ROLE OF THE CENTRAL AMYGDALA IN LOSS-INDUCED EMOTIONAL SELF-MEDICATION

By

SARA GUARINO

Bachelor of Arts, 2016
St. Mary's University
San Antonio, Texas

Submitted to the Graduate Faculty of the
College of Science and Engineering
Texas Christian University
in partial fulfillment of requirements
for the degree of

Master of Science

Fort Worth, Texas

December 2018

ROLE OF THE CENTRAL AMYGDALA IN LOSS-INDUCED EMOTIONAL SELF-MEDICATION

By

Sara Guarino

Thesis approved:

Dr. Mauricio R. Papini, Professor of Psychology

Dr. Kenneth J. Leising, Associate Professor of Psychology

Dr. Anna I. Pétursdóttir, Associate Professor of Psychology

For The College of Science and Engineering

ACKNOWLEDGEMENTS

This research would not have been possible without the support of many people. I would like to acknowledge Mauricio R. Papini for his guidance, encouragement, and advise he has provided throughout my time as his student. I would also like to thank Kenneth Leising and Anna Pétursdóttir for contributing their expertise as members of my thesis committee. Additionally, I would like to thank Shannon E. Conrad and Joanna B. Thompson for their insight and help. Special thanks need to be extended to my undergraduate mentor and research collaborator Heather M. Hill for her constant support and encouragement throughout my graduate school experience. Finally, I would also like to thank my parents, Antonio and Elena, and my boyfriend, Luca Ceresa for their patience and support since the beginning of my graduate experience.

TABLE OF CONTENTS

LIST OF FIGURES

Role of the Central Amygdala on Loss-induced Emotional Self-medication

Emotion regulation involves motivation to engage in behaviors that reduce negative emotional states or increase positive emotional states and adjust the duration or magnitude of the emotional response (Gross, 2013). According to the Anxiety and Depression Association of America (ADAA) and the National Institute of Mental Health (NIMH), 40 million Americans between the ages of 18 and 54 are affected by anxiety disorders, representing about 18% of the nation's population. Of those 40 million people, almost 7 million of them suffer from generalized anxiety disorder, with 15 million suffering from social anxiety disorder, 14.8 million suffering from major depressive disorder, and 7.7 million affected by post-traumatic stress disorder. While normal anxiety is considered an adaptive response to the possible presence of danger, it is susceptible to dysregulation. In individuals with an anxiety disorder or depression, disruption in the emotion-regulating system may lead to higher chances of experiencing negative emotional states relative to healthy individuals. These individuals may respond to abnormal anxiety by engaging in self-regulating behaviors that could include the consumption of substances that lead to an increased positive emotional state (Li, Lu, & Miller, 2012). For example, anxious or depressed individuals may attempt to decrease these negative emotions by increasing the consumption of alcohol due to its anxiolytic effect. As a result, it is possible that the rewarding effect derived from the reduction of negative internal states may contribute to the development of addictive behavior (Torres & Papini, 2016). Alcohol and related substances may have effective short-term emotion-regulating properties similar to the drugs used to treat anxiety disorders pharmacologically (Crum et al., 2013).

Emotional Self-medication Hypothesis

The emotional self-medication (ESM) hypothesis states that some individuals consume substances to relieve undesired symptoms related to psychiatric conditions or to reduce negative

emotional states induced by aversive life events (Khantzian, 1985, 2013; Torres & Papini, 2016; Figure 1). The consumption of these substances is related to their ability to change the intensity of symptoms of psychological distress (e.g., dysphoria, anxiety, stress, frustration). This hypothesis suggests that the reduction of negative internal states following substance intake, involving pharmacological treatments (e.g., benzodiazepines) in psychiatric patients and the self-administration of substances with addictive potential (e.g., alcohol) in healthy individuals who have undergone traumatic events, is at the base of ESM (Darke, 2012).

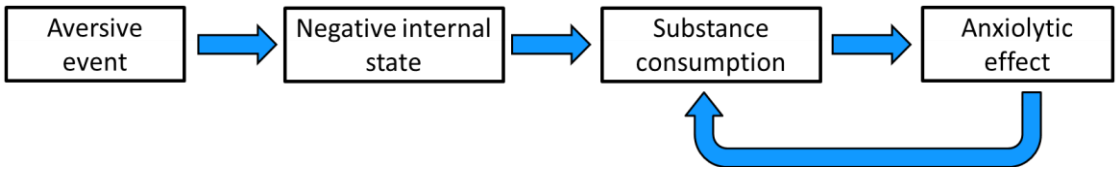

Figure 1. Proposed reinforcement mechanism for the emotional self-medication (ESM) effect induced by reward loss. Negative affect (e.g., frustration) is reduced by the voluntary oral consumption of anxiolytics (e.g., alcohol). (From Torres & Papini, 2016.)

Even though it is not possible to directly transfer results of studies conducted with non-human animals to humans, the presence of homologous neural circuitry and biobehavioral mechanisms conserved across vertebrates have allowed for experimental research on self-medication with animal models (Papini, 2002, 2003). To test the ESM hypothesis using animal models, and generate findings that can be applied to human research, it is necessary to demonstrate that laboratory animals experience negative emotions and that the administration of substances with addictive potential can reduce negative emotions in a way comparable to that of pharmacological treatments (Andrews, Papakosta, & Barnes, 2014; Kumar, Bhat, & Kumar, 2013; Li, 2012).

Anxiolytics. To develop an animal model for ESM, the first step is to expose rodents to stressors, or inject them with anxiogenic substances, and provide opportunities for these animals

to self-administer substances expected to reduce negative emotions. As early as 1956, research suggested that the tension-reducing properties of anxiolytics explain self-medicating behavior in animals (Conger, 1956). The experience of the ameliorating effects on the negative emotional state explains the increased consumption of these substances (Conger, 1956). Consistent with this tension-reducing idea, more recent studies have shown that stress produced by mild foot-shock, forced swim test, and social defeat situations while providing simultaneous or subsequent access to ethanol induced seeking and increased voluntary consumption in rodents (Bertholomey et al., 2011; Caldwell & Riccio, 2010; Spanagel, Noori, & Heilig, 2014). It is also possible that ethanol-seeking behavior is due to the pleasurable state resulting from substance consumption, a hypothesis more consistent with the process of positive reinforcement, and especially present in rats selectively bred to prefer ethanol (Knapp et al., 2011).

Loss-induced ESM

Critical for the proposed study is research providing evidence of the ability of psychoactive substances to reduce negative emotions following a situation involving reward loss. Reward loss is one of the most common factors inducing negative emotions in humans. Emotional responses to reward loss have been described in terms of frustration (Amsel, 1992; Papini & Dudley, 1997) and more generally in terms of psychological pain (Papini, Fuchs, & Torres, 2015). These negative emotional states produced by stressful life events, such as the loss of a loved one, divorce, unemployment, disease diagnoses, or the end of a relationship can deteriorate physical and psychological health, and lead to behavioral and emotional disorders (Papini et al., 2015). Among ways of coping with these unexpected negative events is the consumption of drugs that may result in the initiation and maintenance of addictive behavior (Keyes, Hatzenbuehler, & Hasin, 2011; Torres & Papini, 2016). Non-human animals are also subject to the negative symptoms arising from various forms of loss. In natural habitats, loss of

food resources, loss of mating opportunities, and loss of shelter could influence physiological and behavioral responses important for survival (Papini, 2002, 2003). In the laboratory setting, the effects of reward loss have been assessed through devaluation and omission of an expected reward, triggering the onset of a negative internal state defined as psychological pain (Papini et al., 2015).

Various paradigms, in which the omission of an appetitive event was used as an anxiety-inducing situation, have been employed to test the hypothesis that the loss of a reward results in emotional distress leading to psychobiological responses similar to those induced by an aversive event (Papini & Dudley, 1997), and reduced by psychoactive substances, including anxiolytics and analgesics (Flaherty, 1996; Papini et al., 2015). Research conducted in laboratory settings using contrast procedures shows that a decrease in value of a sucrose solution can induce an anxious internal state similar to removal of the solution (Kamenetzky, Mustaca, & Papini, 2008; Manzo et al., 2015). In a consummatory successive negative contrast (cSNC) procedure, rats receive access to a highly preferred sucrose solution for several consecutive days (i.e., preshift sessions) followed by several days during which these animals receive access to a less preferred sucrose solution (i.e., postshift sessions) that replaces the highly preferred solution. The transition from preshift to postshift sessions marks the sucrose downshift, a reduction in incentive value (Flaherty, 1996; Rowan & Flaherty, 1991). Rats experiencing the downshift showed decreased consumption of the lower concentration sucrose solution compared to controls that received the lower concentration sucrose solution throughout the sessions (i.e., preshift and postshift), without experiencing a downshift. The reduced consumption in the downshifted group relative to the control group is called SNC. This downshift produces a negative emotional state similar to that produced by the unexpected reward omission of the sucrose solution during appetitive extinction sessions (Papini et al., 2015).

Effects of anxiolytics on SNC

Prior research has provided evidence suggesting that benzodiazepine anxiolytics (e.g., CDP) and ethanol have an effective modulating role in reward devaluation tasks. The SNC effect can be prevented by forced administration of alcohol (Kamenetzky et al., 2008) or CDP (Rowan & Flaherty, 1991), prior to exposure to a downshifted sucrose solution, suggesting that SNC is mediated by a negative emotional reaction to the downshifted solution. Interestingly, these two anxiolytics have no effect on cSNC when administered before the first downshift trial, but they attenuate the contrast effect when administered before the second downshift trial (Flaherty, 1996). The finding that benzodiazepine anxiolytics act on the second devaluation session is consistent with the hypothesis that an emotional conflict requires some experience with the downshifted reward (Bentosela et al., 2006).

If the anxiolytic properties of ethanol reduce the SNC effect (Kamenetzky et al., 2008), a rat may seek and consume this substance after a session that produces a negative emotional state (Manzo et al., 2015), which may be comparable to that produced by stressors leading to alcohol seeking in humans. Since SNC produces an aversive emotional state, post-session access to ethanol may provide an opportunity to reduce the negative emotion and increase pleasure. Additionally, evidence of a negative emotional reaction caused by reward reduction comes from measures of the stress hormone corticosterone. The plasma levels of this hormone, released in response to stressors like reward loss, were found to be elevated in rats undergoing downshifted sessions of cSNC after the first reward devaluation session and before and after the second reward devaluation session relative to unshifted controls (Mitchell & Flaherty, 1998; Pecoraro, de Jong, & Dallman, 2009).

Most anxiolytics activate GABAergic neurons by binding to a specific $GABA_A$ receptor and seem to be effective at suppressing the negative contrast on the second postshift session

(Genn, Tucci, Parikh, & File, 2004). Ethanol has anxiolytic effects similar to those of benzodiazepines and also modulates GABAergic function (Tan, Rudolph, & Lüscher, 2011). Consequently, ethanol also affected responding to reward loss by reducing postshift behavioral suppression when administered before the second downshifted session, but had no effect when administered before the first one (Becker & Flaherty, 1982). Manzo and colleagues (2014) tested whether the loss of a reward resulted in ESM behavior, as defined by increased ethanol consumption following a situation involving reward loss. Exposure of rats to a 22% sucrose solution for 10 trials (i.e., acquisition) followed by removal of this sucrose solution for several days (i.e., extinction) led to increased consumption of a 2% ethanol solution in a two-hour, two-bottle ethanol-water preference test administered immediately following extinction sessions relative to the consumption of the same ethanol solution during acquisition sessions and to a group that received access to water in both bottles. This increased consumption of ethanol during the extinction phase was interpreted as ESM relieving the negative emotional reaction caused by the loss of sucrose availability (Manzo et al., 2014).

Additional evidence shows that the benzodiazepine anxiolytic chlordiazepoxide (CDP) administered before downshift sessions reduces negative contrast due to its anxiolytic properties (Becker & Flaherty, 1983; Flaherty, Grigson, & Lind, 1990; Flaherty, Grigson, & Rowan, 1986; Genn, Ahn, & Phillips, 2004). To test the ESM hypothesis, researchers used SNC to induce an anxious state in rats, again followed by access to anxiolytic substances, including either a 2% ethanol solution or 1 mg/kg CDP solution (Manzo et al., 2015). The experimental group of rats had access to a 32% sucrose solution during preshift phase and 4% solution during postshift phase. During postshift, rats decreased consumption of the sucrose solution to levels below that of unshifted controls. Additionally, only rats that showed a contrast effect increased consumption of both ethanol and CDP, whereas no preference for either anxiolytic substance was observed

during preshift sessions. In support to the ESM hypothesis, these findings suggest that animals may regulate an anxious state by consuming anxiolytic substances (i.e., ethanol and CDP, Manzo et al., 2014; Manzo et al., 2015). Notice that, unlike alcohol, CDP lacks caloric value and thus it cannot be argued that rats elevate their consumption because of the loss of calories afforded by a reduction in sucrose concentration during reward downshift sessions.

ESM as an Explanation for Addictive Behavior

Reward loss paradigms have helped to advance the understanding of potential motivational and emotional factors involved in the onset of addictive behavior (Torres & Papini, 2016). In particular, reward processing and emotional dysregulation seem to be controlled by neuropsychological mechanisms mediating the development of drug-seeking behaviors (Egli, Koob, & Edwards, 2012; Koob, 2013). In relation to the ESM hypothesis, studies have suggested the role of stressful life events in triggering the onset of addictive behaviors (Keyes et al., 2011). For example, individuals suffering from substance use disorder (SUD) experience difficulties adjusting to sudden life and environmental changes (Brevers et al., 2015; Ersche et al., 2016), such as unexpected losses (Papini et al., 2006). Consumption of substances that have tension-reducing properties may be the bridge between the negative emotions induced by the loss of a reward, and the development of addictive behavior, one of the most frequent biobehavioral disorders often initiated to cope with negative emotional states caused by traumatic events (Hassanbeigi et al., 2013; Konopka et al., 2013).

Neural Circuitry for Reward Loss, Negative Emotions, and Addiction

In support of the hypothesized relationship among reward loss, negative emotions, and drug addiction is the telencephalic modulatory neural circuitry involved in reward processes, ESM based on consumption of addictive substances, and vulnerability to drug addiction (Ortega, Solano, Torres, & Papini, 2017; Figure 2). Understanding which role each brain structure of this

neural circuitry plays in the modulation of behavioral responses to reward loss, and access to and consumption of substances with addictive potential is critical to formulate hypotheses addressing the development of SUDs. The telencephalic modulatory circuitry has been hypothesized to be sub-divided into two neural circuits: the *reward comparison circuit* and the *negative emotion circuit* (Figure 2). Studies involving irreversible or reversible lesions have examined the function of several of these brain structures in relation to negative emotions in the context of reward loss.

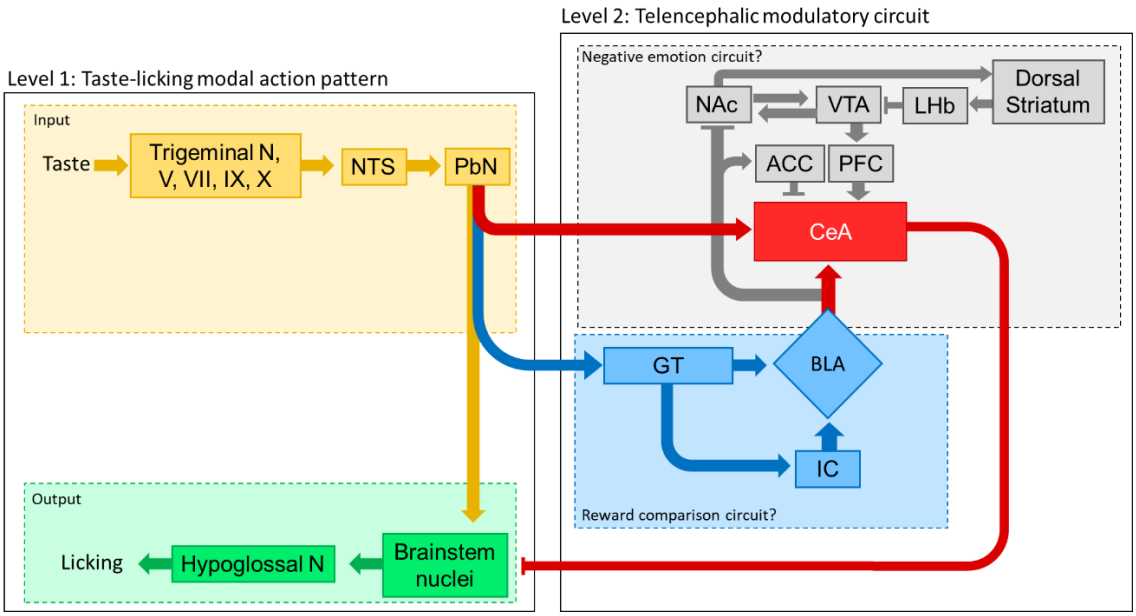

Figure 2. Proposed neural circuitry underlying cSNC. (From Ortega et al., 2017.)

Reward Comparison Circuit

Insular cortex (IC). The role of the IC in reward loss has been suggested by behavioral studies reporting that lesion to this brain structure eliminated the response suppression following reward devaluation, relative to unshifted controls (Lin et al., 2009). In this experiment, IC-lesioned rats showed normal responding to either sucrose (i.e., high-value reward) or saccharin (i.e., low-value reward) during preshift sessions, but animals experiencing the downshift from sucrose to saccharin during postshift sessions displayed higher level of responding to saccharin

compared to unshifted animals. These findings suggested either a failure of the reward devaluation to induce an emotional response or an impairment in IC lesioned animals to recognize the novel substance.

The inclusion of the IC in the reward comparison circuitry seem to be related to the modulating role of this structure in processes involving choices related to psychoactive drug consumption and relapse (Cisler et al., 2013; Naqvi & Bechara, 2009, 2010; Volkow & Baler, 2014). Supporting this view are psychobiological studies reporting that damaged IC affected nicotine craving and relapse. In one experiment, craving was eliminated in smokers with IC damage, whereas craving and relapse tendencies were still present in smokers with damage to other areas of the brain (Naqvi et al., 2007). A similar outcome was reported by another study assessing the effect of granular IC inactivation on nicotine self-administration under fixed and progressive ratio reinforcement schedules, and on reinstatement of nicotine seeking induced by nicotine-associated cues (Forget, Pushparaj, & Le Foll, 2010). When the granular IC was temporarily inactivated by local infusion of GABA agonists mixture (i.e., baclofen/muscimol), nicotine self-administration under both reinforcement schedules decreased relative to food self-administration in the control group. Granular IC inactivation also prevented reinstatement, following extinction of nicotine-seeking, but not food-seeking behaviors. Finally, several studies reported impaired activation of the IC in individuals with anxiety disorders in the presence of salient stimuli. For example, increased cerebral blood flow in bilateral IC was found in patients with obsessive-compulsive disorder, phobias, or posttraumatic stress disorder when symptoms were provoked (Rauch et al., 1997). Also, differential blood flow reduction was found in the IC of individuals with social phobia during public speaking situations (Lorberbaum et al., 2004). Together, these findings suggest a possible connection between this region of the cerebral cortex and components of the negative emotion circuit (Paulus & Stein, 2006).

Basolateral amygdala (BLA). Research exploring the functions of the amygdala has suggested a role for this brain area in establishing learned associations between events involving motivational value, such as situations in which initially neutral stimuli can become signals for an upcoming reward (Everitt, Cardinal, Parkinson, & Robbins, 2003). A key function attributed to the BLA involves maintenance of rewarded behaviors. For example, BLA inactivation by bilateral infusion of the $GABA_A$ agonist muscimol suppressed lever pressing for food in rats, without affecting the consumption of freely available food (Simmons & Neill, 2009), suggesting that anticipatory, but not consummatory behavior, was affected. Further, an experiment involving excitotoxic lesions of the BLA reported that, after reward aversion was established, sham rats displayed a decreased response to reward-toxin pairings relative to when reward and toxin were presented unpaired. In contrast, BLA lesioned animals did not show such response suppression, suggesting that BLA lesions eliminated the effects of reward devaluation (Hatfield et al., 1996), and providing evidence of a role for the BLA in reward processing.

Moreover, research studies have suggested a role for the BLA in comparing current low-value reward with a previously presented high-value reward. In a study involving electrolytic lesions of the lateral amygdala, lesioned animals displayed a reduced cSNC effect (Becker, Jarvis, Wagner, & Flaherty, 1984). The authors suggested that the attenuation of the cSNC effect may have resulted from impairment of the reward comparison mechanism of the lateral amygdala, which led the lesioned animals to adjust to the devalued reward of postshift sessions rather than compare it with the high-value reward of preshift sessions. Consistent with the reward comparison function, c-Fos expression (i.e., indicator of cellular activation) was enhanced in the BLA of rats following the first, but not the second, reward devaluation event (Pecoraro & Dallman, 2005), and pCREB expression (i.e., indicator of synaptic plasticity) was heightened after the second devaluation event relative to the first in both basolateral and central

nuclei (Glueck et al., 2015). A more recent experiment involving excitotoxic lesions of the BLA produced a pattern of results that was also attributed to the reward comparison function of the BLA (Kawasaki et al., 2017). In this case, BLA lesions eliminated the effects of reward devaluation in both consummatory successive negative contrast (cSNC) and anticipatory negative contrast (ANC) tasks, two situations involving transitions in reward magnitude (i.e., reward devaluation and reward omission). However, BLA lesion did not affect activity in the open field (OF), a task not involved in reward comparison, but known to induce negative emotion. According to this view, detection of a reward discrepancy may not involve emotional response. In the cSNC situation, for example, animals may distinguish two sucrose solution concentrations (i.e., reward comparison), but may not show cSNC effect (i.e., emotional response), a pattern that suggests a link between the BLA and other brain structures specifically involved in the modulation of negative emotions.

Negative Emotion Circuit

Anterior cingulate cortex (ACC). The ACC seems to mediate situations involving motivation elicited by stimuli associated with addictive drugs, cognitive challenges resulting from exposure to anxiety-evoking events, and experience of negative emotional states (Kalivas & Volkow, 2007). Research assessing neural activity in individuals undergoing personal losses has reported differential activation of the ACC, caudate, and dorsal striatum, with the observed brain activity patterns associated with diminished control of drug-seeking behavior and negative emotion regulation. For example, a study assessing neural activity using functional magnetic resonance imaging (fMRI) found that, during recall of personal stressful life events, abstinent, cocaine-dependent individuals showed decreased ACC activity, but increased caudate and dorsal striatum activity relative to healthy controls (Sinha et al., 2005). These neurobiological alterations seem to be behind the development of stress-induced cocaine craving.

Furthermore, results from ACC lesion studies have suggested a role for this brain structure in loss-induced behavior regulation. For example, reversible lesions by procaine microinfusions into the ACC disrupted appetitive extinction following acquisition in a Pavlovian conditioning situation (Griffin & Berry, 2004). In this experiment, during the extinction phase, ACC lesioned animals displayed a persistent conditioned response compared to the rapid behavioral suppression of control animals. Another study involving ACC electrolytic lesions reported disrupted recovery from reward devaluation of the cSNC task (Ortega, Uhelski, et al., 2011). Specifically, ACC lesioned rats exposed to a downshifted sucrose solution displayed a slowed recovery relative to downshifted sham rats, suggesting a potential role of the ACC in coping with the loss-induced emotional response.

Orbitofrontal cortex (OFC). In the study by Ortega and colleagues (2013), electrolytic lesions of the OFC indicated a potential role of the orbitofrontal region in situations involving incentive shifts. In the cSNC task, lesioned animals receiving the incentive downshift displayed attenuated behavioral suppression compared to unshifted controls, but only during the first postshift session. Also, in the autoshaping situation, OFC lesions eliminated the enhanced anticipatory behavior during partial reinforcement relative to continuous reinforcement (i.e., partial reinforcement acquisition effect, PRAE). Moreover, inactivation of the OFC by muscimol microinfusion in rats immediately prior to Pavlovian appetitive extinction sessions resulted in impaired extinction learning in a reward omission situation, supporting the OFC role in representing outcome expectancies (Panayi & Killcross, 2014). Further, OFC inactivation resulted in decreased reinstatement of cocaine voluntary consumption in drug seeking rats after exposure to stress-inducing peripheral pain (Capriles, Rodaros, Sorge, & Stewart, 2003). The abnormal functioning of these psychobiological mechanisms suggests a mediating role of the

OFC in the interaction between negative emotion and self-administration of drugs with addictive potential.

Medial prefrontal cortex (mPFC). The mPFC has been associated with impaired psychological process common in individuals with SUDs, including enhanced motivation to seek out stimuli predicting access to drugs of addiction, but decreased motivation towards other stimuli, and enhanced reactivity to stressful situations (Goldstein & Volkow, 2011). Reward omission research showed that inactivation of the infralimbic mPFC by muscimol microinfusion following acquisition resulted in fastened extinction of Pavlovian conditioning (Mendoza, Sanio, & Chaudhri, 2015). Furthermore, a set of experiments assessed the effect of infralimbic mPFC lesions on behavioral phenomena involving an appetitive Pavlovian task (Rhodes & Killcross, 2004, 2007). Specifically, spontaneous recovery of extinguished conditioned fear was enhanced in lesioned animals relative to sham animals. Also, lesioned animals displayed enhanced reinstatement of the extinguished conditioned response following unsignaled presentation of food pellets (Rhodes & Killcross, 2004). Further, infralimbic mPFC lesions resulted in increased renewal of a conditioned response when animals were tested in the acquisition context rather than the extinction context (Rhodes & Killcross, 2007). Finally, neurobiological studies reported that stress hormones affect brain cells of the mPFC causing neural changes and behavioral patterns associated with drug dependence (Lu & Richardson, 2014).

Striatum. The two main regions of the striatum, the nucleus accumbens (NAc; or ventral striatum), and the dorsal striatum, have been proposed to be involved in responses to reward devaluation and reward omission. For example, attenuated efflux of dopamine in the NAc accompanied the negative contrast effect observed in rats exposed to the 32-to-4% sucrose downshift in the cSNC task relative to 4-to-4% controls (Genn et al., 2004). Similarly, low levels of dopamine were found in the NAc of rats after a delay between delivery of reward and

extinction, suggesting that the decrease of dopamine release in the NAc during extinction may be related to an emotional response to the failure to obtain the expected reward (Biesdorf et al., 2015).

Additionally, electrolytic lesions of the NAc resulted in disrupted instrumental reward devaluation. In a runway task, lesioned rats showed a retarded 12-to-1 pellet contrast effect relative to sham rats, and enhanced runway backtracking during both preshift and postshift sessions. These data suggest a role of the NAc in responding to violation of expectancies in situations involving approach or instrumental behavior (Leszczuk & Flaherty, 2000).

Further, excitotoxic lesions of the NAc seem to interfere with responding following surprising reward omission. In one study, after training on a fixed-interval reinforcement schedule, NAc lesioned rats displayed lower response rate during reward omission trials relative to sham animals (Judice-Daher & Bueno, 2013). Also, when rats with dorsomedial striatum lesions were tested in an autoshaping situation, these animals showed suppression of behavior typically displayed during acquisition sessions when lever pressing was partially reinforced (Torres et al., 2016). These findings suggest a possible role of both the ventral and dorsomedial striatum in motivation under conditions of reward uncertainty.

Furthermore, a possible role of the striatum in modulating negative emotional states has been reported by research assessing addictive behaviors. Specifically, the striatum seems to be involved in the transition from the initial approach to addictive drug consumption to the development of drug addiction (Yager, Garcia, Wunsch, & Ferguson, 2015), with the NAc mediating the acute reinforcing effects of these drugs, and both NAc and dorsal striatum regulating the extent to which drug-seeking behavior is influenced by conditioned drug-related stimuli (Everitt & Robbins, 2013). The inputs that the striatum receives from other brain areas, including the ventral tegmental area (VTA) and nuclei of the amygdala, may be critical for the

connecting link between this brain structure and brain areas of the reward comparison circuit (Lanciego, Luquin, & Obeso, 2012).

Lateral habenula (LHb). This brain structure seems to have a crucial role in encoding reward-prediction errors typical of reward-loss situations. As mentioned above, there is a connection between the ventral striatum and the VTA. Evidence suggests that stimuli predicting reward availability or unexpected reward activate the projection of dopaminergic neurons from the VTA to the NAc, whereas the omission of an expected reward results in the inhibition of VTA neurons (Ungless, Argilli, & Bonci, 2010). In reward-loss situations, inputs from the LHb seems to be responsible of this inhibition induced by reward omission (Kimura, Satoh, & Matsumoto, 2007). Further research has suggested that the LHb is also involved in response regulation to anxiety and depression (Pobbe & Zangrossi, 2008; Sartorius et al., 2010), and to behavioral patterns associated with drug seeking and taking, relapse, reinstatement, and extinction.

Central amygdala (CeA). Evidence suggesting the role of the CeA in reward loss is provided by lesions and infusions studies. For example, CeA lesioned rats exposed to incentive downshift displayed a reduced cSNC effect relative to unshifted CeA lesioned controls (Becker et al., 1984). These findings are consistent with research conducted using local CeA infusion of the benzodiazepine diazepam, which also resulted in attenuated cSNC effect (Liao & Chuang, 2003), suggesting a mediating role of the CeA in the recovery effects of benzodiazepines on a reward devaluation situation. Particularly important for the current proposed experiment, Kawasaki et al. (2015) reporting that transient CeA inactivation by lidocaine microinfusion before the first downshifted session resulted in attenuated cSNC effect (Figure 3), and increased locomotion in the central area of a well-lit open field, an effect suggesting reduced innate fear of an open space (Figure 4). However, CeA inactivation did not affect anticipatory negative

contrast (ANC), a procedure providing access to the same rewards of the cSNC task (i.e., 4% and 32% sucrose solutions), but presented in close succession to one another in every session, a situation that does not appear to be disrupted by administration of anxiolytics (Flaherty, 1996). In the ANC task, rats display lower response to the 4% sucrose when immediately followed by access to 32% sucrose compared to when followed by access to the same 4% sucrose. Based on this pattern of results, Kawasaki et al. (2015) suggested that the CeA processes events involving negative emotion. However, the function of the CeA in the neural circuit underlying reward loss, and its specific role in modulating negative emotions requires further research.

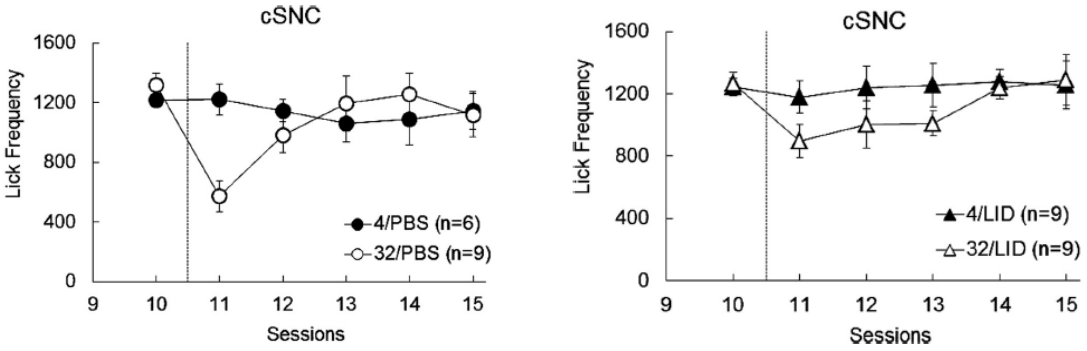

Figure 3. cSNC results from CeA reversible lesions by lidocaine microinfusion. (From Kawasaki et al., 2015.)

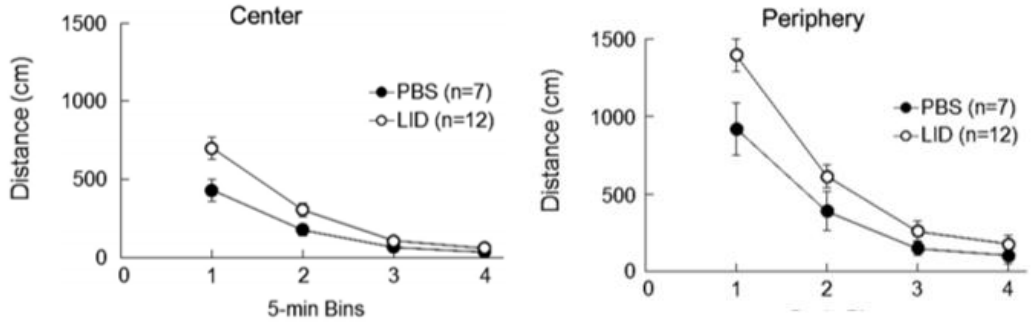

Figure 4. Open field activity results from CeA reversible lesions by lidocaine microinfusions. (From Kawasaki et al., 2015.)

Neuronal Activity Manipulation via Chemogenetics

Various conventional approaches have been used to produce inhibition or excitation of neuronal activity in animal models. These techniques involved permanent lesions or transient inactivation of brain regions achieved via intracranial infusions of chemicals, such as NMDA or lidocaine. More recently, chemogenetic approaches have been employed to remotely control neuronal activity of brain regions, non-invasively. Chemogenetics involves the use of engineered receptors that are activated by otherwise inert substances (Roth, 2016). G protein-coupled receptors are the most common ones to have been chemogenetically engineered to explore brain circuit mechanisms. Designer receptors exclusively activated by designer drugs (DREADDs) are the most widely used class of these engineered receptors employed in chemogenetic research (Urban & Roth, 2015; Roth, 2016).

DREADDs approach. DREADDs are engineered G protein-coupled muscarinic receptors that have been modified to respond specifically to the synthetic compound clozapine N-oxide (CNO), but no longer to their endogenous ligand, acetylcholine (Urban & Roth, 2015). Compared to pretraining irreversible lesions, DREADDs allow training animals under normal brain activity condition before and after their manipulation. Compared to reversible lesions, DREADDs do not require cannula implants for administration of microinfusions prior to behavioral testing. Two steps are necessary to employ the DREADD technique to remotely control neuronal activity: (1) an intracranial infusion of a viral construct to deliver the DREADD, and (2) an intraperitoneal injection of CNO, the activator drug for DREADDs. CNO is a metabolite of clozapine, an antipsychotic drug and agonist of serotonin and dopamine receptors. Concerns about the use of CNO included the potential to be metabolized back to clozapine, which rapidly crosses the blood-brain-barrier (BBB), and has been found to bind DREADDs more potently than CNO (Gomez et al., 2017). However, recent research reported

that no significant effects of CNO up to 10 mg/kg on various motivated behaviors in non-DREADD expressing animals when tested within 30-150 min after CNO systemic injection (Mahler & Aston-Jones, 2018; Roth, 2016). These findings suggest assessing the effects of DREADD activation within 2 h post systemic injection, to use relatively low doses of CNO (0.1 - 3 mg/kg), and to administer CNO to non-DREADD expressing animals as controls (Mahler & Aston-Jones, 2018; Roth, 2016) to ensure that the proportion of clozapine back-metabolized from CNO remained within the levels of specificity for DREADD activation, but below the threshold for altering signaling at endogenous receptors

Current Research

In the present experiment, the DREADD approach was utilized to explore the function of the CeA in the cSNC-ESM effect in a behavioral paradigm involving two tasks, the cSNC (i.e., induction task for negative emotion) and the preference test (assessing the effects of negative emotion on alcohol intake). Specifically, Gi-DREADD (hM4Di) were infused into the CeA of rats to allow for silencing of neuronal activity of this brain region. For the current experiment, this receptor was activated to produce reversible lesions during key reward devaluation sessions of the cSNC task (Trials 11-13). Each day, the cSNC session with sucrose was followed by access to 10% ethanol and deionized water in a free-choice situation. The effects of CeA inactivation were also tested in the open-field situation. This task was chosen because it is known to induce behaviors indicative of negative emotion (Suarez & Gallup, 1981; Pare, 1994; Ramos, 2008). Rats exposed to a well-lit open field show reduced activity in the central area, an indication of heightened unconditioned fear (Bouwknecht et al., 2007). Specifically, one study using microinfusion procedures indicated that, relative to rats receiving PBS microinfusions, rats for which the central amygdala was transiently inhibited using lidocaine displayed higher activity in both center and periphery of the open-field arena (Kawasaki et al., 2015).

Hypotheses

Using the DREADDs technique, the role of the CeA on loss-induced ESM was examined by giving the animals an opportunity to voluntarily consume ethanol, made available for 1 hour after the end of the cSNC task, and expected to reduce negative emotional states. Based on the results of Kawasaki et al. (2015), we predicted that animals in the downshifted group (32/CNO) for which CeA activity was transiently inhibited by DREADD activation via CNO were expected to show a reduced cSNC effect and, therefore, reduced ethanol consumption relative to the downshifted vehicle control (32/VEH), for which the CeA was active during the reward devaluation experience. In contrast, we predicted that the two unshifted groups (2/CNO and 2/VEH) would not differ in their ethanol consumption. This pattern of results would suggest that CeA inactivation reduces the negative emotion induced by reward devaluation, thus blunting the ESM effect. This result would provide support for the role assigned to the CeA as an area responsible for negative emotion processing, and for the voluntary oral consumption of ethanol to produce the ESM effect. The amount of ethanol consumed by the 2/CNO group was important to determine whether CeA inactivation affects ethanol consumption in the absence of reward loss. Also, based on the open field results of Kawasaki et al. (2015), we expected that CeA inactivation prior to open-field testing would enhance activity. This result would confirm prior results obtained via lidocaine microinfusion and provide further support for the use of the DREADDs approach as valuable neuronal manipulation approach for future studies involving behavioral paradigms typically used in our lab.

Method

Subjects

Thirty male Wistar rats experimentally naïve to the procedures administered were the subjects assigned to the current research. Subjects were housed in individual wire-bottom cages

with free access to water. Each cage contained a rodent retreat for enrichment. During the experiment, animals were under a 12 h light/12 h dark schedule (lights on at 07:00 h), in a colony room with constant temperature (22–23 ◦C) and humidity (45–65%). Animals were fed with standard laboratory rat chow. Water was freely available throughout their lives. Food was freely available until animals were approximately 90 days of age. In preparation for surgery, all animals were food deprived to 90% of their average free-food weights estimated as the mean weight from 3 consecutive days. All 30 animals were subject to the surgical procedure for viral infusion. However, two animals assigned to group 2/CNO were excluded from the study due to health-related issues observed on session 12 of the cSNC task.

Design

The 2x2 design of the current experiment was obtained by randomly assigning animals to one of four groups, matched in terms of ad libitum weight. Animals differed in terms of the contrast condition (32-to-2% vs. 2-to-2% sucrose) and the drug administered during i.p. injection (CNO vs. VEH). Two groups of animals were exposed to the reward downshift after either CNO (32/CNO, $n = 8$) or vehicle (32/VEH, $n = 8$) i.p. injections and two groups of animals remained unshifted receiving either CNO (2/CNO, $n = 5$) or vehicle (2/VEH, $n = 7$) i.p. injections.

Viral Vector

Inactivation of the CeA was achieved using the DREADD technique. DREADDs were delivered into the CeA via intracranial infusion of an adeno-associated virus (AAV) capable of infecting cells but shown to be safe for humans due to their inability to replicate, therefore preventing harmful spread. The viral vector construct (pAAV-hSyn-hM4D(Gi)-mCherry, 3×10^{12} virus molecules/ml; Addgene, Cambridge, MA) contains a red fluorescent reporter (i.e., mCherry) and a DNA fragment for an engineered muscarinic receptor (M4) that reacts to CNO, the activator drug for DREADDs, which can produce an effect by binding to its designer

receptor. DREADDs are maximally expressed 2-3 weeks post-infusion and can be selectively activated by systemic administration of CNO.

Surgical Procedure and DREADDs Infusion

Animals were anesthetized with inhalation isoflurane, 5% for induction and 1-2% for maintenance. The area for the incision was shaved, and then animals were positioned in a stereotaxic frame with blunt-tipped ear bars. Prior to incision, the shaved area was swabbed with Betadine (povidone-iodine topical solution, 10%), and each eye was covered with vaseline to prevent eye dryness. Then, a midline incision was made in the scalp, the skull was cleaned, and bregma was located. The viral construct was infused bilaterally at the coordinates for the CeA (-2.2 AP, ±4.2 ML, and -7.1 DV) obtained from the atlas of Paxinos and Watson (2007), using a 10-μl Hamilton syringe mounted on a stereotaxic injector (Quintessential Stereotaxic Injector, Stoelting, Wood Dale, IL) programmed to deliver 1 μl of virus per site at a rate of 0.2 μl/min. Five additional minutes were allowed for the fluid to diffuse in brain tissue. Immediately following surgery, animals received an injection of buprenorphine (1 mg/kg, subcutaneous, sustained release lasting 72 h) to alleviate pain induced by the surgery. Animals were housed individually in polycarbonate cages with food and water continuously available and were allowed a 5-7-day period for recovery from surgery before being placed back in their home cages. Fifteen additional days were allowed before the beginning of behavioral testing to ensure maximal viral expression.

Post-surgery Food Deprivation Procedure

After recovery from surgery and in preparation for behavioral testing, animals were further deprived to an 81-84% of their average original free-food weight, and this deprivation level was maintained throughout the duration of the experiment by feeding the animals a controlled amount of rat chow every day. Food was provided each day at about the same time, at

least 30 min following behavioral testing. Behavioral training began when the weight of all rats was within the target range, usually approximately 5–7 days after the first day of post-surgery food deprivation.

Consummatory Successive Negative Contrast (cSNC)

Apparatus. The cSNC task was conducted in 8 conditioning boxes (MED Associates, St. Albans, VT) made of aluminum and Plexiglas (29.3 × 21.3 × 26.8 cm, L × H ×W). The floor consists of steel rods running parallel to the feeder wall. A tray with corncob bedding is placed below the floor to collect feces and urine. In the feeder wall is a hole 1 cm wide, 2 cm long, and 4 cm from the floor through which a sipper tube, 1 cm in diameter, will be inserted. When fully inserted, the sipper tube is flush against the wall. Diffuse light is provided by a house light located in the center of the box's ceiling. When the rats made contact with the sipper tube, a circuit involving the steel rods in the floor is closed and the lick frequency is automatically recorded. A computer located in an adjacent room controls the presentation and retraction of the sipper tube, and the recording of lick frequency. A trial will last 5 min from the first detection of a sipper tube contact. Each conditioning box is placed in a sound-attenuating chamber containing a speaker to deliver white noise and a fan for ventilation. Together, the speaker and fan produce noise with an intensity of 80.1 dB (SPL, scale C).

Procedure. Rats assigned to the 32-to-2% sucrose groups received daily access to 32% sucrose for ten trials, and then were shifted to a 2% sucrose solution for the following five trials. Rats assigned to the 2-to-2% sucrose groups received daily access to 2% sucrose throughout the experiment. In the typical cSNC task, rats that receive the downshift from 32% to 2% sucrose usually display suppressed consummatory behavior starting on the first downshifted trial (Trial 11), followed by a recovery of the consummatory behavior on the second downshifted trial (Trial

12). In contrast, rats that do not experience the downshift maintain stable levels of consummatory behavior across trials (Flaherty, 1996).

Sucrose solutions. The sucrose solutions (32% and 2%) were prepared on a weight/weight basis, by mixing 68 g (or 98 g) of deionized water for every 32 g (or 2 g) of commercial sugar. The mixture containers were shaken until the sugar was completely dissolved and kept refrigerated between sessions. The sucrose solutions were taken out of the refrigerator at least 30 min before the beginning of the session to be presented to the animals at room temperature.

Preference Test

Following the cSNC task, rats were placed back in their home cages, and transported to a different room for the administration of a free-choice, two-bottle preference test. The enrichment device usually available in the home cages was removed during preference test to avoid any interference with the animals' ability to reach the sipper tubes. Animals received simultaneous access to two solutions, delivered in bottles suspended in their home cages, and continuously available for 1 hour. All animals received access to one bottle containing 10% ethanol, whereas the other bottle contained deionized water. the position of the bottle containing ethanol was exchanged daily to minimize position preferences. The amount of fluid contained in each bottle was recorded by weighing the bottles before and after each preference test session to determine the amount of substance consumed.

The concentration of ethanol was chosen based on the result of experiments conducted in our lab using the two-task paradigm including the cSNC preparation with a 32-to-2% downshift and the preference test with access to 10% ethanol solution and deionized water. These experiments showed a consistent trend in which this concentration produced an increase in ethanol consumption after the second downshifted session of the cSNC task, regardless of the

number of preshift sessions (20 vs. 10; Figure 5), suggesting self-medicating behavior for the modulation of the negative emotional state induced by the reward devaluation. The duration of this task was chosen based on the results a pilot study conducted in our lab, suggesting that beyond 1 h substance consumption remains at low, steady levels (Figure 6).

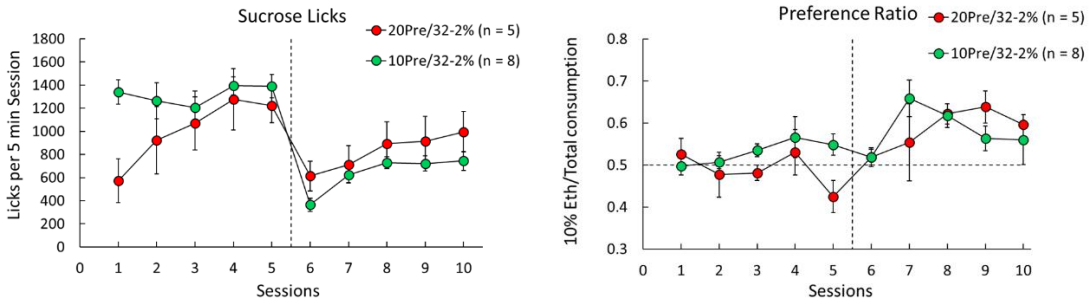

Figure 5. Results of 32-2% reward downshift in rats receiving either 20 (20Pre) or 10 (10Pre) preshift sessions (left panel), and preference ratio results of the 1-h, two-bottle preference test administered immediately after the reward downshift session (right panel). Data for the last five preshift sessions and the first five postshift sessions are represented (Guarino, unpublished data.)

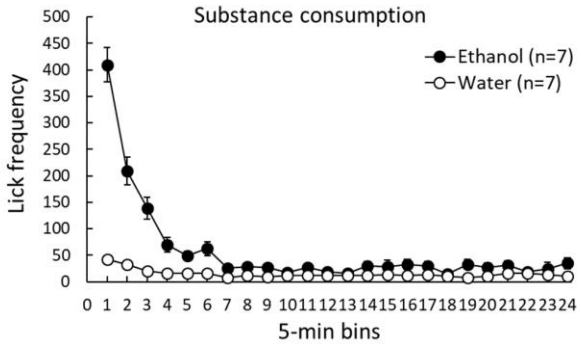

Figure 6. Substance consumption in rats over 1-hour access to water and 8% ethanol in a free-choice situation. (Guarino, unpublished data.)

Open Field Test

Apparatus. Open-field testing was carried out in four units (MED Associates, St. Albans, VT, USA). The dimensions of each chamber were 43x30x43 cm (LxHxW). Rats were tested in squads of four whenever possible. A light bulb (100 W) was suspended on top of each field,

above the central area, to allow for dark vs. light manipulation. The open field was cleaned immediately after each session, before testing the next group of rats.

Procedure. The day after the end of the cSNC task, the same groups of animals were exposed to the open field test for two 20-min sessions, one per day for two consecutive days. One session was lighted whereas the other was in the dark. The order of light and dark sessions was counterbalanced across animals. Immediately before each session, each animal received an i.p. injection of either CNO or vehicle. Of the 13 animals in the CNO condition, seven had been in the 32-to-2% downshifted group (32/CNO), and five in the 2-to-2% unshifted group (2/CNO) during the cSNC task. Of the 15 animals in the vehicle condition, seven had been in the 32-to-2% downshifted group (32/VEH), and six in the 2-to-2% unshifted group (2/CNO) during the cSNC task. At the beginning of the open field session, the animal was placed in the center of the arena and allowed free movement. A computer located in an adjacent room recorder the distance traveled (cm), the dependent measure for this behavioral task.

Clozapine N-oxide (CNO) Preparation and Injection Procedure

CNO (NIDA Drug Supply Program) was dissolved in 5% dimethyl sulfoxide (DMSO) and 95% sterile saline. Based on previous reports, CNO appears to be pharmacologically and behaviorally inert in rats (Ferguson et al., 2011, 2013) when administered at the doses between 0.1–3 mg/kg. A pilot study was conducted in our lab to determine the CNO dose to be used for the current research. As expected, the results of this pilot indicated that i.p. injections of either 1 mg/kg or 3 mg/kg CNO did not disrupt the effects of 32-to-2% sucrose devaluation in the same cSNC preparation used in the current experiment relative to vehicle (5% DMSO, 95% sterile saline) controls (Figure 7). The choice for the highest CNO dose tested (i.e., 3 mg/kg) was made to maximize the chances of activating the receptor.

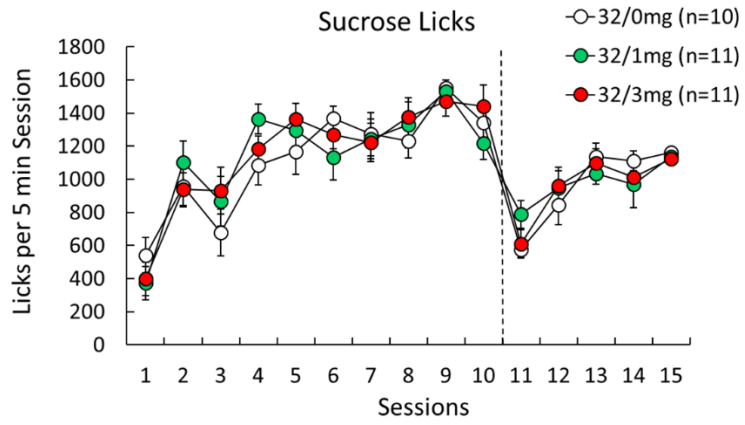

Figure 7. Results of 32-2% reward downshift with i.p. injection on sessions 11 and 12, using 0 mg/kg (i.e., vehicle), 1 mg/kg CNO, or 3 mg/kg CNO. (Guarino, unpublished results.)

Injections were administered in a room different from the rooms in which animals experience the cSNC task or the preference test. Each animal received five total injections: on sessions 11-13 prior to the reward devaluation experience, and prior to each day of open field test (days 16 and 17 of the experiment, after the end of the 15-day cSNC task). A pair of groups (CNO) received i.p. injections of CNO (3 mg/kg). Another pair of groups (VEH) received i.p injections of the vehicle. Thirty minutes after the administration of i.p. injections, animals were placed in the consummatory box for the cSNC task. Immediately after each cSNC session, animals were placed in their home cages where they received the 1-h preference test. Since the effects of CNO can last up to 5 h, the CeA was expected to remain inactive throughout the cSNC and preference test on sessions 11-13 and for the duration of the 20-min session of the open field test.

Virus Localization

Rats were sacrificed with an overdose of CO_2 and the brains were immediately extracted and embedded in 4% paraformaldehyde for at least 3 days. Brains were then embedded in 30% sucrose for at least 2 days. Once fixed, brains were sectioned in 40 µm sections using a cryostat.

Sections were placed onto slides, Fluoromount-G mounting medium was applied to allow for the localization of the fluorescent tag mCherry, and cover slips were applied on the slides to preserve the fluorescent tag and be able to localize the virus at a later time. The location of the virus was assessed via fluorescence microscopy (LSM 710 Confocal microscope, Zeiss, Thornwood, NY).

Statistical Analyses

Behavioral data for the current research were analyzed using between-subject or mixed-model analyses of variance (ANOVA), with an alpha value set at the 0.05 level. Pairwise comparisons using the LSD test were derived from the main analysis whenever justified by appropriate significant interactions. The IBM SPSS package (Version 21) was used to compute all the statistics.

Results

Histology

Figure 8 shows expression of the hM4Di viral construct visualized by the localization of mCherry via fluorescence microscopy. The images indicate that expressions were concentrated primarily in the target region. Two animals assigned to the CNO groups that lacked the viral expression at the region of interest were excluded from all further analysis.

Behavioral Data Analyses

cSNC. cSNC data were transformed according to the following procedure. The average of the last three preshift sessions (sessions 8-10) and the average of the three postshift sessions when CNO was administered (sessions 11-13) were calculated for each animal. Then, a Postshift/Preshift ratio was obtained for each animal. The results are shown in Figure 9, with the final sample size for each group. Using these transformed data, a 2 (Contrast: downshifted vs. unshifted) x 2 (Injection: CNO vs. vehicle) between-subject ANOVA was conducted to assess the effect of reward devaluation and CeA inactivation on consummatory behavior. The results

revealed a significant main effect of Contrast, $F(1, 24) = 18.57$, $p \leq .001$, with downshifted animals exhibiting lower proportion of licks than unshifted animals, $p \leq .001$. The two-way interaction between Contrast and Injection conditions was also significant, $F(1, 24) = 5.42$, $p = .03$. Follow-up tests using LSD pairwise comparisons, found that within the vehicle group, downshifted animals exhibited a significantly lower proportion of licks relative to the unshifted animals, $p \leq .001$. However, there was no evidence of such difference within the CNO group, $p = .19$. Taken together, these results suggest that CeA inactivation via CNO injection eliminated the cSNC effect in the 32/CNO group.

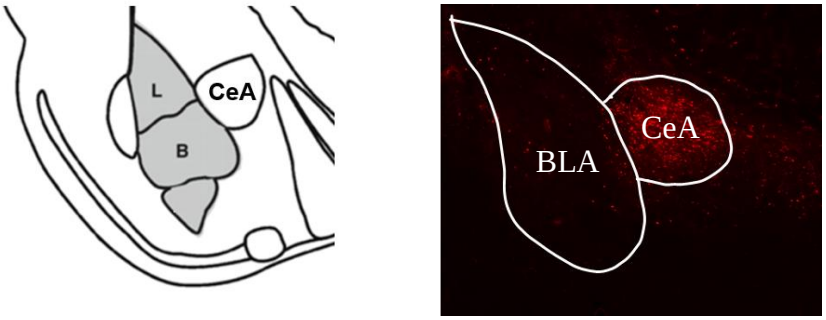

Figure 8. The left panel shows a schematic representation of the amygdala nuclei; lateral (L), basal (B), and central (CeA). The right panel is a representative image of hM4Di viral vector expression for one of the animals in the current study 5 weeks following viral infusions. Viral spread is depicted in bright red; basolateral (BLA) and central (CeA).

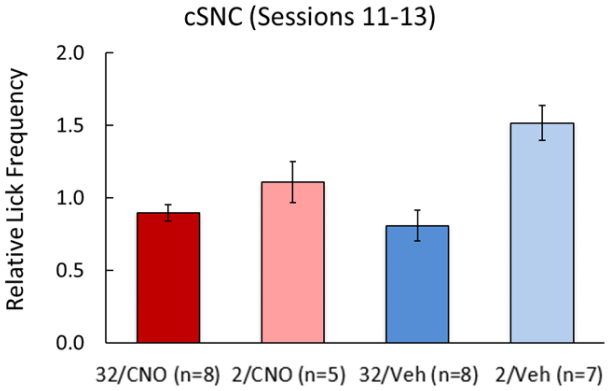

Figure 9. Means (± SEM) of lick proportion in postshift relative to preshift sessions, in groups given access to 32% or 2% sucrose during preshift. All animals received access to 2% sucrose during postshift sessions 11–13. Thus, "32" denotes the 32-to-2% sucrose downshift condition and "2" the 2% sucrose unshifted condition.

Preference test. Figure 10 shows the results of the 1-h two-bottle preference test with the final sample size for each group. A 2 (Contrast: downshifted vs. unshifted) x 2 (Injection: CNO vs. vehicle) x 2 (Bottle: ethanol vs. water) mixed-model ANOVA, with Bottle as the repeated-measure factor, was used to assess the effect of reward devaluation and CeA inactivation on substance consumption. Similar to the cSNC data analysis, preference test data were also transformed to obtained proportion of ethanol and water consumption. No significant main effects or interaction were found, *p*s ≥ .16. Follow-up test using LSD pairwise comparisons showed that within the downshifted groups, animals in the vehicle group exhibited higher proportion of ethanol consumption relative to water, $p = .07$. Even though this trend was not supported statistically, it suggests that a larger sample size may lead to significantly higher ethanol consumption compared to water in the 32/VEH group but not in the 32/CNO group, which would provide evidence for the ESM effect.

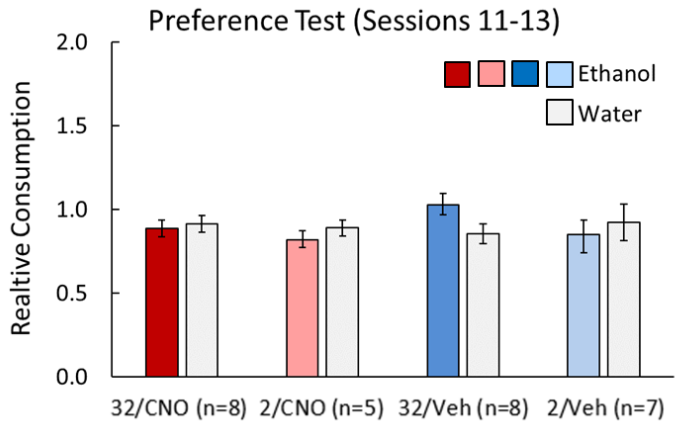

Figure 10. Means (± SEM) of consumption proportion in postshift relative to preshift sessions for the 1-h two-bottle preference test, with one bottle containing 10% ethanol, and the other bottle containing deionized water.

Open field. The same animals assigned to cSNC task followed by the preference test were tested in a two-day open-field task. Figure 11 shows the final sample size for each group and the results of open field activity in terms of distance traveled during the first 5 min of open-

field testing. Infusion (CNO vs. vehicle) x Illumination (dark vs. light) mixed-model ANOVA analysis, with Illumination as repeated-measure factor, was computed for distance traveled. No main effects or interactions were significant, Fs ≤ 1.84, ps ≥ .19. LSD pairwise comparisons derived from the main analysis indicated marginally significant difference between CNO and vehicle animals for the Dark condition, with higher distance traveled in CNO relative to vehicle animals, p = .06. No differences between the groups were found for the Light condition p = .44.

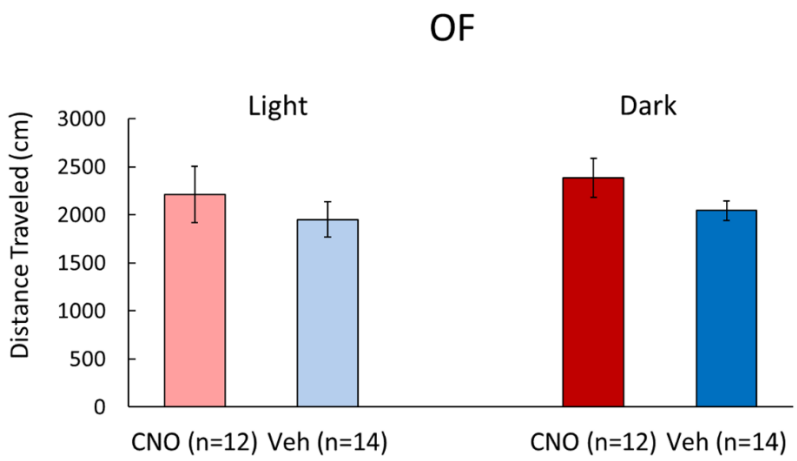

Figure 11. Means (± SEM) of distance traveled during the first 5 min of testing in CNO and vehicle animals under light and dark illumination conditions.

Discussion

The current research explored the role of the CeA in the hypothesized neural circuit underlying reward loss, and its relationship with the emotional self-medication hypothesis utilizing the chemogenetic approach of DREADDs to produce transient inactivation of the CeA on key sessions of our behavioral paradigm involving two successive tasks, cSNC and preference test. Groups of animals received insertion of a viral construct carrying the hM4Di inhibitory DREADD, the most common type of DREADD used as a medium for silencing neuronal activity via CNO systemic injections. Animals were exposed to surprising reward devaluation (e.g., incentive downshift) in the cSNC situation, the induction task, immediately followed by 1-h

access to ethanol and deionized water, made available to the animals simultaneously in a free-choice situation. At the end of this 15-day two-task behavioral paradigm, animals were tested on the open-field task for two consecutive days in alternate dark and light conditions. The results of this experiment can be summarized as follows: (1) CeA inactivation prior to reward devaluation session eliminated the cSNC effect, (2) a hint of the ESM effect was present in animals that experienced the reward devaluation under normal CeA activity, but not in animals for which the CeA was inhibited, and (3) open-field activity showed a trend, albeit nonsignificant, toward increased activity in animals with inhibited CeA activity.

After being trained for ten consecutive days, during which rats were given access to either a 32% or a 2% sucrose solution, CNO administration eliminated the suppression of consummatory behavior induced by a 32% to 2% sucrose downshift (32/CNO). In contrast, vehicle administration in downshifted animals (32/VEH) resulted in decreased consummatory behavior compared to preshift sessions. When compared to the respective unshifted controls, the proportion of consummatory behavior within animal groups administered with vehicle was significantly lower in the downshifted group, an indicator of the cSNC effect. This difference was not detected in animals treated with CNO. It should be noted that unshifted animals also carry the DREADD receptor, and therefore allowing CNO administration to produce neuronal silencing. Even though CNO administration in the unshifted group was not expected to affect consummatory behavior, it is possible that DREADD activation may trigger other behavioral responses in the absence of reward devaluation. A closer examination of the viral expression for all animals included in the experiment will help determine whether a different region was affected. Overall, these findings corroborated the results from a previous study conducted in our lab, indicating that the transient inactivation of the centromedial amygdala via lidocaine microinfusion resulted in a reduction of the cSNC effect (Kawasaki et al., 2015). The results we

obtained in the open-field test provided inconclusive information about the role of the CeA in this behavioral task. Open-field testing in a well-lit or dark arena did not affect ambulatory behavior, resulting in similar distance traveled for the two illumination conditions, for the two groups. Results reported higher levels of locomotor activity in CNO animals relative to vehicle animals when tested in the dark, but this trend was not supported statistically. Significance may be achieved with a larger sample size.

The current study extended our knowledge of the CeA function in situation involving negative emotions by providing an opportunity to the animals to consume ethanol immediately after the cSNC task in a free-choice situation. Despite the lack of significance, the negative emotion induced by the reward devaluation of the cSNC task seems to lead to an increase of preference for ethanol in the free-choice test. During sessions of the preference test following the preshift phase of the cSNC task, the consumption of ethanol and deionized water was similar across all four groups. However, downshifted animals that experienced the reward devaluation under normal CeA condition (32/VEH), showed higher proportion of ethanol consumption compared to water. No indication of this response was present in unshifted animals (2/VEH and 2/CNO) or in downshifted animals for which the CeA was inactivated (32/CNO).

One important contribution of this experiment consists of the use of the DREADD approach to achieve transient inactivation of brain regions, which was tested for the first time in our lab, and was able to produce behavioral consequences in the cSNC task similar to those obtained using lidocaine microinfusions (Kawasaki et al., 2015). Based on the results of this study and on the results of a couple of ongoing DREADD projects from our lab, the DREADD approach seems to be a valuable method to further explore our hypothesized brain circuitry underlying reward loss. DREADDs have been employed in a variety of studies to investigate neuronal circuits via activation or inhibition of target brain regions (Roth, 2016), and have

identified two engineered muscarinic receptors successfully utilized for remote neuronal manipulation, both of which respond only to CNO. The human M4 muscarinic receptor (hM4) has been engineered as inhibitory Gi-DREADD (hM4Di), and its activation facilitates an overall reduction in cellular activity via suppression of neuronal firing and synaptic transmission (Armbruster et al., 2007; Ferguson et al., 2011; Cheng et al., 2017b). The human M3 muscarinic receptor (hM3) has been engineered as excitatory Gq-DREADD (hM3Dq), and its activation facilitates an overall increase in cellular activity, increasing neuronal excitability and firing (Alexander et al., 2009; Krashes et al., 2014) and neurotransmitter release (Cheng et al., 2017b).

The inhibitory Gi-DREADD (hM4Di) is the only one we used in our research thus far. However, one possible way to employ the DREADD approach to carry on our understanding of the reward loss circuitry is to utilize both inhibitory and excitatory DREADDs. For example, if the CeA is an important component of the circuit activated by reward devaluation involving negative emotion, would the excitation of the CeA prior to the sucrose downshift of the cSNC situation result in enhanced cSNC effect? And if so, would this enhanced negative emotion also result in enhanced ethanol consumption? These questions could be assessed using the DREADD approach by inserting the excitatory Gq-DREADD (hM3Dq) into the CeA and exposing the animals to the behavioral tasks used in the current study. Additionally, the design could be improved by adding intact groups, receiving either CNO or vehicle administration, but not subject to viral infusion.

Another alternative involves the nucleus accumbens (NAc) as target brain region. Previous research showed that the negative emotion induced by 32-to-4% sucrose downshift in the cSNC task was accompanied by decreased dopamine release in the NAc in rats (Genn et al., 2004). Would inhibition or excitation of the NAc prior to reward devaluation have any effects on the cSNC? And based on the association of the NAc with dopamine release, would excitation of

the NAc result in enhanced ethanol consumption? The investigation of these questions may provide evidence supporting the hypothesis that ethanol-seeking behavior may be more related to the pleasurable state resulting from substance consumption (Knapp et al., 2011), rather than to the anxiolytic properties of ethanol.

The assessment of the cSNC effect and ethanol consumption under different neural activity and reward devaluation conditions will help formulate new questions regarding brain pathways involved in triggering, controlling, and reducing the intensity of loss-induced negative emotions.

References

Acevedo, M. B., Nizhnikov, M. E., Molina, J. C., & Pautassi, R. M. (2014). Relationship between ethanol-induced activity and anxiolysis in the open field, elevated plus maze, light-dark box, and ethanol intake in adolescent rats. *Behavioural Brain Research, 265*, 203-215.

Amsel, A. (1992). *Frustration theory: An analysis of dispositional learning and memory*. Cambridge University Press.

Andrews, N. A., Papakosta, M., & Barnes, N. M. (2014). Discovery of novel anxiolytic agents: The trials and tribulations of pre-clinical models of anxiety. *Neurobiology of Disease, 61*, 72-78.

Becker, H. C., & Flaherty, C. F. (1982). Influence of ethanol on contrast in consummatory behavior. Psychopharmacology, 77, 253-258.

Becker, H. C., & Flaherty, C. F. (1983). Chlordiazepoxide and ethanol additively reduce gustatory negative contrast. *Psychopharmacology*, *80*(1), 35-37.

Becker, H. C., Jarvis, M. F., Wagner, G. C., & Flaherty, C. F. (1984). Medial and lateral amygdalectomy differentially influences consummatory negative contrast. *Physiology & Behavior*, *33*, 707-712.

Bentosela, M., Ruetti, E., Muzio, R.N., Mustaca, A.E., Papini, M.R. (2006). Administration of corticosterone after the first downshift trial enhances consummatory successive negative contrast. *Behavioral Neuroscience, 120*, 371-376.

Bertholomey, M., West, C., Jensen, M., Li, T., Stewart, R., Weiss, J., & Lumeng, L. (2011). Genetic propensities to increase ethanol intake in response to stress: studies with selectively bred swim test susceptible (SUS), alcohol-preferring (P), and non-preferring (NP) lines of rats. *Psychopharmacology*, *218*(1), 157-167.

Biesdorf, C., Wang, A. L., Topic, B., Petri, D., Milani, H., Huston, J. P., & de Souza Silva, M. A. (2015). Dopamine in the nucleus accumbens core, but not shell, increases during signaled food reward and decreases during delayed extinction. *Neurobiology of Learning and Memory, 123,* 125-139.

Bouwknecht, J. A., Spiga, F., Staub, D. R., Hale, M. W., Shekhar, A., & Lowry, C. A. (2007). Differential effects of exposure to low-light or high-light open-field on anxiety-related behaviors: Relationship to c-Fos expression in serotonergic and non-serotonergic neurons in the dorsal raphe nucleus. *Brain Research Bulletin, 72*(1), 32-43.

Brevers, D., Bechara, A., Hermoye, L., Divano, L., Kornreich, C., Verbanck, P., Noël, X., 2015. Comfort for uncertainty in pathological gamblers: an fMRI study. *Behavioural Brain Research, 278,* 262-270.

Caldwell, E. E., & Riccio, D. C. (2010). Alcohol self-administration in rats: Modulation by temporal parameters related to repeated mild social defeat stress. *Alcohol, 44,* 265-274.

Capriles, N., Rodaros, D., Sorge, R. E., & Stewart, J. (2003). A role for the prefrontal cortex in stress-and cocaine-induced reinstatement of cocaine seeking in rats. *Psychopharmacology, 168,* 66-74.

Cisler, J. M., Elton, A., Kennedy, A. P., Young, J., Smitherman, S., James, G. A., & Kilts, C. D. (2013). Altered functional connectivity of the insular cortex across prefrontal networks in cocaine addiction. *Psychiatry Research: Neuroimaging, 213,* 39-46.

Conger, J. J. (1956). Reinforcement theory and the dynamics of alcoholism. *Quarterly Journal of Studies on Alcohol, 17,* 296-305.

Crum, R. M., La Flair, L., Storr, C. L., Green, K. M., Stuart, E. A., Alvanzo, A. A. H., & Mojtabai, R. (2013). Reports of drinking to self-medicate anxiety symptoms: Longitudinal assessment for subgroups of individuals with alcohol dependence. *Depression & Anxiety, 30,* 174-183.

Darke, S. (2012). Pathways to heroin dependence: Time to re-appraise self-medication. *Addiction, 108,* 659-667.

Egli, M., Koob, G.F., Edwards, S. (2012). Alcohol dependence as a chronic pain disorder. *Neuroscience & Biobehavioral Reviews, 36,* 2179-2192.

Ersche, K.D., Gillan, C.M., Jones, P.S., Williams, G.B., Ward, L.H., Luijten, M., Wit, S., Sahakian, B., Bullmore, E.T., & Robbins, T.W. (2016). Carrots and sticks fail to change behavior in cocaine addiction. *Science, 352,* 1468-1471.

Everitt, B. J., Cardinal, R. N., Parkinson, J. A., & Robbins, T. W. (2003). Appetitive behavior. *Annals of the New York Academy of Sciences*, *985*(1), 233-250.

Everitt, B. J. & Robbins, T. W. (2013). From the ventral to the dorsal striatum: devolving views of their roles in drug addiction. *Neuroscience & Biobehavioral Reviews, 37,* 1946-1954.

Ferguson, S. M., Eskenazi, D., Ishikawa, M., Wanat, M. J., Phillips, P. E., Dong, Y., ... & Neumaier, J. F. (2011). Transient neuronal inhibition reveals opposing roles of indirect and direct pathways in sensitization. *Nature neuroscience*, *14*(1), 22-24.

Ferguson, S. M., Phillips, P. E., Roth, B. L., Wess, J., & Neumaier, J. F. (2013). Direct-pathway striatal neurons regulate the retention of decision-making strategies. *Journal of Neuroscience, 33,* 11668-11676.

Flaherty, C. F. (1996). *Incentive relativity*. Cambridge, UK: Cambridge University Press.

Flaherty, C. F., Grigson, P. S., & Lind, S. (1990). Chlordiazepoxide and the moderation of the initial response to reward reduction. *The Quarterly Journal of Experimental Psychology*, *42*(1), 87-105.

Flaherty, C. F., Grigson, P. S., & Rowan, G. A. (1986). Chlordiazepoxide and the determinants of negative contrast. *Animal Learning & Behavior*, *14*, 315-321.

Forget, B., Pushparaj, A., & Le Foll, B. (2010). Granular insular cortex inactivation as a novel therapeutic strategy for nicotine addiction. *Biological Psychiatry*, *68*, 265-271.

Genn, R. F., Ahn, S., & Phillips, A. G. (2004). Attenuated dopamine efflux in the rat nucleus accumbens during successive negative contrast. *Behavioral Neuroscience*, *118*, 869-873.

Genn, R. F., Tucci, S., Parikh, S., & File, S. E. (2004). Effects of nicotine and a cannabinoid receptor agonist on negative contrast: distinction between anxiety and disappointment? *Psychopharmacology*, *177*, 93-99.

Glueck, A. C., Dennis, T. S., Perrotti, L. I., Torres, C., & Papini, M. R. (2015). Brain expression of pCREB in rats exposed to consummatory successive negative contrast. *Neuroscience Letters*, *587*, 93-97.

Goldstein, R. Z., & Volkow, N. D. (2011). Dysfunction of the prefrontal cortex in addiction: Neuroimaging findings and clinical implications. *Nature reviews neuroscience*, *12*, 652-669.

Gomez, J. L., Bonaventura, J., Lesniak, W., Mathews, W. B., Sysa-Shah, P., Rodriguez, L. A., Ellis, R. J., Richie, C. T., Harvey, B. K., Dannals, R. F., Pomper, M. G., Bonci, A., & Michaelides, M. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science*, *357*, 503-507.

Griffin, A. L., & Berry, S. D. (2004). Inactivation of the anterior cingulate cortex impairs extinction of rabbit jaw movement conditioning and prevents extinction-related inhibition of hippocampal activity. *Learning & Memory, 11*, 604-610.

Gross, J. J. (2013). Emotion regulation: Taking stock and moving forward. *Emotion, 13*, 359-365.

Hassanbeigi, A., Askari, J., Hassanbeigi, D., Pourmovahed, Z. (2013). The relationship between stress and addiction. *Procedia-Social and Behavioral Sciences, 84,* 1333-1340.

Hatfield, T., Han, J. S., Conley, M., Gallagher, M., Holland, P. (1996). Neurotoxic lesions of basolateral, but not central, amygdala interfere with Pavlovian second-order conditioning and reinforcer devaluation effects. *Journal of Neuroscience, 16,* 5256-5265.

Judice-Daher, D. M., & Bueno, J. L. O. (2013). Lesions of the nucleus accumbens disrupt reinforcement omission effects in rats. *Behavioural Brain Research, 252,* 439-443.

Kalivas, P. W., & Volkow, N. D. (2007). The neural basis of addiction: A pathology of motivation and choice. *Focus, 5*, 208-219.

Kamenetzky, G. V., Mustaca, A. E., & Papini, M. R. (2008). An analysis of the anxiolytic effects of ethanol on consummatory successive negative contrast. *Avances En Psicología Latinoamericana, 26,* 135-144.

Kamenetzky, G. V., Mustaca, A. E., Pedron, V. T., Cuenya, L., & Papini, M. R. (2009). Ethanol facilitates consummatory extinction. *Behavioural Processes, 82*, 352-354.

Kawasaki, K., Annicchiarico, I., Glueck, A. C., Morón, I., & Papini, M. R. (2017). Reward loss and the basolateral amygdala: A function in reward comparisons. *Behavioural Brain Research, 331,* 205-213.

Kawasaki, K., Glueck, A. C., Annicchiarico, I., & Papini, M. R. (2015). Function of the centromedial amygdala in reward devaluation and open-field activity. *Neuroscience, 303*, 73-81.

Keyes, K. M., Hatzenbuehler, M. L., & Hasin, D. S. (2011). Stressful life experiences, alcohol consumption, and alcohol use disorders: the epidemiologic evidence for four main types of stressors. *Psychopharmacology*, *218*(1), 1-17.

Khantzian, E. J. (1985). The self-medication hypothesis of addictive disorders: Focus on heroin and cocaine dependence. *The American Journal of Psychiatry, 142*, 1259-1264.

Khantzian, E. J. (2013). Addiction as a self-regulation disorder and the role of self-medication. *Addiction, 108*, 668-669.

Kimura, M., Satoh, T., & Matsumoto, N. (2007). What does the habenula tell dopamine neurons?. *Nature Neuroscience*, *10*, 677-678.

Knapp, D., Overstreet, D., Huang, M., Wills, T., Whitman, B., Angel, R., & ... Breese, G. (2011). Effects of a stressor and corticotrophin releasing factor on ethanol deprivation-induced ethanol intake and anxiety-like behavior in alcohol-preferring P rats. *Psychopharmacology*, *218*(1), 179-189.

Koob, G.F. (2013). Addiction is a reward deficit and stress surfeit disorder. *Frontiers in Psychiatry, 4*, 1-18.

Konopka, A., Pełka-Wysiecka, J., Grzywacz, A., Samochowiec, J. (2013). Psychosocial characteristics of benzodiazepine addicts compared to not addicted benzodiazepine users. *Progress in Neuro-Psychopharmacology and Biological Psychiatry, 40*, 229-235.

Kumar, V., Bhat, Z. A., & Kumar, D. (2013). Animal models of anxiety: A comprehensive review. *Journal of Pharmacological and Toxicological Methods, 68*, 175-183.

Lanciego, J. L., Luquin, N., & Obeso, J. A. (2012). Functional neuroanatomy of the basal ganglia. *Cold Spring Harbor Perspectives in Medicine, 2*, 1-21.

Leszczuk, M. H., & Flaherty, C. F. (2000). Lesions of nucleus accumbens reduce instrumental but not consummatory negative contrast in rats. *Behavioural Brain Research, 116*(1), 61-79.

Li, X. (2012). Using the conditioned fear stress (CFS) animal model to understand the neurobiological mechanisms and pharmacological treatment of anxiety. *Shanghai Archives of Psychiatry*, *24*, 241-249.

Li, X., Lu, Q., & Miller, R. (2012). Self-medication and pleasure seeking as dichotomous motivations underlying behavioral disorders. *Journal of Business Research, 66*, 1598-1604.

Liao, R. M., & Chuang, F. J. (2003). Differential effects of diazepam infused into the amygdala and hippocampus on negative contrast. *Pharmacology Biochemistry and Behavior, 74*, 953-960.

Lin, J. Y., Roman, C., & Reilly, S. (2009). Insular cortex and consummatory successive negative contrast in the rat. *Behavioral Neuroscience*, *123*, 810-814.

Lorberbaum, J. P., Kose, S., Johnson, M. R., Arana, G. W., Sullivan, L. K., Hamner, M. B., ... & George, M. S. (2004). Neural correlates of speech anticipatory anxiety in generalized social phobia. *Neuroreport, 15*, 2701-2705.

Lu, Y. L., & Richardson, H. N. (2014). Alcohol, stress hormones, and the prefrontal cortex: A proposed pathway to the dark side of addiction. *Neuroscience, 277*, 139-151.

Mahler, S. V., & Aston-Jones, G. (2018). CNO Evil? Considerations for the use of DREADDs in behavioral neuroscience. *Neuropsychopharmacology, 43*, 1-9.

Manzo, L., Donaire, R., Sabariego, M., Papini, M. R., & Torres, C. (2015). Anti-anxiety self-medication in rats: Oral consumption of chlordiazepoxide and ethanol after reward devaluation. *Behavioural Brain Research, 278,* 90-97.

Manzo, L., Gómez, M. J., Callejas-Aguilera, J. E., Fernández-Teruel, A., Papini, M. R., & Torres, C. (2014). Anti-anxiety self-medication induced by incentive loss in rats. *Physiology & Behavior, 123,* 86-92.

Martin, J. H., Ghez, C. (1999). Pharmacological inactivation in the analysis of the central control of movement. *Journal of Neuroscience Methods, 86,* 145-159.

Mendoza, J., Sanio, C., & Chaudhri, N. (2015). Inactivating the infralimbic but not prelimbic medial prefrontal cortex facilitates the extinction of appetitive Pavlovian conditioning in Long-Evans rats. *Neurobiology of Learning and Memory, 118,* 198-208.

Mitchell, C. & Flathery, C. F. (1998). Temporal dynamics of corticosterone elevation in successive negative contrast. *Physiology and Behavior, 64,* 287-292.

Naqvi, N. H., & Bechara, A. (2009). The hidden island of addiction: the insula. *Trends in Neurosciences, 32,* 56-67.

Naqvi, N. H., & Bechara, A. (2010). The insula and drug addiction: An interoceptive view of pleasure, urges, and decision-making. *Brain Structure & Function, 214,* 435-450.

Naqvi, N. H., Rudrauf, D., Damasio, H., & Bechara, A. (2007). Damage to the insula disrupts addiction to cigarette smoking. *Science, 315,* 531-534.

Ortega, L. A., Glueck, A. C., Uhelski, M., Fuchs, P. N., & Papini, M. R. (2013). Role of the ventrolateral orbital cortex and medial prefrontal cortex in incentive downshift situations. *Behavioural Brain Research, 244,* 120-129.

Ortega, L. A., Solano, J. L., Torres, C., & Papini, M. R. (2017). Reward loss and addiction: Opportunities for cross-pollination. *Pharmacology Biochemistry and Behavior, 154,* 39-52.

Ortega, L. A., Uhelski, M., Fuchs, P. N., & Papini, M. R. (2011). Impairment of recovery from incentive downshift after lesions of the anterior cingulate cortex: emotional or cognitive deficits?. *Behavioral Neuroscience, 125,* 988-995.

Panayi, M. C., & Killcross, S. (2014). Orbitofrontal cortex inactivation impairs between-but not within-session Pavlovian extinction: An associative analysis. *Neurobiology of Learning and Memory, 108,* 78-87.

Papini, M. R. (2002). Pattern and process in the evolution of learning. *Psychological Review, 109,* 186-201.

Papini, M. R. (2003). Comparative psychology of surprising nonreward. *Brain, Behavior and Evolution, 62,* 83-95.

Papini, M. R., & Dudley, R. T. (1997). Consequences of surprising reward omissions. *Review of General Psychology, 1,* 175-197.

Papini, M. R., Fuchs, P. N., & Torres, C. (2015). Behavioral neuroscience of psychological pain. *Neuroscience & Biobehavioral Reviews, 48,* 53-69.

Papini, M. R., Wood, M., Daniel, A. M., & Norris, J. N. (2006). Reward loss as psychological pain. *International Journal of Psychology and Psychological Therapy, 6,* 189-213.

Pare, W. P. (1994). Open field, learned helplessness, conditioned defensive burying, and forced -swim tests in WKY rats. *Physiology & Behavior, 55,* 433-439.

Parent M. B., McGaugh, J. L. (1994). Posttraining infusion of lidocaine into the amygdala basolateral complex impairs retention of inhibitory avoidance training. *Brain Research, 661,* 97-103.

Paulus, M. P., & Stein, M. B. (2006). An insular view of anxiety. *Biological Psychiatry, 60,* 383-387.

Paxinos, G., & Watson, C. (2007). The rat brain in stereotaxic coordinates. 6th ed. New York: Academic Press.

Pecoraro, N., & Dallman, M. F. (2005). c-Fos after incentive shifts: Expectancy, incredulity, and recovery. *Behavioral Neuroscience, 119,* 366-387.

Pecoraro, N., de Jong, H., & Dallman, M. F., (2009). An unexpected reduction in sucrose concentration activated the HPA axis on successive post shift days without attenuation by discriminative contextual stimuli. *Physiology & Behavior, 96,* 651-661.

Pobbe, R. L., & Zangrossi, H. (2008). Involvement of the lateral habenula in the regulation of generalized anxiety- and panic-related defensive responses in rats. *Life Sciences, 82,* 1256-1261.

Ramos, A. (2008). Animal models of anxiety: Do I need multiple tests?. *Trends in Pharmacological Sciences, 29,* 493-498

Rauch, S. L., Savage, C. R., Alpert, N. M., Fischman, A. J., Jenike, M. A. (1997): The functional neuroanatomy of anxiety: a study of three disorders using positron emission tomography and symptom provocation. *Biological Psychiatry, 42,* 446-452.

Rhodes, S. E., & Killcross, S. (2004). Lesions of rat infralimbic cortex enhance recovery and reinstatement of an appetitive Pavlovian response. *Learning & Memory, 11,* 611-616.

Rhodes, S. E. V., & Killcross, A. S. (2007). Lesions of rat infralimbic cortex enhance renewal of extinguished appetitive Pavlovian responding. *European Journal of Neuroscience, 25,* 2498-2503.

Rowan, G. A., & Flaherty, C. F. (1987). The effects of morphine in the consummatory contrast paradigm. *Psychopharmacology, 93*(1), 51-58.

Rowan, G. A., & Flaherty, C. F. (1991). Behavior of Maudsley reactive and nonreactive rats (*Rattus norvegicus*) in three consummatory contrast paradigms. *Journal of Comparative Psychology, 105*, 115-124.

Roth, B. L. (2016). DREADDs for neuroscientists. *Neuron, 89*, 683-694.

Sartorius, A., Kiening, K. L., Kirsch, P., von Gall, C. C., Haberkorn, U., Unterberg, A. W., ... & Meyer-Lindenberg, A. (2010). Remission of major depression under deep brain stimulation of the lateral habenula in a therapy-refractory patient. *Biological psychiatry, 67*, e9-e11.

Simmons, D.A. Neill, D.B. (2009). Functional interaction between the basolateral amygdala and the nucleus accumbens underlies incentive motivation for food reward on a fixed ratio schedule. *Neuroscience, 159*, 1264-1273.

Sinha, R., Lacadie, C., Skudlarski, P., Fulbright, R. K., Rounsaville, B. J., Kosten, T. R., & Wexler, B. E. (2005). Neural activity associated with stress-induced cocaine craving: A functional magnetic resonance imaging study. *Psychopharmacology, 183*, 171-180.

Spanagel, R., Noori, H. R., & Heilig, M. (2014). Stress and alcohol interactions: Animal studies and clinical significance. *Trends in neurosciences, 37*, 219-227.

Suarez, S. D., & Gallup, G. G. (1981). An ethological analysis of open-field behavior in rats and mice. *Learning & Motivation, 12*, 342-363.

Tan, K. R., Rudolph, U., & Lüscher, C. (2011). Hooked on benzodiazepines: GABA A receptor subtypes and addiction. *Trends in Neurosciences, 34*, 188-197.

Torres, C., Glueck, A.C., Conrad, S.E., Moron, I., & Papini, M.R. (2016). Dorsomedial striatum lesions affect adjustment to reward uncertainty, but not to reward devaluation or omission. *Neuroscience, 332*, 13-25.

Torres, C., & Papini, M. R. (2016). Emotional self-medication and addiction. In V. R. Preedy (Ed.), *Neuropathology of drug addiction and substance misuse,* Vol. 1 (pp. 71-81). New York: Elsevier.

Ungless, M. A., Argilli, E., & Bonci, A. (2010). Effects of stress and aversion on dopamine neurons: Implications for addiction. *Neuroscience & Biobehavioral Reviews, 35*, 151-156.

Urban, D. J., & Roth, B. L. (2015). DREADDs (designer receptors exclusively activated by designer drugs): Chemogenetic tools with therapeutic utility. *Annual Review of Pharmacology and Toxicology*, *55*, 399-417.

Volkow, N. D., & Baler, R. D. (2014). Addiction science: Uncovering neurobiological complexity. *Neuropharmacology, 76*, 235-249.

Yager, L. M., Garcia, A. F., Wunsch, A. M., & Ferguson, S. M. (2015). The ins and outs of the striatum: Role in drug addiction. *Neuroscience, 301,* 529-541.

VITA

PERSONAL Sara Guarino

EDUCATION Bachelor of Arts, Psychology, St. Mary's University, San Antonio, Texas, 2016

PUBLICATIONS

Hill, H. M., Dietrich, S., **Guarino, S.,** Banda, M., & Lacy, K. (in press). Preliminary observations of an unusual mouth interaction between beluga calves (*Delphinapterus leucas*). *Zoo Biology.*

Yeater, D., **Guarino, S.,** Lacy, S., Dees, T., Hill, H. (2017). Do belugas (*Delphinapterus leucas*), bottlenose dolphins (*Tursiops truncatus*), and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) display lateralized eye preference when presented with familiar or novel objects?. *International Journal of Comparative Psychology, 30,* 1-13.

Guarino, S., Yeater, D., Lacy, S., Dees, T., & Hill, H. M. (2017). Responses to familiar and unfamiliar objects by belugas (*Delphinapterus leucas*), bottlenose dolphins (*Tursiops truncatus*), and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*). *Animal Cognition, 20,* 823-827.

Hill, H. M., **Guarino, S.,** Geraci, C., Sigman, J., & Noonan, M. (2017). Developmental changes in the resting strategies of killer whale (*Orcinus orca*) calves and their mothers from birth to 36 months. *Behaviour, 154,* 435-466.

Hill, H. M., **Guarino, S.,** Calvillo, A., Gonzalez, A., Zuniga, K., Bellows, C., Polasek, L., & Sims, C. (2017). Lateralized swim positions are conserved across environments for beluga (*Delphinapterus leucas*) mother-calf pairs. *Behavioural Processes, 138,* 22-28.

Hill, H., **Guarino, S.,** Dietrich, S. (2017). Field of comparative psychology. In T.K. Shackelford, & V.A. Weekes-Shackelford (Eds.), *Encyclopedia of Evolutionary Psychological Science*. Springer International Publishing AG.

Guarino, S., Hill, H., & Sigman, J. (2016). Development of sociality and emergence of independence in a killer whale (*Orcinus orca*) calf from birth to 36 months. *Zoo Biology*, *36*(1), 11-20.

Harder, J., Hill, H., Dudzinski, K., Sanabria, K., **Guarino, S.,** & Kuczaj, S. (2016). The development of echolocation in bottlenose dolphins (*Tursiops truncatus*). *International Journal of Comparative Psychology*, *29*, 1-19.

Hill, H., Yeater, D., Gallup, S., **Guarino, S.,** Lacy, S., Dees, T., & Kuczaj, S. (2016). Responses to familiar and unfamiliar humans by belugas (*Delphinapterus leucas*), bottlenose dolphins (*Tursiops truncatus*), and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*): A replication and extension. *International Journal of Comparative Psychology*, *29*(1), 1-21.

Hill, H. M., **Guarino, S.,** Dietrich, S., & St Leger, J. (2016). An inventory of peer-reviewed articles on killer whales (*Orcinus orca*) with a comparison to bottlenose dolphins (*Tursiops truncatus*). *Animal Behavior and Cognition, 3,* 135-149.

Guarino, S., & Hill, H. (2016, September). Marine mammal behavior: Captivity vs. natural habitats. *Eye on Psi Chi, 21*(1), 12-15. http://www.psichi.org/resource/resmgr/eye_pdf/21

Hill, H. M., **Guarino, S.,** Crandall, S., Lenhart, E., & Dietrich, S. (2015). Young belugas diversify adult beluga (*Delphinapterus leucas*) behavior. *Animal Behavior and Cognition, 2*, 267-284.

EXPERIENCE Teaching Assistant**,** Texas Christian University, 2016 – present
Research Assistant, St. Mary's University, 2013 – 2016

MEMBERSHIP AND PROFESSIONAL ASSOCIATIONS

American Psychological Association (APA)
Society for Marine Mammalogy (SMM)
Animal Behavior Society (ABS)
Psi Chi, International Honor Society in Psychology
Southwestern Psychological Association (SWPA)

ABSTRACT

ROLE OF THE CENTRAL AMYGDALA IN LOSS-INDUCED EMOTIONAL SELF-MEDICATION

By Sara Guarino, B.A., 2016
Department of Psychology
Texas Christian University

Keywords: Reward Loss, Central Amygdala, Emotional Self-medication

Thesis Advisor: Mauricio R. Papini, Professor of Psychology

The present experiment was designed to explore the role of the central amygdala (CeA) in the hypothesized neural circuit underlying reward loss, and its relationship with the emotional self-medication (ESM) hypothesis utilizing the DREADD technique to remotely control neural activity. Rats received intracranial infusion of inhibitory DREADDs to allow for transient inactivation of the CeA, obtained via systemic injection of clozapine N-oxide (CNO), the activator drug for DREADDs. Animals were exposed to a 32-to-2% sucrose downshift in the consummatory successive negative contrast (cSNC) situation. After each cSNC session, animals were given simultaneous access to ethanol and water in a 1-h, two-bottle preference test. During the preshift phase (sessions 1-10), animals had access to either 32% (32/CNO and 32/VEH) or 2% (2/CNO and 2/VEH) sucrose. During the postshift phase (sessions 1-15), 32% groups were downshifted to 2% sucrose, whereas 2% groups were unshifted. Prior to downshifted sessions 11-13, animals received an i.p. injection of either CNO or vehicle. At the end of the 15-day two-task behavioral paradigm, animals were tested on the open-field task for two consecutive days in alternate dark and light conditions. The results indicated that CeA inactivation prior to reward devaluation session eliminated the cSNC effect (32/CNO), a hint of ESM effect was present in animals that experienced the reward devaluation under normal CeA activity (32/VEH), but not in animals for which the CeA was inhibited (32/VEH), and open-field activity showed a trend, albeit nonsignificant, toward increased activity in animals with inhibited CeA activity (32/CNO). One important contribution of this experiment involves the use of the DREADD technique to achieve transient inactivation of brain regions. This approach produced behavioral consequences in the cSNC task similar to those obtained in previous research using lidocaine microinfusions. The results of this study suggest that the DREADD approach is a valuable method to manipulate neural activity to further explore the role of these brain regions in our hypothesized reward loss circuitry.