that cocaine administration both prior and immediately after Pavlovian cued fear conditioning impaired fear memory acquisition and fear memory consolidation. Our results suggest that cocaine's impairment of fear memory consolidation is mediated through interactions with dopamine D2 receptors in the ventral hippocampus. Though future studies are necessary to uncover the specific underlying mechanism through which D2 receptors in the VH are mediating cocaine's impairing effect on fear memory consolidation, these results further emphasize the deleterious effects that cocaine use has on memory processes. Future studies should focus on identifying D2-expressing neurons in the ventral hippocampus to further understand the signaling pathways between the VH and other fear-related brain structures like the amygdala and the PL. Additionally, manipulation of dopaminergic input from the VTA, LC, and SN could prove beneficial to uncover how dopamine signaling between these brain regions regulates fear memory acquisition and expression.

The present findings offer translational use in the context of cocaine use disorder, as continued exposure to cocaine might lead individuals to engage in increased risky behavior with potentially fatal consequences due to their inability to consolidate aversive memories. Increases in impulsive and risk-taking behavior have been observed in cocaine use due to alterations in the decision-making circuitry [6]. In fact, cocaine users show increased propensity to engage in risk-taking behavior when compared to users of other illicit drugs [7]. While more research is needed to understand why cocaine users show greater impulsive behavior, leading to decision-making without conscious judgment, the results of this study suggest that an impairment in fear memory acquisition and consolidation by cocaine may reinforce risk-taking behavior. It is possible that, under the influence of cocaine, an individual that encounters a threatening or dangerous situation is not able to properly consolidate an aversive memory, thus leading to repeated exposure to said threat. These findings provide a potential explanation as to why cocaine users often engage in increased risk-taking and impulsive behavior, which could lead to dangerous consequences that pose a threat to the individual's life.

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