Chronic Variable Stress Induces Avolition and Disrupts Corticoaccumbens Encoding of Approach Cues

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CHRONIC VARIABLE STRESS INDUCES AVOLITION AND DISRUPTS CORTICOACCUMBENS ENCODING OF APPROACH CUES

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ABSTRACT
CHRONIC VARIABLE STRESS INDUCES AVOLITION AND DISRUPTS CORTICOACCUMBENS ENCODING OF APPROACH CUES

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Disorders in the ability to process, evaluate, and interact with rewards are hallmarks of a range of mental illnesses. Such disorders are multi-faceted and arise from altered activity throughout diffuse brain regions. Chronic variable stress (CVS) is an oft-used tool for modeling reward-related disorders in preclinical research because it impairs the function of multiple brain regions and causes a range of severe hedonic and motivational deficits. While much research has focused on the former, the latter is poorly characterized.

A panel of behavioral tests was used to characterize the effect of CVS exposure on different facets of reward related behaviors in Sprague-Dawley rats. In a subset of animals, *in vivo* electrophysiology was used to assess the impact of CVS on reward encoding in a primary reward processing region, the nucleus accumbens (NAc). Behavioral deficits occurred in motivational, rather than hedonic, domains, and stress altered the encoding of primary rewards in the Shell subregion of the NAc, an area responsible for encoding value.

The prelimbic region of the prefrontal cortex (PL) is known to be sensitive to stress and responsive to reward-predictive cues. The extent to which this area encodes the incentive value of cues has not been characterized. Pavlovian autoshaping is a behavior in which trained animals transfer the incentive value of a primary reward to an associated cue. *In vivo* electrophysiological recordings of single units in the PL of Sprague-Dawley rats demonstrated that this region was attuned to incentivized cues in the autoshaping paradigm.

A projection pathway from the PL targeting the NAc Core (NAcC) subregion has a significant role in promoting motivated approach. However, little is known about how activity in this pathway (1.) changes during associative learning to encode incentivized cues or (2.) may be altered by stress. An intersectional fiber photometry approach used in male Sprague Dawley rats engaged in autoshaping demonstrated that the rapid acquisition of conditioned approach was associated with cue-induced PL-NAcC activity. Prior stress reduced both cue-directed behavior and associated cortical activity.

These results support the interpretation that stress disrupts reward processing by altering the attribution of incentive to associated cues.

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I. INTRODUCTION

What is Reward?

A historical understanding

The usage of the term “reward” in psychology and neuroscience has changed subtly over its long history. It was initially defined in psychological literature as something given in exchange for completing a behavior, which agreed with its etymological definition. Its first usage, according to a Pubmed keyword search for “Reward”, was a 1946 article titled, appropriately, “‘Reward’ and ‘Punishment’ in Learning” (Dand, 1946). While occasional reference was made in the following years to “reward value”, the primary focus remained on the strength of a chosen “reward”—typically a sucrose solution, palatable food, or the opportunity to mate—in promoting learning or directing behavior, which is understandable given the technical restraints of the time; if all that can be observed is an animal’s behavior, all that can be concluded ought to be grounded in that behavior. However, something changed with the serendipitous advent of intracranial self-stimulation: researchers could now study the neural correlates of reward. In this technique, a chronically implanted electrode delivers electrical stimulation to a targeted region of a rodent’s brain when the animal engages in particular behaviors, such as pressing a lever (Olds & Millner, 1954). The degree to which an animal will press a lever to receive this stimulation varies with the electrode’s location; stimulation of the septal region is particularly reinforcing (Olds & Millner, 1954; Sidman, Brady, Boren, Conrad, & Schulman, 1955).

Even more interesting, Olds’ body of work provided evidence for different reward systems (e.g. food vs. sex) and countered the prevailing theory of the day, drive-reduction (Olds, 1958). Drive-reduction theory postulated that pursuit of rewards was mediated exclusively by the motivation to alleviate aversive states (represented as

excitation in certain brain areas) induced by the lack of that reward. By defining “reward” as the absence of punishment, this theory left no space for the concept of hedonic pleasure. Intracranial self-stimulation demonstrated that punishment and reward were in fact dissociable constructs by inducing them separately in different brain areas (Olds, 1958). Building on this work, later researchers would go on to propose that not only were reward and punishment dissociable, but so too were motivation and hedonics (though they did not use this terminology; Deutsch, Howarth, Ball, & Deutsch, 1962). This advancement in how reward was understood, as not just a physical reinforcer such as a sugar pellet but rather a multifaceted neural representation, has profoundly shaped neuroscientific research. The fewer than 100 annual publications regarding “reward” in the 1950s and ‘60s has grown to over 3,000 per annum for the past 6 years. Given how central reward has become as a concept across disciplines in the field today, it is more important than ever to understand the different facets of “reward” and their representation in neural systems.

Reward is multifaceted

Modern conceptualizations of the neural representation of “reward” identify three discrete components: learning-related, motivational, and hedonic (Berridge & Robinson, 2003). All three constructs are interrelated and critical to understand reward, but the first two are particularly interconnected because of the overlap in methodologies available to study them. As such, discussing hedonics alone is a convenient starting point for parsing reward. To begin, it should be noted that, as in the case of “reward”, the term “hedonics” sees somewhat inconsistent usage in the field. That is, while the term is occasionally used to refer specifically to positive affective processing (in agreement with the word’s etymology), it also often refers to affective processing generally, encompassing both

positive and negative states (Berridge, 2000). Usage in the present document will comport with the latter, more popular, usage.

Using food to study reward

While there are many domains of reward, such as palatable food or social interaction, food is, arguably, the default reinforcer in much neuroscientific research, likely due to the ease with which its quantity, quality, and characteristics (e.g. the volume, concentration, and flavor, respectively, of a Kool-aid™ reward) can be manipulated. As such, "reward" often refers specifically to food reward, and many commonly studied reward-related behaviors are therefore consummatory in nature. The present review will focus on food-rewards, in part because they are common-place, but primarily because such focus allows for over a century of research into parsing the components of consummatory behaviors to be leveraged in the parsing of reward's role in those behaviors. For example, Thomsen and colleagues (2015) present a compelling model which maps primary components of reward onto the principal steps of consummatory behaviors (Craig, 1917): an initial appetitive phase is characterized by motivation to obtain the reward and active seeking towards that end. Once the reward has been obtained, it is consumed and its hedonic impact experienced. Following consumption, the animal enters a state of satiety, during which the consequences of consumption are evaluated (e.g. Did it cause illness? Was it worth the effort necessary to obtain it? etc.). Such a mapping enables specifically consummatory behaviors to be used to study general reward systems (e.g. Kelley & Berridge, 2002). One particularly powerful expression of this has been the development of the taste reactivity test and its use in studying the representation of disgust and pleasure in rodents.

Measurements of orofacial "taste reactivity" (i.e. stereotyped movements of the mouth and face made in response to certain tastes) were first characterized in human

infants by Jacob Steiner (1973), who made the key observation that responses elicited by many different tastes fell along a spectrum. Had these responses reflected merely the sensory characteristics of the tastes used (i.e. sweetness, salinity, acidity, or bitterness), one might have expected to see 4 different classes of responses corresponding to the different taste categories. However, that the actual responses instead existed along a continuum and scaled with the expected hedonic impact of the tastes suggested that the reactivity itself was a product of hedonic, rather than sensory, processing. Shortly after this phenomenon was first described in humans, homologous behaviors were characterized in rodents (Grill & Norgren, 1978a).

The claim of homology between rodents and humans is based on not only similarities in the mechanical behavior exhibited in response to certain kinds of tastes, but also on the ability of similar manipulations in rodents and humans to produce like results. For example, infants with congenitally low potassium (due to their mothers experiencing frequent morning sickness during pregnancy) tend to show less aversion when presented with concentrated salt solutions than do infants without this deficit (Crystal & Bernstein, 1998). Placing rodents on a salt-deprivation diet produces similar results (Berridge, Flynn, Schulkin, & Grill, 1984). While this phenomenon is partially explained by alterations in deprivation-induced changes of peripheral-nerve sensitivity (e.g. Contreras & Frank, 1979), taste reactivity can also be modulated through learning. In both rats and humans, pairing a palatable taste with illness makes the taste aversive (Breslin, Spector, & Grill, 1992; Rozin & Schulkin, 1990), which suggests the alteration of something more abstract and higher-order than peripheral sensory encoding. While much could be (and has been: see Berridge, 2000) written on the history and homology of taste reactivity, its import in this dissertation is its use in rodents to map neural representations of hedonics.

Where is Reward?

Using taste processing pathways to understand the neural representation of hedonics

A complex network of reciprocally connected areas is involved in taste processing. Taste information is carried from the tongue to the central nervous system via the corda tympani, the neurons of which synapse in the brainstem at the nucleus of the solitary tract (NTS). From there, the canonical taste processing pathway ultimately reaches gustatory cortex by way of (in ascending order) another brainstem nucleus, the parabrachial nucleus (PBN), and the ventral posterior medial thalamic nucleus (Lundy Jr. & Norgren, 2015). The brainstem nuclei are all that is required for the basic motor expression of taste reactivity, as evidenced by the observations that surgically decerebrate rats (Grill & Norgren, 1978b) and congenitally acephalic human infants (Steiner, 1973) still display it. However, pharmacological manipulations within certain forebrain areas, specifically the nucleus accumbens (NAc) and ventral pallidum (VP), are sufficient to alter both consumption and taste reactivity (Castro & Berridge, 2014; Hanlon, Baldo, Sadeghian, & Kelley, 2004).

A proposed resolution to this apparent disconnect is that brainstem areas respond to basic sensory characteristics of a taste, and the NAc and VP encode hedonic valuation. Taste information reaches the NAc via direct projections from the NTS and gustatory cortex, and the accumbens in turn projects to the VP (Kelley et al., 2002). However, unlike preceding areas in the chain, the accumbens is not simply a sensory relay; rather it appears to both integrate limbic information and link limbic systems to motor outputs (Mogenson, Jones, & Yim, 1980).

Opioidergic signaling in basal forebrain nuclei represents hedonics

Subregions in the NAc have distinct roles in reward processing. Broadly speaking, the Core (NAcC) serves to associate rewards with predictive environmental

cues, while the Shell (NAcSh) is responsible for representing reward value (West & Carelli, 2016). Pharmacological inactivation via $GABA_A$ agonism or AMPA antagonism induces general hyperphagia in the Shell but not the Core (Basso & Kelley, 1999; Maldonado-Irizarry, Swanson, & Kelley, 1995). By contrast, μ-opioid receptor (MOR) agonists induce hyperphagia when administered in either region (Zhang & Kelley, 2000) but only for highly palatable (i.e. fat or carbohydrate rich) foods. Furthermore, MOR stimulation in a segregated region of the rostral NAcSh promotes not only feeding, but also appetitive taste reactivity (Castro & Berridge, 2014). One of the primary projection targets of the rostral NAcSh, the caudal VP, contains another such hotspot in which MOR agonism promotes appetitive taste reactivity (Castro & Berridge, 2014).

GABAergic medium spiny neurons (MSNs) are the principal neuronal subtype and the primary output neuron of the NAc (Gerfen & Surmeir, 2011). Two large subpopulations are distinguished by the differential expression of dopamine receptor subtypes, D1 and D2 (Gerfen & Surmeir, 2011). While D1 and D2 MSNs of the dorsal striatum are further distinguished by their highly-segregated projection targets, NAc D1 and D2 MSNs do not adhere as closely to equivalent ‘direct’ and ‘indirect’ output pathways (Kupchick et al., 2015). Direct and indirect refer to whether neurons reach the midbrain “directly” via monosynaptic connections or “indirectly” via di-synaptic connections through the VP. Nevertheless, D1 and D2 MSNs are functionally distinct, such that optogenetically targeted excitation of D1 or D2 neurons promotes approach and avoidance, respectively (Kravitz, Tye, & Kreitzer, 2012).

While MOR activation profoundly regulates hedonic perception, precisely how MOR activation accomplishes this by regulating D1 and D2 MSN activity remains unclear. Mu-opioid receptors are expressed on dendrites throughout the accumbens (Gracy, Svingos, & Pickel, 1997; Mansour, Khachaturian, Lewis, Akil, & Watson, 1988). The likely endogenous agonist of MORs, enkephalin, is produced in D2 MSNs (Gerfen et

al., 1990) and is likely released directly onto D1 MSNs via axonal collaterals (Burke, Rotstein, & Alvarez, 2017; Dobbs et al., 2017). While anatomical data enable speculation about endogenous MOR stimulation, the exact circumstances are unknown. Interestingly, the general activity patterns of MSNs following the experience of a palatable taste align with what would be expected to result from MOR stimulation in the region (Hakan & Henriksen, 1989; McCarthy, Walker, & Woodruff, 1977). Most taste-responsive neurons in the NAc display decreases in activity during the experience of a palatable taste (Roitman, Wheeler, & Carelli, 2005). Unpalatable (e.g. bitter quinine) tastes induce the opposite pattern of activity. That is, most taste-responsive neurons increase in firing rate, as do once-palatable tastes paired with illness (Roitman et al., 2005; Roitman, Wheeler, Tiesinga, Roitman, & Carelli, 2010). Thus, the ratio of taste-excited to taste-inhibited neurons tracks the hedonic value of experienced rewards just as taste reactivity does.

In summary, single neurons in the NAc respond to the hedonic value of delivered tastes rather than their sensory characteristics. Further, while numerous neurotransmitter systems within the accumbens mediate consumption itself, μ-opioid signaling within a restricted region of the medial NAcSh encodes the hedonic impact of palatable tastes; outside of that region, opioids, dopamine and amino acids regulate the motivational components of reward (Caref & Nicola, 2018; Castro & Berridge, 2014; Kelley et al., 2002).

Parsing the learning and motivational components of reward

In taste reactivity studies, rats are given direct intraoral infusions of solutions that may be either palatable or unpalatable. These infusions are neither cued nor contingent on the rat's behavior because the goal of such studies is to separate "pure" hedonic processing from the myriad other processes involved in reward seeking. In the "real

world"–insofar as that term applies to rodents–environmental cues predict food availability and direct the animal to engage certain behavioral repertoires to acquire food; most non-hedonic behavioral designs reflect this process. Progressing backwards from reward consumption, internal representations of motivation must interface with motor output systems to produce reward seeking. Prior to this approach, there must be systems in place that select the appropriate motor output to maximize the likelihood or magnitude of reward receipt. Knowing what actions will produce a reward requires that the animal recognizes environmental predictors of the reward. Thus, during previous exposures to an environmental cue, the animal must have been able to form an association between the cue's presence and the reward's delivery and, during the current presentation of the cue, must be able to recall that association. At minimum there are three broad categories of behavioral processes that permit successful reward seeking: (1.) learning, (2.) action selection, and (3.) motivated approach. It must be noted that these can be further subdivided almost *ad infinitum*.

Even as these three constructs may be further subdivided, it can be difficult to separate them experimentally. Behavior in most experimental designs is the product of both learning and action-selection. The inverse is also true: both learning and action-choice are quantified by observing an animal's behavior. Thus, parsing the circuitry involved in each requires careful interpretation of behavioral paradigms and consideration of how each process may contribute to observed behavioral alterations.

Learning refers to the formation of many different types of association. Its use herein has referred to the acquisition of an instrumental contingency between an action and the receipt of a reward. The ability to acquire this contingency is often studied using operant contingency degradation, in which animals are trained to perform separate operant behaviors (e.g. lever pressing and nose-poking) in exchange for separate rewards. Repeated, unearned delivery of one of these rewards selectively decreases

engagement in the behavior that produces that reward (Dickinson & Mulatero, 1989; Hammond, 1980; Williams, 1989). The rate of unearned reward delivery can be matched to that at which the same reward can be earned through operant responding, thereby removing the contingency of reward receipt on action; however, because the reinforcement rate of the operant response does not change, the decrease in behavior cannot be said to be due to a pure "law of effect", which proposes simply that behaviors that are rewarded will be performed, while those that are not will not (Thorndike, 1932). These experiments were critical in identifying the existence of an action-outcome contingency as a construct distinct from stimulus-response associations.

The ability of animals to understand the contingency between the presence of cues with the appearance of rewards, as in Pavlovian or classical conditioning, constitutes another style of learning. A formal description of this process is this: unexpected presentation of a primary reinforcer (the unconditioned stimulus, or US) elicits an innate, unconditioned response (UR) that is specific to both the reinforcer and the animal's biology. Over successive pairings of a neutral predictive cue (the conditioned stimulus, or CS) with the US, the CS itself comes to elicit the same response as the US. This conditioned response (CR) has multiple components. At a very basic learning level, the CR reflects the ability of an animal to predict a reward's appearance following CS presentation. However, the CR also often suggests that some aspects of the value of the US, particularly its incentive value, have been transferred to the CS. Though presented here as distinct from instrumental conditioning, most learning designs likely have aspects of both Pavlovian and Operant conditioning (Moore, 2004).

The role of the prefrontal cortex in learning and action selection

The rodent prelimbic (PL) prefrontal cortex (PFC) is critically involved in the formation of action-outcome contingencies (Corbit & Balleine, 2003). Lesions to this area

impair performance in contingency degradation tasks (Corbit & Balleine, 2003; Coutureau, Esclassan, Di Scala, & Marchand, 2012), but do not universally impair behavioral flexibility (Coutureau et al., 2012). Dopaminergic signaling within the PL plays a critical role in this process, given that dopaminergic lesions (via targeted microinfusion of 6-hydroxydopamine) or D1/D2 blockade impair contingency degradation but not outcome devaluation (Naneix, Marchand, Di Scala, & Coutureau, 2009; though see Lex & Hauber, 2010). Interestingly, while the corticoaccumbens pathway (i.e. projections from the PL to the NAcC; PL-NAcC) has been implicated in behavioral activation (McFarland, Lapish, & Kalivas, 2003; McGlinchey, James, Mahler, Pantazis, & Aston-Jones, 2016), PL connectivity with the dorsal but not ventral striatum is necessary for instrumental outcome learning (Hart, Bradfield, & Balleine, 2018a; Hart, Bradfield, Fok, Chieng, & Balleine, 2018b). Ventral striatial projections, however, are necessary for the expression of conditioned responses in Pavlovian tone conditioning (Otis et al., 2017).

Outcome-devaluation studies, in which animals trained to respond instrumentally for two separate rewards are pre-fed with one of those rewards, also demonstrate the role of the PL in *learning* reward values. Lesioning the PL prior to devaluation training impairs an animal's ability to differentiate between the devalued and non-devalued reward (Couterau, Marchand, & Di Scala, 2009; Ostlund & Balleine, 2005) but does not alter behavior when performed after training (Ostlund & Balleine, 2005). Therefore, the PL appears to be required for the acquisition, but not expression, of cue-triggered reward expectations. Such expression appears to be the domain of the orbitofrontal cortex (OFC), specifically projections from its medial subregion to the amygdala's basolateral subregion (BLA) (Malvaez, Shieh, Murphy, Greenfield, & Wassum, 2019).

While the PL is not required for the recall of reward value, it continues to play a role after learning in action-selection. Single neurons in the PL encode behavior only when performed in a context where that behavior has previously resulted in reward

delivery (Muldur, Nordquist, Örgüt, & Pennartz, 2003). Trask and colleagues (2017) demonstrated that PL inhibition impairs renewal of a previously extinguished operant behavior only when it occurs in the same context as the original learning; animals exposed to an entirely novel context for renewal show absolutely no effect of PL inhibition. Cortical projections to the NAcC are especially important for cue-directed reward seeking behavior (Ishikawa, Ambroggi, Nicola, & Fields, 2008; McFarland et al., 2003; Stefanik et al., 2014). This importance extends beyond instrumental behavior and applies to Pavlovian anticipatory behavior as well (Otis et al., 2017), suggesting that this projection provides a common pathway that modulates cue-directed approach.

The nucleus accumbens is a limbic-motor interface

Once a cue has been perceived and an action selected, these abstract internal representations must be linked to motor output. As previously discussed, the nucleus accumbens appears to be an important hub at which this linkage occurs (Mogenson et al., 1980). Dopaminergic signaling, specifically, encodes motivational drive. Hyper-dopaminergic mice display significant increases in their motivation to pursue a reward, but no apparent increase in their hedonic enjoyment of it (Peciña, Cagniard, Berridge, Aldridge, & Zhuang, 2003). When physical cues (e.g. a lever) are paired with reward delivery in a Pavlovian conditioning design, animals develop two types of conditioned responses: approach towards either the cue or the site of reward delivery (Meyer et al., 2012). The former type of approach reflects the transfer of incentive value to the cue and requires intact dopaminergic signaling, while the latter appears to reflect the formation of a basic stimulus-response contingency and can occur in the absence of dopamine (Flagel et al., 2011b; Robinson & Flagel, 2009). Dopamine's ability to drive behavior is most likely mediated via the activation of D1-expressing MSNs, which is itself reinforcing (Kravitz et al., 2012).

A basic circuit model of reward-seeking

Thus, a basic circuit-based working model for goal-directed reward seeking may be sketched as follows: over repeated exposures to cue-reward pairings, prelimbic and orbitofrontal connections with limbic structures, the NAc and BLA particularly, form contingencies and representations of the reward. The appearance of the cue triggers the representation of outcomes in these same structures and activates a behavioral response that involves the activity of PL projections to the NAcC. Simultaneously, cue elicited mesolimbic dopamine signaling encodes the incentive value of the cue/paired reward. The convergence of cortical glutamatergic inputs and mesolimbic dopaminergic inputs drives the selective activation of D1 MSNs. These neurons project to midbrain dopaminergic cells and the thalamus, at which point their activity becomes integrated into thalamo-sensorimotor cortical loops to direct motor output. Ultimately, the animal approaches and consumes the reward, whereupon basal forebrain nuclei encode its hedonic impact.

These processes are not the only ones that enable animals to obtain rewards in an environment. So-called “goal-directed reward seeking” may also be described as “outcome-sensitive” motivated behavior, which sets them in contrast to “outcome-*in*sensitive” behaviors. Note that each step of the process laid out above involves some form of reward prediction, and this prediction is ultimately causal in the animal’s behavior. If certain events weaken the predictive strength of cues or the association between an action and the ultimate receipt of reward, the behavior itself will become less likely to occur. Other types of behavior, such as those that are reflexive or habitual, do not depend on this prediction, and are thus insensitive to their ultimate outcomes.

Unsurprisingly, these different types of behavior rely on different neural circuits than do outcome-sensitive behaviors. As areas within outcome-insensitive circuits were not probed in the present body of work, they will not be described here. Of note, there is

reason to believe that stressful experiences may bias reward processing towards these alternate circuits (e.g. Dias-Ferreira et al., 2009), and as such they are the focus of greater speculation in the Discussion of this document (see Figure 5.3). For now, the focus of both this introductory section and the experiments themselves will remain on the myriad ways in which chronic stress impairs the function outcome-sensitive circuitry and related behavior.

Dysregulation of Reward Processes by Stress

There is inherent value to studying the neural processes that comprise reward because doing so provides a better understanding of normal human behavior. There is additional value in this study because there is considerable evidence that these same processes are compromised in disease states, especially disease states exacerbated by stress. Chronic Variable Stress (CVS)–in which animals are repeatedly exposed to a battery of physical and psychological stressors over an extended, typically multi-week, period–has been used as a model of many different disorders, most commonly major depressive disorder (MDD), due to its ability to induce an anhedonia-like phenotype that is reversed by treatment with tricyclic antidepressants (Willner, Towell, Sampson, Sophokleous, & Muscat, 1987).

As the name itself suggests, “anhedonia” traditionally refers to the inability to experience pleasure (Ribot, 1896). However, patients with MDD do not reliably display hedonic deficits (Berlin, Givry-Steiner, Lecrubier, & Puech, 1998; Dichter, Smoski, Kampov-Plevoy, Gallop, & Garbutt, 2010), and commonly used animal models, including the two-bottle sucrose preference test, are also sensitive to changes in incentive and learning processes (Meyerolbersleben, Winter, & Bernhardt, 2020; Rizvi, Pizzagalli, Sproule, & Kennedy, 2016). Motive (Morgado, Silva, Sousa, & Cerqueira, 2012; Sherdell, Waugh, & Gotlib, 2012; Treadway, Buckholtz, Schwartzman, Lambert, & Zald,

2009) and learning deficits (Dias-Ferreira et al., 2009; Landes et al., 2018) have been repeatedly observed in both human MDD and rodent CVS models. In human MDD, these deficits have been identified in the absence of concurrent deficits in hedonic enjoyment (Landes et al., 2018; Sherdell et al., 2012). Thus, while it is clear that CVS is linked to disease and disruptions in reward processes, it is not at all clear which reward-related constructs are affected.

Diagnosing behavioral deficits following stress requires precision

Common behavioral tests used to diagnose the effects of CVS are poorly suited to parse deficits in reward processing. The two-bottle sucrose preference test is particularly popular (Willner, 2017) and has long been used to identify “anhedonia” following CVS (Willner et al., 1987). The popularity of this test is almost certainly due in part to its simplicity. In this test, consumption of a low concentration (typically 1 to 2%) sucrose solution is measured relative to simultaneously available water. Reduced sucrose intake relative to a “healthy” baseline is often interpreted as indicating reduced sensitivity to the rewarding nature of sucrose. However, as previously discussed, consummatory behaviors require more than hedonic processing. An observed deficit in sucrose consumption *could* reflect a deficit in hedonic sensitivity, but it may also reflect alterations in incentive value or action-selection.

The same holds true for other common tests of hedonic sensitivity. In place preference conditioning, reward delivery is paired with exposure to a certain area of a conditioning chamber. During testing, animals are placed into the chamber with access to both the reward-paired area and an area never paired with reward. The preference that animals show for the reward-paired context, as measured by the amount of time they spend in that context, has been interpreted as reflecting the hedonic value of the reward and is reduced following CVS (Muscat, Papp, & Willner, 1991). As in the case of

the two-bottle test, this reduction can be reversed using pharmacological antidepressants (Muscat, Papp, & Willner, 1991). Obviously, the ability of an animal to learn to associate a reward with a context and mount an approach toward that context relies on more diffuse constructs than hedonics. Some hint as to the specific constructs impacted by stress may be found by focusing on the neural systems altered following CVS, many of which are outlined above in the discussion of reward-processing.

Chronic variable stress disrupts dopaminergic signaling pathways

Chronic variable stress induces degeneration of VTA dopamine neurons (Sugama & Kakinuma, 2016) and alters the activity of those that remain (Bambico et al., 2019; Tye et al., 2013). Dopaminergic neurons in the caudal VTA display reduced burst firing (Bambico et al., 2019; Tye et al., 2013), which should be expected to reduce dopaminergic release in the region's target nuclei, including the NAc, PFC, and BLA (Grace, 1991). Interestingly, dopaminergic neurons in the rostral VTA display elevated burst-firing following CVS; these rostral neurons project only to the lateral NAcSh and appear to participate in coding unsigned "salience" (de Jong et al., 2019; Lammel, Ion, Roeper, & Malenka, 2011; Lammel, Lim, & Malenka, 2014).

Evidence of reduced dopamine transmission in the PFC has been observed over long time scales using microdialysis following chronic stress (Mizoguchi, Shoji, Ikeda, Tanaka, & Tabira, 2008). The reduced activity of dopaminergic neurons in the ventral tegmental area has been causally related to behavioral effects following CVS (Tye et al., 2013), and optogenetically stimulating these neurons reverses the disruptive effects of stress in the two-bottle sucrose preference test (Tye et al., 2013). Note that dopamine depletion in non-stressed animals alters only overall consumption, not expressed preference, in this design (Meyerolbersleben et al., 2020). Given the diffuse projections of these neurons (Lammel et al., 2014) and their myriad roles in behavior (de Jong et al.,

2019), the behavioral consequences of CVS-induced alterations in the dopaminergic system require further study.

Stress differentially modulates excitability in NAc sub-populations

Chronic stress also disrupts the function of the NAc at multiple levels. Dopamingergic inputs (as discussed above) are likely disrupted (Tye et al., 2013), as are both excitatory inputs (Brancato et al., 2017) and post-synaptic plasticity (Aceto et al., 2020). Stress induced atrophy within the accumbens (Anacker et al., 2016) is likely due to both a loss of myelination (Liu, Dietz, Hodes, Russo, & Cassacia, 2018) and a reduction of dendritic complexity in the NAcSh, specifically (Taylor et al., 2014). Disturbances in NAcSh function, as may be expected with alterations to cyto-architecture, would be predicted to produce deficits in the ability of animals to appropriately valuate rewards (West & Carelli, 2016). Alterations in MSN activity following stress are pathway specific, such that reduced D1 MSN activity and increased D2 MSN activity following stress is associated with the behavioral impact of stress (Francis et al., 2015; Lim, Huang, Grueter, Rothwell, & Malenka, 2012), and consistent with the predicted effect of D1 MSN under-activation or D2 MSN over-activation (Kravitz et al., 2012).

Consequences of stress-induced atrophy in the PFC

Chronic stress also disrupts the function of the PFC. Regressive plasticity in the medial PFC (mPFC) has been well characterized following chronic stress (Anderson et al., 2019; Radley, Anderson, Hamilton, Alcock, & Romig-Martin, 2013; Radley et al., 2006; Radley et al., 2008). This plasticity likely contributes to the reduced activity seen in the mPFC of stressed animals (Covington et al., 2010). In part, stress-induced atrophy within the PL has been linked to a reduced ability to provide inhibitory control over the hypothalamic-pituitary-adrenal (HPA) axis (Radley et al., 2013; Radley, Gosselink, &

Sawchenko, 2009). However, HPA-regulation does not appear to be the only role of the PL neurons that are disrupted by stress. Stimulation of the PL reverses the behavioral deficits induced by a chronic social defeat stress (Covington et al., 2010). Such deficits have been causally linked to induced expression of a specific second messenger in the PFC (Becker et al., 2008), and are similarly reversed by stimulation of the PL-NAcC pathway (Vialou et al., 2014). Therefore, atrophy of PL neurons may serve as another possible substrate for stress induced behavioral deficits.

In sum, many neural processes contribute to the construct of reward. Chronic stress disrupts the structure and function of many brain areas associated with reward processing and, not surprisingly, impairs reward-related behaviors. However, it is clear that there is much to be gained by more closely examining the precise circuits and psychological constructs that contribute to reward and the damaging effects of stress. Such efforts may yield more effective strategies for correcting stress-induced mental illness. The work presented in this dissertation combines behavioral assessments with *in vivo* electrophysiology and fiber photometry to (1.) Characterize the nature of stress induced behavioral deficits; (2.) Characterize the encoding of conditioned approach behavior in the PL; and (3.) Characterize stress induced alterations in the corticoaccumbens pathway during conditioned approach.

II. DIFFERENTIAL EFFECTS OF CHRONIC VARIABLE STRESS ON FACETS OF REWARD-RELATED BEHAVIOR AND ENCODING IN NUCLEUS ACCUMBENS SUBREGIONS

Abstract

Chronic variable stress is a popular model of rodent “anhedonia” because of its ability to disrupt signaling in reward-related networks. However, the extent to which the behavioral deficits observed in rats used in this model reflect true deficits in hedonic processing, rather than some other facet of reward such as motivation or learning, has come under scrutiny. In this study, a panel of behavioral tests–two-bottle sucrose preference, taste reactivity, fixed ratio acquisition, and progressive ratio training–were used to characterize the effect of stress on different facets of reward related behaviors. Observed behavioral deficits primarily occurred in motivational, rather than hedonic, domains. The nucleus accumbens serves many functions within reward-processing that involve the processing of limbic information and conveying it to motor outputs. The two subregions of the nucleus accumbens, Core and Shell, subserve different aspects of this function. *In vivo* electrophysiology was used to assess the impact of chronic stress on hedonic encoding of single units within the Core and Shell. Prior to stress, the pattern of unit responses (as categorized by the positive or negative modulation of firing rates) agreed with what has previously been reported: appetitive tastes generally reduced activity in responsive units. However, stress altered this pattern such that fewer units encoded a sweet taste with reduced firing rate. Stress’ effect was selectively present in the Shell, which is consistent with an impact on value-encoding. In combination with the findings from the behavioral studies, it is concluded that CVS selectively impairs the neural representation of incentive value.

Introduction

As described in the prior chapter, reward processing is a complex, adaptive phenomenon that involves many brain circuits and shows great plasticity in response to different experiences. The Wheeler lab has traditionally examined the modification of brain activity and affective state in response to acutely aversive stimuli that influences behavior. However, the experience of chronic stress has been more directly linked to long lasting modifications of brain physiology and human mental illness. Thus, chronic stress models present a powerful way to study these circuits.

The chronic variable stress model (CVS) of depression entails repeatedly exposing animals to daily, unpredictable stressors over the course of multiple weeks to induce reversible (by anti-depressants) behavioral deficits (Willner et al., 1987). In recent years, there has been remarkable proliferation of the technique (see Willner, 2017). There have also been developments in how to best use and interpret CVS and models like it. Namely, the National Institutes of Mental Health (NIMH) released their Research Domain Criteria Initiative, which, put simply, calls for researchers to focus on symptoms rather than syndromes. This call is built on the recognition that while many human disorders, such as Major Depression, rely on myriad combinations of subjectively diagnosed behavioral changes that do not easily translate to rodents, the discrete symptoms present in those disorders not only can be identified in rodents, but also serve as more productive starting points for research questions, as they can be both grounded in specific circuits and applied across disorders (Insel & Cuthbert, 2015). The most common adverse effect of the CVS paradigm in rodents and Major Depressive Disorder (MDD) in humans is anhedonia. The term anhedonia is classically defined as an inability to experience pleasure (Ribot, 1896), though it is often used as an all-inclusive term to describe any reward related deficits following CVS. In agreement with the NIMH's new guidelines on taking greater care in defining symptoms in behavioral models, there have

been numerous calls to recognize the presence of incentive as well as hedonic deficits following CVS (Olney, Warlow, Naffziger, & Berridge, 2018; Rizvi, Lambert, & Kennedy, 2018; Thomsen et al., 2015).

As discussed in the introduction, food is the most common substrate of basic reward in rodent research; therefore, "reward-related" behaviors, more often than not, are consummatory behaviors. Multiple discrete processes, subserved by dissociable neural circuits, motivate consumption in rodents. The NAc, in its role as a limbic-motor interface, sits at the heart of many of these otherwise distinct circuits. Homeostatic feeding processes, which enable animals to regulate their internal energy balance, involve hypothalamic connectivity with the NAcSh (e.g. Kelley, 2004). Hedonic processes, however, involve signaling within the NAc itself (Castro & Berridge, 2014). Dopaminergic inputs to the NAc, arising from the ventral tegmental area, encode incentive salience, and provide a mechanism for modulating the vigor with which rewards are pursued that is separable from both hedonic enjoyment and homeostatic hunger (e.g. Berridge, Robinson, & Aldridge, 2009). Thus, while CVS exposure may impair any one of the described reward-processes, evidence of this disruption will likely be seen in the NAc.

This chapter employs a panel of behavioral tests and *in vivo* electrophysiological recordings of reward processing in the NAc to characterize the precise nature of the deficit induced by CVS. These findings should be valuable to researchers working to understand the specific psychological domains that are affected by chronic stress.

Methods

Animals

Adult male Sprague-Dawley rats (300-350 g; Harlan Laboratories, St. Louis, Missouri) were used in all experiments. Animals were individually housed on a reverse 12:12 light-dark cycle in a temperature- and humidity-controlled, Association for Assessment and Accreditation of Laboratory Animal Care accredited vivarium. One cohort of animals (N=29) was used to assess the effect of chronic stress on behavior in a panel of tests (two-bottle sucrose preference, FR1 acquisition, and PR breakpoints; (Stress, n=15; Control n=14)). Food and water were provided *ad libitum* prior to and during chronic stress. Following the post-stress sucrose preference test, animals were food restricted to maintain 90% bodyweight prior to beginning operant behaviors.

A separate cohort was used to examine the effect of stress on Taste Reactivity (N=28; Stress n=18; Control n=10), with a subset of animals being used for electrophysiological recordings in the nucleus accumbens (Stress n=13; Control n=10). Food and water were provided *ad libitum* for the duration of experiments.

Surgeries

Intraoral Catheters

Rats were anesthetized with a ketamine (100 mg/kg) xylazine (20 mg/kg) mixture. Two ~8 cm lengths of PE-100 tubing, phalanged at one end with a Teflon washer, were implanted bilaterally. The cannula was inserted just lateral to the first maxillary molar with the Teflon washer flush against the molar. The other end was exteriorized out of an incision made just behind the ipsilateral ear and held in place with a second washer and tape wrapped around the tubing.

Electrophysiology

Following intraoral cannulation, a subset of animals received eight-wire microelectrode arrays (NB Labs; Denison, TX) implanted bilaterally in the NAc Core [AP: +1.3 mm, ML: ±1.3 mm, DV: -6.2 mm @ 0º] or Shell [AP: +1.7 mm, ML: ±0.8 mm, DV: -6.2 mm @ 0º]. Each array was grounded by wrapping a wire around a stainless-steel screw implanted in the skull.

For all surgical procedures, rats were treated with the anti-inflammatory meloxicam (1% oral suspension) the day of surgery and for 4 d following surgery to reduce inflammation and postoperative pain.

CVS

The CVS regimen was a 14 day procedure consisting of exposure to two of the following stressors per day: forced swim (4 °C water for 20 min), cold room (4°C, 2 hrs, alone or in combination with other stressors), novel environment (different novel environments for 1-3 hrs; including wet bedding in cages, ½ inch of water in cages, or no bedding in cages), motion (cage without bedding which is placed on an orbital shaker and rotated for 2 hrs; 1 revolution/sec), noise (continuous 60-68 dB noise such as radio static for 1 h), open field (alone or in groups in a 1-meter diameter circular brightly-illuminated field for 45 min), restraint (plastic restraint tubes for 30 min), and cage tilt (30° for 6-12 hours with food and water available). For repeating stressors, variables such as light, temperature, and noise were varied to maintain novelty. On each day over the 14-day period, one of the stressors from the battery was presented at 0800h and the other stressor was be presented at 1700h. Control rats were handled and weighed daily at the evening timepoint.

Sucrose preference testing, taste reactivity, and electrophysiological recordings began the day following the end of the CVS paradigm. For animals involved in further behavioral experiments, food deprivation began after sucrose preference testing.

Behavior and Analysis

Taste Reactivity

Tastant delivery occurred in Plexiglass operant chambers (MED-Associates; St. Albans, VT) housed within sound-attenuating boxes (Stanley Vidmar; Allentown, PA). Sucrose solution (0.3 M) and Quinine solution (0.001 M) were delivered intra-orally via implanted cannulae. For each tastant, a dedicated, single speed syringe pump (MED-Associates) delivered 30 infusions (6s; 200 µL), via plastic, one-channel swivels (Instech Laboratories; Plymoth Meeting, PA) with an inter-infusion interval of 60 seconds. Animals received all sucrose infusions and were then connected to a separate fluid-delivery line to receive quinine infusions. Animals were placed in the delivery chamber and connected to an empty fluid delivery line one day prior to testing to habituate them to testing conditions.

Tastant delivery sessions were recorded on DVD with a camera fixed below the testing chamber. The chamber floor was clear acrylic glass, and a house light was positioned on the door of the sound attenuating chamber to ensure recording quality. Videos were converted to digital files for frame-by-frame analysis of appetitive taste reactivity according to the technique of Grill & Norgren (1978). Instances in which the tongue protruded and crossed the midline were counted as appetitive. Taste reactivity was quantified as events/trial. Trials in which the mouth was obstructed were excluded from analysis; animals that did not contribute at least 10 unobstructed trials to both pre and post stress were excluded as well. Outliers remained in these data, as did a

tendency towards a baseline difference between Stress and Control animals. As such, fold-changes [(Post-Score – Pre-Score) / Pre-Score] were calculated for each animal and compared with a between subjects ANOVA.

Sucrose Preference Test

Animals were pre-exposed for 30 minutes to 1% Sucrose solution one day prior to sucrose preference testing. Pre-exposure occurred during the dark cycle in novel cages filled with fresh bedding in the animals' home vivarium. Sucrose consumption was monitored visually by experimenter (under red light) for all animals. Animals that were not observed to consume any sucrose during pre-exposure received a small volume of 1% sucrose placed on their face around their mouth to ensure exposure and were left in the novel cage for an additional 10 minutes. Preference testing occurred in a new set of freshly filled housing cages in the colony room one day prior to entry into the CVS paradigm and one day following the end of the CVS paradigm. During testing, animals were given access to 1% sucrose and water in two identical bottles placed on the opposite sides at the same end of the testing cage for 30 minutes. Bottles were weighed prior to testing and at 10-minute intervals during testing. After each weighing, bottles were replaced in the cage on alternating sides (i.e. for a given animal the sucrose bottle was switched from left to right to left over the course of the session). Consumption was quantified as the volume consumed from each bottle over the course of the session. Sucrose preference was calculated as [Sucrose consumed / Total liquid consumed]. A baseline difference in sucrose preference was observed between Stress and Control animals, so fold-changes were calculated as in Taste Reactivity analysis and compared using mixed ANOVA.

Fixed and Progressive Ratio Training

Operant training occurred in Plexiglass operant chambers (MED-Associates) housed within sound-attenuating boxes (Stanley Vidmar) 3 days after the cessation of chronic stress. A retractable lever flanked a centrally located, recessed food cup on one wall of the operant chamber. An illuminated cue light located on the wall directly above the lever indicated reward availability for the duration of the session. Operant training sessions consisted of 50 trials. In each trial, the lever was extended for up to two minutes. In response to a lever-press by the animals, the lever retracted and a sucrose pellet (45 mg; Bio-Serv) was delivered into a food cup. If the animals did not press the lever within two minutes of its extension, a pellet delivery occurred as though there had been a response. Following pellet delivery in either case, a 20 second timeout separated the end of a trial from the beginning of the next. The session cue light was extinguished at the end of the 50th trial. Animals were trained daily until they responded on all 50 trials for two consecutive days. Two animals, both from the Stress condition, failed to acquire any amount of lever responding and were excluded from subsequent behavior and analysis. Separate Analyses of Variance were used to compare days to criteria and average trial length (i.e. response latency) between Stress and Control groups.

After reaching criteria in the FR1 task, animals were trained daily (1 session/day for 3 days) in a progressive ratio task. Lever and light presentation indicated the availability of a sucrose pellet reward. Rewards were delivered on a modified PR2 schedule, such that the response requirement for pellet delivery began the session at 1 and doubled every 10 deliveries. Pellet delivery coincided with lever retraction, and a two second timeout preceded the beginning of the next trial. Sessions were terminated after two minutes without a lever-response. Note, this means that an animal could take well over two minutes to receive a single reward as long as it consistently made at least one

lever response within the two-minute window. As in the FR1 paradigm, an illuminated cue light above the lever indicated continued reward availability and was extinguished at session's end. A mixed ANOVA compared breakpoints (i.e. the response requirement at which an animal stopped pressing) between Stress and Control animals across three days of training. Family-wise error was maintained at $\alpha=0.05$ for post-hoc contrasts using the Holm method.

Electrophysiology

Recording

Recordings were conducted in conjunction with taste reactivity testing with microelectrode arrays featuring eight stainless steel wires (50 μm diameter) arranged in a 2 × 4 configuration (NB Labs; Denison, TX). To familiarize the rats with the recording situation, they were connected to a flexible recording cable (Plexon; Dallas, TX) attached to a commutator (Crist Instruments; Hagerstown, MD) one day prior to recording. Habituation for taste reactivity occurred during this same session.

The recording headstage contained 16 miniature unity-gain field effect transistors. NAc activity was recorded differentially between each active wire and an inactive wire chosen for an absence of neuronal activity. Online isolation and discrimination were accomplished using a commercially available neurophysiological system (OmniPlex system; Plexon). Multiple-window discrimination modules and high-speed analog-to-digital signal processing in conjunction with computer software enabled isolation of neuronal signals based on waveform analysis. The neurophysiological system incorporated an array of digital signal processors (DSPs) for continuous spike recognition. The DSPs provided a continuous parallel digital output of neuronal events to a computer. Another computer controlled behavioral events of the experiment (Med

Associates) and sent digital outputs corresponding to each event to the OmniPlex to be timestamped along with the neural data. Criteria for identifying different neurons on a single wire were described previously in detail (Roitman et al., 2005). Briefly, discrimination of individual waveforms corresponding to a single neuron was accomplished using template and principle component analysis procedures provided by the PlexControl software system. The template analysis procedure involves taking a sample of the waveform and building a template of that extracellular waveform. Subsequent neurons that match this waveform are included as the same neuron. Cell sorting was further accomplished after the experiment concluded using additional principle components analysis in Offline Sorter v3.3.5 (Plexon).

Histology

After electrophysiology testing, subjects were killed with CO_2. To verify placements of recording electrodes, a current (20 A) was run through the implanted microwires. Following retrieval, brains were incubated in a 10% formaldehyde/4% potassium ferrocyanide solution for one week prior to being transferred to 30% sucrose for 1-2 days in preparation for freezing in 100% ethanol cooled with dry ice. Frozen brains were then sliced into 40 µm sections and mounted. Lesion sites were visualized on an Olympus light microscope under the 10X objective. The NAcC and Shell were identified using Paxinos and Watson (4^{th}. Ed.).

Placements were unable to be confirmed for some animals. Two animals could not receive electrolytic lesions and ferrocyanide staining to visualize electrode tip placements. Attempts to identify electrode tracks in tissue collected from these animals (both in the Stress condition) were unsuccessful. A further group of animals (5 Stress, 2 Control) were unable to undergo histological placement because following sectioning,

their tissue was lost when the freezer in which the tissue was stored lost power. Of the 22 arrays (comprising 11 animals) that were included in the histological analysis, only two were off-target between subregions; in one Stress animal a Shell-targeted array terminated in the Core, and in one Control animal a Core-targeted array terminated in the Shell. Because the majority of placements that could be confirmed were on target, electrodes that could not be confirmed were still included in the analysis as “putative” Core and Shell placements.

Data Analysis

Firing rates of individual units were aligned to sucrose delivery onset, such that each trial comprised a 10 second baseline and a 10 second tastant period. A 10 second analysis period was chosen because although the fluid delivery pump only operates for 6 seconds, delivery continues for at least another 4 seconds due to pressure built up in the fluid line. Histograms (1 second bins) were created to summarize unit activity (spikes/second) across all 30 trials of tastant delivery. Units with an average pre-sucrose firing rate of greater than 15 Hz or less than 0.1 Hz were excluded from all subsequent analyses to filter out non-medium spiny neurons and units with too little activity to be characterized, respectively. A Z-Normalized histogram was created for each unit based on the mean and standard deviation of the 10 pre-sucrose bins. Phasic cells were identified by analyzing each unit’s z-transformed histogram.

A unit was classified as phasic if 2 consecutive one-second bins were at least 1.5 standard deviations from the mean or if a single one-second bin exceeded 2.37 absolute standard deviations; the chosen Z-Score thresholds correspond to $p_{two.tailed} = 0.018$ and were selected based on their sensitivity to small but visible changes in activity. In certain units with a sufficiently low firing rate at baseline, it was possible for a firing rate of 0

spikes/second to be above –1.5 standard deviations. In the case of these units, the z-score corresponding to 0 spikes/second was used as the lower threshold for 2 consecutive bins of activity; these units were only classified based on the presence of two consecutive bins without any activity. Phasic units with activity during sucrose delivery above baseline were classified as having “Excitatory” responses, while those displaying reduced activity were classified as having “Inhibitory” responses. A unit’s response magnitude was quantified as the absolute average z-score in the 10 seconds following sucrose delivery onset.

The effect of Stress on response type was assessed by using the number of Excitatory and Inhibitory units counted during the pre- and post-tests in Control animals to calculate expected values of those same counts in Stress animals using the following formula: $[(U_{r\_Control}/U_{total\_Control})*(U_{total\_Stress})]$, where “$U_{r\_Control}$” is the count of a particular response type at a particular recording timepoint in Control Animals, and $U_{total\_Control/Stress}$ are the total numbers of units displaying that response across timepoints in the Control or Stress groups, respectively. Observed and expected counts were compared using Chi-Square Goodness of Fit Tests(α=0.05). Separate analyses were carried out for putative Shell and putative Core populations.

Within the putative population of phasic units from each location, a mixed ANOVA was used to assess the effect of chronic stress, recording timepoint, and response type on response magnitude. Statistical analyses were performed with R (https://www.r-project.org/).

Results

CVS does not alter the hedonic impact of sucrose

The two-bottle sucrose preference test is commonly used to assess the effect of chronic stress on hedonic processing. As the name suggests, in this test animals are

given simultaneous access to 1% sucrose and water and allowed to consume either freely. Consumption may be quantified as either a preference ratio (Fig. 2.1A) or the gross volume consumed (Fig. 2.1B). However, because the present study was not concerned with the particular value of either metric, but rather the effect of stress on each metric, a fold-change was calculated for both metrics for both animals.

The transformation of both preference ratio and consumption to a normalized metric further allowed them to be analyzed together (Fig. 2.1C). A 2 (Stress condition) X 2 (Metric) mixed ANOVA found a significant interaction between stress and performance metric [$F(1, 27)=78.6456$, $p=1.749e-9$]. Single-DF contrasts were used to assess the effect of stress on each metric. While no difference was found between Stress and Control groups for sucrose preference [$F(1, 27)=0.004$, $p=0.951$, Holm-adjusted], total consumption changed in significantly different directions for Stress and Control animals [$F(1, 27)=72.13$, $p=4.18e-9$]. Control animals tended to consume more fluid during their second test (Mean ± SEM: 0.378 ± 0.081), while animals in the Stress condition showed a change of similar magnitude in the opposite direction (-0.402 ± 0.047).

The change in fluid consumption was further assessed using a 2 (Stress condition) X 2 (Fluid) mixed ANOVA to compare changes in sucrose and water consumption (Fig. 2.1D). There was a significant main effect of fluid type [$F(1, 27)=16.1462$, $p=0.000422$], such that the change in Sucrose consumption (0.166±0.120) was significantly higher than that of water consumption (-0.298±0.070). There was also a significant main effect of stress, such that, as already shown in Figure 2.1C, fluid consumption increased in Control animals (0.308±0.11) across fluid types, while consumption decreased in Stress animals (–0.415 ± 0.050). Further, while there was not a significant interaction between fluid type and stress condition at $\alpha=0.05$ [$F(1, 27)=3.149$, $p=0.0873$], it would be needlessly doctrinarian to ignore that the increase in fluid consumption observed in Control animals is driven entirely by the increase in

sucrose consumption (0.648±0.148), whereas water consumption does not truly change from the first preference test to the second (-0.0315±0.097). These data suggest that chronic variable stress altered the motivation to consume fluid generally but did not specifically reduce the hedonic impact of 1% sucrose.

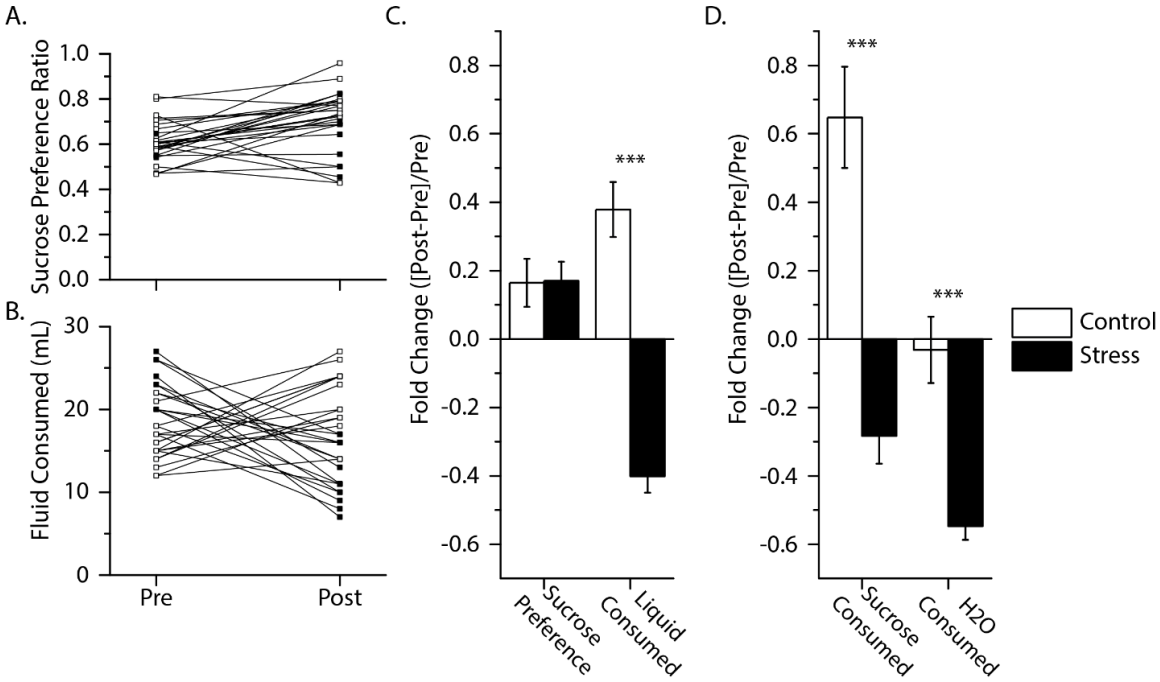

Figure 2.1. Chronic stress reduces liquid consumption but not sucrose preference. (A.) Chronic stress significantly reduced overall fluid consumption relative to controls [F(1, 27)=72.13, p=4.18e-9], but did not alter the relative preference for a 1% sucrose solution [F(1,27)=0.004, p=0.951]. (B.) Stress significantly decreased consumption of both 1% sucrose and H2O [F(1, 27)=100.23, p=1.385e-10]. (C.) Stress and Control groups for showed similar sucrose preference [F(1, 27)=0.004, p=0.951] but differed significantly in total consumption [F(1, 27)=72.13, p=4.18e-9] (D.) While sucrose consumption tended to increase from the first to the second test (0.166±0.120) and H2O consumption decreased (-0.298±0.070), there was not a significant interaction between stress condition and fluid type [F(1,27)=3.149, p=0.0873).

The effect of stress on hedonics was more directly assessed by measuring orofacial responses to 0.3M sucrose in a separate cohort of animals. In response to palatable stimuli, animals exhibit stereotyped responses, such as lateral tongue protrusions (Fig. 2.2A); the type and frequency of these responses are thought to track the hedonic value of a taste stimulus (see Berridge, 2000). The number of lateral tongue protrusions made before and after stress was quantified as events/trial (Fig. 2.2B), and

converted into a fold change (Fig. 2.2C), as in the analysis above, to preserve the possible effects of stress experience while enabling the application of a simple non-parametric test. Because of the presence of an outlier in the stress group (identified using Grubbs Test, $p<0.05$), the Mann-Whitney U-Test was used to compare fold-changes between Stress and Control. No difference was found between the groups [$U=42$, $p=0.897$].

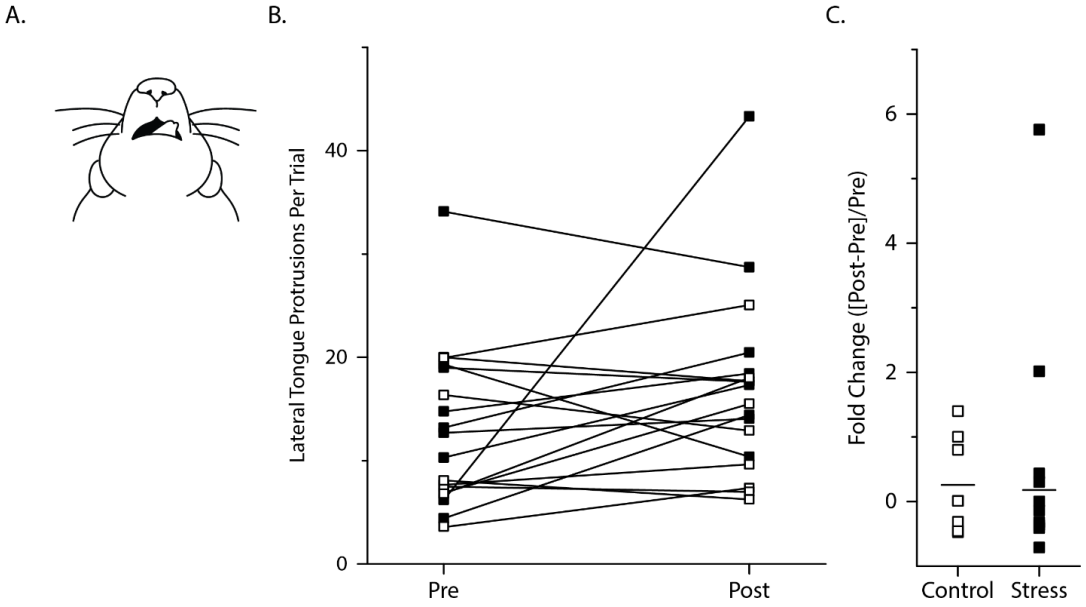

Figure 2.2. Chronic stress does not alter hedonic taste reactivity to 0.3M sucrose. (A.) An illustration of a lateral tongue protrusion, the orofacial response to a palatable solution. (B.) Lateral tongue protrusions were counted per trial at two time points for Stress (black squares) and Control (white squares) animals. These time points were separated by 2 weeks of either CVS or daily handling. (C.) Lateral tongue protrusions from the two time points were converted into a single fold change metric for each animal. Stress did not significantly alter hedonic taste reactivity [$W=42$, $p=0.8968$; Mann-Whitney U test]. Square markers indicate the fold change from pre-stress to post-stress for individual animals. Horizontal bars indicate group means.

CVS selectively impairs effortful instrumental action

Based on the findings of the sucrose preference test and taste-reactivity experiments, stress appeared to affect some aspect of behavioral execution rather than hedonic processing. To determine the nature of this behavioral effect, animals (from the same cohort used in sucrose preference testing) were tested for their ability to acquire

an operant association. Animals were trained to lever-press for a sucrose pellet reward on a fixed ratio (FR1) schedule over successive days of 50-trial sessions. Stress did not delay the acquisition of FR1 responding [F(1, 25)=1.149, p=0.294; Fig. 2.3A]. Press latencies on the first or last (i.e. the second day with 100% responding) day of FR1 training were also tested for potential differences in the nature of lever-pressing between Stress and Control animals (Fig. 2.3B). Because trials could be terminated by either a lever-press or a failure to do so for a set amount of time (i.e. 2 minutes), “latencies” on trials in which no press occurred were counted as 120 seconds. A 2 (Stress condition) X 2 (Training day) mixed ANOVA found a significant main effect of training [(F(1, 25)=55.755, p=8.068e-8], such that trials became much shorter (indicating faster lever-pressing) following training (First Day: 60.512 ± 7.82; Last Day: 1.055 ± 0.117). There was neither a main effect of stress [F(1, 25)=0.1233, p=0.7285] nor an interaction between stress and training [F(1, 25)=0.1101, p=0.7428], further indicating that Stress animals show no deficit in forming and acting upon basic contingencies required for lever-pressing.

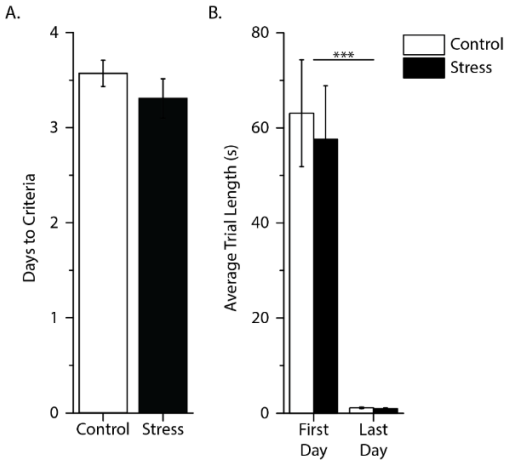

Figure 2.3. Chronic stress does not impair acquisition of fixed ratio responding. (A.) Animals were trained to lever press for sucrose on an FR1 schedule. Criteria was defined as two successive days with 50 responses; stress did not significantly affect the amount of training required to reach this [F(1, 25)=1.149, p=0.294]. Two Stress animals were excluded for failing to ever acquire lever pressing. (B.) Stress and Control animals did not perform differently in FR1 trials, such that training significantly reduced latency to lever press in both groups [F(1, 25)]=55.755, p=8.068e-8].

To test whether Stress alters the general motivational state of an animal, the same cohort of animals was introduced to a progressive ratio design following FR1 acquisition. The response requirement for reward delivery now doubled each time an animal obtained ten rewards. Animals were given the opportunity to earn sucrose pellets on this schedule for three days, and the requirement at which they “broke” (i.e. passed two minutes without a lever press) was recorded each day (Fig. 2.4). A 2 (Stress condition) X 3 (Training Day) mixed ANOVA found a significant interaction between Stress and Training, such that Control animals reached significantly higher breakpoints than Stress animals on Day 3 of training (Stress: 97.231±11.8; Control: 155.429±18.634; p=0.032, Holm adjusted), but not on either Day 1 (Stress: 70.15±17.33; Control: 33.14±6.08; p=0.096, Holm adjusted) or Day 2 (Stress: 90.462±18.901; Control: 74.571±19.282; p=0.56, Holm adjusted).

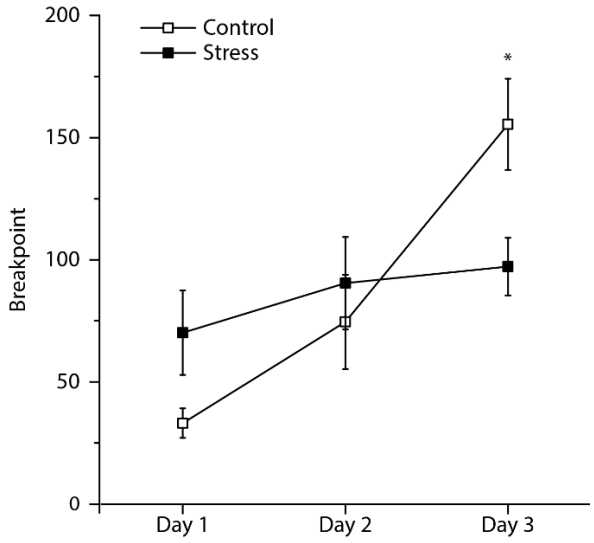

Figure 2.4. Chronic stress reduces breakpoint on progressive ratio schedule. Following FR1 training, animals were placed on a progressive ratio schedule. Successive days of training in this schedule had different effects on Stress and Control animals [F(2, 50)=8.0592, p=0.00093], such that Stress animals had significantly lower breakpoints by Day 3 of training [p=0.048], but did not differ from Control animals on either Day 1 [p=0.096] or Day 2 [p=0.56]. Pairwise Stress vs. Control comparisons at each level of Day report Holm-adjusted p-values.

CVS differentially alters reward encoding in the NAc Core and Shell

In vivo electrophysiological recordings of putative NAc Core and Shell neurons (Fig. 2.5, but see methods for notes on placement confirmations) were performed during 0.3 M sucrose delivery to assess the effect of stress on the neural representation of reward.

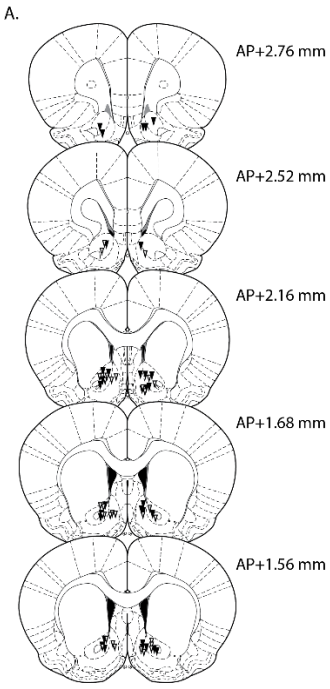

Figure 2.5. Electrode tip placements in NAc Shell and Core. Stress (black triangles) and Control (white triangles) groups shown.

Table 2.1 presents a complete summary the population characteristics of putative NAcSh units that were recorded at each timepoint in Stress and Control animals. This table includes the proportion of these units that were classified as having excitatory (E) or Inhibitory (I) responses. Statistical analyses of sucrose encoding were restricted to phasic responses (Fig. 2.6) The unit counts observed in Control animals formed the basis for calculating expected unit counts in Stress animals (see methods for details). A chi-square for goodness of fit was used to assess how well the observed unit counts in

Stress animals adhered to what would be expected based on the distribution of response types in Control animals. The distribution of putative NAcSh units in Stress animals was found to deviate significantly from that of units in Control animals [$\chi^2(1)=5.948$, $p=0.0147$; Table 2.1, Fig. 2.6]. Counts of putative NAcC units and the analysis of phasic responses are likewise presented in Table 2.2 and Figure 2.7. A Chi-Square Goodness of Fit test found that the distribution of response types in Stress animals fit the distribution expected based on response types in Control animals for putative NAcC units [Table 2.2; $\chi^2(1)=3.43$, $p=0.0641$].

Table 2.1. Putative NAc Shell Unit Sucrose Response Categorization

		Total Units	# Phasic	% Phasic	# I	% I	# E	% E
Control	Pre	**58**	32	55%	16	50%	16	50%
	Post	**57**	32	56%	20	63%	12	38%
Stress	Pre	**60**	41	68%	25	61%	16	39%
	Post	**53**	31	58%	15	48%	16	52%

Table 2.2. Putative NAc Core Unit Sucrose Response Categorization

		Total Units	# Phasic	% Phasic	# I	% I	# E	% E
Control	Pre	**48**	30	63%	12	40%	18	60%
	Post	**46**	28	61%	10	36%	18	64%
Stress	Pre	**80**	49	61%	33	67%	16	33%
	Post	**81**	56	69%	32	57%	24	43%

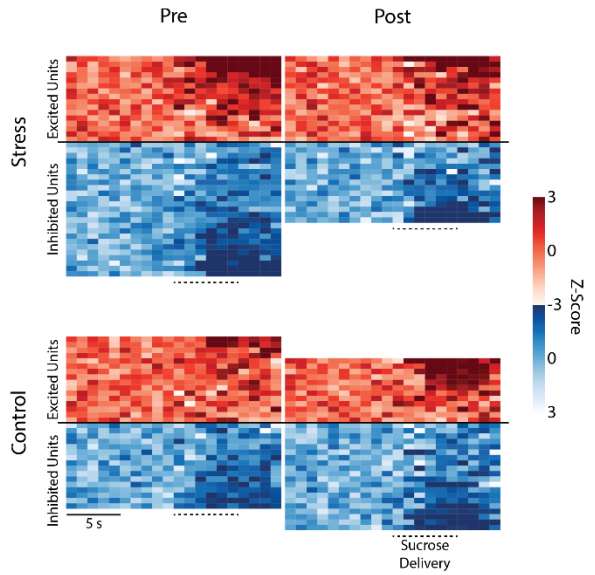

Figure 2.6. Chronic stress reduces sucrose-induced inhibtion in NAcSh. Positively (red) and negatively (blue) modulated units were identified by the presence of at least two consecutive seconds of activity during sucrose delivery (dashed line) exceeding 1.5 standard deviations above or below baseline (10 seconds), or a single second exceeding 2.37 standard deviations. The proportion of positively and negatively modulated units in Stress animals significantly differed from what would be expected based on the proportions seen in Control animals [$\chi^2(1)=5.948$, $p=0.0147$].

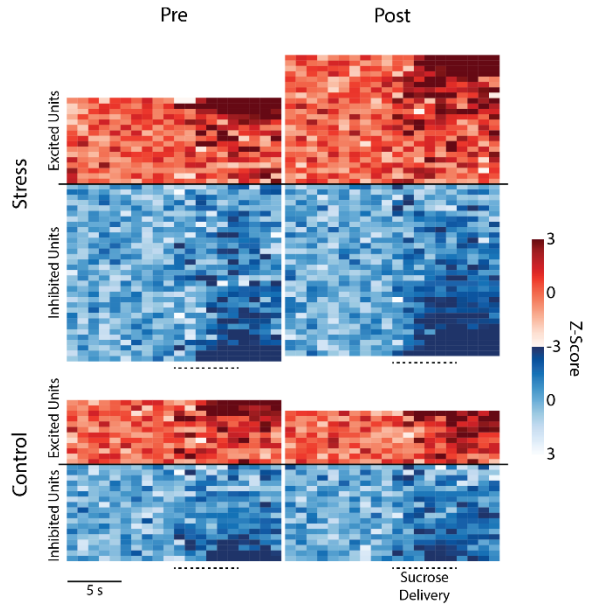

Figure 2.7. Chronic stress does not alter NAcC sucrose response. Unit classification was performed as in Figure 2.5. Units from Stress animals did not deviate from expectations based on the pattern of responses observed in Control animals [$\chi^2(1)=3.43$, $p=0.0641$].

The magnitudes of phasic responses were summarized as the absolute value of normalized activity during sucrose delivery. A 2 (Stress condition) X 2 (Recording timepoint) X 2 (Response type) mixed ANOVA on the response magnitudes in putative NAcSh units (Fig. 2.8A) found a significant main effect of Stress condition on encoding magnitude, such that the units from Stress animals had larger responses (1.518±0.152) on average than those from Control animals (1.123±0.138) [$F(1, 128)=4.217$, $p=0.0421$]. There was no effect of either Recording time [$F(1, 128)=0.210$, $p=0.6473$] or Response type [$F(1, 128)=0.273$, $p=0.6021$]. Though there were no significant interactions [Stress X Recording: $F(1, 128)=2.261$, $p=0.1351$; Stress X Response: $F(1, 128)=0.338$, $p=0.5620$; Recording X Response: $F(1, 128)=0.814$, $p=0.3688$; Stress X Recording X Response: $F(1, 128)=1.684$, $p=0.1968$], the magnitude of Excitatory responses from the units in Control animals during the first recording time-point is surprisingly low (0.614±0.146), which may account for the observed main effect of Stress condition. An identically structured mixed ANOVA performed on magnitudes from putative NAcC units (Fig. 2.8B) found neither significant main effects [Stress condition: $F(1, 155) = 0.833$, $p=0.363$; Recording timepoint: $F(1, 155)=0.740$, $p=0.391$; Response type: $F(1, 155)=1.792$, $p=0.183$] nor interactions [Stress X Recording: $F(1, 155)=1.031$, $p=0.311$; Stress X Response: $F(1, 155)=0.031$, $p=0.860$; Recording X Response: $F(1, 155)=1.459$, $p=0.229$; Stress X Recording X Response: $F(1, 155)=0.082$, $p=0.775$].

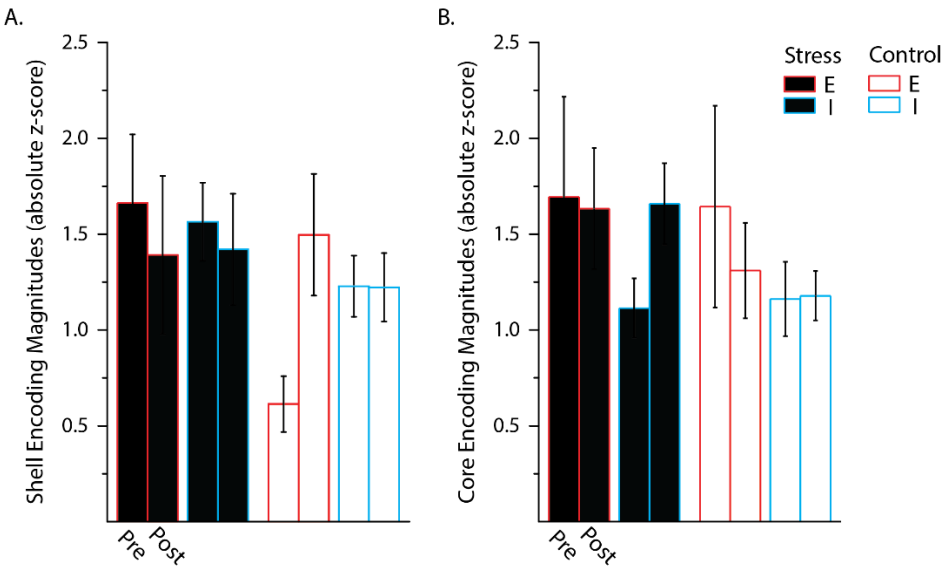

Figure 2.8. Chronic stress does not alter encoding magnitude of sucrose delivery. Unit response magnitudes (i.e. absolute z-normalized activity during a 10 second period beginning at the onset of sucrose delivery) were averaged in the Shell (A.) and Core (B.) for both excitations and inhibitions. A 2 (Stress Condition) X 2 (Recording Timepoint) X 2 (Response Type) was used to assess the effect of stress experience on response magnitude. (A.) Within the Shell, there was a main effect of stress on magnitude ($F(1, 128)=4.217$, $p=0.0421$). There were no other significant main effects, nor were there interactions between any of the factors. (B.) There were no significant main effects or interactions in the Core.

The analysis strategy above was likewise applied to phasic activity during quinine delivery (Tables 2.3 and 2.4; Figs. 2.9-2.11). Note that the total number of units recorded within each Stress-Time-Location sub-group is the same between sucrose and quinine delivery with the exception of putative NAcSh units recorded post-Stress; a single Stress animal experienced complications following the completion of all sucrose deliveries that prevented further recording during quinine delivery. This animal contributed four phasic units to the sucrose-response dataset. The distribution of phasic response types in Stress animals deviated significantly from that observed in Control animals for putative NAcSh units [Fig. 2.9; $\chi^2(1)=12.132$, $p=0.000496$] but not putative NAcC units [Fig. 2.10; $\chi^2(1)=3.368$, $p=0.066464$]. A 2 (Stress condition) X 2 (Recording timepoint) X 2 (Response type) mixed ANOVA revealed no significant effects of Stress, Recording time, or Response type on absolute response magnitude in either putative NAcSh

[Stress condition: F(1, 134) = 1.547, p=0.0.216; Recording timepoint: F(1, 134)=2.438, p=0.121; Response type: F(1, 134)=2.181, p=0.142] or Core units [Stress condition: F(1, 144)=0.035, p=0.852; Recording timepoint: F(1, 144)=0.407, p=0.524; Response type: F(1, 144)=1.046, p=0.308]. Likewise, the analysis found no significant interactions between Stress X Recording time [Shell: F(1, 134)=0.020, p=0.888; Core: F(1,144)=0.186, p=0.667], Stress X Response type [Shell: F(1, 134)=0.028, p=0.867; Control: F(1, 144)<0.001, p=0.990], Recording time X Response type [Shell: F(1, 134)=1.261, p=0.264; Core: F(1, 144)=0.220, p=0.639], or Stress X Recording time X Response type [Shell: F(1, 134)=0.086, p=0.769; Core: F(1, 144)=0.854, p=0.357].

Table 2.3. Putative NAc-Shell Unit Quinine Response Categorization

		Total Units	# Phasic	% Phasic	# I	% I	# E	% E
Control	Pre	**58**	38	66%	13	34%	25	66%
	Post	**57**	33	58%	16	48%	17	52%
Stress	Pre	**60**	43	72%	17	40%	26	60%
	Post	**49**	28	57%	5	18%	23	82%

Table 2.4. Putative NAc-Core Unit Quinine Response Categorization

		Total Units	# Phasic	% Phasic	# I	% I	# E	% E
Control	Pre	**48**	32	67%	10	31%	22	69%
	Post	**46**	33	72%	14	42%	19	58%
Stress	Pre	**80**	38	48%	14	37%	24	63%
	Post	**81**	49	60%	16	33%	33	67%

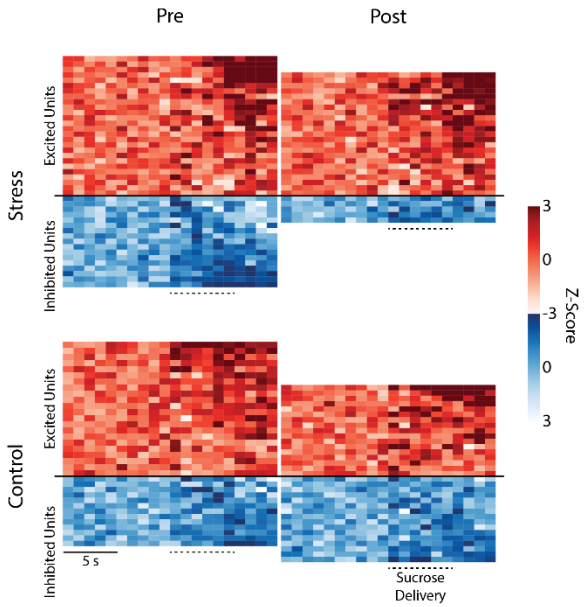

Figure 2.9. Chronic stress reduces quinine-induced inhibition the NAc Shell. The proportion of excitatory and inhibitory responses in Stress animals significantly deviated from the distribution observed in Control animals [$\chi^2(1)=12.132$, $p=0.000496$].

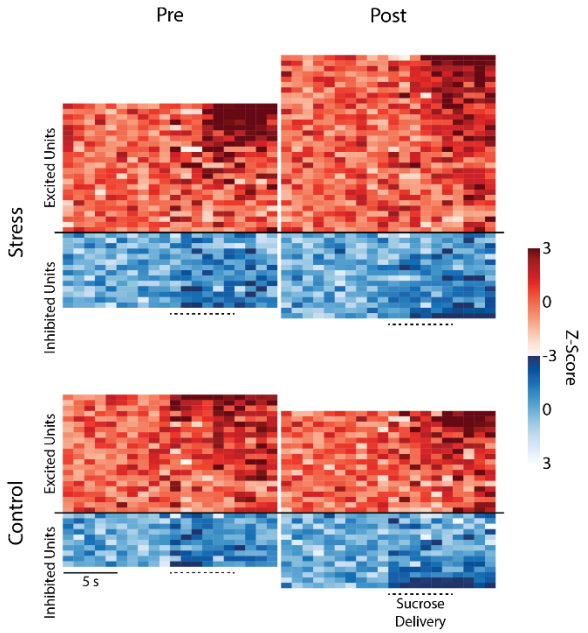

Figure 2.10. Chronic stress does not alter NAcC quinine response [$\chi^2(1)=3.368$, $p=0.066464$].

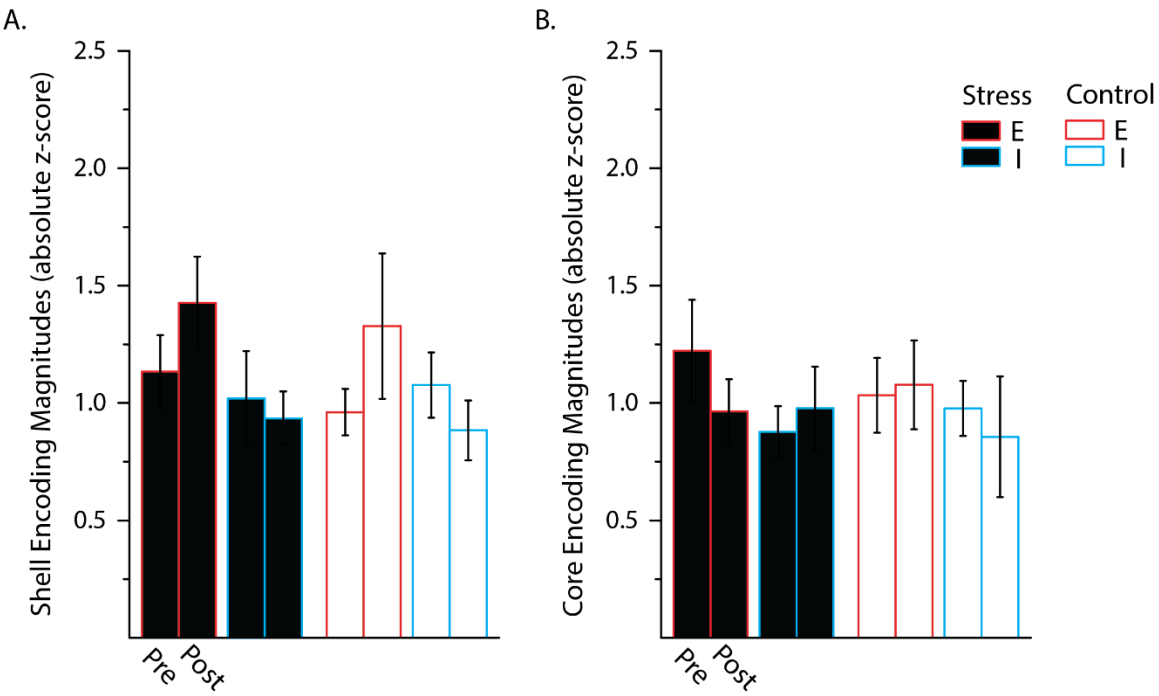

Figure 2.11. Chronic stress does not alter encoding magnitude of quinine delivery. Magnitude quantification occurred as in Figure 2.8. A 2 (Stress Condition) X 2 (Recording Timepoint) X 2 (Response Type) was used to assess the effect of stress experience on response magnitude. There were no significant effects of Stress, Recording timepoint, or Response type on absolute encoding magnitude in either putative NAc-Shell (A.) or putative NAc-Core (B.) units.

Discussion

These studies present an assay of chronic stress' effects on reward related behavior in specific contexts and the impact of stress on hedonic encoding in the NAc. The overall profile of behavioral effects suggests that this stress paradigm produced deficits in motivation rather than hedonic processing.

The sucrose preference test has been regularly used to assess "anhedonia" following chronic stress for over three decades (Willner et al., 1987). Consummatory behaviors have been recognized to include an appetitive approach component for over a century (Craig, 1917). Yet, only recently has the latter understanding been applied to interpreting the former test (Scheggi, De Montis, & Gambarana, 2018; Rizvi et al., 2018). Under the revised framework for interpreting the two-bottle sucrose preference test, the reduction in fluid intake, but not sucrose preference, observed in the current study

indicated motivational deficits. The absence of any effect on sucrose taste reactivity further supports the interpretation that CVS did not alter hedonic processing.

The chronic stress paradigm used in this study also induced differential deficits in two types of operant behavior. Stressed animals readily learned to approach and interact with a lever to acquire a reward. It should be noted that two animals failed to acquire any lever approach, and both had stress experience. While it is possible that stress produced a pronounced associative deficit in these animals, their behavior was so qualitatively different than the other animals in both groups that they were excluded from analysis. It seems likely that their unusual behavior was an artifact of the task design. Reward delivery occurred in the absence of a lever-press during early training, making lever retraction a Pavlovian cue predicting reward delivery. Stress exposure can bias animals away from cue-interactions and towards the site of reward delivery in a similar, but purely Pavlovian, task (see Ch. 4 of this document or Fitzpatrick et al., 2019). Thus, the failure of these two animals to acquire lever pressing may also be evidence of a particularly strong bias towards reward-approach rather than cue-approach. In any case, the FR1 task required animals to expend very little effort, and animals that acquired lever approach (which were, again, the majority) did so with apparent alacrity regardless of their stress experience, as evidenced by equally short lever-response times in both groups.

In contrast, a deficit became apparent in the same stressed animals when they were required to increase their effort expenditure in a progressive ratio design. Though breakpoints in such designs are sensitive to the value of the reward that is received (Reilly, 1999), it is unlikely that hedonic deficits explain the reduced breakpoints given the absence of any change in sucrose preference in the same cohort of animals. Further, the above report that detailed decreased breakpoints with lower-valued rewards also described a much greater effect on responding induced by dopamine inhibition by

haloperidol (Reilly, 1999). Since dopaminergic manipulations do not alter hedonic perception (Peciña, Berridge, & Parker, 1997; Peciña et al., 2003), the most likely explanation for the observed reduction in breakpoints is a reduction in some aspect of motivation or willingness to expend effort. Indeed, this agrees with the alteration that *was* observed in the sucrose preference test: a reduction in overall consumption, which can also be induced by dopaminergic manipulations (Meyerolbersleben et al., 2020). The overall picture of deficits observed in this battery of behaviors, then, is one centered around an apparent deficiency in the ability to translate sensory pleasure into incentive drive.

Single unit activity in the Core and Shell subregions of the NAc was recorded during intraoral delivery of pleasurable or aversive tastants[1] following chronic stress. In stressed animals, the profile of unit responses shifted towards excitation, specifically in the Shell. In response to a pleasurable tastant (such as sucrose) the majority (~65-75%, historically) of responsive units display a reduction in firing rate (Roitman et al., 2005; Wheeler et al., 2008), and pharmacological manipulations in the accumbens suggest that this pattern reflects the neural representation of a reward's hedonic and incentive values (Castro & Berridge, 2014). Likewise, the predominance of elevated firing rate responses induced by aversive tastes (Roitman et al., 2005; Wheeler et al., 2008) aligns with the observation that pharmacological excitation of this area (either by GABA antagonism or Glutamate agonism) reduces the experience of pleasure and augments aversion (see Carlezon & Thomas, 2009).

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[1] N.B. The interpretation of electrophysiological data presented herein should be undertaken with caution and restraint due to the failure to fully confirm the placements of electrode arrays. Nevertheless, as discussed in the Methods, most confirmed placements were accurate, and the following discussion will assume the same to be true for all other placements.

The altered encoding profile was observed specifically in the Shell but not the Core. While both subregions are similarly responsive to simple reward delivery (Roitman et al., 2005), these areas process different components of reward. Specifically, while the Core appears to be involved in learning and action in the context of goal directed behavior, the Shell seems to process the hedonic and motivational values of rewards themselves (Kelley, 2004; Saddoris, Cacciapaglia, Wightman, & Carelli, 2015; West & Carelli, 2016). The location and direction of the change in reward encoding both suggest that stress impairs the ability of the animal to appropriately valuate the reward.

Stereotypical NAc encoding profiles during sucrose or quinine delivery have been consistently reported (Roitman et al., 2005; Wheeler et al., 2008; Roitman et al., 2010). Palatable tastants cause a reduction in the firing rates of most responsive NAc neurons, while unpalatable tastants cause an increase in firing rates of most responsive NAc neurons. Oddly, this typical population response was not consistently observed in this study, making the interpretation of the effect of stress difficult. This result was unexpected since the criteria for determining a phasic excitation or inhibition was adopted from prior published work. Close examination of the activity of individual units reveals a number of weak responses, particularly in the group with the lowest response magnitude (i.e. Control group Shell units classified as excitatory at the first recording timepoint). This may be suggestive of the unusual inclusion of an atypically large number of weaker excitatory responses in this group. This would explain the observed deviations from the usual 3:1 Inhibitory:Excitatory ratio in non-stressed groups. The choice to use these same groups as the baseline for comparison with stress groups was a relatively conservative one; the deviations from “normal” observed at some timepoints in control and pre-stress conditions made alterations in the response profile in post-stress animals less likely to appear significant. This approach was chosen because it better represents within group variability due to sampling (via pre-post measurements) and environmental

factors (i.e. all surgeries, recordings, unit-sorts, and unit categorizations were performed by one lab using one set of techniques).

Directly comparing the distribution of Post-Stress response types from the present study to that expected based on the literature would arguably change some of the conclusions drawn about these data, though only for quinine data. The distribution of inhibitory and excitatory responses to sucrose observed in putative NAcSh units differs from the 3:1 ratio seen in the literature, while the distribution of responses in the NAcC does not. In response to quinine, the proportion of excitatory units in the Core did not change appreciably from pre (63%) to post-stress (67%), and neither ratio was different from what would be expected based on the literature. In the Shell, however, the proportion of excitatory responses increased from only 60% pre-stress to 82% post stress, and this change was driven by a reduction in the number of inhibitory responses. When compared to the historical ratios it was the *pre*-stress timepoint that appeared aberrant. This kind of conflict was a central driver in the choice to *not* use the historical ratios as the main comparator in the present study, as to do so ignores the individual variability within samples.

As a final caveat, some unusual differences between groups were observed in response magnitudes. It is likely that these differences also resulted from the somewhat unusual representation of phasic units. Given that, a defense of the criteria chosen to classify a unit as phasic seems appropriate. “Phasic”, as used here, is synonymous with “responsive” and describes neuronal activity that consistently changes in the same direction in response to a given stimulus. This change is inferred to reflect the neuronal representation of that stimulus. The difficulty in identifying units as phasic arises from the fact that there appears to be a thin line between a slight, but consistent, response and no response at all, and the statistical thresholds that researchers rely on to make that distinction are not what the brain uses to code information in the form of firing rate

change. That is, neural circuits do not check whether $p<0.05$ when integrating information. So, while stricter criteria for classification could be used to reduce false-positives, this would also lead to an increase in false-negatives. Moreover, the presence of false-negatives would be arguably more problematic for interpreting this dataset than false-positives because the most interesting effects are *reductions* in the number of negatively modulated units. Observing such reductions is more meaningful when the most likely kind of classification error increases unit counts. In short, classifying neuronal types based on reliable, statistically significant changes in firing rate is somewhat arbitrary on its face, and has the potential be influenced by unknown experimental conditions. Unfortunately, this appears to have occurred in the current experiment, thereby hindering stronger interpretations.

Despite the shortcomings of these experiments, these results are valuable. First, the data clearly align the deficits that result from CVS in the approach motivation domain. There was no evidence that chronic stress altered either the perceived palatability of a reward or the ability of animals to learn how to obtain it, but it did reduce the effort an animal would expend in their pursuit of the reward. Second, the data indicate that even though perceived palatability was not altered by chronic stress, the encoding of a reward by NAc neurons was altered. The specificity of the behavioral effects observed in the present studies, along with the localization and direction of the electrophysiological effects, suggest that CVS impaired the representation of motivational value. Thus, rather than inducing “anhedonia” as classically defined, the CVS paradigm used in these studies induced something better described as “avolition.” Since the NAc is a limbic/motor integrator and encodes both hedonic valence and incentive, it is likely that the altered encoding reflects altered incentive.

The integrative nature of this nucleus may make the disrupted encoding pattern difficult to interpret. Therefore, it may prove fruitful to pivot to an examination of an input

to the NAc that has been more specifically implicated in providing regulatory control of motivated approach. The PL is an excellent candidate. It has long been implicated in regulating conditioned approach, and it is susceptible to the damaging effects of stress. In addition, examining a robust approach behavior, such as Pavlovian autoshaping, can better illustrate the ability of a reward-predictive cue to incentivize behavior. We hypothesize that the PL encodes cues that elicit approach, and that the cortical projections that modulate NAc activity become compromised following chronic stress and are associated with diminished approach. Experiments detailed in the subsequent chapters will test both hypotheses.

III. PRELIMBIC PREFRONTAL CORTICAL ENCODING OF STIMULI THAT PROMOTE CONDITIONED APPROACH

Abstract

Animals attribute incentive value and learn to approach otherwise behaviorally inert stimuli if these stimuli come to predict the delivery of reward. Interestingly, this adaptive Pavlovian learning process has been implicated in behavioral control disorders, such as drug addiction. One brain region implicated in directing conditioned approach behavior is the prelimbic region of the prefrontal cortex. However, activity patterns in this region have not been characterized in response to incentivized cues that induce Pavlovian approach behavior. The present study employed *in vivo* electrophysiology in the prelimbic cortex to characterize the distribution of neural responses to the presence of a cue that had acquired incentive value after being associated with a primary reward. Rats were trained in a Pavlovian autoshaping task in which a lever was presented prior to reward delivery. Following repeated pairings of lever availability and reward delivery, rats pressed the lever even though reward delivery was not contingent on any interaction with the lever. Neurons in the prelimbic cortex selectively encoded the presentation of the reward-predicting lever. Although the response was heterogeneous, most responsive neurons decreased their firing rate in response to the presence of the lever. These findings characterize the varied responses of prelimbic cortical neurons to cues that elicit approach and are consistent with evidence that the role of neurons in the prelimbic cortex in attributing incentive value depends on their downstream target.

Introduction

An environmental cue that predicts the availability of a pleasurable reward can become a powerful incentive unto itself. The process by which this this occurs is important to characterize not just because it is essential for normal behavior, but also because it may be involved in impulse-control disorders and addiction (Colaizzi et al., 2020, Tomie, Badawy, & Rutyna, 2016). Furthermore, as described in the previous chapter, the behavioral effects of stress are likely in the incentive, rather than hedonic, domain. One useful model for the study of acquired incentive is conditioned approach (i.e., Pavlovian autoshaping; Brown & Jenkins, 1968), which assesses an animal's tendency to approach an otherwise motivationally-neutral cue that predicts a rewarding outcome, often while ignoring the location of the actual reward (Flagel & Robinson, 2017).

Incentive learning involves many structures implicated in learning, including a complex role for the mPFC. Many experiments have demonstrated a role for the mPFC in behaviors that require the use of cues to pursue specific rewards (Balleine & Dickinson, 1998; Killcross & Couterrau, 2003; Mulder, et al., 2003; Otis et al., 2017), and neurons in the mPFC encode cue-evoked reward-seeking behaviors (Homayoun & Moghaddam, 2009; Horst & Laubach, 2013; Petykó et al., 2015). However, the relationship between mPFC activity and Pavlovian autoshaping is complex. Although Pavlovian autoshaping induces glutamate, norepinephrine, and serotonin release in the mPFC (Batten, Pomerleau, Quintero, Gerhardt, & Beckman, 2018; Tomie, Tirado, Yu, & Phorecky, 2004), and lesions of the mPFC reduce cue-approach behavior (Serrano-Barroso, Vargas, Diaz, O'Donnell, & López, 2019), there is little direct evidence that the mPFC encodes such incentivized Pavlovian cues.

In the present experiment, we characterized the mPFC encoding of a cue that had acquired incentive value. Using *in vivo* electrophysiological techniques, we recorded

single unit activity in the prelimbic mPFC during a Pavlovian autoshaping task and describe cue-selective activity patterns that likely impact downstream processing to promote autoshaping behavior.

Methods

Animals

Male, Sprague-Dawley rats (n=16; Envigo, Indianapolis, IN) weighing 300-350g were individually housed with a 12:12h light:dark cycle. Body weights were maintained at 90% of free feeding weight during testing. All procedures were approved by the Marquette University Institutional Animal Care and Use Committee.

Pavlovian Conditioned Approach Training

Pavlovian autoshaping occurred in Plexiglass operant chambers (MED-Associates; St. Albans, VT). Two retractable levers flanked a centrally-located, recessed, food cup. Cue lights were located above each lever. Daily 1-hour training sessions comprised 25 CS+ trials and 25 CS– trials. During CS+ trials, the lever and light on one side of the food hopper were extended and illuminated, respectively, for 10 seconds, after which a sucrose pellet (45 mg; Bio-Serv) was delivered to the food cup. During CS– trials the lever and light on the other side were presented in the same manner but were not followed by sucrose delivery. Because the goal was to determine if mPFC neurons encoded conditioned approach behavior, criterion for inclusion was the acquisition of selective CS+ approach in the autoshaping task. This was determined by the demonstration of CS+ approach probability > 80% on day 10 of conditioning. Six rats failed to achieve this criterion, were excluded from behavioral analyses, and did not receive electrode implantation.

Electrophysiology

Surgery

The 10 remaining subjects received electrode implantation surgery following conditioning. Under isoflurane anesthesia, 8-wire stainless steel microelectrode arrays (NB Labs; Denison, TX) were implanted bilaterally in the PL at AP: +3.0mm, ML: ±0.6mm @ 0º; ±1.6mm @ 15º, DV: -4.0mm @ 0º; -4.1mm @ 15º. Recordings were conducted using a commercially available neurophysiological system (Plexon; Dallas TX), a commutator (Crist Instruments; Hagerstown, MD), and unit isolation software described previously (Wheeler et al., 2015). Animals received an additional training session in the recording chamber to verify recovery from surgery. Electrophysiological recordings occurred over 2 days of autoshaping training, with units recorded on the day with the most robust signal used for analysis.

Data Analysis

After testing, subjects were euthanized and microwire placements were verified as described in Chapter 2, with one small procedural change. Tissue sections were counterstained with Neutral Red to aid in the visual identification of lesions. Units recorded from wires outside of the PL were excluded from analysis. Firing rates of individual cells were aligned to CS+ and CS– onset. Spike histograms (1s bins) were created. Phasic cells were identified using ANOVA ($\alpha = .05$) to analyze the average firing rate within the following levels: 10 second pre-CS period, 5 second early CS period, 5 second late CS period. Differences were used to identify phasic responses. Histogram bins were normalized to the area under the receiver operating characteristic (auROC). This analysis approach differs from that used to characterize responses to tastant

delivery in Chapter 2. This deviation was intentional. The methods used in this analysis were chosen for their sensitivity to a range of different response types and were thus more appropriate for the exploratory nature of this work. The firing rates within each time bin across trials was compared to the firing rates throughout the baseline. A receiver operating characteristic was created from this comparison by plotting the probability of the firing rate during the window of interest exceeding a given value against the probability that the baseline firing rate exceeded that same value. This comparison was made for the range of values from zero through the maximum firing rate of a given unit. Unit auROC normalizations were also used for assessing the magnitude of unit responses by calculating the absolute deviation from 0.5 for each bin of the effect period and further calculating the area under the resulting curve.

Comparisons of signal intensity or behavior were made using ANOVA, T-tests, or non-parametric tests ($\alpha s = .05$) using Python and R. In the event of sphericity violations in repeated measures ANOVA, the Greenhouse-Geisser corrected p value is reported.

Results

To study the involvement of prelimbic neural activity in approach behavior, animals were trained to discriminate between two compound lever/light cues that either predicted non-contingent sugar pellet delivery (CS+) or did not (CS–; Fig. 3.1). A within subjects 2 (CS type) X 10 (Day) ANOVA on CS approach found an interaction between CS type and Day, [$F(9, 81) = 15.76$, $p = 1.95e\text{-}8$]. Planned contrasts between the first day of training and the last day of training found that while CS+ approach increased, [$t(9) = 5.65$, $p = .0002$] CS– approach fell [$t(9) = 2.9$, $p = .015$].

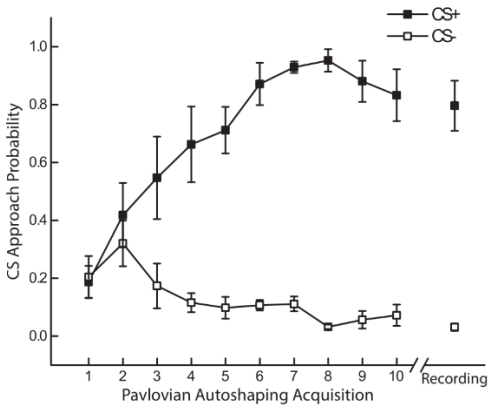

Figure 3.1. Autoshaping Behavior. Approach probability (mean ± SEM) for the CS+ (filled squares) and CS– (open squares) across training. Rats interacted with the CS+ on more trials on Day 10 relative to Day 1 ($p < .001$) but interacted with CS– on fewer trials ($p = .015$). X-axis break indicates microelectrode surgery.

Single-unit activity in the PL (Fig. 3.2A) was recorded during a conditioning session following acquisition. The majority of neurons responded to the CS+ (45/70), with a plurality doing so selectively (31/45). Only 11% (8/70) of units significantly altered their firing rate during CS– presentation (Fig. 3.2B), demonstrating predominantly selective encoding of the reward predicting cue. Consistent with this, units that responded to both the CS+ and CS– (14/70) did not do so equally (Fig. 3.2C): CS– responses were significantly weaker than CS+ responses (ANOVA on areas under the auROC during the effect; $F(1, 13) = 17.164$, $p = .01046$).

In addition to characterizing the selectivity of the prelimbic response to a salient CS+, we also detected a directionality in the encoding (Fig. 3.2D). Of the neurons that encoded the CS+, a large majority (36/45) showed a firing rate reduction. Only 20% (9/45) exhibited an increase in activity when the reward-predicting cue was presented.

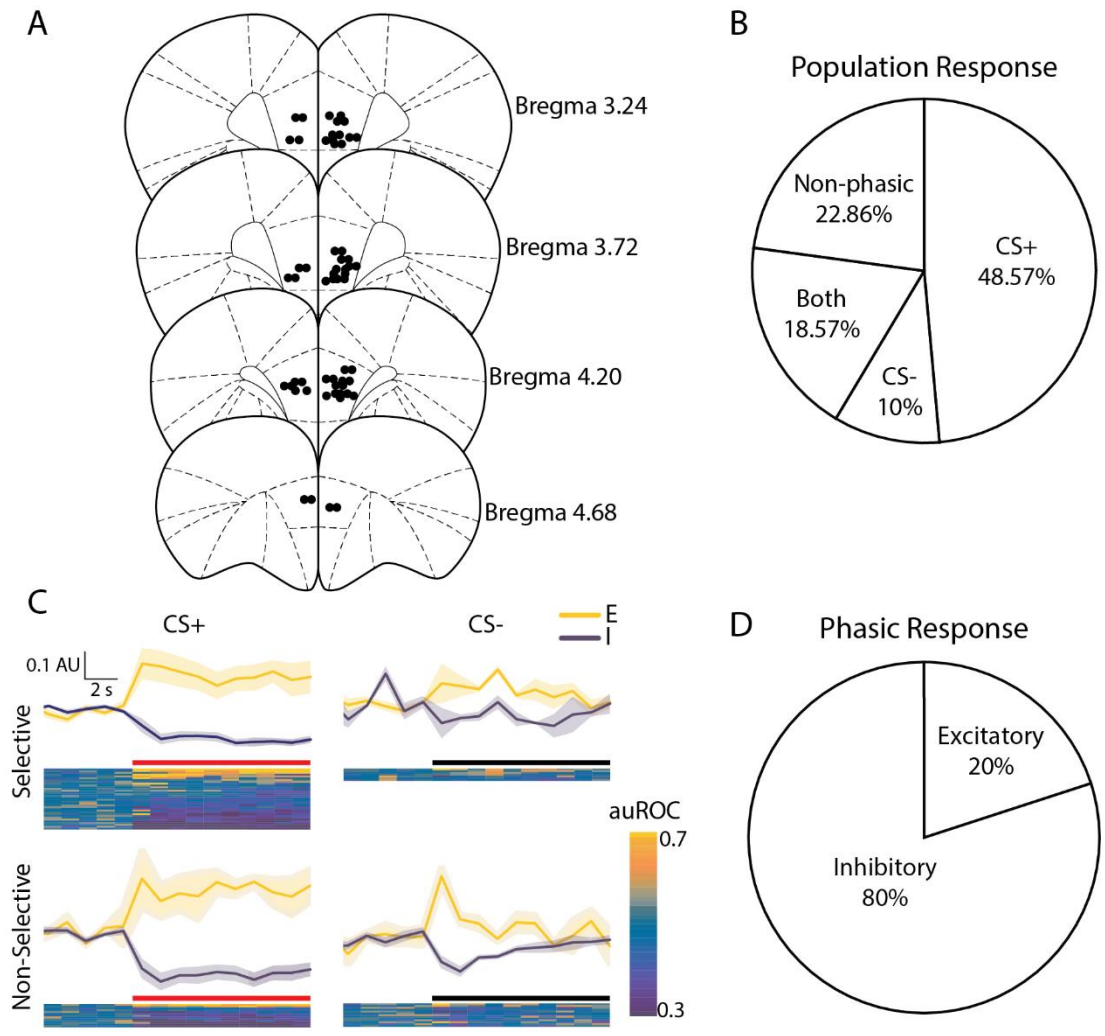

Figure 3.2. Electrophysiology Recordings. (A) Electrode placements in the PL. (B) Response distribution of PFC neurons to the CS+ and CS–. (C) auROC (mean ± SEM) for selective and non-selective units that displayed excitatory (E) or inhibitory (I) encoding of the CS. Horizontal line indicates CS duration. Colorplot: individual unit auROC normalizations for CS–responsive units. Deviations above and below 0.5 depict increased and decreased firing rates. (D) Distribution of CS+ responsive neurons.

Discussion

This brief characterization found that PL neurons preferentially encode cues that have acquired incentive value, and a plurality of these units encode these cues with a reduction in firing rate. These results are consistent with the complex role of the mPFC in regulating approach behavior. Prelimbic neural activity has been identified as a top-

down inhibitor of autoshaping behavior that helps an animal to maintain focus on the primary reward rather than the cue (Campus et al., 2019; Paolone, Angelakos, Meyer, Robinson, & Sarter, 2013). However, other studies show that autoshaping behavior is associated with glutamate release in the mPFC and that lesions of the mPFC can reduce autoshaping behavior (Batten et al., 2018; Serrano-Barroso et al., 2019). The fact that we observed a heterogeneous response to the CS+ is consistent with these *prima facie* incongruent findings. For example, it is possible that cue-inhibited neurons predominantly project to areas such as the paraventricular thalamus, which has been implicated in the pursuit of the primary reward rather than the predictive cue (Campus et al., 2019; Haight, Fraser, Akil, & Flagel, 2015). If these prelimbic glutamatergic projections reduce downstream drive during CS+ presentations, animals may be more likely to attend to the cue rather than the primary goal. In contrast, cue-excited PFC neurons may project to structures that promote conditioned approach, such as the NAc or amygdala (Chang, Wheeler, & Holland, 2012; Chow, Nickell, Darna, & Beckmann, 2016). While *in vivo* electrophysiology does not easily allow for the distinction of efferent pathways, calcium imaging techniques do.

The advent of genetically encoded calcium indicators (GECI) has permitted the targeted monitoring of projection specific neural populations. This technique has been applied to two PL subpopulations–defined by their projection targets, the NAcC and paraventricular thalamus (PVT)–in Pavlovian conditioning of head fixed mice (Otis et al., 2017). Consistent with the heterogeneity of single-unit responses reported here, these divergent pathways display opposite responses to a reward-paired tone. Further, activity in NAcC projecting PL neurons was necessary for the expression of conditioned-anticipatory licking in response to the tone cue. However, as conditioning occurred under head-fixed conditions, animals could not express responses other than licking thereby limiting the applicability of this study to the current work.

The following chapter describes the application of *in vivo* fiber-photometric measurement of GECI fluorescence to monitor NAcC projecting PL neurons during autoshaping. This system permitted examination of a specific PL projection's role in sign- and goal-tracking behaviors. Further, this approach is combined with CVS to probe stress' impact on both autoshaping behavior itself and signaling within the PL-NAcC pathway.

IV. CHRONIC STRESS PREVENTS CORTICOACCUMBENS CUE ENCODING AND ALTERS CONDITIONED APPROACH

Abstract

Chronic stress impairs the function of multiple brain regions and causes severe hedonic and motivational deficits. One brain region known to be susceptible to these effects is the prefrontal cortex. Neurons in this region, specifically neuronal projections from the PL to the NAcC, have a significant role in promoting motivated approach. However, little is known about how activity in this pathway changes during associative learning to encode cues that promote approach. Less is known about how activity in this pathway may be altered by stress. In this study, an intersectional fiber photometry approach was used in male Sprague Dawley rats engaged in a Pavlovian autoshaping design to characterize the involvement of the PL-NAcC pathway in the typical acquisition of learned approach (directed at both the predictive cue and the goal), and its potential alteration by stress. Specifically, the hypotheses that neural activity in PL-NAcC would encode a Pavlovian approach cue and that prior exposure to chronic stress would disrupt both the nature of conditioned approach and the encoding of a cue that promotes approach were tested. Results of the study demonstrated that the rapid acquisition of conditioned approach was associated with cue-induced PL-NAcC activity. Prior stress both reduced cue-directed behavior and impaired the associated cortical activity. These findings demonstrate that prior stress diminishes the task-related activity of a brain pathway that regulates approach behavior. In addition, the results support the interpretation that stress disrupts reward processing by altering the attribution of incentive to associated cues.

Introduction

Mood disorders are debilitating, in part, because they involve severe hedonic and motivational deficits. These same symptoms are associated with several types of regressive neuroplasticity induced by chronic stress exposure (Price & Duman, 2020). This close relationship makes stress a useful procedural tool for invasive studies attempting to identify the dysfunctional brain circuits that produce depressive-like symptoms (Willner, 2017). Unfortunately, it can be difficult to disentangle hedonic and motivational deficits that are caused by stress. As discussed in previous chapters, anhedonia is traditionally defined as the inability to experience pleasure (Ribot, 1896) and considered a hallmark of both major depressive disorder (MDD) and the efficacy of a stress procedure (Drysdale et al., 2017; Rizvi et al., 2018; Willner, 2017). However, anhedonia is not universally observed in MDD (Rizvi et al., 2018; Thomsen et al., 2015) and is present in a much wider range of pathologies (Insel & Cuthbert, 2015). Adding to this complexity is the fact that tests of compromised hedonic processing are often also sensitive to disruptions in motivation (as demonstrated by the experiments in Chapter 2), suggesting that behavioral disruptions assumed to be signs of anhedonia may instead be the result of impaired approach motivation. Focusing on symptoms rather than disorders, then, is critical; and expanding approaches to better characterize these symptoms is critical as well. For this reason, conditioned approach designs may be useful for characterizing the disruptive effects of stress.

Conditioned approach behavior (i.e., approach elicited by a reward-paired cue) is an essential behavior for the navigation of an animal's environment. Conditioning parameters have a significant effect on the nature of this approach. Purely visual or auditory Pavlovian cues promote approach directed toward the site of reward delivery. However, when a physical cue is used in conditioning, as in Pavlovian autoshaping, animals display parameter-dependent variability in the direction of approach (Meyer et

al., 2012; Robinson & Flagel, 2009). Some animals display reward-site directed behavior. Others express a remarkable degree of cue interaction, often going well beyond approach behavior, appearing to attempt to “consume” the cue as though it were the reward (Davey & Cleland, 1982). The difference between the two types of learned responses is thought to reflect a difference in reward value that is attached to the physical cue, with the transfer of conditioned incentive leading to vigorous interaction with the otherwise neutral cue (Robinson & Flagel, 2009). The development of conditioned approach has been used to assess the effects of circuit manipulation on hedonic vs. incentive valuation processes (e.g., Berridge et al., 2009) and can be impaired by exposure to prolonged stress (Fitzpatrick et al., 2019).

Chapter 3 demonstrated that neurons in the PL subregion of the PFC encode incentivized cues. This population of neurons is also susceptible to the regressive neuroplasticity caused by stress (Dias-Ferreira et al., 2009; Radley et al., 2006). A subpopulation of PL projection neurons that target the NAcC serves as a critical substrate for motivated approach (McFarland et al., 2003; Vialou et al., 2014). Activity in this pathway is causally related to conditioned appetitive responses elicited by a reward-predictive cue (Otis et al., 2017). Although the pathway itself has not been studied extensively in autoshaping designs, cue presentation has been shown to promote glutamatergic signaling in both the PL and NAcC of sign-tracking rats (Batten et al., 2018). Given the sensitivity of the PFC to stress and the involvement of the PL-NAcC pathway in directing motivated approach, this study isolated and characterized its involvement in the acquisition of conditioned approach. Specifically, this study tested the hypothesis that neural activity in PL-NAcC would encode a Pavlovian approach cue, and that prior exposure to chronic stress would disrupt both the nature of conditioned approach and the encoding of a cue that promotes approach.

Methods

Animals

Adult male Sprague-Dawley rats (300-350 g; Harlan Laboratories, St. Louis, Missouri) were used in all experiments. Animals were individually housed on a reverse 12:12 light-dark cycle in a temperature- and humidity-controlled, Association for Assessment and Accreditation of Laboratory Animal Care accredited vivarium. All procedures were approved by the Marquette University Institutional Animal Care and Use Committee. All animals were trained in autoshaping (N=59), with half being exposed to chronic variable stress (n=30). A subset of animals received fiber photometry surgery and those animals with both confirmed fiber placement and GCaMP expression contributed data to both the behavior and photometry analyses (Stress: n=8; Control: n=13). During autoshaping training and for three days prior, animals were fed standard chow (TekLad) once daily to maintain 90% body weight. Water was available *ad libitum* for the duration of all experiments.

Chronic Variable Stress (CVS)

The CVS regimen used here was identical to that previously described in Chapter 2. Briefly, it was a 14-day procedure consisting of exposure to 2 of the following stressors per day: forced swim (4 °C water for 20 min), cold room (4°C, 2 h, alone or in combination with other stressors), novel environment (different novel environments for 1-3 h; including wet bedding in cages, ½ inch of water in cages, or no bedding in cages), motion (cage without bedding which is placed on an orbital shaker and rotated for 2 h; 1 revolution/sec), noise (continuous 60-68 dB noise such as radio static for 1 h), open field (alone or in groups in a 1-meter diameter circular brightly-illuminated field for 45 min), restraint (plastic restraint tubes for 30 min), and cage tilt (30° for 6-12 h with food and water available). For repeating stressors, variables such as light, temperature, and noise

were varied to maintain novelty. On each day over the 14-day period, one of the stressors from the battery was presented at 0800h and the other stressor was presented at 1700h. Control rats were handled and weighed daily at the evening timepoint.

Pavlovian Conditioned Approach Training

Pavlovian autoshaping took place in Plexiglass operant chambers (MED-Associates; St. Albans, VT) housed within sound-attenuating boxes (Stanley Vidmar; Allentown, PA). Two retractable levers flanked a centrally located food cup on one wall of the operant chamber. For animals that did not contribute photometry data, this food cup was recessed; for animals in photometry experiments, this food cup extended into the cage to prevent the optic fiber from interfering with pellet retrieval. This minor chamber adjustment prevented automated photobeam detection of goal approach behavior for the subset of animals that contributed photometric data. For these animals, video analysis provided goal approach behavioral measures. Cue lights were located above each lever. A house light placed on the opposite wall illuminated the chamber.

Daily 1-hour training sessions comprised 50 trials. For 25 trials, the lever and light on one side of the food hopper were extended and illuminated for 10 seconds, after which a sucrose pellet (45 mg; Bio-Serv) was delivered to the food cup (CS+ trials). In another 25 trials the lever and light on the other side of the cup were presented in the same manner but were not followed by sucrose delivery (CS- trials). CS presentations occurred in pseudorandom order such that no more than two trials of a single type occurred sequentially. Random inter-trial intervals, with an average duration of 60 seconds, separated CS trials. During each session, behavioral data (including lever interactions, head entries into the goal box, and pellet consumption) were collected. The food hopper was checked at the beginning and end of each session to verify pellet

delivery. On the rare occasion in which pellet delivery was interrupted due to an equipment malfunction, data from that day of training were omitted from analyses.

Behavioral Analysis

Autoshaping acquisition was first characterized by calculating the probability of lever approach. This probability was calculated as the [number of trials of a given type (CS+ or CS-) in which at least one lever contact was made] / [number of trials of corresponding type]. For all animals, lever contacts were recorded automatically upon lever deflection. Head entry information was also scored for all animals. Automated photobeam detection of head entries into the goal box was not possible for animals that contributed photometry data. For these animals, video-recording (10 frames/second) of behavior was used to score head entries. For animals used exclusively for behavioral analyses, automatically registered beam breaks were used to calculate metrics.

Pavlovian Conditioned Approach (PCA) index was calculated to assess the degree of “sign-tracking” and “goal-tracking” exhibited by animals. The calculation of this metric was taken from Meyer et al. (2012). The index comprises three components, which are averaged together. The three metrics used in PCA index calculation are as follows: CS+ over Goal approach preference: [(CS+ approaches – Goalbox head entries)/(CS+ approaches + Goalbox head entries)]; Probability of CS+ over Goal approach: [Pr(CS+ Approach) – Pr(Goal Approach)]; Latency to approach: [mean((latency to goal approach) – (latency to CS approach))/10]. The difference in latency to approach was divided by 10 seconds, the length of CS presentation, to place it on the same scale of -1 to 1 as the previous two metrics. Due to a computer error, CS Latency data failed to be recorded for 3 (of 59) animals on Day 7. These animals were omitted from the PCA analysis.

Since the primary hypothesis being tested was that stress experience would alter learned approach behavior, some behavioral analyses compare “Early Training” to “Late Training.” For most animals, Early Training included all conditioning trials on Day 1 and Late Training included all conditioning trials on Day 7. There were 4 occurrences on Day 1 and on Day 7 in which an equipment malfunction prevented either proper behavioral or photometric recordings. In these cases, a subsequent conditioning day was used in the analyses. For the Day 7 timepoint, this required 4 rats to be run in an additional conditioning session, Day 8, which was used to obtain Late Training data for analysis.

An analysis of variance (ANOVA) was used for all comparisons. In cases with multiple levels of a repeated measure factor (i.e., analysis of CS approach over multiple days), sphericity assumptions were tested using Mauchly’s test. Where this assumption was violated, the p-value of the affected test-statistic was adjusted using the Hyun-Feldt estimated epsilon. Holm corrections were used to preserve family-wise error rate for all multiple comparisons. Statistical analyses were performed with R (https://www.r-project.org/).

Fiber Photometry

A subset of animals that experienced the CVS procedure first received surgery for photometric recording in order to characterize the PL-NAcC activity patterns associated with the acquisition of conditioned approach.

Surgery

Animals to be used for photometry experiments were anesthetized under isoflurane (2.0 - 2.5%) and head-fixed for stereotaxic implantation of an optic fiber targeting the PL and viral injection of GCaMP6f. Selective expression of the Ca^{2+} indicator GCaMP6f in PL-NAcC neurons was accomplished using a dual viral approach.

First, retrograde AAV2-CAG-Cre (University of North Carolina Vector Core) was injected into the Core at two sites (6°; AP: +1.2/+0.7 mm; ML: +2.4 mm; DV: -5.0 mm; 0.3µL/3 min/site; titer = 8.1 x 10^{12} molecules/mL). Next, AAV1-hSyn-FLEX-GCaMP6f-WPRE.SV40 (University of Pennsylvania Vector Core) was injected into the PL (8°; AP: +2.8 mm; ML: 1.0 mm; DV: -4.0 mm; 0.5 µL/5 min; 6.5 x 10^{12} molecules/mL) followed by optic fiber (5 mm length, 400 µm core/430 µm outer diameter, 0.48 numerical aperture, flat tip; Doric) implantation (0°; AP: +2.8 mm; ML: +0.6 mm; DV: -3.7 mm) at the same site. Rats were treated with the anti-inflammatory drug, meloxicam (1% oral suspension) the day of surgery and for 4 d following surgery to reduce inflammation and postoperative pain.

Recording

Simultaneous recording of GCaMP6f fluorescence and background was accomplished using two separate wavelengths of light (465 nm and 405 nm, respectively) provided by two single wavelength LEDs (Doric; Quebec, QC) controlled by an external dual channel driver (Doric), which itself was driven by an RZ5P processor (Tucker Davis Tech; Alachua, FL). Both wavelengths were routed through a dichroic mirror (4-port fluorescence mini cube, Doric) and combined into a single 2-meter jacketed patch cord (400 µm core, 0.48 numerical aperture; Doric). This fiber was secured to the optic fiber implanted in the animal using a ceramic sleeve (Precision Fiber Products; Milpitas, CA) and custom-made thumb screw clamp (University of Illinois, Chicago Machine Shop). This fiber carried both the excitation and emission fluorescence, which were separated by a dichroic mirror that delivered the GCaMP fluorescence to a Newport Visible Femtowatt photoreceiver (Doric; delivered by 600 µm core/630 µm outer diameter, 0.48 numerical aperture patch cord, Doric). Recordings

occurred using commercially available software (Synapse; Tucker Davis Tech) at 1017.2 Hz on each day of Pavlovian conditioning. Signal was recorded for at least 10 minutes prior to the beginning of each behavioral session to permit early signal decay. Behavior was video recorded (10 frames/second) using a high definition webcam (Logitech; Lausanne, Switzerland).

Data Analysis

Data were extracted using scripts generously provided by the Lerner Lab (Lerner et al., 2015; https://github.com/talialerner/Photometry-Analysis-Shared). A 40 Hz lowpass butterworth filter was first applied to the 405 nm (isosbestic) signal. Then, both the 405 and 465 nm signals were downsampled by a factor of 10 from the original sampling rate. The processed isosbestic signal was fitted to the excitation signal using a linear fit to correct for signal decay. The GCaMP excitation signal was then normalized by subtracting the fitted isosbestic from it and dividing the difference by the fitted isosbestic, yielding the ΔF/F.

The CS response was visualized by aligning the ΔF/F to CS events (10 s prior to CS onset and 20 s following). The signal during each trial was normalized relative to the baseline of that trial using a robust median Z-score ($Z = (X - \tilde{x}) / (MAD)$; where $MAD = Median(X - \tilde{x})$) and $\tilde{x}$ = the median ΔF/F during the 10 second pre-CS period for a given trial. Differences in activity around the presentation of the CS were calculated by examining different time epochs (10 s prior to CS presentation, 10 s during CS presentation, and 10 s post CS presentation). Aggregate activity for a given day (i.e., across 25 trials of a single type) was summarized as the area under the curve (trapezoidal estimation) of the average signal during these epochs.

Naturally occurring transient activity prior to a conditioning session was quantified by transient identification. Transients were counted as events in which activity exceeded 2.91 MADs as in (Calipari et al., 2016). Transients were counted for the 5 minutes that immediately preceded the initiation of autoshaping training.

Investigator-scored time stamps of CS or Goal approach were used to compare activity patterns during individual CS and goal approach events. For each animal, the z-normalized ΔF/F signal during all approaches lasting at least 400 ms was extracted and averaged. 400 ms was selected following the qualitative assessment that the majority of approaches briefer than that threshold appeared incidental to an orienting response rather than an approach per se. On the last day of conditioning, most animals displayed both Goal-directed and CS-directed approach; however, three animals (2 Control, 1 Stress) made only CS approaches and had to be excluded from the analysis.

Statistical analyses were performed on CS type, stress condition, and training, and were conducted using mixed ANOVAs. All statistics were performed in R.

Experimental Design

All animals involved in photometry experiments recovered from surgery for 5 days prior to the initiation of CVS (or handling) procedures. From this timepoint, the experimental timeline was identical for both stressed and non-stressed animals. CVS was administered for 14 days. Following the cessation of CVS, animals were left alone in their home cages with food and water available *ad libitum* for 7 days to allow for weight recovery in CVS animals. Food restriction to 90% body-weight began three days prior to the initiation of autoshaping. Autoshaping training was conducted for at least 7 days for all animals.

Results

Chronic stress impairs conditioned CS+ approach

Acquisition of conditioned cue approach was quantified as the probability of approach, calculated as the number of trials of a given type (CS+ or CS–) in which the animal contacted the cue at least once divided by the total number of trials of that type. A 2 (stress condition) X 2 (CS type) X 7 (day) mixed ANOVA was used to analyze the effect of conditioning, stress, and reward pairing on CS approach probability (Fig. 4.1A). There was a significant 3-way interaction ($F(6,342)=4.5127$, $p=0.002036$; sphericity violated, Hyun-Feldt (HF) corrected $p=0.004332$). To interpret the 3-way interaction, 2 (stress) X 7 (day) mixed ANOVAs were run at both levels of CS. A significant interaction between stress condition and day was found for CS+ approach ($F(6, 342)=2.9237$, $p=0.008553$, sphericity violated, HF-adjusted $p=0.03542$), but not CS– approach ($F(6, 342)=2.0488$, $p=0.05876$, sphericity violated, adjusted $p=0.1100$). This interaction is explained by differences in the degree to which Stress and Control animals differed in their approach across days of training. Comparisons of approach on the first day of conditioning (Day 1) and after conditioning (Day 7) found there was no effect of stress on Day 1 approach probability ($p=0.197$, Holm adjusted) but, following 7 days of conditioning, Control animals (mean±SEM: 0.69±0.07) were significantly more likely to approach the CS+ than Stress animals (0.44±0.08; ($p=0.0358$).

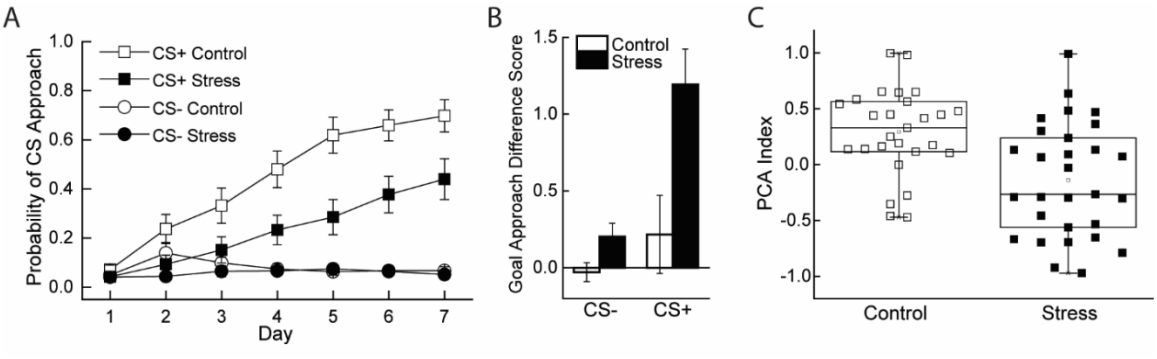

Figure 4.1. Chronic stress impairs conditioned approach directed at the CS+. (A.) Approach probability directed at the CS across daily training sessions. Chronic stress (n=30) reduced CS+ approach on Day 7 compared to the Control condition (n=29) [F(1, 57)=5.949,p=0.0358, Holm corrected]. The CS– failed to promote approach in either condition. (B.) On day 7, conditioned goal approach was quantified by calculating a difference score between goal approaches during the CS and goal approaches 10 s prior to the CS. Compared to the Control condition, chronic stress significantly increased goal approaches in response to the CS+ [F(1,57)=9.493, p=0.00317]. (C.) PCA index was calculated for all animals using CS+ and goal approach behavior in the manner of (Meyer et al., 2012). A positive score indicates CS+-directed behavior while a negative score indicates goal-directed behavior. This metric confirms the observation of a range of behavior in both groups, but a significant stress-induced change in approach behavior [F(1,54)=12.63, p=0.000797].

Chronic stress enhances conditioned goal approach

Conditioned goal approach was also examined. Head entries into the area of the food cup were counted during both the 10 s CS presentation period and the preceding 10 seconds for each trial. The goal approach difference score for a given trial was calculated by subtracting the number of pre-CS head entries from head entries within the CS period during that trial. The count for all trials on the last day of conditioning was then averaged across all trials of the same CS type. A 2 (stress condition) X 2 (CS type) mixed ANOVA found a significant interaction between stress condition and CS type on relative head entries ($F(1, 57)=6.1811$, $p=0.01586$; Fig. 4.1B). Animals in the stress condition made more relative head entries than Control animals during CS+ presentation ($F(1,57)=9.493$, $p=0.00317$, partial $\eta^2=0.143$) and CS– presentation ($F(1,57)=4.698$, $p=0.0344$ partial $\eta^2=0.076$), but this effect was much larger on CS+ trials (Stress: 1.29±0.24; Control: 0.22±0.26) than CS– trials (Stress: 0.20±0.09; Control: –0.03±0.06).

Individual differences in the tendency to engage in conditioned CS+ or Goal approach behavior were examined by calculating a composite PCA index in the manner of Meyer et al. (2012). This score was calculated as the average of three metrics: CS+ over Goal approach preference; Probability of CS+ over Goal approach; and Latency to approach. This index falls on a scale between -1, indicating exclusively Goal approach, and +1, indicating exclusively CS+ approach. A 1-way ANOVA compared the PCA Indices of Stress and Control animals on the last day of conditioning. There was a significant effect of stress experience ($F(1,54)=12.63$, $p=0.000797$), such that Control animals as a group displayed more CS+ approach behavior (0.29±0.07) while Stress animals engaged in more goal approach behavior (-0.14±0.09; Fig. 4.1C).

Chronic stress does not alter naturally occurring, non-task related, PL-NAcC activity

Selective expression of GCaMP6(f) in PL-NAcC neurons combined with optic-fiber implantation in the PL was used to monitor PL-NAcC activity during autoshaping conditioning (Fig. 4.2).

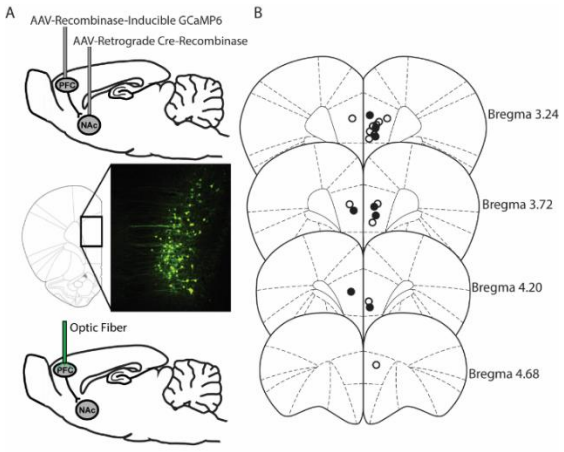

Figure 4.2. Technical approach for fiber-photometric monitoring of PL-NAcC activity. (A.) Viral strategy for selective expression of GCaMP6(f) and example micrograph. NAcC: retrograde AAV2-CAG-Cre; PL: AAV1-hSyn-FLEX-GCaMP6f-WPRE.SV40. Fiber implanted in PL. (B.) Optical fiber placement in animals used for recording (Control: n=13; Stress: n=8). Circles indicate histologically verified fiber tips that terminated in a region of the PL in which GCaMP6(f) expression was also verified. Open circles indicate placements in Control animals, while closed circles indicate placements in Stress animals.

Signal was recorded for the duration of the behavioral session and for at least 5 minutes prior to the first CS presentation on each conditioning day. Non-task related, naturally occurring coordinated neural activity was examined by identifying and comparing transient activity in Control and Stress conditions. A transient was defined as any period in which the ΔF/F exceeded 2.91 median absolute deviations (Fig. 4.3 A and B). A 2 X 2 mixed ANOVA was conducted to compare the effect of conditioning on transient activity during this baseline period in both Stress and Control animals. Neither main effects (stress condition: $F(1,19)=0.0849$, $p=0.7739$; day: $F(1,19)=0.9465$, $p=0.3428$) nor an interaction ($F(1,19)=1.6156$, $p=0.2190$) were found (Fig. 4.3C).

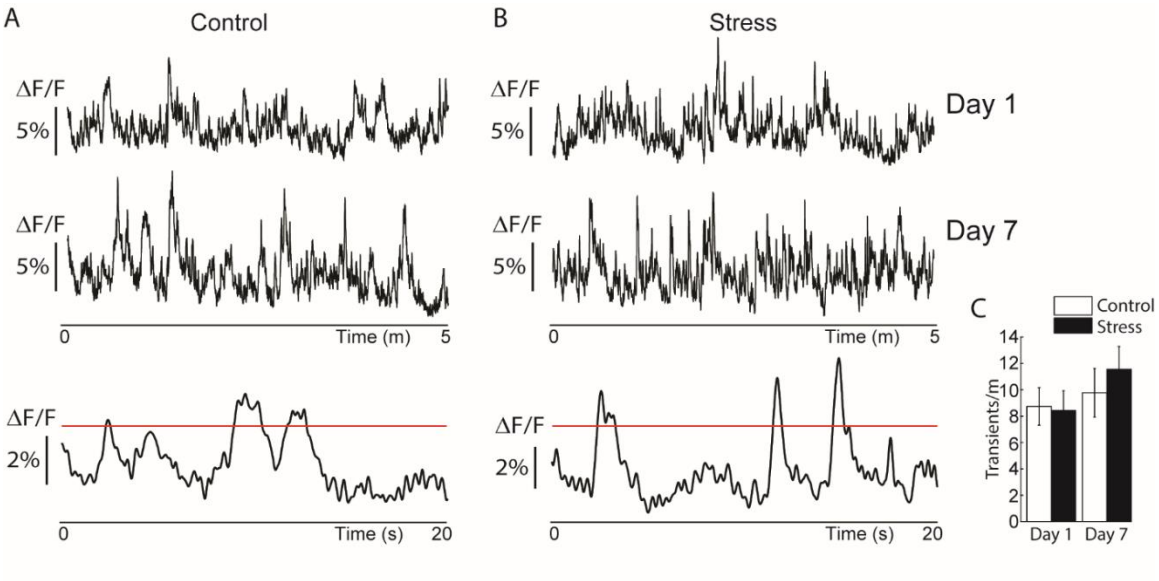

Figure 4.3. Chronic stress does not alter non-task related transient activity. Naturally occurring, non-task related activity from a single Control (A.) and Stress (B.) animal. Each recording occurred five-minutes prior to the onset of the autoshaping session on the first day (top trace) and last day (middle trace) of conditioning. Recordings were used to identify and count transients (activity peaks greater than 2.91 median absolute deviations, MAD). The lower trace depicts a 20 s segment of an above trace with the MAD illustrated as a red horizontal line. (C.) Non-task related activity was quantified for all animals. There was neither an effect of conditioning [$F(1,19)=0.9465$, $p=0.3428$] nor an effect of stress [$F(1,19)=0.0849$, $p=0.7739$] on transient frequency.

Chronic stress attenuates CS+ encoding in PL-NAcC neurons

Task-related activity on each conditioning trial was examined across days for each animal. Representative colorplots of activity illustrate differences in the development of activity related to the CS and reward in Control and Stress animals (Fig. 4.4A). This difference was analyzed at the beginning (Early Training) and last day of conditioning (Late Training, Fig. 4.4B) to test the hypothesis that stress disrupts conditioned cue encoding. A 2 (stress condition) X 2 (Early vs. Late Training) X 2 (CS type) mixed ANOVA analyzed the area under the curve (AUC) of the signal during the 10 s CS presentation (Fig. 4.4C). This analysis found a significant interaction between Stress experience and Training ($F(1,19)=5.8014$, $p=0.02633$) and a significant main effect of CS type ($F(1,19)=8.2782$, $p=0.00965$). To test the hypothesis that stress experience would interfere with the acquisition of CS+ encoding, the planned comparison did not include the CS– response. One-way (Early vs. Late Training) ANOVAs at each level of Stress found a significant effect of Training on signal magnitude for Control animals ($F(1,12)=5.3627$, $p=0.0391$; Early Training: 11.16±2.24; Late Training: 19.53±4.47) but not Stress animals ($F(1,7)=0.1515$, $p=0.709$; Early Training: 4.47±1.19; Late Training: 5.32±2.72).

Similar analyses examined the 10 s period following the termination of the CS. This period coincides with reward delivery on CS+ trials. For this period, a significant interaction was found between Stress experience and CS type ($F(1,19)=4.7677$, $p=0.0417$) as was a significant main effect of Training ($F(1,19)=9.1781$, $p=0.006895$), such that autoshaping increased pathway activity in the period following CS termination. The interaction was interpreted by collapsing across Training and performing separate one-way ANOVAs on CS type for Control and Stress animals. Only Control animals showed a significant increase in activity during the post-CS+ period relative to the post-CS– period ($F(1,12)=6.9632$, $p=0.0216$; CS+: 17.81±3.19; CS–: 7.50±1.65). Stress

animals did not display a difference in their encoding (F(1,7)=1.1305, p=0.322; CS+: 7.99±2.88; CS–: 4.88±1.88), indicating that, unlike in Control animals, pathway activity in these animals did not discriminate between reward delivery and the absence thereof.

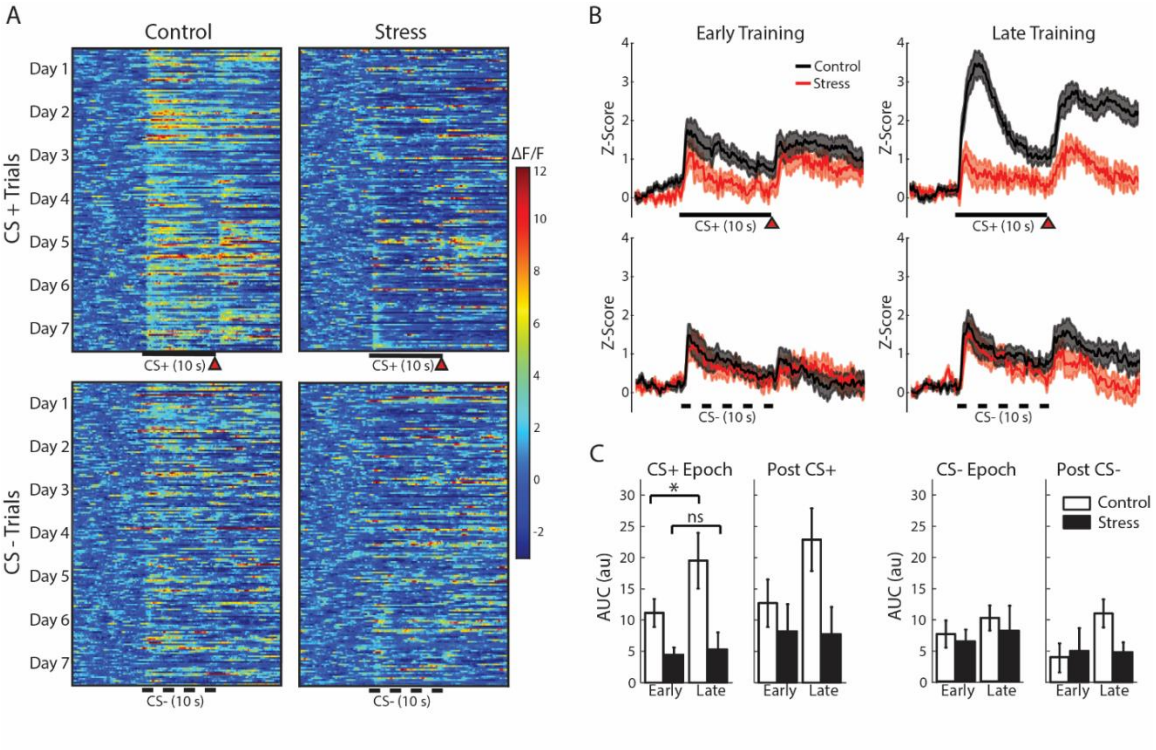

Figure 4.4. Chronic stress reduces CS+ evoked PL-NAcC Activity. (A.) Representative fiber photometric monitoring of trial by trial CS+ and CS– encoding in PL-NAcC neurons over 7 days of conditioning in a Control and Stress animal. Thirty second traces of ΔF/F (represented in pseudo-color) are aligned to 175 CS+ (solid line) and CS– (dashed line) trials for representative Control and Stress animals. Reward delivery is indicated by a red triangle. (B.) Mean (±SEM in shaded area) PL-NAcC activity during the beginning (Early Training) and last day (Late Training) of conditioning during CS+ (solid line) and CS– (dashed line) trials. Reward delivery is indicated by a red triangle. (C.) Activity during the 10 s CS period and 10 s post CS period was quantified as area under the curve (AUC) for each animal in each condition. In Control animals, conditioning significantly increased pathway activity during CS+ [F(1,12)=5.3627, p=0.0391], but not CS–, trials. Stress prevented this effect. Control animals also showed significant increase in activity during the post CS+ period following conditioning, but not during the post-CS– period, trials [F(1, 12)=6.9632, p=0.0216]. Animals in the stress condition did not significantly alter pathway activity following training.

PL-NAcC activity does not predict the direction of conditioned approach

The possibility that quantitatively different PL-NAcC activity patterns could be associated with different types of approach behavior (CS+ vs. Goal) was also examined

on the last day of conditioning (Fig. 4.5A). An approach was defined as the animal contacting either the CS or food cup. Timestamps of both initiation and cessation of approach were marked and the average signal during these types of approach was calculated. A 2 (Stress vs. Control) X 2 (CS vs. Goal) ANOVA found a significant main effect of Stress experience ($F(1,16)=8.617$, $p=0.009699$), but no effect of Approach Type ($F(1,16)=2.16$, $p=0.161$) nor an interaction ($F(1,16=0.0032$, $p=0.955$; Fig. 4.5B). To assess the possibility that variability in signal magnitude related to individual behavioral variability, Pearson correlations were performed between average signal during CS+ Approach and PCA Index within both Stress and Control groups (Fig. 4.5C). Neither Stress ($r = 0.395$, $p = 0.333$) nor Control ($r = –0.176$, $p = 0.565$) groups showed a significant correlation between signal and preferred direction of approach behavior.

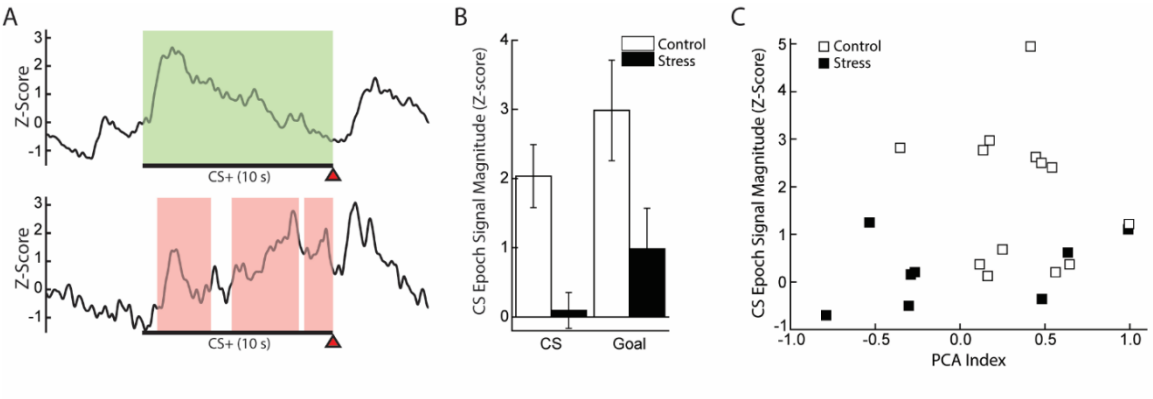

Figure 4.5. PL-NAcC activity is present during both CS+ and Goal approach behavior. (A.) Representative traces from individual trials in which Control animals displayed either a single CS+ approach (top, behavior for the duration of green overlay) or 3 separate Goal approaches (bottom, behavior for the duration of each red overlay). Reward delivery is indicated by a red triangle. (B.) Mean (±SEM) PL-NAcC activity during different types of approach. Activity during CS vs. Goal approaches did not differ [($F(1,19)=1.39$, $p=0.252$]. Stress induced a general reduction in activity regardless of approach type [$F(1,19)=6.6037$, $p=0.01875$]. (C.) Individual differences in approach behavior and PL-NAcC activity in response to the CS+. Variability in the average signal during CS-prompted approaches is not explained by individual variation in the propensity to exhibit CS+ vs. Goal approach behavior (Stress $r = 0.395$, $p = 0.395$; Control $r = 0.176$, $p = 0.565$).

Discussion

The results presented in this chapter demonstrate that prior chronic stress exposure both disrupts the encoding of a cue that promotes approach behavior and alters the nature of conditioned approach. Pavlovian autoshaping is a behavioral design in which a physical cue (CS+) predicts the delivery of a reward, usually food. Animals trained in this design develop a conditioned approach during cue presentation directed toward either the cue itself or the site of reward delivery. These two types of conditioned responses are thought to reflect different kinds of cue learning: reward port-approaching animals, referred to as goal trackers, appear to assign only predictive value to the CS+, while for cue-approaching animals, referred to as sign trackers, the CS+ appears to also take on incentive value (Robinson & Flagel, 2009). Most animals in this report displayed mixed approach. A given animal's relative likelihood to approach the cue over the food-cup can be described using a compound metric, the PCA index (Meyer et al., 2012), that averages the relative preference for CS+ over goal approach, the relative likelihood of CS+ vs. goal approach, and the relative latency of CS+ to goal approach. Using this metric, most non-stressed animals were found to develop a preference for the CS+ (as indicated by PCA index values above 0). However, experience with CVS not only reduced the tendency of animals to develop sign-tracking, but also significantly elevated their propensity to goal-track. Because both types of responses to the cue are learned, stress did not impair the ability to either learn the predictive nature of a stimulus or develop and express a conditioned response to that stimulus. Instead, animals that are most susceptible to stress appear to have a specific deficit in *incentive* learning and are capable of ascribing only *predictive* value to the reward-paired cue. This interpretation is consistent with prior research reporting intact associative learning, but disrupted cue-incentivized responding in stress exposed animals (Morgado et al., 2012).

Though chronic stress procedures have long been used to induce dysfunction in reward processing (Willner, 2017), the precise nature of that dysfunction has been the subject of recent debate. (Olney et al., 2018; Rizvi et al., 2018). As reported in Chapter 2, animals exposed to this same CVS procedure do not display hedonic deficits as measured by either taste reactivity or even the 2-bottle sucrose-preference test. Additionally, it was noted that all animals consumed the sucrose reward on all trials in the current study. Therefore, these data are consistent with the view that stress disrupts reward processing by interfering with the ability of rewarding stimuli to properly motivate behavior. In this case, rewarding stimuli failed to support the attribution of incentive to a conditioned stimulus. More work will be needed to identify the specific nature of the incentive deficit, and whether it arises at the level of perception, representation, or transfer to cues. The altered encoding of an unanticipated reward observed in the NAcSh reported in Chapter 2 suggests that the representation of incentive may be a promising place to start.

It should be noted that the tendency of most animals in this study to develop cue approach behavior is different than what has been observed by others using different parameters that produce a more even distribution of sign- and goal-tracking phenotypes (Meyer et al., 2012). One possible explanation for the distribution observed in this study is the use of food restriction in training. Deprivation states increase levels of homeostatic hormones such as ghrelin, which is known to increase the mesolimbic dopamine response to food-predictive cues (Cone, Roitman, & Roitman, 2015). Dopaminergic signaling is both associated with, and necessary for, the development of sign-tracking (Chow et al., 2016; Day, Roitman, Wightman, & Carelli, 2007; Flagel et al., 2011b). Consistent with this, chronic stress has been shown to disrupt the basal firing patterns of dopamine neurons (Tye et al., 2013). It should also be noted that the effects of stress on sign-tracking appear to depend on the nature or timing of the stress. A similar prolonged

stress procedure produced behavioral effects similar to those observed herein (Fitzpatrick et al., 2019), while social isolation during adolescence was shown to increase sign-tracking in adulthood (Beckman & Bardo, 2011), which has been associated with the development of sensitization thought to promote compulsive drug seeking (Berridge & Robinson, 1995). Thus, neither sign-tracking nor goal-tracking should be interpreted as evidence of pathology *per se*. Instead, these behaviors are an enormously valuable tool for understanding how experience modifies specific neural pathways that regulate motivated behavior, as some of these modifications may align with specific symptoms of psychopathology.

The mPFC has a particular role in learning reward contingencies (Balleine & Dickinson, 1998), and the PL subregion (in particular via its efferents to the NAcC) is necessary for cue-directed motivated behavior (McFarland et al., 2003; Otis et al., 2017). Glutamate in this pathway tracks CS+ presentation in sign-tracking animals (Batten et al., 2018). The present study employed fiber photometric recording of PL-NAcC projection neurons to monitor activity in this pathway during autoshaping training in stressed and non-stressed animals. Activity in this pathway emerged as the CS+ came to predict reward delivery only in non-stressed animals. Nonetheless, PL-NAcC neurons did not become quiescent following stress; transient analysis of pre-session baseline activity found no difference in the rate of naturally occurring, non-task related, activity. This finding recalls the context-specific, rather than resting-state, deficits observed in corticolimbic connectivity within people who suffer from MDD (Young et al., 2016). The lack of a difference in non-task related activity suggests that, following stress, neurons in this pathway may be insensitive to drive from other inputs, these inputs may themselves be compromised, or both.

In this autoshaping task the activity of PL-NAcC neurons did not appear to predict the likelihood that a given approach was directed at the CS+ or the goal. This

may reflect the complex role of the PFC in regulating conditioned approach behavior. Prelimbic neural activity has been proposed to provide top-down inhibition of autoshaping behavior that helps the animal to maintain focus on the primary reward rather than the cue (Campus et al., 2016; Paolone et al., 2013). However, autoshaping behavior is associated with glutamate release in the PL, and lesions of the mPFC can reduce autoshaping behavior (Batten et al., 2018; Serrano-Barroso et al., 2019). While the PL-NAc pathway was significantly less active during this task in stressed animals, they continued to goal-track and consume the reward, suggesting that PL-NAcC activity is not necessary for those behaviors.

The similar activity patterns in non-stressed sign- and goal-tracking animals suggests that PL-NAcC activity is not sufficient to cause the acquisition or expression of conditioned incentive directed toward the cue. It is likely, then, that PL-NAcC activity contributes to, but is not required for, this incentive. This complexity may reflect the function of other PFC projection targets, such as the PVT, that act to inhibit conditioned cue approach behavior. Increased activity of the PL-PVT pathway interferes with cue-directed behavior, while disruption of this pathway promotes attending to the cue in a similar design (Campus et al., 2019). It is possible that PL-PVT activity competes with PL-NAcC activity to direct behavior toward the goal or cue, respectively. However, when PL-NAcC activity is compromised, as after chronic stress, the balance for behavioral control is shifted.

Alternatively, these findings may indicate that stress induces a fundamental change in the processes by which animals learn and engage in behavior. Chronic stress induces atrophy in mPFC neurons (Radley et al., 2006; Dias-Ferreira et al., 2009), which are associated with goal-directed action, while simultaneously leading to hypertrophy of sensorimotor cortices (Dias-Ferreira et al., 2009), which are associated with decidedly more rigid, reflexive behaviors. Consistent with other Pavlovian approach designs, non-

stressed rats learned to associate the CS+ with reward delivery and express a conditioned response via a circuit that includes PL-NAcC projections (Otis et al., 2017). That stressed animals continued to express a conditioned response in the absence of PL-NAcC activity may indicate that learning or behavioral execution in these animals relied on separate neural circuits.

This study contributes to an emerging understanding of both how a stressful experience interferes with the acquisition of learned approach and how stress changes brain circuits involved in approach behavior. Using an intersectional approach, this report characterizes the involvement of the PL-NAcC pathway in the typical acquisition of learned approach directed at both the incentivized cue and the goal. Further, the data characterize the reduction in cue-directed behavior that accompanies stress and is associated with severely impaired cortical activity. These findings support the interpretation that stress disrupts reward processing by altering the attribution of incentive to cues. Perhaps most interesting is the possibility that the emergent behavior may be rooted in altered circuitry available for learning. Future work may characterize the mechanisms by which typically used brain circuits are dysregulated by stress and how the roles of these circuits are transferred to other areas.

V. CONCLUSIONS

Summary of Results

The neural representation of reward involves intersecting psychological constructs and brain systems that become compromised by stress. Though traditionally thought to induce a specific deficit in pleasure processing, CVS influences multiple facets of reward. The present experiments describe a behavioral deficit in incentive and motivational, rather than hedonic, domains. Concurrent with those deficits, *in vivo* electrophysiology and fiber photometry identified stress-induced alterations in neural encoding of rewards and associated cues in the NAc and PL-NAcC projection, respectively. These brain areas are critically involved in representing reward values and directing behavioral responses towards reward-paired cues. In sum, the results presented in this document are consistent with a failure of neural pathways to respond appropriately to incentive value and a corresponding impairment of motivated behavior.

Revisiting the Value of Considering the Hedonic and Motivational Components of Reward

Clinical researchers have considered the utility of dissecting the components of reward, such as hedonic “liking” and motivational “wanting”, and applying them to human disorders, including PTSD (Nawijn et al., 2015), compulsive gambling (Wölfling et al., 2011), eating disorders (Berridge, 2009; Finlayson, King, & Blundell, 2007), and beyond (Olney et al., 2018). However, this application has not been without controversy (Finlayson & Dalton, 2012; Havermans, 2011; 2012; Tibboel et al., 2011; for review see Pool, Sennwald, Delplanque, Brosch, & Sander, 2016). Some skeptics of the approach argue that these constructs cannot be separated in normal consumption because even in rodents they are primarily separated in pathology or via neural manipulations (Havermans, 2012). Indeed, this line of argument is grounded in the reasoning of one of

the leading voices advocating the value of the “liking”-“wanting” framework. In speculating as to why separate systems for these processes may have developed, Berridge (2009) ultimately concludes that “[t]he important point is that ‘liking’ and ‘wanting’ normally go together, but they can be split apart under certain circumstances, especially by certain brain manipulations.” Thus, detractors of applying “liking” and “wanting” to human behaviors have a point when leveling their critiques at studies attempting to dissociate the constructs in healthy humans (Epstein, Truesdale, Wojcik, Paluch, & Raynor, 2003; Finlayson & Blundell, 2007). This line of criticism does not, however, apply to pathologies in which hedonics and incentive may be separated. Further, some of the inconsistency in the human literature appears to relate to a failure to consistently operationalize the definitions of “liking” and “wanting” (Havermans, 2012; Pool et al., 2016). Both processes have been assessed using tasks that rely on participants’ representing expected values of future rewards, which is itself a fundamentally different process.

Another reasonable criticism of applying these constructs in human research is that they were identified using consummatory behaviors in rodents. Can a rat’s internal experience while consuming sugar water *really* be compared to the emotions a person experiences when listening to a favorite song? Indeed, is even the pleasure experienced by a person drinking sweet lemonade comparable to the pleasure that same person derives from music? Philosophers have separated rewards into “higher” and “lower” pleasures since Socrates, and this distinction is subjectively appealing. Such questions illustrate the limitations of “liking” as a purely psychological construct. However, “liking” finds more solid ground as a description of the neural processes that underlie pleasure; the same can be said for other aspects of reward processing. There appear to be core circuitry involved in processing all rewards, higher and lower, that serve as a “common

currency” for representing pleasure, incentive, and expected value (Crisp & Kringelbach, 2018).

Brain areas that contribute to reward

The neural circuitry that underly discrete facets of reward processing in rodents align to brain areas so far identified in humans as being important for pleasure, motivation, and expected pleasantness. In rodents, a chief mediator of hedonic processing is opioidergic signaling in the basal forebrain (Castro & Berridge, 2014; Peciña et al., 2003; Smith & Berridge et al., 2005; Fig. 5.1). The same region in the human brain appears to be sufficient for the experience of positive affect (Damasio, Damasio, & Tranel, 2012) and responds to preferred music (Koelsch, 2014) and “erotic pictures” (Buchel, Miedl, & Sprenger, 2018). Paralleling rodent experiments, this signaling is disrupted by opioid antagonists only during the actual experience of such stimuli, and only when those stimuli are primary reinforcers (Buchel et al., 2018). Thus, opioidergic signaling in the NAc appears to be involved in representing pleasure in humans as well. Similarly, dopaminergic signaling in the mesoaccumbens pathway appears to play a selective role in motivational “wanting” in both rodents (Peciña et al., 2003; Fig. 5.1) and humans (Evans et al., 2006; ; Guitart-Masip et al., 2012; Leyton et al., 2002; Liggins, Pihl, Benkelfat, & Leyton, 2012). Expected pleasantness in humans corresponds to representations of expected rewards and likewise appears to rely on activity in the orbitofrontal cortex and amygdala (Malvaez et al., 2019; O’Doherty, Deichmann, Critchley, & Dolan, 2002; Fig. 5.1). Behavioral control, which in this context refers to the selection and execution of volitional acts to distinguish it from both reflexes and the broader constructs so far discussed, relies on corticostriatial connectivity in both rats (Hart et al., 2018) and humans (Keeley et al., 2020). Connections between specific

subregions, the mPFC and the ventral striatum, also enable the expression of learned responses to reward associated cues (Otis et al., 2017; Fig. 5.1).

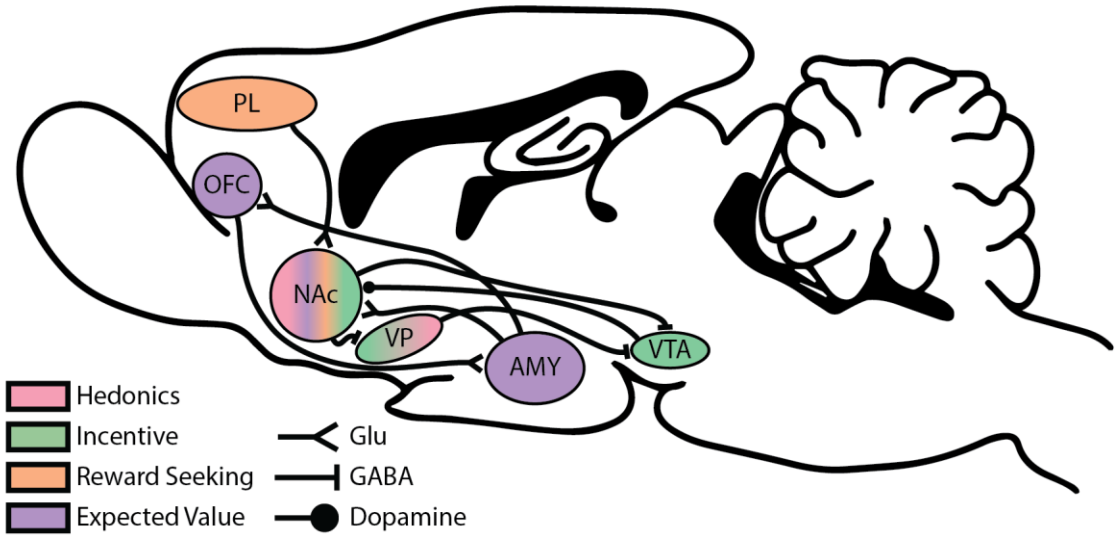

Figure 5.1 Simplified circuits for processing different facets of reward. The fill-colors of nuclei indicate their role in reward-processing and representation. The Nucleus Accumbens (NAc) and Ventral Pallidum (VP) contain hotspots for representing hedonic value. Dopamingergic inputs to the NAc arising from the Ventral Tegmental Area (VTA) encode the incentive value of rewards and controls the vigor with which rewards are pursued. Connections between the Amgydala (AMY) and Orbito-Frontal Cortex (OFC) represent the expected values of rewards associated with perceived cues, while the connection between the Prelimbic cortex (PL) and NAc enables behavioral direction in response to such cues.

Autoshaping Is a Behavioral Design That Facilitates the Discrimination of Reward Processes

The involvement of reward-related brain areas in guiding behavior can be studied using a variety of methods. Several were used in the experiments detailed herein, with an autoshaping design proving useful for understanding the regulation of motivated approach. Animals engaged in autoshaping exhibited a wide range of behavior, with some animals primarily exhibiting cue-directed approach, and others goal-directed approach. As stated earlier, animals at the ends of this continuum are often referred to as sign-trackers and goal-trackers, respectively. Both sign- and goal-trackers learn behavioral responses to reward-paired cues. In sign-trackers, the cue-directed response

indicates that the cue has taken on incentive value particular to the paired reward (Davey & Cleland, 1981), while goal-trackers appear to assign the cue only predictive value.

These behaviors rely on divergent neural activity patterns (Flagel et al., 2011a). The circuit that supports sign-tracking is organized around promoting dopaminergic release in the NAc (Fig. 5.2). Dopamine signaling is necessary for the acquisition of this behavior (Flagel et al., 2011b), specifically in the NAc (Chow et al., 2016). Prelimbic projections to the PVT are a critical *negative* regulator of dopamine release in the NAcSh (Campus et al., 2019), and stimulation of the PL-PVT pathway decreases sign-tracking in trained animals (Campus et al., 2019). Either inhibiting this same pathway or lesioning the PVT both *increase* the propensity to sign-track (Campus et al., 2019; Haight et al., 2015). In Pavlovian tone conditioning, CS+ presentation inhibits both PL-PVT and PVT-NAc activity, with disruption of the former preventing the expression of the latter (Otis et al., 2017; Otis et al., 2019). Excitation of glutamatergic PVT terminals in the NAcSh elevates population activity in the VTA (Perez & Lodge, 2018). It is speculated that this reflects a disinhibition mediated by active NAc neurons inhibiting GABAergic VP neurons that in turn project to the VTA. While this speculation was not tested, it is consistent with both the anatomy of the striato-pallidal pathway (Kupchik et al., 2015) and the necessity of a functional VP in the development of sign-tracking (Chang, Todd, Bucci, & Smith, 2015).

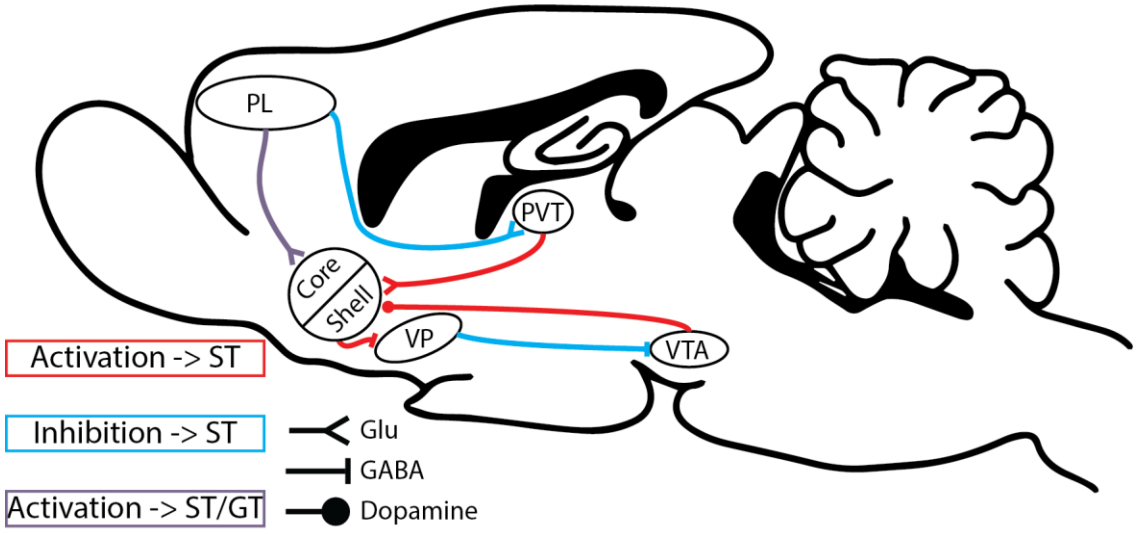

Figure 5.2 Circuitry underlying Sign-Tracking behavior. Projection pathways are color-coded based on their involvement in sign tracking. Sign-tracking requires activation of glutamatergic projections from the Paraventricular Nucleus of the Thalamus (PVT) to the Shell subregion of the Nucleus Accumbens (NAcSh). Stimulation of this pathway increases activity in Ventral Tegmental Area (VTA) neurons and dopaminergic release into the NAcSh. PVT-NAcSh modulation of VTA-NAcSh dopamine is thought to be accomplished by the inhibition (via GABAergic projections from the NAcSh) of GABAergic Ventral Pallidal (VP) neurons that project to the VTA. Activation of PVT-NAcSh neurons during the presentation of reward paired cues requires the inhibition of PVT projections arising in the Prelimbic cortex (PL). Activity in PL neurons that project to the NAc Core is proposed to mediate conditioned responding, generally.

Is all sign-tracking identical?

Differences in conditioning paradigms may lead to differences in the nature of "sign-tracking" behavior. The design used for most of the circuit-mapping described above produces a relatively even distribution of sign- and goal-trackers (Meyer et al., 2012), with the majority of animals displaying an "intermediate" phenotype (i.e. they engage in both CS and Goal approach with only a weak bias, at best, towards one over the other). However, many other research groups (including ours) find a different distribution entirely, one dominated by sign-trackers (e.g. Batten et al., 2018; Derman, Schneider, Juarez, & Delameter, 2018; K. S. Smith, personal communication, July 6, 2020; Chs. 3 and 4 of this dissertation). In some cases, the very nature of the observed "sign-tracking" conflicts with how it is commonly understood. Sign-trackers are typically

considered to be guided by “model-free” learning processes, in which behavior is directed by primitive, outcome-insensitive systems, separate from those involved in goal-tracking (Flagel et al., 2011a; Flagel et al., 2011b). However, sign-trackers produced by conditioning that primarily produces sign-tracking display both effective outcome-devaluation (Amaya, Stott, & Smith, 2020; Derman et al., 2018) and evidence that learning processes in sign- and goal-tracking overlap (Derman et al., 2018). Given that the sign-trackers produced in the present body of work come from a conditioning paradigm that primarily produces sign-trackers, it is important to consider how their behavior, and by extension the underlying circuits generating it, may differ from the more canonical sign- vs. goal-tracking circuits.

What is the role of PL-NAcC connections in sign-tracking?

Specifically, the work presented in Chapter 4 of this dissertation suggests that PL-NAcC activity may be involved in both non-canonical sign- and goal-tracking. Stress reduces both PL-NAcC encoding of reward-paired cues and the propensity of animals to approach those cues. However, this encoding is not selectively present in non-stressed animals that engage in sign-tracking. Otis and colleagues (2017) observe that inhibition of PL-NAcC neurons prevents the expression, but not acquisition, of a Pavlovian conditioned-anticipatory response. Work from instrumental tasks implicates the PL-NAcC pathway in mounting a behavioral response to reward paired cues (Woon, Sequeira, Barbee, & Gourley, 2003). Further, the PL appears to be involved in representing the availability of rewards in an environment (Mulder al., 2003). In non-appetitive learning designs, the PL is necessary for the expression of Kamin blocking effects, indicating again that it is not important for the acquisition of Pavlovian learning, but rather the representation of salience in the environment (Furlong, Cole, Hamlin, & McNally, 2010). The PL-NAcC pathway, then, may be involved in signaling that the

animal is in a context where reward is available without being involved in the specific behavior that this signal leads to, be it sign-tracking or goal-tracking.

This hypothesis is consistent with the importance of PL-NAcC activity in cue-induced reinstatement behaviors (McFarland et al., 2003; McGlinchey et al., 2016) and may suggest that Pavlovian and Instrumental behaviors are not necessarily as far apart as their distinction in some literature would suggest. Moore (2004) posits that autoshaping is an evolutionary predecessor of instrumental learning. This suggestion is founded on the fact that even early researchers of operant behavior (and acolytes of B.F. Skinner himself) noted that, with enough operant training, animals tended to "regress" to behavior that looked remarkably like sign-tracking (Breland & Breland, 1961).

Thus, activity in the PL-NAcC pathway may be related to behavioral engagement as a green light is to a car driving through an intersection: it signals that now would be an appropriate time for such an action to occur, but a separate process, a metaphorical "stepping on the gas pedal", causes the behavior. The processes responsible for selecting and engaging in the behavior determine the nature of it (e.g. sign- vs. goal tracking or volitional vs. reflexive, depending on the context). Dopamine likely has an important role in coordinating both signals. While VTA-PL activity is not itself reinforcing in the way that VTA-NAc activity is (Han et al., 2017), it promotes activity in the PL (Buctha, Mahler, Harlan, Aston-Jones, & Riegel, 2017) and is necessary for PL-NAcC control over behavioral responses to cues (McGlinchey et al., 2016). Conceiving of the PL-NAcC projection as having a much broader role than directly driving behavior is also consistent with the understanding of the role of the ventromedial PFC in humans, of which the PL is the rodent homologue (Euston, Gruber, & McNaughton, 2012). Specifically, Euston and colleagues (2012) "… propose that the function of the mPFC is to learn associations between context, locations, events, and corresponding adaptive

responses, particularly emotional responses." Activity within the PL-NAcC pathway may reflect an aspect of that broader role that is specific to motivationally salient contexts.

Stress Preferentially Impairs Volitional Reward Processes

The behavioral disturbances observed following CVS in the reported experiments are consistent with an alteration of volitional rather than hedonic processes. Stressed animals do not display any reduction in either the apparent hedonic impact of, or their preference for, a sucrose solution, even as the neural encoding of the reward is altered and less of it is consumed. Further, stress does not impair either Pavlovian or Operant learning but reduces breakpoints in a progressive ratio design and the propensity of animals to transfer incentive value to reward-paired cues. Finally, the failure to assign incentive value to reward-paired cues occurs alongside a reduction in the activity of PL-NAcC projection neurons during the presentation of those cues.

Stress impacts the neural encoding of both primary rewards and reward paired cues. With respect to the altered encoding of primary rewards, there are two interesting details to note. First, NAc single unit activity differentially encoded reward even as the hedonic impact of that reward was unaltered. Second, the difference in encoding is specific to the Shell. The NAc integrates affective information from various limbic nuclei and incorporates it into motor systems. Neurons in the Shell subregion of the NAc track the "value" of a reward (West & Carelli, 2016). As previously discussed, this value representation comprises both incentive and hedonic components. The GABAergic output neurons of the NAc, Core and Shell, may be categorized by both dopamine receptor expression (D1 vs. D2) and projection target (VP vs. mesencephalon). A subpopulation of D1 expressing neurons in the Shell projects to the lateral hypothalamus (LH) (O'Connor et al., 2015).

A hypothalamic hypothesis of stress' effects on reward encoding

One appealing, albeit speculative, interpretation of stress' effects on reward encoding focuses on the NAcSh-LH projection population of D1 MSNs. From first blush, this is an interesting population to consider because it, like the effect of stress on sucrose encoding, exists only in the NAcSh. Further, it uniformly encodes consummatory behaviors with a reduction in firing activity and inhibiting this response prematurely ceases consumption (O'Connor et al., 2015). Just such a disruption in consummatory behavior was observed in the sucrose preference test of Chapter 2. NAc activity was not monitored in this group, but it is likely that a similar change in activity was also present in this separate cohort exposed to the same stressors. In this theory the lost "inhibitory" sucrose responses seen following stress come from a selective disruption of this NAcSh-LH pathway, which results in a reduction in the drive to consume sucrose without altering the hedonic enjoyment thereof.

A dopaminergic hypothesis of stress' effects on reward encoding

Another speculative hypothesis considers the observed alteration in NAcSh activity through a dopaminergic framework. Chronic stress reduces activity in dopaminergic neurons (Tye et al., 2013) and alters NAc MSNs themselves, such that D1 MSNs both become less excitable (Francis et al., 2015) and receive less excitatory input (Lim et al., 2012) while D2 MSNs become more excitable (Francis et al., 2015). The D1 and D2 receptors meaningfully differ in their affinity for dopamine and their respective signal transduction pathways. First, D2 receptors exist primarily in a high affinity state that is likely occupied at basal levels of dopamine (Berke & Hyman, 2000; Kawagoe, Garris, Wiedemann, & Wightman, 1992; Richfield, Penney, & Young, 1989; Ross, 1991). The lower affinity D1 receptors (Richfield et al., 1989), however, are more likely to become occupied when dopamine levels increase dramatically following reward induced

burst firing of VTA neurons (Dreyer, Herrik, Berg, & Hounsgaard, 2010; Kawagoe et al., 1992; Overton and Clark, 1992; Roitman, Stuber, Phillips, Wightman, & Carelli, 2004; Shultz, Dayan, & Montague, 1997; Wheeler et al., 2015). Thus, the distinct populations of “excited” and “inhibited” neurons observed in the NAc during the delivery of a palatable taste (Roitman et al., 2005; Ch. 2) may be the distinct D1 and D2 populations displaying opposite responses to the dopamine released during that taste (Wheeler et al., 2015). Previous, unpublished work from the Wheeler lab observed that targeted inhibition of VTA dopamine neurons (via G_i DREADDs expressed in TH^+ neurons of the VTA) altered the single unit encoding of a palatable taste in much the same direction as CVS did. Finally, the totality of the behavioral studies presented here are consistent with what might be expected following dopamine depletion (Peciña et al., 1997; Flagel et al., 2011b).

While these observations strongly suggest that there is some dopaminergic involvement in the reported effect of stress on NAc activity, it remains unlikely that dopamine is the only mediator of this effect. However, the very things that makes this so unlikely–the extensive glutamatergic innervation of the NAc (Ma, Chen, Yu, & Han, 2020) and its complex microcircuitry (Burke et al., 2017)–also make it difficult to determine what the mechanistic explanation for the effect of stress on NAc reward encoding might be. That is, there are many possibilities and, without concrete knowledge vis a vis the identity of “excited” and “inhibited” palatability encoding neurons, few ways to winnow them at present. Techniques enabling targeted monitoring or manipulation of cell-type (e.g. D1 or D2 expressing MSNs) or projection specific (e.g. VTA or VP directed) populations have significantly progressed in the past decade, and this advancement gives hope for untangling previously knotty questions in the future. There are active plans in the Wheeler lab to leverage rat strains with genetically identifiable D1

or D2 expressing MSNs to identify neuronal sub-populations involved in processing the hedonic and incentive components of reward.

Hypotheses on the role of PL-NAc projections in stress' effect on sign-tracking

Stress impairs PL-NAcC encoding of a reward-paired cue and approach towards that cue. While PL-NAcC activity cannot be causally linked to sign-tracking, due its presence in non-stressed goal-trackers, the effect of stress suggests a potential correlation with the propensity of animals to sign-track. The observation that animals continue to display a conditioned response (i.e. goal-tracking) in the absence of PL-NAcC activity has multiple possible interpretations. The first (and entirely unsatisfying) possibility is that PL-NAcC activity is epiphenomenal in autoshaping. The majority of neural responses within the PL to the CS+ are reductions in firing rate (Chapter 3), suggesting that Core projecting neurons, which display an aggregate *increase* in activity (Chapter 4), are likely a small part of the PL response to the incentive cue. In this interpretation, the PL-NacC does selectively respond to some aspect of reward paired cues, but this neural signal is not involved in the behavior being studied. This possibility is unlikely because, in other Pavlovian designs, conditioned responses do not occur without PL-NAcC activity (Otis et al., 2017), suggesting that the CS+ induced activity observed herein should not be written off entirely.

Another possibility is that while PL-NAcC activity is not a causal factor in sign-tracking, it plays a permissive role in the behavior. That is to say, the presence of PL-NAcC activity in non-stressed animals may allow other processes to direct conditioned responses towards the CS+ without necessarily participating in that direction. This is an extension of the "Green-Light" theory of PL function advanced earlier. Thus, the absence of PL-NAcC activity in stressed animals would impair the ability of these hypothesized "other processes" to direct behavior towards anything other than the goal-box.

Though not mutually exclusive with the previous theory, it may also be the case that by impairing the ability of PL-NAcC neurons to participate in conditioned responding, stress biases animals towards entirely separate neural systems for learning and behavioral engagement (Dias-Ferreira et al., 2009). The systems underlying outcome dependent vs. independent behaviors are a possible substrate of this change (e.g. Balleine, 2019).

Stress May Facilitate the Engagement of Outcome-Insensitive Circuits

The concepts of outcome-dependence and outcome-independence are applied to behaviors in numerous contexts. Instrumental behaviors that are sensitive to changes in the value of outcomes are commonly referred to as “goal-directed” and considered to be based on “action-outcome” contingencies (Balleine, 2019). With sufficient overtraining, these behaviors become “habits”, which are insensitive to outcomes and based on “stimulus-response” contingencies (Smith and Graybiel, 2016). For the purposes of this discussion, the defining feature of different behaviors will be whether they are or are not sensitive to alterations in outcome value; the parameters under which they emerge–be it from Operant vs. Pavlovian conditioning or overtraining of an instrumental behavior–are of less import than this fundamental difference in flexibility.

Dissociable networks for outcome sensitivity and insensitivity

There is evidence that these different types of behavior involve dissociable neural systems. Outcome-sensitive action depends upon circuits involving the PFC, dorsomedial striatum (DMS), and NAc (Belin, Jonkman, Dickinson, Robbins, & Everitt, 2009; Yin, Ostlund, & Balleine, 2008). Outcome-insensitive circuits are rooted in motoric systems, such as sensorimotor cortex and the dorsolateral striatum (DLS) (Yin et al., 2008). Traditional models, using the frameworks of “goal-directed” vs. “habit” behavior, view outcome-insensitivity as the product of over-trained outcome-sensitive behaviors,

with the “dorsalization” of processing underlying this transition (Belin et al., 2009; Smith & Graybiel, 2013; Fig. 5.3A). However, recent evidence suggests greater complexity in the relationship between these two classes of behavior (e.g. Dezfouli & Balleine, 2013). Inhibiting circuitry necessary for learning action-outcome contingencies does not necessarily prevent the development of *prima facie* appropriate instrumental responses (Tran-Tu-Yen, Marchand, Pape, Di Scala, & Coutureau, 2009; Fig. 5.3B). In other words, in the absence of overtraining, animals can acquire outcome-insensitive behavior. A newer model proposes that contingencies mediating outcome-sensitive and outcome-insensitive behaviors develop simultaneously but are hierarchically organized such that the outcome-sensitivity dominates behavioral control (Dezfouli & Balleine, 2013). In circumstances where outcome-sensitive circuitry is unable to participate in learning, outcome-insensitive circuitry takes over and the resultant behavior reflects this (Fig. 5.3C).

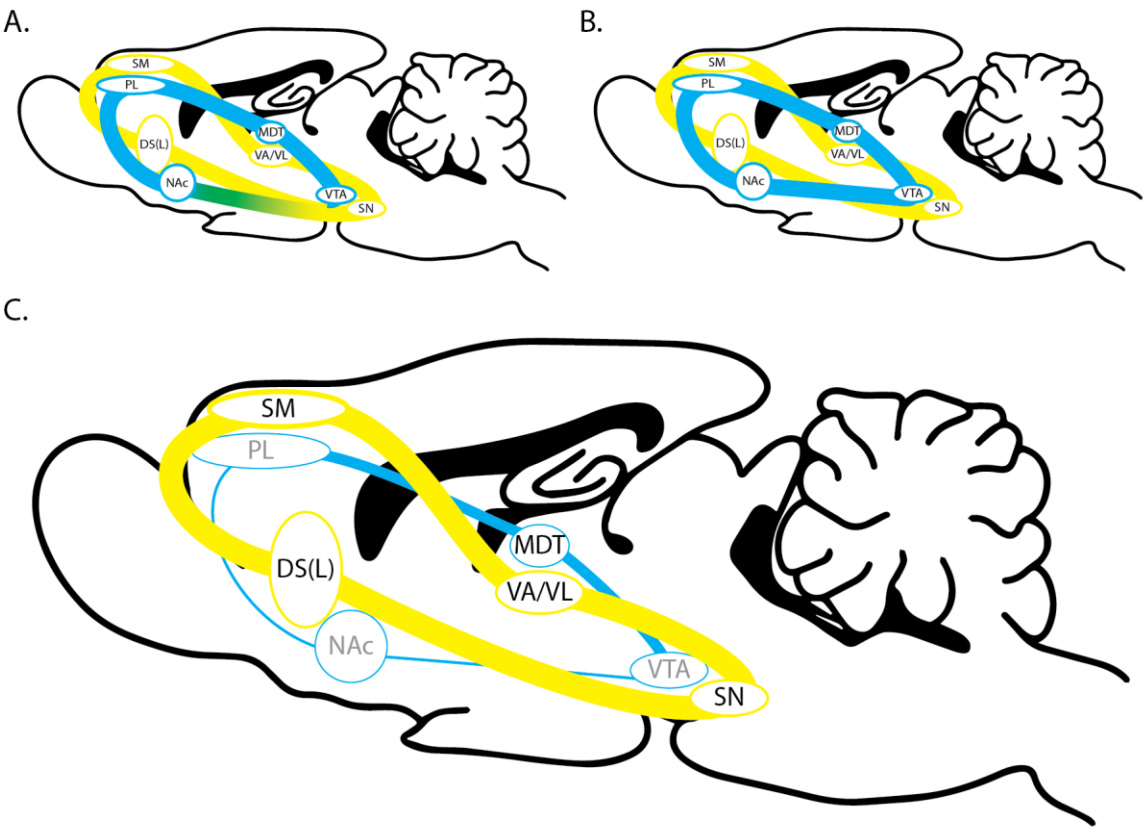

Figure 5.3 Models of the relationship between Outcome-Sensitive (OS) and Outcome-Insensitive (OI) Networks. (A.) In the "dorsalization" model, behaviors are initially acquired via processing within an OS network (cyan). Overtraining of the behavior engages the OI network (yellow), thereby altering the nature of the behavior from outcome oriented to reflexive. (B.) The "parallel" model proposes that both networks are active during the acquisition of a behavior. Within this framework, animals simultaneously acquire OS and OI contingencies. The "dorsalization" model provides a mechanism for the process by which goal-directed behaviors become habitual. The "parallel" model explains the observation that perturbations within the OS network (such as by lesion of the PL) prevents the development of only goal-directed behavior, while sparing the acquisition of inflexible, OI behavior. (C.) Stress is proposed to bias processing towards OI networks by perturbing the function both within and between the VTA, NAc, and PL . VTA: Ventral Tegmental Area; MDT: Medio-Dorsal Thalamic Nucleus; PL: Prelimbic Cortex; NAc: Nucleus Accumbens; SN: Substantia Nigra; VA/VL: Ventral-Anterior/Ventral-Lateral Thalamic Nuclei; SM: Sensorimotor Cortex; DS(L): Dorsal Striatum, Lateral Subregion.

Applying an outcome-insensitivity model of CVS effects to Pavlovian autoshaping

Chronic stress produces specific deficits in outcome sensitivity without impairing the ability of animals to acquire an instrumental response (Dias-Ferreira et al., 2009). Furthermore, chronic stress atrophies nodes within the outcome-sensitive network, while those within the outcome-insensitive network become hypertrophic (Anacker et al., 2016;

Dias-Ferreira et al., 2009). However, it is reasonable to question the applicability of this framework to Pavlovian conditioned approach, which is typically understood within a stimulus-response (i.e. outcome-insensitive) framework. Such an understanding may be overbroad: as previously discussed, sign-tracking is sometimes sensitive to changes in outcome value (Amaya et al., 2020; Derman et al., 2018).

The extent to which conditioning in the present studies produced outcome-sensitive behavior was, regrettably, never fully tested. From a neural-systems perspective, however, the presence of PL-NAcC activity during behavior in non-stressed animals is evidence that these animals engage outcome-sensitive systems during conditioned responding. Stressed animals behave without activating this circuitry. That these animals perhaps engage an alternate, outcome-insensitive circuit instead is both a reasonable and testable hypothesis. I will go further and say that, based in part on the existing literature discussed above, I propose this as a working model for understanding the consequences of PL-NAcC atrophy following stress. Identifying the circuits brought to prominence by stress experience, and the mechanisms by which this happens, may prove fruitful avenues of future inquiry.

The final question raised by these data is this: was autoshaping the best behavioral read-out for studying the effect of stress on PL-NAcC function? In retrospect, other behavioral designs may have been better suited to this end. Again, stress reduces sign-tracking, but PL-NAcC activity is not sufficient for animals to engage in sign-tracking. Unreported pilot studies from the Wheeler lab now suggest that PL-NAcC activity is not even *necessary* for sign-tracking. Of course, hindsight is 20/20, and this is not to say that the choice to use autoshaping in these experiments was a mistake. The present work enabled the determination that, in the future, a different learning design may prove more useful in the study of PL-NAcC function in health and disease.

Final Thoughts

Chronic variable stress has long been used to disrupt reward related behavior. The data presented herein offer evidence that this disruption cannot be assumed to represent “anhedonia”. These data also provide evidence that the PL-NAcC pathway, most often associated with instrumental behavior, is engaged in Pavlovian conditioning as well. Finally, stress-induced impairment of PL-NAcC activity during conditioning coincides with behavioral alterations. Characterizing the precise relationship between aberrant PL-NAcC activity and altered behavior will continue to be a promising direction for future research aiming to characterize the effects of chronic stress on reward processing and better treat debilitating psychiatric conditions.

BIBLIOGRAPHY

Aceto, G., Colussi, C., Leone, L., Fusco, S., Rinaudo, M., Scala, F., … Grassi, C. (2020). Chronic mild stress alters synaptic plasticity in the nucleus accumbens through GSK3β-dependent modulation of Kv4.2 channels. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(14), 8143–8153. https://doi.org/10.1073/pnas.1917423117

Amaya, K. A., Stott, J. J., & Smith, K. S. (2020). Sign-tracking behavior is sensitive to outcome devaluation in a devaluation context-dependent manner: Implications for analyzing habitual behavior. *Learning and Memory*, *27*(4), 136–149. https://doi.org/10.1101/lm.051144.119

Anacker, C., Scholz, J., O'Donnell, K. J., Allemang-Grand, R., Diorio, J., Bagot, R. C., … Meaney, M. J. (2016). Neuroanatomic Differences Associated with Stress Susceptibility and Resilience. *Biological Psychiatry*, *79*(10), 840–849. https://doi.org/10.1016/j.biopsych.2015.08.009

Anderson, R. M., Johnson, S. B., Lingg, R. T., Hinz, D. C., Romig-Martin, S. A., & Radley, J. J. (2019). Evidence for Similar Prefrontal Structural and Functional Alterations in Male and Female Rats Following Chronic Stress or Glucocorticoid Exposure. *Cerebral Cortex*, *30*(1), 353–370. https://doi.org/10.1093/cercor/bhz092

Balleine, B. W. (2019). The Meaning of Behavior: Discriminating Reflex and Volition in the Brain. *Neuron*, *104*(1), 47–62. https://doi.org/10.1016/j.neuron.2019.09.024

Balleine, B. W., & Dickinson, A. (1998). Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. *Neuropharmacology*, *37*, 407–419.

Bambico, F. R., Li, Z., Oliveira, C., McNeill, S., Diwan, M., Raymond, R., & Nobrega, J. N. (2019). Rostrocaudal subregions of the ventral tegmental area are differentially impacted by chronic stress. *Psychopharmacology*, *236*(6), 1917–1929. https://doi.org/10.1007/s00213-019-5177-8

Basso, A. M., & Kelley, A. E. (1999). Feeding induced by GABA(A) receptor stimulation within the nucleus accumbens shell: regional mapping and characterization of macronutrient and taste preference. *Behavioral Neuroscience*, *113*(2), 324–336. https://doi.org/10.1037//0735-7044.113.2.324

Batten, S. R., Pomerleau, F., Quintero, J., Gerhardt, G. A., & Beckman, J. J. (2018). The role of glutamate signaling in incentive salience: second-by-second glutamate recordings in awake Sprague-Dawley rats. *Journal of Neurochemistry*, *145*, 276–286. https://doi.org/10.1111/jnc.14298

Becker, C., Zeau, B., Rivat, C., Blugeot, A., Hamon, M., & Benoliel, J. J. (2008). Repeated social defeat-induced depression-like behavioral and biological alterations in rats: Involvement of cholecystokinin. *Molecular Psychiatry*, *13*(12), 1079–1092. https://doi.org/10.1038/sj.mp.4002097

Beckmann, J. S., & Bardo, M. T. (2012). Environmental enrichment reduces attribution of incentive salience to a food-associated stimulus. *Behavioural Brain Research*, *226*(1), 331–334. https://doi.org/10.1016/j.bbr.2011.09.021

Belin, D., Jonkman, S., Dickinson, A., Robbins, T. W., & Everitt, B. J. (2009). Parallel and interactive learning processes within the basal ganglia: Relevance for the understanding of addiction. *Behavioural Brain Research*, *199*(1), 89–102. https://doi.org/10.1016/j.bbr.2008.09.027

Berke, J. D., & Hyman, S. E. (2000). Addiction, Dopamine, and the Molecular Mechanisms of Memory. *Neuron*, *25*(3), 515–532. https://doi.org/https://doi.org/10.1016/S0896-6273(00)81056-9

Berlin, I., Givry-Steiner, L., Lecrubier, Y., & Puech, A. J. (1998). Measures of anhedonia and hedonic responses to sucrose in depressive and schizophrenic patients in comparison with healthy subjects. *European Psychiatry*, *13*(6), 303–309. https://doi.org/10.1016/S0924-9338(98)80048-5

Berridge, K. C., Flynn, F. W., Schulkin, J., & Grill, H. J. (1984). Sodium depletion enhances salt palatability in rats. *Behavioral Neuroscience*, *98*(4), 652–660. https://doi.org/10.1037/0735-7044.98.4.652

Berridge, K. C. (2000). Measuring hedonic impact in animals and infants: microstructure of affective taste reactivity patterns. *Neuroscience & Biobehavioral Reviews*, *24*, 173–198.

Berridge, K. C. (2009). "Liking" and "wanting" food rewards: Brain substrates and roles in eating disorders. *Physiology and Behavior*, *97*(5), 537–550. https://doi.org/10.1016/j.physbeh.2009.02.044

Berridge, K. C., & Robinson, T. E. (2003). Parsing reward. *Trends in Neurosciences*, *26*(9), 507–513. https://doi.org/10.1016/S0166-2236(03)00233-9

Berridge, K. C., & Robinson, T. E. (1995). The Mind of an Addicted Brain: Neural Sensitization of Wanting Versus Liking. *Current Directions in Psychological Science*, *4*(3), 71–76. https://doi.org/10.1111/1467-8721.ep10772316

Berridge, K. C., Robinson, T. E., & Aldridge, J. W. (2009). Dissecting components of reward: "liking", "wanting", and learning. *Current Opinion in Pharmacology*, *9*(1), 65–73. https://doi.org/10.1016/j.coph.2008.12.014

Brancato, A., Bregman, D., Ahn, H. F., Pfau, M. L., Menard, C., Cannizzaro, C., … Hodes, G. E. (2017). Sub-chronic variable stress induces sex-specific effects on glutamatergic synapses in the nucleus accumbens. *Neuroscience*, *350*, 180–189. https://doi.org/10.1016/j.neuroscience.2017.03.014

Breland, K., & Breland, M. (1961). The misbehavior of organisms. *American Psychologist*, *16*(11), 681–684. https://doi.org/10.1037/h0040090

Breslin, P. A. S., Spector, A. C., & Grill, H. J. (1992). A Quantitative Comparison of Taste Reactivity Behaviors to Sucrose Before and After Lithium Chloride Pairings: A Unidimensional Account of Palatability. *Behavioral Neuroscience*, *106*(5), 820–836. https://doi.org/10.1037/0735-7044.106.5.820

Brown, P. L., & Jenkins, H. M. (1968). Auto-shaping of the pigeon's key-peck. *Journal of the Experimental Analysis of Behavior*, *11*(I), 1–8.

Buchel, C., Miedl, S., & Sprenger, C. (2018). Hedonic processing in humans is mediated by an opioidergic mechanism in a mesocorticolimbic system. *ELife*, *7*, 1–16. https://doi.org/10.7554/eLife.39648

Buchta, W. C., Mahler, S. V., Harlan, B., Aston-Jones, G. S., & Riegel, A. C. (2017). Dopamine terminals from the ventral tegmental area gate intrinsic inhibition in the

prefrontal cortex. *Physiological Reports*, *5*(6), 1–13. https://doi.org/10.14814/phy2.13198

Burke, D. A., Rotstein, H. G., & Alvarez, V. A. (2017). Striatal Local Circuitry: A New Framework for Lateral Inhibition. *Neuron*, *96*(2), 267–284. https://doi.org/10.1016/j.neuron.2017.09.019

Calipari, E. S., Bagot, R. C., Purushothaman, I., Davidson, T. J., Yorgason, J. T., Peña, C. J., … Nestler, E. J. (2016). In vivo imaging identifies temporal signature of D1 and D2 medium spiny neurons in cocaine reward. *Proceedings of the National Academy of Sciences*, *113*(10), 2726–2731. https://doi.org/10.1073/pnas.1521238113

Campus, P., Covelo, I. R., Kim, Y., Parsegian, A., Kuhn, B. N., Lopez, S. A., … Flagel, S. B. (2019). The paraventricular thalamus is a critical mediator of top-down control of cuemotivated behavior in rats. *ELife*, *8*, 1–25. https://doi.org/10.7554/eLife.49041

Caref, K., & Nicola, S. M. (2018). Endogenous opioids in the nucleus accumbens promote approach to high-fat food in the absence of caloric need. *ELife*, *7*, 1–25. https://doi.org/10.7554/eLife.34955

Carlezon, W. A., & Thomas, M. J. (2009). Biological substrates of reward and aversion: A nucleus accumbens activity hypothesis. *Neuropharmacology*, *56*(SUPPL. 1), 122–132. https://doi.org/10.1016/j.neuropharm.2008.06.075

Castro, D. C., & Berridge, K. C. (2014). Opioid Hedonic Hotspot in Nucleus Accumbens Shell : Mu , Delta , and Kappa Maps for Enhancement of Sweetness " Liking " and " Wanting ." *The Journal of Neuroscience*, *34*(12), 4239–4250. https://doi.org/10.1523/JNEUROSCI.4458-13.2014

Chang, S. E., Todd, T. P., Bucci, D. J., & Smith, K. S. (2015). Chemogenetic manipulation of ventral pallidal neurons impairs acquisition of sign-tracking in rats. *European Journal of Neuroscience*, *42*(12), 3105–3116. https://doi.org/10.1016/j.physbeh.2017.03.040

Chang, S. E., Wheeler, D. S., & Holland, P. C. (2012). Roles of nucleus accumbens and basolateral amygdala in autoshaped lever pressing. *Neurobiology of Learning and Memory*, *97*(4), 441–451. https://doi.org/10.1038/jid.2014.371

Chow, J. J., Nickell, J. R., Darna, M., & Beckmann, J. S. (2016). Toward isolating the role of dopamine in the acquisition of incentive salience attribution. *Neuropharmacology*, *109*, 320–331. https://doi.org/10.1016/j.neuropharm.2016.06.028

Colaizzi, J. M., Flagel, S. B., Joyner, M. A., Gearhardt, A. N., Stewart, J. L., & Paulus, M. P. (2020). Mapping sign-tracking and goal-tracking onto human behaviors. *Neuroscience and Biobehavioral Reviews*, *111*(July 2019), 84–94. https://doi.org/10.1016/j.neubiorev.2020.01.018

Cone, J. J., Roitman, J. D., & Roitman, M. F. (2015). Ghrelin regulates phasic dopamine and nucleus accumbens signaling evoked by food-predictive stimuli. *Journal of Neurochemistry*, *133*(6), 844–856. https://doi.org/10.1111/jnc.13080

Contreras, R. J., & Frank, M. (1979). Sodium deprivation alters neural responses to gustatory stimuli. *Journal of General Physiology*, *73*(5), 569–594. https://doi.org/10.1085/jgp.73.5.569

Corbit, L. H., & Balleine, B. W. (2003). The role of prelimbic cortex in instrumental conditioning. *Behavioural Brain Research*, *146*(1–2), 145–157. https://doi.org/10.1016/j.bbr.2003.09.023

Coutureau, E., Esclassan, F., Di Scala, G., & Marchand, A. R. (2012). The role of the rat medial prefrontal cortex in adapting to changes in instrumental contingency. *PLoS ONE*, *7*(4). https://doi.org/10.1371/journal.pone.0033302

Coutureau, E., Marchand, A. R., & Di Scala, G. (2009). Goal-Directed Responding Is Sensitive to Lesions to the Prelimbic Cortex or Basolateral Nucleus of the Amygdala but Not to Their Disconnection. *Behavioral Neuroscience*, *123*(2), 443–448. https://doi.org/10.1037/a0014818

Covington, H. E., Lobo, M. K., Maze, I., Vialou, V., Hyman, J. M., Zaman, S., … Nestler, E. J. (2010). Antidepressant effect of optogenetic stimulation of the medial prefrontal cortex. *Journal of Neuroscience*, *30*(48), 16082–16090. https://doi.org/10.1523/JNEUROSCI.1731-10.2010

Craig, W. (1917). Appetites and Aversions As Constituents of Instincts. *Proceedings of the National Academy of Sciences of the United States of America*, *3*(12), 685–688. https://doi.org/10.2307/1536346

Crystal, S. R., & Bernstein, I. L. (1998). Infant salt preference and mother's morning sickness. *Appetite*, *30*(3), 297–307. https://doi.org/10.1006/appe.1997.0144

Damasio, A., Damasio, H., & Tranel, D. (2012). Persistence of feelings and sentience after bilateral damage of the insula. *Cerebral Cortex*, *23*(4), 833–846. https://doi.org/10.1093/cercor/bhs077

Dand, A. (1946). "Reward" and "Punishment" in Learning. *British Journal of Psychology*, *36*(2), 83–87.

Davey, G. C. L., & Cleland, G. G. (1982). Topography of Signal-Centered Behavior in the Rat: Effects of Deprivation State and Reinforcer Type. *Journal of the Experimental Analysis of Behavior*, *38*(3), 291–304. https://doi.org/10.1901/jeab.1982.38-291

Day, J. J., Roitman, M. F., Wightman, R. M., & Carelli, R. M. (2007). Associative learning mediates dynamic shifts in dopamine signaling in the nucleus accumbens. *Nature Neuroscience*, *10*(8), 1020–1028. https://doi.org/10.1038/nn1923

de Jong, J. W., Afjei, S. A., Dorocic, I. P., Peck, J. R., Liu, C., Kim, C. K., … Lammel, S. (2019). A neural circuit mechanism for encoding aversive stimuli in the mesolimbic dopamine system. *Neuron*, *101*(1), 133–151. https://doi.org/10.1016/j.neuron.2018.11.005.A

Derman, R. C., Schneider, K., Juarez, S., & Delamater, A. R. (2018). Sign-tracking is an expectancy-mediated behavior that relies on prediction error mechanisms. *Learning and Memory*, *25*(10), 550–563. https://doi.org/10.1101/lm.047365.118

Deutsch, J. A., Howarth, C. I., Ball, G. C., & Deutsch, D. (1962). Threshold Differentiation of Drive and Reward in the Olds Effect. *Nature*, *196*, 699–700. https://doi.org/10.1017/CBO9781107415324.004

Dezfouli, A., & Balleine, B. W. (2013). Actions, Action Sequences and Habits: Evidence That Goal-Directed and Habitual Action Control Are Hierarchically Organized. *PLoS Computational Biology*, *9*(12). https://doi.org/10.1371/journal.pcbi.1003364

Dias-Ferreira, E., Sousa, J. C., Melo, I., Morgado, P., Mesquita, A. R., Cerqueira, J. J., … Sousa, N. (2009). Chronic Stress Causes Frontostriatial Reorganization and Affects Decision-Making. *Science*, *325*, 621–625.

Dichter, G. S., Smoski, M. J., Kampov-Polevoy, A. B., Gallop, R., & Garbutt, J. C. (2010). Unipolar depression does not moderate responses to the sweet taste test. *Depression and Anxiety*, *27*(9), 859–863. https://doi.org/10.1002/da.20690.Unipolar

Dickinson, A., & Mulatero, C. W. (1989). Reinforcer Specificity of the Supression of Instrumental Performance on a Non-Contingent Schedule. *Behavioural Processes*, *19*, 167–180.

Dobbs, L. K., Kaplan, A. R., Lemos, J. C., Matsui, A., Rubinstein, M., & Alvarez, V. A. (2017). Dopamine regulation of lateral inhibition between striatial neurons gates the stimulant actions of cocaine. *Neuron*, *90*(5), 1100–1113. https://doi.org/10.1016/j.physbeh.2017.03.040

Dreyer, J. K., Herrik, K. F., Berg, R. W., & Hounsgaard, J. D. (2010). Influence of Phasic and Tonic Dopamine Release on Receptor Activation. *Journal of Neuroscience*, *30*(42), 14273–14283. https://doi.org/10.1523/JNEUROSCI.1894-10.2010

Drysdale, A. T., Grosenick, L., Downar, J., Dunlop, K., Mansouri, F., Meng, Y., … Liston, C. (2017). Resting-state connectivity biomarkers define neurophysiological subtypes of depression. *Nature Medicine*, *23*(1), 28–38. https://doi.org/10.1038/nm.4246.Resting-state

Epstein, L. H., Truesdale, R., Wojcik, A., Paluch, R. A., & Raynor, H. A. (2003). Effects of deprivation on hedonics and reinforcing value of food. *Physiology and Behavior*, *78*(2), 221–227. https://doi.org/10.1016/S0031-9384(02)00978-2

Euston, D. R., Gruber, A. J., & McNaughton, B. L. (2012). The Role of Medial Prefrontal Cortex in Memory and Decision Making. *Neuron*, *76*(6), 1057–1070. https://doi.org/10.1016/j.neuron.2012.12.002

Evans, A. H., Pavese, N., Lawrence, A. D., Tai, Y. F., Appel, S., Doder, M., … Piccini, P. (2006). Compulsive drug use linked to sensitized ventral striatal dopamine transmission. *Annals of Neurology*, *59*(5), 852–858. https://doi.org/10.1002/ana.20822

Finlayson, G., & Dalton, M. (2012). Current progress in the assessment of "liking" vs. "wanting" food in human appetite. Comment on " 'You Say it's Liking, I Say it's Wanting...' On the difficulty of disentangling food reward in man." *Appetite*, *58*(1), 373–378. https://doi.org/10.1016/j.appet.2011.10.011

Finlayson, G., King, N., & Blundell, J. E. (2007). Is it possible to dissociate "liking" and "wanting" for foods in humans? A novel experimental procedure. *Physiology and Behavior*, *90*(1), 36–42. https://doi.org/10.1016/j.physbeh.2006.08.020

Fitzpatrick, C. J., Jagannathan, L., Lowenstein, E. D., Robinson, T. E., Becker, J. B., & Morrow, J. D. (2019). Single prolonged stress decreases sign-tracking and cue-induced reinstatement of cocaine-seeking. *Behavioural Brain Research*, *359*(June 2018), 799–806. https://doi.org/10.1016/j.bbr.2018.07.026

Flagel, S. B., Cameron, C. M., Pickup, K. N., Watson, S. J., Akil, H., & Robinson, T. E. (2011a). A food predictive cue must be attributed with incentive salience for it to induce c-fos mRNA expression in cortico-striatal-thalamic brain regions. *Neuroscience*, *196*, 80–96. https://doi.org/10.1016/j.neuroscience.2011.09.004

Flagel, S. B., & Robinson, T. E. (2017). Neurobiological basis of individual variation in stimulus-reward learning. *Current Opinion in Behavioral Sciences*, *13*, 178–185. https://doi.org/10.1016/j.cobeha.2016.12.004

Flagel, S. B., Clark, J. J., Robinson, T. E., Mayo, L., Czuj, A., Willuhn, I., … Akil, H. (2011b). A selective role for dopamine in stimulus-reward learning. *Nature*, *469*(7328), 53–59. https://doi.org/10.1038/nature09588

Francis, T. C., Chandra, R., Friend, D. M., Finkel, E., Dayrit, G., Miranda, J., … Lobo, M. K. (2015). Nucleus accumbens medium spiny neuron subtypes mediate depression-related outcomes to social defeat stress. *Biological Psychiatry*, *77*(3), 212–222. https://doi.org/10.1016/j.biopsych.2014.07.021

Furlong, T. M., Cole, S., Hamlin, A. S., & McNally, G. P. (2010). The role of prefrontal cortex in predictive fear learning. *Behavioral Neuroscience*, *124*(5), 574–586. https://doi.org/10.1037/a0020739

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., & Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, *250*(4986), 1429–1432. https://doi.org/10.1126/science.2147780

Gerfen, C. R., & Surmeier, D. J. (2011). Modulation of Striatal Projection Systems by Dopamine. *Annual Review of Neuroscience*, *34*(1), 441–466. https://doi.org/10.1146/annurev-neuro-061010-113641

Grace, A. A. (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: a hypothesis for the etiology of schizophrenia. *Neuroscience*, *41*, 1–24.

Gracy, K. N., Svingos, A. L., & Pickel, V. M. (1997). Dual ultrastructural localization of μ-opioid receptors and NMDA-type glutamate receptors in the shell of the rat nucleus accumbens. *Journal of Neuroscience*, *17*(12), 4839–4848. https://doi.org/10.1523/jneurosci.17-12-04839.1997

Grill, H. J., & Norgren, R. (1978a). The Taste Reactivity Test. I. Mimetic Responses to Gustatory Stimuli in Neurologically Normal Rats. *Brain Research*, *143*, 263–279.

Grill, H. J., & Norgren, R. (1978b). The Taste Reactivity Test. II. Mimetric Responses to Gustatory Stimuli in Chronic Thalamic and Chronic Decerebrate Rats. *Brain Research*, *143*, 281–297.

Guitart-Masip, M., Huys, Q. J. M., Fuentemilla, L., Dayan, P., Duzel, E., & Dolan, R. J. (2012). Go and no-go learning in reward and punishment: Interactions between affect and effect. *NeuroImage*, *62*(1), 154–166. https://doi.org/10.1016/j.neuroimage.2012.04.024

Haight, J. L., Fraser, K. M., Akil, H., & Flagel, S. B. (2015). Lesions of the paraventricular nucleus of the thalamus differentially affect sign- and goal-tracking conditioned responses. *European Journal of Neuroscience*, *42*(7), 2478–2488. https://doi.org/10.1111/ejn.13031.Lesions

Hakan, R. L., & Henriksen, S. J. (1989). Opiate influences on nucleus accumbens neuronal electrophysiology: Dopamine and non-dopamine mechanisms. *Journal of Neuroscience*, *9*(10), 3538–3546. https://doi.org/10.1523/jneurosci.09-10-03538.1989

Hammond, L. J. (1980). The Effect of Contingency Upon the Appetitive Conditioning of Free-Operant Behavior. *Journal of the Experimental Analysis of Behavior*, *34*(3), 297–304. https://doi.org/10.1901/jeab.1980.34-297

Han, X., Jing, M.-Y., Zhao, T.-Y., Wu, N., Song, R., & Li, J. (2017). Role of dopamine projections from ventral tegmental area to nucleus accumbens and medial prefrontal

cortex in reinforcement behaviors assessed using optogenetic manipulation. *Metabolic Brain Disease*, *32*(5), 1491–1502. https://doi.org/10.1007/s11011-017-0023-3

Hanlon, E. C., Baldo, B. A., Sadeghian, K., & Kelley, A. E. (2004). Increases in food intake or food-seeking behavior induced by GABAergic , opioid , or dopaminergic stimulation of the nucleus accumbens : is it hunger ?, 241–247. https://doi.org/10.1007/s00213-003-1654-0

Hart, G., Bradfield, L. A., & Balleine, B. W. (2018a). Prefrontal corticostriatal disconnection blocks the acquisition of goal-directed action. *Journal of Neuroscience*, *38*(5), 1311–1322. https://doi.org/10.1523/JNEUROSCI.2850-17.2017

Hart, G., Bradfield, L. A., Fok, S. Y., Chieng, B., & Balleine, B. W. (2018b). The Bilateral Prefronto-striatal Pathway Is Necessary for Learning New Goal-Directed Actions. *Current Biology*, *28*(14), 2218-2229.e7. https://doi.org/10.1016/j.cub.2018.05.028

Havermans, R. C. (2011). "You Say it's Liking, I Say it's Wanting ...". On the difficulty of disentangling food reward in man. *Appetite*, *57*(1), 286–294. https://doi.org/10.1016/j.appet.2011.05.310

Havermans, R. C. (2012). How to tell where "liking" ends and "wanting" begins. *Appetite*, *58*(1), 252–255. https://doi.org/10.1016/j.appet.2011.10.013

Homayoun, H., & Moghaddam, B. (2009). Differential Representation of Pavlovian-Instrumental Transfer by Prefrontal Cortex Subregions and Striatum. *European Journal of Neuroscience*, *29*(7), 1461–1476. https://doi.org/10.1038/jid.2014.371

Horst, N. K., & Laubach, M. (2013). Reward-related activity in the medial prefrontal cortex is driven by consumption. *Frontiers in Neuroscience*, *7*(7 APR), 1–15. https://doi.org/10.3389/fnins.2013.00056

Insel, T. R., Cuthbert, B. N. (2015). Brain Disorders? Precisely. *Science*, *348*(6234), 499–500. https://doi.org/10.1126/science.aaa9102

Ishikawa, A., Ambroggi, F., Nicola, S. M., & Fields, H. L. (2008). Dorsomedial Prefrontal Cortex Contribution to Behavioral and Nucleus Accumbens Neuronal Responses to Incentive Cues. *Journal of Neuroscience*, *28*(19), 5088–5098. https://doi.org/10.1523/JNEUROSCI.0253-08.2008

Kawagoe, K. T., Garris, P. A., Wiedemann, D. J., & Wightman, R. M. (1992). Regulation of transient dopamine concentration gradients in the microenvironment surrounding nerve terminals in the rat striatum. *Neuroscience*, *51*(1), 55–64. https://doi.org/10.1016/0306-4522(92)90470-M

Keeley, R. J., Hsu, L. M., Brynildsen, J. K., Lu, H., Yang, Y., & Stein, E. A. (2020). Intrinsic differences in insular circuits moderate the negative association between nicotine dependence and cingulate-striatal connectivity strength. *Neuropsychopharmacology*, *45*(6), 1042–1049. https://doi.org/10.1038/s41386-020-0635-x

Kelley, A. E. (2004). Ventral striatal control of appetitive motivation: Role in ingestive behavior and reward-related learning. *Neuroscience and Biobehavioral Reviews*, *27*(8), 765–776. https://doi.org/10.1016/j.neubiorev.2003.11.015

Kelley, A. E., Bakshi, V. P., Haber, S. N., Steininger, T. L., Will, M. J., & Zhang, M. (2002). Opioid modulation of taste hedonics within the ventral striatum. *Physiology and Behavior*, *76*(April), 365–377.

Kelley, A. E., & Berridge, K. C. (2002). The neuroscience of natural rewards: relevance to addictive drugs. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *22*(9), 3306–3311. https://doi.org/20026361

Killcross, S., & Coutureau, E. (2003). Coordination of actions and habits in the medial prefrontal cortex of rats. *Cerebral Cortex*, *13*(4), 400–408. https://doi.org/10.1093/cercor/13.4.400

Koelsch, S. (2014). Brain correlates of music-evoked emotions. *Nature Reviews Neuroscience*, *15*(3), 170–180. https://doi.org/10.1038/nrn3666

Kravitz, A. V., Tye, L. D., & Kreitzer, A. C. (2012). Distinct roles for direct and indirect pathway striatal neurons in reinforcement. *Nature Neuroscience*, *15*(6), 816–818. https://doi.org/10.1038/nn.3100

Kupchik, Y. M., Brown, R. M., Heinsbroek, J. A., Lobo, M. K., Schwartz, D. J., & Kalivas, P. W. (2015). Coding the direct/indirect pathways by D1 and D2 receptors is not valid for accumbens projections. *Nature Neuroscience*, *18*(9), 1230–1232. https://doi.org/10.1038/nn.4068

Lammel, S., Ion, D. I., Roeper, J., & Malenka, R. C. (2011). Projection-Specific Modulation of Dopamine Neuron Synapses by Aversive and Rewarding Stimuli. *Neuron*, *70*(5), 855–862. https://doi.org/10.1016/j.neuron.2011.03.025

Lammel, S., Lim, B. K., & Malenka, R. C. (2014). Reward and aversion in a heterogeneous midbrain dopamine system. *Neuropharmacology*, *76*(PART B), 351–359. https://doi.org/10.1016/j.neuropharm.2013.03.019

Landes, I., Bakos, S., Kohls, G., Bartling, J., Schulte-Körne, G., & Greimel, E. (2018). Altered neural processing of reward and punishment in adolescents with Major Depressive Disorder. *Journal of Affective Disorders*, *232*(January), 23–33. https://doi.org/10.1016/j.jad.2018.01.017

Lerner, T. N., Shilyansky, C., Davidson, T. J., Evans, K. E., Kevin, T., Zalocusky, K. A., … Deisseroth, K. (2015). Intact-Brain Analyses Reveal Distinct Information Carried by SNc Dopamine Subcircuits. *Cell*, *162*(3), 635–647.

Lex, B., & Hauber, W. (2010). The role of dopamine in the prelimbic cortex and the dorsomedial striatum in instrumental conditioning. *Cerebral Cortex*, *20*(4), 873–883. https://doi.org/10.1093/cercor/bhp151

Leyton, M., Boileau, I., Benkelfat, C., Diksic, M., Baker, G., & Dagher, A. (2002). Amphetamine-induced increases in extracellular dopamine, drug wanting, and novelty seeking: A PET/[11C]raclopride study in healthy men. *Neuropsychopharmacology*, *27*(6), 1027–1035. https://doi.org/10.1016/S0893-133X(02)00366-4

Liggins, J., Pihl, R. O., Benkelfat, C., & Leyton, M. (2012). The dopamine augmenter l-dopa does not affect positive mood in healthy human volunteers. *PLoS ONE*, *7*(1), 0–5. https://doi.org/10.1371/journal.pone.0028370

Lim, B. K., Huang, K. W., Grueter, B. A., Rothwell, P. E., & Malenka, R. C. (2012). Anhedonia requires MC4R-mediated synaptic adaptations in nucleus accumbens. *Nature*, *487*(7406), 183–189. https://doi.org/10.1038/nature11160

Liu, J., Dietz, K., Hodes, G. E., Russo, S. J., & Casaccia, P. (2018). Widespread transcriptional alternations in oligodendrocytes in the adult mouse brain following chronic stress. *Developmental Neurobiology*, *78*(2), 152–162. https://doi.org/10.1002/dneu.22533

Lundy, R. F., & Norgren, R. (2015). Chapter 26 - Gustatory System. In G. B. T.-T. R. N. S. (Fourth E. Paxinos (Ed.), *The Rat Nervous System (4th Ed.)* (pp. 733–760). San Diego: Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-374245-2.00026-7

Ma, L., Chen, W., Yu, D., & Han, Y. (2020). Brain-Wide Mapping of Afferent Inputs to Accumbens Nucleus Core Subdomains and Accumbens Nucleus Subnuclei. *Frontiers in Systems Neuroscience*, *14*(March), 1–16. https://doi.org/10.3389/fnsys.2020.00015

Maldonado-Irizarry, C. S., Swanson, C. J., & Kelley, A. E. (1995). Glutamate receptors in the nucleus accumbens shell control feeding behavior via the lateral hypothalamus. *Journal of Neuroscience*, *15*(10), 6779–6788. https://doi.org/10.1523/jneurosci.15-10-06779.1995

Malvaez, M., Shieh, C., Murphy, M. D., Greenfield, V. Y., & Wassum, K. M. (2019). Distinct cortical-amygdala projections drive reward value encoding and retrieval. *Nature Neuroscience*, *22*(5), 762–769. https://doi.org/10.1038/s41593-019-0374-7.Distinct

Mansour, A., Khachaturian, H., Lewis, M. E., Akil, H., & Watson, S. J. (1988). Anatomy of CNS opioid receptors. *Trends in Neurosciences*, *11*(7). https://doi.org/10.1016/0166-2236(88)90093-8

McCarthy, P. S., Walker, R. J., & Woodruff, G. N. (1977). Depressant actions of enkephalins on neurones in the nucleus accumbens [proceedings]. *The Journal of Physiology*, *267*(1), 40P-41P.

McFarland, K., Lapish, C. C., & Kalivas, P. W. (2003). Prefrontal Glutamate Release into the Core ofthe Nucleus Accumbens Mediates Cocaine-Induced Reinstatement of Drug-Seeking Behavior. *The Journal of Neuroscience*, *23*(8), 3531–3537.

McGlinchey, E. M., James, M. H., Mahler, S. V., Pantazis, C., & Aston-Jones, G. (2016). Prelimbic to accumbens core pathway is recruited in a dopamine-dependent manner to drive cued reinstatement of Cocaine Seeking. *Journal of Neuroscience*, *36*(33), 8612–8623. https://doi.org/10.1523/JNEUROSCI.1291-15.2016

Meyer, P. J., Lovic, V., Saunders, B. T., Yager, L. M., Flagel, S. B., Morrow, J. D., & Robinson, T. E. (2012). Quantifying individual variation in the propensity to attribute incentive salience to reward cues. *PLoS ONE*, *7*(6). https://doi.org/10.1371/journal.pone.0038987

Meyerolbersleben, L., Winter, C., & Bernhardt, N. (2020). Dissociation of wanting and liking in the sucrose preference test in dopamine transporter overexpressing rats. *Behavioural Brain Research*, *378*(October 2019), 112244. https://doi.org/10.1016/j.bbr.2019.112244

Mizoguchi, K., Shoji, H., Ikeda, R., Tanaka, Y., & Tabira, T. (2008). Persistent depressive state after chronic stress in rats is accompanied by HPA axis dysregulation and reduced prefrontal dopaminergic neurotransmission. *Pharmacology, Biochemistry, and Behavior*, *91*, 170–175. https://doi.org/10.1016/j.pbb.2008.07.002

Mogenson, G. J., Jones, D. L., & Yim, C. Y. (1980). From motivation to action: functional interface between the limbic system and the motor system. *Progress in Neurobiology*, *14*, 69–97. https://doi.org/10.1016/0301-0082(80)90018-0

Moore, B. R. (2004). The evolution of learning. *Biological Reviews*, *79*, 301–335.

Morgado, P., Silva, M., Sousa, N., & Cerqueira, J. J. (2012). Stress transiently affects pavlovian-to-instrumental transfer. *Frontiers in Neuroscience*, *6*(JUN), 1–6. https://doi.org/10.3389/fnins.2012.00093

Mulder, A. B., Nordquist, R. E., Örgüt, O., & Pennartz, C. M. A. (2003). Learning-related changes in response patterns of prefrontal neurons during instrumental conditioning. *Behavioural Brain Research*, *146*(1–2), 77–88. https://doi.org/10.1016/j.bbr.2003.09.016

Muscat, R., Papp, M., & Willner, P. (1992). Reversal of stress-induced anhedonia by the atypical antidepressants, fluoxetine and maprotiline. *Psychopharmacology*, *109*(4), 433–438. https://doi.org/10.1007/BF02247719

Naneix, F., Marchand, A. R., Di Scala, G. D., Pape, J. R., & Coutureau, E. (2009). A role for medial prefrontal dopaminergic innervation in instrumental conditioning. *Journal of Neuroscience*, *29*(20), 6599–6606. https://doi.org/10.1523/JNEUROSCI.1234-09.2009

Nawijn, L., van Zuiden, M., Frijling, J. L., Koch, S. B. J., Veltman, D. J., & Olff, M. (2015). Reward functioning in PTSD: A systematic review exploring the mechanisms underlying anhedonia. *Neuroscience and Biobehavioral Reviews*, *51*, 189–204. https://doi.org/10.1016/j.neubiorev.2015.01.019

O'Connor, E. C., Kremer, Y., Lefort, S., Harada, M., Pascoli, V., Rohner, C., & Lüscher, C. (2015). Accumbal D1R Neurons Projecting to Lateral Hypothalamus Authorize Feeding. *Neuron*, *88*(3), 553–564. https://doi.org/10.1016/j.neuron.2015.09.038

O'Doherty, J. P., Deichmann, R., Critchley, H. D., & Dolan, R. J. (2002). Neural responses during anticipation of a primary taste reward. *Neuron*, *33*(5), 815–826. https://doi.org/10.1016/S0896-6273(02)00603-7

Olds, J. (1958). Self-Stimulation of the Brain. *Science*, *127*(3294), 315–324.

Olds, J., & Milner, P. (1954). Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *Journal of Comparative and Physiological Psychology*. US: American Psychological Association. https://doi.org/10.1037/h0058775

Olney, J. J., Warlow, S. M., Naffziger, E. E., & Berridge, K. C. (2018). Current perspectives on incentive salience and applications to clinical disorders. *Current Opinion in Behavioral Sciences*, *22*(Mdd), 59–69. https://doi.org/10.1016/j.cobeha.2018.01.007

Ostlund, S. B., & Balleine, B. W. (2005). Lesions of medial prefrontal cortex disrupt the acquisition but not the expression of goal-directed learning. *Journal of Neuroscience*, *25*(34), 7763–7770. https://doi.org/10.1523/JNEUROSCI.1921-05.2005

Otis, J. M., Namboodiri, V. M. K., Matan, A. M., Voets, E. S., Mohorn, E. P., Kosyk, O., … Stuber, G. D. (2017). Prefrontal cortex output circuits guide reward seeking through divergent cue encoding. *Nature*, *543*(7643), 103–107. https://doi.org/10.1038/nature21376

Otis, J. M., Zhu, M., Rodriguez-romaguera, J., Anton, E. S., Stuber, G. D., Otis, J. M., … Matan, A. M. (2019). Paraventricular Thalamus Projection Neurons Integrate Cortical and Hypothalamic Signals for Cue- Report Paraventricular Thalamus Projection Neurons Integrate Cortical and Hypothalamic Signals for Cue-Reward Processing. *Neuron*, *103*, 1–9. https://doi.org/10.1016/j.neuron.2019.05.018

Overton, P. G., & Clark, D. (1997). Burst firing in midbrain dopaminergic neurons. *Brain Research Reviews*, *25*(3), 312–334. https://doi.org/https://doi.org/10.1016/S0165-0173(97)00039-8

Paolone, G., Angelakos, C. C., Meyer, P. J., Robinson, T. E., & Sarter, M. (2013). Cholinergic control over attention in rats prone to attribute incentive salience to reward cues. *Journal of Neuroscience*, *33*(19), 8321–8335. https://doi.org/10.1523/JNEUROSCI.0709-13.2013

Peciña, S., Berridge, K. C., & Parker, L. A. (1997). Pimozide does not shift palatability: Separation of anhedonia from sensorimotor suppression by taste reactivity. *Pharmacology Biochemistry and Behavior*, *58*(3), 801–811. https://doi.org/10.1016/S0091-3057(97)00044-0

Peciña, S., Cagniard, B., Berridge, K. C., Aldridge, J. W., & Zhuang, X. (2003). Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *23*(28), 9395–9402. https://doi.org/10.1097/00008877-200409000-00094

Perez, S. M., & Lodge, D. J. (2018). Convergent inputs from the hippocampus and thalamus to the nucleus accumbens regulate dopamine neuron activity. *Journal of Neuroscience*, *38*(50), 10607–10618. https://doi.org/10.1523/JNEUROSCI.2629-16.2018

Petykó, Z., Gálosi, R., Tóth, A., Máté, K., Szabó, I., Szabó, I., … Lénárd, L. (2015). Responses of rat medial prefrontal cortical neurons to Pavlovian conditioned stimuli and to delivery of appetitive reward. *Behavioural Brain Research*, *287*, 109–119. https://doi.org/10.1016/j.bbr.2015.03.034

Pool, E., Sennwald, V., Delplanque, S., Brosch, T., & Sander, D. (2016). Measuring wanting and liking from animals to humans: A systematic review. *Neuroscience and Biobehavioral Reviews*, *63*, 124–142. https://doi.org/10.1016/j.neubiorev.2016.01.006

Price, R. B., & Duman, R. (2020). Neuroplasticity in cognitive and psychological mechanisms of depression: An integrative model. *Molecular Psychiatry*, *25*(3), 530–543. https://doi.org/10.1038/s41380-019-0615-x.Neuroplasticity

Radley, J. J., Anderson, R. M., Hamilton, B. A., Alcock, J. A., & Romig-Martin, S. A. (2013). Chronic stress-induced alterations of dendritic spine subtypes predict functional decrements in an hypothalamo-pituitary-adrenal-inhibitory prefrontal circuit. *Journal of Neuroscience*, *33*(36), 14379–14391. https://doi.org/10.1523/JNEUROSCI.0287-13.2013

Radley, J. J., Gosselink, K. L., & Sawchenko, P. E. (2009). A discrete GABAergic relay mediates medial prefrontal cortical inhibition of the neuroendocrine stress response. *Journal of Neuroscience*, *29*(22), 7330–7340. https://doi.org/10.1523/JNEUROSCI.5924-08.2009

Radley, J. J., Rocher, A. B., Miller, M., Janssen, W. G. M., Liston, C., Hof, P. R., … Morrison, J. H. (2006). Repeated stress induces dendritic spine loss in the rat medial prefrontal cortex. *Cerebral Cortex*, *16*(3), 313–320. https://doi.org/10.1093/cercor/bhi104

Radley, J. J., Rocher, A. B., Rodriguez, A., Douglas, B., Dammann, M., Mcewen, B. S., … Hof, P. R. (2008). Repeated Stress Alters Dendritic Spine Morphology in the Rat Medial Prefrontal Cortex. *Journal of Comparative Neurology*, *507*(1), 1141–1150. https://doi.org/10.1002/cne.21588.REPEATED

Reilly, S. (1999). Reinforcement value of gustatory stimuli determined by progressive ratio performance. *Pharmacology Biochemistry and Behavior*, *63*(2), 301–311. https://doi.org/10.1016/S0091-3057(99)00009-X

Ribot, T. (1896). La Psychologie des Sentiments. *Ancient Philosophy*, *7*, 287.

Richfield, E. K., Penney, J. B., & Young, A. B. (1989). Anatomical and affinity state comparisons between dopamine D1 and D2 receptors in the rat central nervous system. *Neuroscience*, *30*(3), 767–777. https://doi.org/https://doi.org/10.1016/0306-4522(89)90168-1

Rizvi, S. J., Lambert, C., & Kennedy, S. (2018). Presentation and Neurobiology of Anhedonia in Mood Disorders: Commonalities and Distinctions. *Current Psychiatry Reports*, *20*(2), 6–9. https://doi.org/10.1007/s11920-018-0877-z

Rizvi, S. J., Pizzagalli, D. A., Sproule, B. A., & Kennedy, S. H. (2016). Assessing anhedonia in depression: Potentials and pitfalls. *Neuroscience & Biobehavioral Reviews*, *65*(1), 21–35. https://doi.org/10.1002/adma.201403943.Evaluating

Robinson, T. E., & Flagel, S. B. (2009). Dissociating the Predictive and Incentive Motivational Properties of Reward-Related Cues Through the Study of Individual Differences. *Biological Psychiatry*, *65*(10), 869–873. https://doi.org/10.1016/j.biopsych.2008.09.006

Roitman, M. F., Stuber, G. D., Phillips, P. E. M., Wightman, R. M., & Carelli, R. M. (2004). Dopamine Operates as a Subsecond Modulator of Food Seeking. *Journal of Neuroscience*, *24*(6), 1265–1271. https://doi.org/10.1523/JNEUROSCI.3823-03.2004

Roitman, M. F., Wheeler, R. A., & Carelli, R. M. (2005). Nucleus accumbens neurons are innately tuned for rewarding and aversive taste stimuli, encode their predictors, and are linked to motor output. *Neuron*, *45*(4), 587–597. https://doi.org/10.1016/j.neuron.2004.12.055

Roitman, M. F., Wheeler, R. A., Tiesinga, P. H. E., Roitman, J. D., & Carelli, R. M. (2010). Hedonic and nucleus accumbens neural responses to a natural reward are regulated by aversive conditioning. *Learning and Memory*, *17*(11), 539–546. https://doi.org/10.1101/lm.1869710

Ross, S. B. (1991). Synaptic Concentration of Dopamine in the Mouse Striatum in Relationship to the Kinetic Properties of Dopamine Receptors and Uptake Mechanism. *Journal of Neurochemistry*, *56*(1), 22–29.

Rozin, P. N., & Schulkin, J. (1990). Food selection. In E. M. Stricker (Ed.), *Neurobiology of food and fluid intake* (pp. 297–328). New York, NY: Plenum Press.

Saddoris, M. P., Cacciapaglia, F., Wightman, R. M., & Carelli, R. M. (2015). Differential Dopamine Release Dynamics in the Nucleus Accumbens Core and Shell Reveal Complementary Signals for Error Prediction and Incentive Motivation. *The Journal*

of Neuroscience, *35*(33), 11572–11582. https://doi.org/10.1523/JNEUROSCI.2344-15.2015

Scheggi, S., De Montis, M. G., & Gambarana, C. (2018). Making sense of rodent models of anhedonia. *International Journal of Neuropsychopharmacology*, *21*(11), 1049–1065. https://doi.org/10.1093/ijnp/pyy083

Schultz, W., Dayan, P., & Montague, P. R. (1997). A Neural Substrate of Prediction and Reward. *Science*, *275*(5306), 1593–1599. https://doi.org/10.1126/science.275.5306.1593

Serrano-Barroso, A., Vargas, J. P., Diaz, E., O'Donnell, P., & López, J. C. (2019). Sign and goal tracker rats process differently the incentive salience of a conditioned stimulus. *PLoS ONE*, *14*(9), 1–16. https://doi.org/10.1371/journal.pone.0223109

Sherdell, L., Waugh, C. E., & Gotlib, I. H. (2012). Anticipatory pleasure predicts motivation for reward in major depression. *Journal of Abnormal Psychology*, *121*(1), 51–60. https://doi.org/10.1037/a0024945

Sidman, M., Brady, J. V., Boren, J. J., Conrad, D. G., & Schulman, A. (1955). Reward Schedules and Behavior Maintained by Intracranial Self-Stimulation. *Science*, *122*, 830–831.

Smith, K. S., & Berridge, K. C. (2005). The ventral pallidum and hedonic reward: Neurochemical maps of sucrose "liking" and food intake. *Journal of Neuroscience*, *25*(38), 8637–8649. https://doi.org/10.1523/JNEUROSCI.1902-05.2005

Smith, K. S., & Graybiel, A. M. (2016). Habit formation coincides with shifts in reinforcement representations in the sensorimotor striatum. *Journal of Neurophysiology*, *115*(3), 1487–1498. https://doi.org/10.1152/jn.00925.2015

Smith, K. S., & Graybiel, A. M. (2013). A dual operator view of habitual behavior reflecting cortical and striatal dynamics. *Neuron*, *79*(2), 361–374. https://doi.org/10.1016/j.neuron.2013.05.038

Stefanik, M. T., Moussawi, K., Kupchik, Y. M., Smith, K. C., Miller, R. L., Huff, M. L., … Lalumiere, R. T. (2013). Optogenetic inhibition of cocaine seeking in rats. *Addiction Biology*, *18*(1), 50–53. https://doi.org/10.1111/j.1369-1600.2012.00479.x

Steiner, J. E. (1973). The gustofacial response: observation on normal and anencephalic newborn infants. *Symposium on Oral Sensation and Perception*, (4), 254–278.

Sugama, S., & Kakinuma, Y. (2016). Loss of dopaminergic neurons occurs in the ventral tegmental area and hypothalamus of rats following chronic stress: Possible pathogenetic loci for depression involved in Parkinson's disease. *Neuroscience Research*, *111*, 48–55. https://doi.org/https://doi.org/10.1016/j.neures.2016.04.008

Taylor, S. B., Anglin, J. M., Paode, P. R., Riggert, A. G., Olive, M. F., & Conrad, C. D. (2014). Chronic struss may facilitate the recruitment of habit- and addiction-related neurocircuitries through neuronal restructuring of the striatum. *Neuroscience*, *280*, 231–242. https://doi.org/10.1038/jid.2014.371

Thomsen, K. R., Whybrow, P. C., & Kringelbach, M. L. (2015). Reconceptualizing anhedonia: novel perspectives on balancing the pleasure networks in the human brain. *Frontiers in Behavioral Neuroscience*, *9*(May). https://doi.org/10.3389/fnbeh.2015.00049

Thorndike, E. L. (1932). *The fundamentals of learning. The fundamentals of learning.* New York, NY, US: Teachers College Bureau of Publications. https://doi.org/10.1037/10976-000

Tibboel, H., De Houwer, J., Spruyt, A., Field, M., Kemps, E., & Crombez, G. (2011). Testing the validity of implicit measures of wanting and liking. *Journal of Behavior Therapy and Experimental Psychiatry*, *42*(3), 284–292. https://doi.org/10.1016/j.jbtep.2011.01.002

Tomie, A., Badawy, N., & Rutyna, J. (2016). Sign-Tracking Model of Loss of Self-Control of Drug-Taking. Berlin, Germany: Avid Science.

Tomie, A., Tirado, A. D., Yu, L., & Pohorecky, L. A. (2004). Pavlovian autoshaping procedures increase plasma corticosterone and levels of norepinephrine and serotonin in prefrontal cortex in rats. *Behavioural Brain Research*, *153*(1), 97–105. https://doi.org/10.1016/j.bbr.2003.11.006

Tran-Tu-Yen, D. A. S., Marchand, A. R., Pape, J. R., Di Scala, G., & Coutureau, E. (2009). Transient role of the rat prelimbic cortex in goal-directed behaviour. *European Journal of Neuroscience*, *30*(3), 464–471. https://doi.org/10.1111/j.1460-9568.2009.06834.x

Trask, S., Shipman, M. L., Green, J. T., & Bouton, M. E. (2017). Inactivation of the prelimbic cortex attenuates context-dependent operant responding. *Journal of Neuroscience*, *37*(9), 2317–2324. https://doi.org/10.1523/JNEUROSCI.3361-16.2017

Treadway, M. T., Buckholtz, J. W., Schwartzman, A. N., Lambert, W. E., & Zald, D. H. (2009). Worth the "EEfRT"? The effort expenditure for rewards task as an objective measure of motivation and anhedonia. *PLoS ONE*, *4*(8), 1–9. https://doi.org/10.1371/journal.pone.0006598

Tye, K. M., Mirzabekov, J. J., Warden, M. R., Ferenczi, E. A., Tsai, H.-C., Finkelstein, J., … Deisseroth, K. (2013). Dopamine neurons modulate neural encoding and expression of depression-related behaviour. *Nature*, *493*(7433), 537–541. https://doi.org/10.1038/nature11740

Vialou, V., Bagot, R. C., Cahill, M. E., Ferguson, D., Robison, A. J., Dietz, D. M., … Nestler, E. J. (2014). Prefrontal Cortical Circuit for Depression- and Anxiety-Related Behaviors Mediated by Cholecystokinin: Role of FosB. *Journal of Neuroscience*, *34*(11), 3878–3887. https://doi.org/10.1523/JNEUROSCI.1787-13.2014

West, E. A., & Carelli, R. M. (2016). Nucleus Accumbens Core and Shell Differentially Encode Reward-Associated Cues after Reinforcer Devaluation. *Journal of Neuroscience*, *36*(4), 1128–1139. https://doi.org/10.1523/JNEUROSCI.2976-15.2016

Wheeler, D. S., Robble, M. A., Hebron, E. M., Dupont, M. J., Ebben, A. L., & Wheeler, R. A. (2015). Drug Predictive Cues Activate Aversion-Sensitive Striatal Neurons That Encode Drug Seeking. *Journal of Neuroscience*, *35*(18), 7215–7225. https://doi.org/10.1523/JNEUROSCI.4823-14.2015

Wheeler, R. a., Twining, R. C., Jones, J. L., Slater, J. M., Grigson, P. S., & Carelli, R. M. (2008). Behavioral and Electrophysiological Indices of Negative Affect Predict Cocaine Self-Administration. *Neuron*, *57*, 774–785. https://doi.org/10.1016/j.neuron.2008.01.024

Williams, B. A. (1989). The effects of response contingency and reinforcement identity on response suppression by alternative reinforcement. *Learning and Motivation*, *20*(2), 204–224. https://doi.org/10.1016/0023-9690(89)90018-0

Willner, P. (2017). The chronic mild stress (CMS) model of depression: History, evaluation and usage. *Neurobiology of Stress*, *6*, 78–93. https://doi.org/10.1016/j.ynstr.2016.08.002

Willner, P., Towell, A., Sampson, D., Sophokleous, S., & Muscat, R. (1987). Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. *Psychopharmacology*, *93*(3), 358–364.

Wölfling, K., Mörsen, C. P., Duven, E., Albrecht, U., Grüsser, S. M., & Flor, H. (2011). To gamble or not to gamble: At risk for craving and relapse - learned motivated attention in pathological gambling. *Biological Psychology*, *87*(2), 275–281. https://doi.org/10.1016/j.biopsycho.2011.03.010

Woon, E. P., Sequeira, M. K., Barbee, B. R., & Gourley, S. L. (2020). Involvement of the rodent prelimbic and medial orbitofrontal cortices in goal-directed action: A brief review. *Journal of Neuroscience Research*, *98*(6), 1020–1030. https://doi.org/10.1002/jnr.24567

Yin, H. H., Ostlund, S. B., & Balleine, B. W. (2008). Reward-guided learning beyond dopamine in the nucleus accumbens: The integrative functions of cortico-basal ganglia networks. *European Journal of Neuroscience*, *28*(8), 1437–1448. https://doi.org/10.1111/j.1460-9568.2008.06422.x

Young, C. B., Chen, T., Nusslock, R., Keller, J., Schatzberg, A. F., & Menon, V. (2016). Anhedonia and general distress show dissociable ventromedial prefrontal cortex connectivity in major depressive disorder. *Translational Psychiatry*, *6*(5), e810. https://doi.org/10.1038/tp.2016.80

Zhang, M., & Kelley, A. E. (2000). Enhanced intake of high-fat food following striatal mu-opioid stimulation: Microinjection mapping and Fos expression. *Neuroscience*, *99*(2), 267–277. https://doi.org/10.1016/S0306-4522(00)00198-6