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Probing a hypothalamic-midbrain circuit for model-based learning and aberrant decision-making following methamphetamine

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Probing a hypothalamic-midbrain circuit for model-based learning and aberrant decision-making

following methamphetamine

A dissertation submitted in partial satisfaction of the requirements for the degree Doctor of

Philosophy in Psychology

by

Ivy Belin Hoang

2024

ABSTRACT OF THE DISSERTATION

Probing a hypothalamic-midbrain circuit for model-based learning and aberrant decision-making following methamphetamine

by

Ivy Belin Hoang

Doctor of Philosophy in Psychology

University of California, Los Angeles, 2024

Professor Melissa Sharpe, Co-Chair

Professor Alicia Izquierdo Edler, Co-Chair

Our decisions often involve consideration of prospective outcomes, which allow us to assess the consequences of our possible responses before deciding on a course of action. This process requires a detailed representation of how events are related so we can recall this information when appropriate. While normally adaptive, disruptions in this learning process can give rise to maladaptive behaviors underpinning neuropsychiatric disorders, such as with substance abuse. This dissertation investigates the neural substrates for the formation of associative maps between rewarding cues and outcomes, and how these neural circuits are changed with drug experience to contribute to maladaptive decision-making.

Inhibition of GABAergic neurons in the lateral hypothalamus (LH) revealed the LH to be important for helping to learn detailed associations between cues and rewards that can be used to influence behavior. Targeted inhibition and stimulation of dopaminergic projections from the ventral tegmental area (VTA) to the LH were identified as facilitating learning about detailed cue-reward associations in LH. A history of methamphetamine experience was shown to increase the

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control that reward cues have over decision-making and to strengthen LH-VTA circuits. To examine endogenous dopamine activity in the LH and how this might change following drug exposure, we measured dopamine release across learning of cue-reward associations in rats with or without a prior history of methamphetamine self-administration. Dopamine release in the LH was shown to increase to cues and rewards across learning, which constitutes a unique profile of dopamine activity. Importantly, prior methamphetamine self-administration was found to amplify dopamine release to reward cues that emerges across learning. Altogether, these data characterize a circuit between the hypothalamus and the midbrain that supports the acquisition of detailed cue-reward associations that are strengthened with prior drug experiences to heighten the control that reward cues have over behavior.

The dissertation of Ivy Belin Hoang is approved.

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2024

This dissertation is dedicated to my 婆婆 (po^4 po^4). Get the mahjong table ready, we have a ton of rounds to make up.

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Chapter 1: Introduction

Decisions are thought to be governed by two primary cognitive strategies: model-free learning and model-based learning (Daw et al., 2005; Dayan & Berridge, 2014). A model-free strategy promotes behavior by using arbitrary, scalar values accumulated to reward cues and actions, whereas a model-based agent employs associations developed from past experiences to influence decision-making. A healthy balance of both cognitive approaches is necessary for maximizing the efficiency and the flexibility of our day-to-day decisions. Routine tasks such as brewing yourself a cup of coffee in the morning as you get ready for work should not require mental deliberation, making a model-free approach the most optimal strategy to save you cognitive resources for more taxing decisions. However, a recent detection of prediabetes might require you to adapt to the situation and switch to a model-based approach instead – foregoing your usual vanilla creamer and using plain milk to lighten up your coffee as a sugar-free alternative. Countless examples of this balance between model-free and model-based learning can be found at every turn of our daily lives, which illustrates the importance of this process. Indeed, an imbalance in these cognitive strategies can lead to the maladaptive behaviors that arise with psychopathologies such as substance use disorders (Belin et al., 2013; Everitt & Robbins, 2005, 2016; Nelson & Killcross, 2006; Sebold et al., 2014; Sebold et al., 2017; Vandaele & Ahmed, 2021; Voon et al., 2017).

Learning components that dictate model-based behavior

Conceptually, model-based behavior is based on consideration of future outcomes and is comprised of the two fundamental building blocks of associative learning: Pavlovian learning and instrumental learning. Pavlovian learning is famously coined after Pavlov and his work classically conditioning a dog to salivate to a food-paired bell. Here, you passively experience the cues in your environment to form associations with other environmental cues. These associations provide

you with consequential or predictive information, but do not require you to act on your environment in order for the consequence to occur. Taking Pavlov's famous conditioning experiment, the dog is taught that if a bell is rung, a bowl of food follows. After enough conditioning, the bell is able to elicit a conditioned salivation response from the dog, despite food delivery being independent of whether the dog salivates or not. On the other hand, instrumental learning requires that you take a more active role in your environment for certain outcomes to occur. For example, if we were to revise Pavlov's experiment to demonstrate instrumental learning, in lieu of a bell predicting food, perhaps Pavlov's dog had to wag its tail twice in order to receive food. Here, the delivery of food is contingent on the action of the dog and is considered instrumental behavior.

Often, Pavlovian and instrumental components of learning interact (Rescorla & Solomon, 1967). In the lab, this can be studied using Pavlovian-to-Instrumental Transfer (PIT), a behavioral phenomenon in which Pavlovian cues are able to invigorate instrumental actions (Davidson et al., 1988; Rescorla & Solomon, 1967). In this procedure, subjects undergo Pavlovian conditioning to learn about cues predictive of rewards (e.g., tone→food; click→sucrose) and then, separately, instrumental training to perform actions that earn those rewards (e.g., left lever press→food; right lever press→sucrose). During the transfer test, the response pattern of instrumental lever presses is observed in the presence of the reward-predictive cues, without reward feedback. Expression of the PIT effect can be thought of as a behavioral measure for how Pavlovian cues are able to influence instrumental responding (Davidson et al., 1988; Rescorla & Solomon, 1967). There are two main types of PIT expression (Cartoni et al., 2013): general PIT, in which cues can invigorate actions towards either the same or different reward (i.e., the food-paired tone elevates pressing on the food-paired left and sucrose-paired right levers), and specific PIT, which occurs when cues specifically invigorate responding for the same reward (i.e., the food-paired tone elevates responding more on the food-paired left lever). As the Pavlovian cue-reward and instrumental action-reward components take place separately, PIT allows us to investigate how cue-evoked representations can influence actions.

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Computational reinforcement learning frameworks have mapped general PIT and specific PIT effects onto the use of model-free and model-based strategies, respectively (Dayan & Berridge, 2014). Model-free strategies explain reinforcement learning through the accumulation of scalar values across events, leading to efficient, yet inflexible responding. On the other hand, model-based strategies suggest that learning builds an internal representation of an associative network of different states and events, which can be accessed to inform appropriate responses. The general PIT effect has often been regarded as following a model-free strategy, such that the ability of cues to generally excite actions is due to value assignment of cues and responses based on previously rewarding experiences (Daw et al., 2005; Dayan & Berridge, 2014). For example, a food-paired tone cue may be arbitrarily encoded with a positive value of 1. Similarly, pressing on the left lever, which leads to food, will also have a positive value of 1. Accordingly, you might elevate responding on the left lever when the tone is presented. However, you're equally like to elevate responding on the right lever that leads to sucrose, which also has a value of 1. Thus, the expression of general PIT for the food-paired tone to promote lever-pressing non-selectively on either the left and right levers is thought to occur because cues and actions match in value, even though the cue and actions are associated with different outcome identities. Meanwhile, the specific PIT effect more closely resembles the use of a model-based approach for decision-making. Here, instrumental actions are selected based on whether their expected outcome matches the specific outcomes predicted by the Pavlovian cues, thus requiring navigation through a cognitive model encompassing associative links between cues, rewards, and actions (Daw et al., 2005; Dayan & Berridge, 2014). For example, the food-paired tone promotes more lever-pressing on the left lever, because the left lever also was paired with the same food. Additionally, less lever presses would be made on the right lever during the food-paired tone because the right lever was paired with a different outcome, sucrose. Thus, the PIT procedure allows experimenters to dissociate model-free and model-based influences in how cues exert control over instrumental behavior.

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The cognitive role of lateral hypothalamus in associative learning

The lateral hypothalamus (LH) has long been considered as the feeding center of the brain. Early studies demonstrated that lesions made in the LH would reduce spontaneous feeding behavior, suggesting the necessity of this region for food consumption and the prevention of starvation (Anand & Brobeck, 1951). Indeed, animals with lesions in their LH would often become anorexic and had to be tube-fed in order to be kept alive, a phenomenon previously described as the 'lateral hypothalamic syndrome' (Teitelbaum & Epstein, 1962). Similarly, electrical stimulation of the LH was shown to increase food intake in sated rats (Anand & Brobeck, 1951; Delgado & Anand, 1953; Hoebel & Teitelbaum, 1962; Margules & Olds, 1962). These seminal findings have been taken as evidence to suggest that the LH functions as a regulator for metabolic homeostasis. In addition to inducing feeding, the LH itself supports intracranial self-stimulation, such that subjects will perform an action to receive electrical or optical stimulation of LH (Hoebel & Teitelbaum, 1962; Margules & Olds, 1962; Olds, 1962; Urstadt & Berridge, 2020; Wise, 1974). Further, neural recordings in LH show that it responds to rewards, such as glucose and stimulation (Noritake & Nakamura, 2019; Ono et al., 1986; Otis et al., 2019). This is argued to reflect an appetitive role for this region in processing primary rewards, like food, by regulating the motivation to approach, seek, and obtain them (Olds, 1962; Stuber & Wise, 2016).

The LH is rich in cell populations that release both neurotransmitters (e.g., glutamate and GABA) and neuromodulatory peptides (e.g., orexin, leptin, melanin-concentrating hormone, agouti-related peptide, etc.) (Bonnavion et al., 2016). Given the metabolic role of the LH, these cell types have primarily been defined in modulating various homeostatic processes, such as feeding, wakefulness, and arousal (Jennings et al., 2013; Jennings et al., 2015; Lee et al., 2023; Leinninger et al., 2009; Navarro et al., 2016; Nieh et al., 2015). Two prominent classes of neurons in the LH are GABAergic and glutamatergic populations, both of which exert opposing effects on feeding (Stanley et al., 2011; Stuber & Wise, 2016). Paradoxically, inhibitory GABAergic neurons in LH (LH$_{GABA}$) stimulate feeding behaviors (Jennings et al., 2013; Lee et al., 2023), while

excitatory glutamatergic neurons in LH (LH_{glu}) inhibit feeding (Stamatakis et al., 2016). In line with this, optical stimulation studies to determine the appetitive nature of these two neuron classes found that LH_{GABA} neurons are readily self-stimulated (Jennings et al., 2015), but stimulation of LH_{glu} neurons is aversive and avoided (Jennings et al., 2013). In addition to homeostatic feeding, distinct neuronal populations within the LH have also been examined and implicated in reward-motivated behaviors, such as reward-seeking (Alonso-Lozares et al., 2024; Aston-Jones et al., 2009; Ha et al., 2023; Harris et al., 2005; Lee et al., 2023; Petrovich, 2018; Siemian et al., 2021).

Other work has investigated the output circuitry of these cell types in the LH with the midbrain, namely to the ventral tegmental area (VTA), in supporting feeding behaviors and reward motivation (Korotkova et al., 2003; Stuber & Wise, 2016; Tyree & de Lecea, 2017). Stimulation and inhibition of LH_{GABA} terminal projections in VTA have been shown to elicit or suppress feeding, respectively. In contrast, inhibition of LH_{glu} projections to VTA do not appear to play a role in these behaviors (Barbano et al., 2016; Nieh et al., 2016). In determining the appetitive nature of LH→VTA circuits, Gigante et al. (2016) first found that animals would perform instrumental responses to earn optogenetic stimulation of LH terminal projections in the VTA, replicating the original studies where the LH alone was enough to support self-stimulation (Hoebel & Teitelbaum, 1962; Margules & Olds, 1962; Olds, 1962; Urstadt & Berridge, 2020; Wise, 1974). However, this study did not specify which population of cells in the LH projecting to the VTA were mediating this effect. This was later clarified by others using a more pathway-specific optogenetic viral approach as likely mediated by GABAergic projections from the LH to the VTA (Barbano et al., 2016; Nieh et al., 2016; Schiffino et al., 2019; Siemian et al., 2021), in line with previous work showing LH_{GABA} neurons alone were rewarding (Jennings et al., 2015). Additionally, glutamatergic projections from the LH to the VTA were shown to promote aversion to a context paired with stimulation of this pathway (Nieh et al., 2016). Thus, research demonstrates the diverse cellular architecture of the LH, positioning this structure as a critical node in reward circuitry, particularly in regard to the GABAergic neurons in the LH and their output to VTA.

Building upon the role of the LH in increasing reward-motivated behavior, new research has considered a more complex role for LH beyond reward motivation in cognitive processing (Alonso-Lozares et al., 2024; Nieh et al., 2015; Sharpe, 2024; Sharpe et al., 2021; Sharpe, Marchant, et al., 2017; Siemian et al., 2021). In particular, recent work has revealed the necessity of GABAergic neurons in LH (LH$_{GABA}$) for learning about cues that predict food rewards (Sharpe et al., 2021; Sharpe, Marchant, et al., 2017). Taking advantage of the temporal precision of optogenetics, Sharpe, Marchant, et al. (2017) first demonstrated that optogenetically inhibiting LH$_{GABA}$ neurons across a reward-predictive cue during sessions of Pavlovian conditioning reduces learning about the cue. Critically, optogenetic inhibition was restricted to cue presentation and ceased before rewards were delivered, and responding for the rewards was not reduced. Thus, all rats could hear the auditory cue and experienced the food in close succession but were unable to use the cue to predict arrival of rewards. This was confirmed in an extinction test where LH$_{GABA}$ neurons were not inhibited and cues were presented without reward. Here, rats continued to show low responding to the cue, indicating an attenuation of learning and not performance. Finally, when a separate group of rats were allowed to acquire the cue-reward associations normally and optogenetic inhibition across the cue occurred during the extinction test, rats also showed reduced responding to the reward-predictive cue. This demonstrated that LH$_{GABA}$ neurons are necessary for acquisition and expression of cue-reward associations, beyond a role in homeostatic feeding.

Surprisingly, the opposite effects of LH$_{GABA}$ inhibition were seen during acquisition of cue-cue associations. Sharpe et al. (2021) used two conditioning procedures (sensory preconditioning and second-order conditioning) to determine if LH$_{GABA}$ neurons were also involved in cue-cue learning. In sensory preconditioning, two neutral cues are first paired together, where neither carry any motivational significance (e.g., A→B). Subsequently, cue B is paired with a food reward (i.e., B→food), endowing this cue with motivational significance. Learning about the food reward can be inferred by presentation of cue A (i.e., A→B→food) and is assessed in a probe test where cue A is presented alone. When GABAergic neurons in LH were optogenetically inhibited during initial

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pairing of A→B, subjects showed elevated responding toward the preconditioned cue, A, after its associate B had been paired with food. This demonstrated that inhibition of LH$_{GABA}$ neurons during cue-cue learning increased the association between the neutral cues. However, it was possible that these effects were either due to the fact that cues A and B did not hold any motivational significance at the time of learning, or that LH opposes any learning that is not directly relevant to predicting food during a session. Thus, a separate group of rats were trained using a second-order conditioning design, which is similar to sensory preconditioning except that A→B pairings are learned after cue B has been established as predictive of food. When LH$_{GABA}$ neurons were inhibited during cue-cue learning in this task, responding for cue A in the probe test was again enhanced. Thus, the motivational significance of cue A at the time of learning was not relevant in either procedure. Rather, inhibition of LH$_{GABA}$ neurons enhances learning of cues that are not directly paired with primary reward. Altogether, these findings implicate a critical role for LH$_{GABA}$ cells in Pavlovian learning for cues proximal to primary reinforcers, and away from distal predictors that are one-step removed from primary reinforcers.

Dopamine in lateral hypothalamus

The wider circuit involved in helping the LH to learn about cues and rewards is currently unknown. One potential circuit involves midbrain dopamine neurons that may project to LH (Aransay et al., 2015; Lerner et al., 2021; Nasser et al., 2017; Yang et al., 2019; Yonemochi et al., 2019). Unsurprisingly, much of what is known about dopaminergic mechanisms in this region has been with regards to the canonical function of LH for regulating the motivation to feed (Chen et al., 2014; Fetissov et al., 2000; Fetissov et al., 2002; Ikeda et al., 2018; Legrand et al., 2015; Meguid et al., 2000; Meguid et al., 1995; Parada et al., 1988; Parada et al., 1990; Parada et al., 1991; Sato et al., 2001; Yang et al., 2019; Yonemochi et al., 2019). Here, it has been argued that dopamine plays an inhibitory role in food take. That is, increases in dopamine activity is associated with lower levels of eating. D$_1$ and D$_2$ dopamine receptor subtypes are expressed in the LH

(Fetissov et al., 2002; Meguid et al., 2000; Sato et al., 2001; Yang et al., 2019). It has been shown that blockade of both D_1 and D_2 receptor subtypes with a non-selective antagonist in LH increases food intake (Ikeda et al., 2018). However, selective blockade of D_1 or D_2 receptors alone does not seem to impact food intake (Ikeda et al., 2018; Parada et al., 1991; Yonemochi et al., 2019); but see: (Chen et al., 2014; Parada et al., 1988). Based on these findings, food intake generally appears to decrease with increases in dopamine receptor activity in the LH. Microdialysis studies provide converging evidence for an inhibitory role of hypothalamic dopamine in feeding. Specifically, dopamine levels in the LH have been shown to rise when animals consume food (Fetissov et al., 2000; Ikeda et al., 2018; Legrand et al., 2015; Meguid et al., 2000; Yonemochi et al., 2019), and this increase scales with meal size (Meguid et al., 1995). Further, basal dopamine levels in the LH are also greater in fed or sated conditions relative to fasted or food-restricted conditions (Fetissov et al., 2000). Altogether, these data indicate that dopamine in the LH increases to signal food consumption, which decreases the homeostatic need for feeding and food intake.

Less is known about dopaminergic mechanisms in the LH for more cognitive processes like learning. However, some evidence that implicates this neural substrate in learning can be found with conditioned taste preference and aversion procedures (Booth, 1985; Welzl et al., 2001). Much like the Pavlovian conditioning described earlier, these procedures pair a gustatory cue (e.g., saccharin) with a reward like intragastric sugar injections (for taste preference) or lithium chloride-induced nausea (for taste aversion). Acquisition of these cue-outcome associations is typically seen if subjects continue to consume or stop consuming the gustatory cue, depending on whether it is a conditioned taste preference or taste aversion procedure, respectively. Studies have shown that D_1 and D_2 dopamine receptors in LH differentially contribute to conditioned taste preference and conditioned taste aversion (Sclafani et al., 2011; Touzani et al., 2010). Selective blockade of D_1 receptors in the LH prevents the acquisition, but not expression, of conditioned taste preference and conditioned taste aversion (Amador et al., 2014; Caulliez et al., 1996;

Sclafani et al., 2011; Touzani et al., 2009). Meanwhile, results for the selective blockade of D_2 receptors in the LH find no effects on the acquisition and expression of conditioned taste preference (Amador et al., 2014). Together, these provide some supporting evidence that dopamine contributes to learning in LH, though the specific nature of this role is not known.

Given the ventral tegmental area (VTA) is a region rich in dopamine neurons (Beier et al., 2015; Cohen et al., 2012; Morales & Margolis, 2017; Watabe-Uchida et al., 2012) and a key neural substrate heavily involved in associative learning (Keiflin & Janak, 2015; Lerner et al., 2021; Nasser et al., 2017; Schultz et al., 1997; Steinberg et al., 2013), this midbrain structure is a likely source candidate for dopamine input to the LH. As described in the previous section, an abundance of research has been dedicated to study the LH projections to VTA (primarily GABAergic connections) in reward-motivated behaviors (Aston-Jones et al., 2009; Barbano et al., 2016; Gigante et al., 2016; Harris et al., 2005; Korotkova et al., 2003; Mahler & Aston-Jones, 2012; Nieh et al., 2015; Nieh et al., 2016; Petrovich, 2018; Schiffino et al., 2019; Sharpe, Marchant, et al., 2017; Stuber & Wise, 2016; Tyree & de Lecea, 2017). However, few have examined the input from dopamine neurons in the VTA to the LH. Neuroanatomical evidence for this projection exists, yet the topography as well as the density of this projection remains unclear (Aransay et al., 2015; Bubser et al., 2005; Yonemochi et al., 2019). Further, it is unknown how this circuit might contribute to reward learning occurring in the LH (Harada et al., 2023).

Maladaptive cue-guided behavior in substance use disorder

The influence of cues in directing behavior is a relatively adaptive process under normal circumstances. However, this process can go awry with psychopathology such as in substance use disorders (Everitt et al., 2001; Hogarth et al., 2013; Keiflin & Janak, 2015; Lamb et al., 2016; Pickens et al., 2011; Volkow et al., 2010; Voon et al., 2017). It is well-documented that patients with a substance use disorder exhibit high susceptibility to drug- and other reward-predictive cues in influencing their decision-making (Carter & Tiffany, 1999a, 1999b; Volkow et al., 2010). This

vulnerability is thought to arise through long-term exposure to addictive substances and is considered one of the biggest determinants of risk for relapse (Bossert et al., 2013; Everitt et al., 2001; Everitt & Robbins, 2005; Kauer & Malenka, 2007; Koob & Volkow, 2016; Marchant et al., 2013; Pickens et al., 2011; Robinson & Berridge, 1993; Volkow et al., 2019; Volkow & Morales, 2015). The National Institute on Drug Abuse reports continued rises in reported drug overdose deaths over the last decade, primarily from synthetic opioids (e.g., fentanyl) and psychostimulants (e.g., methamphetamine and cocaine) (National Institute on Drug Abuse, 2023). In particular, the greatest percentage increase in overdose deaths resulted mainly from psychostimulants (Han et al., 2021). Given the prevalence of substance use disorders such as stimulant use disorder, it is of utmost concern to pinpoint the underlying causes and correlates for maladaptive cue-induced drug use.

Heightened responding to reward-paired cues in individuals with a history of substance use can be modeled and measured in the laboratory (Cartoni et al., 2016; Lamb et al., 2016; Marchant et al., 2013; Venniro et al., 2016). In clinical settings, we see that patients with a substance use disorder show increased desire or subjective craving in response to relevant drug-associated cues in cue reactivity paradigms (Carter & Tiffany, 1999a, 1999b; Yalachkov et al., 2012). Furthermore, the intensity of drug craving elicited by drug-related cues in these patients, measured using cue-induced relapse models, grows across abstinence, a phenomenon termed "incubation of drug craving" (Grimm et al., 2001; Liu et al., 2023; Lu et al., 2004; Marchant et al., 2013; Pickens et al., 2011; Venniro et al., 2021). Preclinical reinstatement models for the incubation of drug craving first have animals self-administer a drug paired with a discrete cue by responding on a lever (or nosepoke) to earn the drug and cue. These animals are then tested for relapse (i.e., reinstatement of responding on drug-associated lever without drug reinforcement) with this cue at different time points of abstinence. It has been shown that cue-evoked reinstatement of drug seeking becomes enhanced with protracted withdrawal. Importantly, this effect is enduring in animals across both forced abstinence (i.e., animals removed from drug

context and then later re-exposed for relapse test) and forms of voluntary abstinence (e.g., punishment-based, electric barrier around the drug-paired lever, etc.) (Venniro et al., 2016).

Despite evidence for the importance of Pavlovian cues in drug addiction, the dominating theory for this increased sensitivity to drug- and other reward-paired cues in individuals with a substance use disorder has attributed this vulnerability to the maladaptive development of instrumental behavior into model-free habits (Belin et al., 2013; Everitt & Robbins, 2005, 2016; Sebold et al., 2017; Vandaele & Ahmed, 2021). That is, individuals with a substance use disorder engage in persistent drug seeking and drug taking because these behaviors, which have repeatedly been reinforced with drug use, have now become automatic, reflexive, and despite the negative consequences. However, much of the research to support the habit theory of addiction has primarily been in the context of instrumental conditioning (Furlong et al., 2018; Furlong et al., 2017; LeBlanc et al., 2013; Leong et al., 2016; Nelson & Killcross, 2006; Schoenberg et al., 2022; Wassum et al., 2009; Zapata et al., 2010). In these studies, subjects are often put in training procedures meant to measure and test the transition from model-based actions, which are dependent on an outcome representation, to model-free habits that do not contain these representations (Furlong et al., 2018; Furlong et al., 2017; LeBlanc et al., 2013; Leong et al., 2016; Nelson & Killcross, 2006; Schoenberg et al., 2022; Wassum et al., 2009; Zapata et al., 2010). In rodents, this is usually shown in the context of devaluation studies. Here, rats are trained to perform two instrumental responses (e.g., a left or right lever press) for two food rewards (e.g., grain and sucrose pellets). Next, devaluation for one of the rewards occurs in which consumption of the reward is paired with lithium-chloride induced nausea. Following devaluation, rats are given a test to determine sensitivity to devaluation by examining their response patterns on the levers. Prior exposure to drugs of abuse, including amphetamine, have shown to render rats insensitive to devaluation, such that they continue to respond on the lever that was paired with the devalued outcome (Furlong et al., 2018; Furlong et al., 2017; LeBlanc et al., 2013; Leong et al., 2016; Nelson & Killcross, 2006; Zapata et al., 2010). Thus, the current theory for persistent drug-seeking

and drug-taking behaviors in perpetuating the ongoing cycle of addiction places an overreliance on model-free habits, making people with substance use disorders insensitive to future outcomes that can influence their decision-making. However, human studies using analogous instrumental devaluation procedures in substance-dependent individuals present mixed results (Ersche et al., 2016; Hogarth, 2012; Hogarth & Chase, 2011; Hogarth et al., 2012; Hogarth et al., 2019; Luijten et al., 2020; Sjoerds et al., 2013). Because of these discrepancies in characterizing drug-induced changes to instrumental responses across rodent and human literature, this makes it difficult to draw a definitive conclusion that maladaptive drug use is driven by model-free habits.

While changes in instrumental learning following drug exposure have been well-documented (Belin et al., 2013; Di Chiara et al., 1999; Everitt et al., 2001; Everitt & Robbins, 2005, 2016; LeBlanc et al., 2013; Lipton et al., 2019; Nordquist et al., 2007; Ostlund & Balleine, 2008; Sebold et al., 2017; Vandaele & Ahmed, 2021), the impact of drug exposure on Pavlovian cue-evoked behavior is less explored. In one study conducted in rats that received repeated injections of cocaine, subjects showed intact responding to Pavlovian cues previously paired with a devalued reward (i.e., insensitivity to devaluation), suggesting the use of model-free habits to respond to the cue (Schoenbaum & Setlow, 2005). However, prior cocaine sensitization took place in the same chambers as Pavlovian conditioning and testing, which could have confounded the results having previously associated the training/testing context with drug. Another study that tested the effects of devaluation in drug exposed animals used sensory-specific satiety of rewards instead of pairing rewards with lithium-chloride induced illness. In a sensory-specific satiety procedure, animals are conditioned to associate two distinct cues with two different rewards (e.g., grain vs. chocolate pellets). Prior to test, rats are sated on only one of these outcomes, thus selectively reducing the motivational state for that reward (i.e., devaluation), while the other is left intact. Rats that received repeated injections of fentanyl after conditioning, but before satiety and testing, also showed insensitivity to devaluation (Pickens et al., 2024). However, because drug exposure took place *after* Pavlovian conditioning, the results from this study only conclude that

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exposure to drugs of abuse promotes the expression of model-free habits, but how it affects the acquisition of Pavlovian associations is still an open question. Furthermore, inconsistent with rodent work, human studies show the opposite result and instead find devaluation sensitivity to cues in drug-dependent subjects (Ersche et al., 2016; Luijten et al., 2020). Thus, the impacts of previous drug exposure on Pavlovian processes remain unclear.

To better understand the reinforcement learning mechanisms that drive the influence of drug and other reward-predictive cues on the actions of individuals with a substance use disorder, researchers utilized the aforementioned PIT procedure to model this in humans and rodents. Previous work in this domain has demonstrated that drug-paired cues are able to invigorate instrumental responses (Corbit & Janak, 2007, 2016; Glasner et al., 2005; Hogarth et al., 2007; Krank, 2003; Krank et al., 2008; Lamb et al., 2016; LeBlanc et al., 2012). Additionally, several studies across various addictive substances such as amphetamine, cocaine, and alcohol, have shown that drug exposure can enhance the ability of non-drug reward-predictive cues to energize instrumental responding (LeBlanc et al., 2013; Ostlund et al., 2014; Pecina & Berridge, 2013; Pecina et al., 2006; Saddoris et al., 2011; Shields & Gremel, 2021; T. T. Takahashi et al., 2019; Wyvell & Berridge, 2000, 2001), though some work suggests drug exposure has the opposite effect (Hall & Gulley, 2011; Ripley et al., 2004). It should be noted, however, that most of these studies have examined the impact of drug exposure on heightening the *overall* ability of reward-predictive cues to invigorate instrumental actions, without characterizing the specific nature. In other words, these studies only use one cue and one action that are paired with a reward (e.g., click→sucrose, lever press→sucrose) to show that animals with prior exposure to drugs of abuse show a greater PIT effect than drug-naïve animals (i.e., greater instrumental responses elicited by a reward cue). Overall, these results align with clinical findings showing the heightened susceptibility to drug- and other reward-related cues seen in individuals with substance use disorders (Garbusow et al., 2016; Hogarth & Chase, 2012; Manglani et al., 2017). However, it is unclear whether drug-induced enhancements in the PIT effect are driven by the general influence

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of cues to invigorate actions that predict a different outcome than the cue (i.e., general PIT) or the ability of cues to direct behavior specifically towards actions that predict the same outcome as the cue (i.e., specific PIT). That is, it is not clear from these studies whether there is a model-free or model-based enhancement of cue control on behavior following drug exposure.

A few studies have investigated whether drug exposure also heightens the specificity of cue-guided behavior (Alarcon & Delamater, 2019; Corbit & Janak, 2007, 2016; Shiflett, 2012). For example, Corbit and Janak (2007) found that ethanol-paired cues promote specificity in responding for ethanol-paired actions, and in later work, showed that the magnitude of this specificity increases with extended instrumental training (Corbit & Janak, 2016). However, the latter study compared ethanol cues with sucrose cues, and also had an ethanol-paired lever as the only response option available, making it unclear whether this design biased ethanol-related responding. The effects found may also be confounded by caloric competition in using both ethanol and sucrose as rewards in these experiments. Further, other work by Shiflett (2012) showed that with repeated injections of amphetamine after Pavlovian and instrumental trainings but prior to the PIT test, animals do not show a specific PIT effect. This has been taken as evidence to support model-free, habitual responding. However, amphetamine-exposed animals do not show general enhancements in responding, which might suggest a failure to replicate previous work that do find overall increases in cue-evoked responses following drug exposure. Based on these studies, it is unclear whether the influence of drug cues (or enhancement in reactivity to reward cues prior drug exposure) is driven by model-based processes or model-free habits.

Objective and approach

Though the homeostatic role of the LH in feeding and reward motivation has been exhaustively studied, the cognitive role of this region is still relatively new with much uncharted territory. For example, what is the specific associative nature of the role the LH plays in reward

learning? The LH is necessary for cue-reward learning (Sharpe et al., 2021; Sharpe, Marchant, et al., 2017), but whether it is involved because it uses model-free scalar values or because it acquires model-based representations for conditioned responding is currently unknown. Furthermore, the wider circuits that support the LH in learning is another outstanding question. The LH has prominent efferent connections to the VTA in the midbrain (Berk & Finkelstein, 1982; Kallo et al., 2015; Saper et al., 1979), and this pathway has been heavily implicated in reward-motivated behaviors (Barbano et al., 2016; Gigante et al., 2016; Mahler & Aston-Jones, 2012; Nieh et al., 2015; Nieh et al., 2016; Schiffino et al., 2019; Sharpe, Marchant, et al., 2017; Siemian et al., 2021). Thus, the LH is well-positioned to receive input from midbrain dopamine, a critical neural substrate for reward learning (Aransay et al., 2015; Bubser et al., 2005; Yonemochi et al., 2019). Importantly, dopamine signaling has been shown to be capable of supporting both model-free (Bayer & Glimcher, 2005; Chang et al., 2016; Daw & Touretzky, 2002; Eshel et al., 2015; Glimcher, 2011; Maes et al., 2020; Niv et al., 2005; Schultz et al., 1997; Steinberg et al., 2013; Suri & Schultz, 2001; Tsai et al., 2009) and model-based types of learning (Chang et al., 2017; Engelhard et al., 2019; Gardner et al., 2018; Howard & Kahnt, 2018; Jeong et al., 2022; Keiflin et al., 2019; Langdon et al., 2018; Nasser et al., 2017; Seitz et al., 2022; Sharpe et al., 2017; Sharpe et al., 2020; Stalnaker et al., 2019; Takahashi et al., 2017), making it a promising candidate to facilitate the role of the LH in learning regardless of the specific way in which the LH contributes to reward learning. Furthermore, studies have shown that activity in the LH and its projections to the VTA becomes strengthened following exposure to drugs of abuse. (Ahmed et al., 2005; Aston-Jones et al., 2009; Cornish et al., 2012; Espana et al., 2010; Harris et al., 2005; James et al., 2019; Mahler & Aston-Jones, 2012; Marchant et al., 2012; McPherson et al., 2007). Putting these two lines of research together, it may be that the cognitive role that the LH plays in learning could be enhanced following exposure to drugs of abuse, which may underlie the heightened control that reward cues have over behavior in substance use disorders. These two lines of research, however, are still largely independent and have yet to be directly tested.

The overarching goal of this dissertation is to characterize the role of the LH and related circuits in learning and to understand how these circuits may be changed in substance use disorders. Thus, our studies probe the specific contribution of the LH to Pavlovian cue-reward learning and to investigate the impacts of drug exposure on this region that may give rise to maladaptive decision-making. First, in Chapter 2, we reveal the role of the LH and its connections with midbrain dopamine neurons in cue-reward learning and show the reinforcement learning mechanism by which it is driven (i.e., model-free and/or model-based). In Chapter 3, we evaluate the specific nature of heightened cue-controlled behavior in rats with previous methamphetamine experience and examine how LH circuits are changed following methamphetamine exposure. Finally, in Chapter 4, we characterize the temporal profile of endogenous dopamine release in the LH during cue-reward learning and assess how previous methamphetamine experience influences these dopamine dynamics.

Chapter 2: A novel hypothalamic-midbrain circuit for model-based learning

The LH has long been shown to contribute to motivated behaviors such as feeding (Anand & Brobeck, 1951; Hoebel & Teitelbaum, 1962; Jennings et al., 2013; Morgane, 1961; Olds, 1962; Petrovich, 2018; Stuber & Wise, 2016). Most recently, our lab has expanded on this function by demonstrating that GABAergic populations in this region are necessary for cue-reward learning (Sharpe, Marchant, et al., 2017). However, the content of cue-reward associations being learned about by LH is still undetermined. Thus, we were interested in uncovering whether the learning occurring in LH is model-free, which lacks representation of prospective outcomes, or whether the LH learns and stores a model-based associative map that details the cue-reward contingency. To do this, we used outcome devaluation, a procedure that requires the use of an associative map to derive a representation of the specific outcome. In this procedure, subjects first learn that a cue leads to a rewarding outcome. Then, they receive pairings of the reward outside of the experimental context with injections of lithium chloride, which induces nausea. At test, we can examine whether subjects were able to use a model-based associative map by measuring how much they respond to the reward-predictive cue. If the cue is presented and elicits a representation of the rewarding outcome that was paired with illness, responding to the cue will reduce because they no longer desire the reward associated with it. By inhibiting LH$_{GABA}$ neurons at the time when these reward representations are elicited and need to be used (i.e., during cue presentation), we can determine whether LH$_{GABA}$ neurons are necessary for accessing model-based reward information. This approach will inform us of the associative structure being represented in LH as well as how they are used to influence behavior.

Given the newfound role of LH in associative learning (Sharpe et al., 2021; Sharpe, Marchant, et al., 2017), we were also interested in understanding the wider learning circuits in the brain that are contributing to learning in this region. One possibility would be receipt of dopamine

prediction errors from the ventral tegmental area (VTA). Indeed, the projections from the LH to the VTA have been well-studied in reward motivation (Aston-Jones et al., 2009; Barbano et al., 2016; Gigante et al., 2016; Harris et al., 2005; Mahler & Aston-Jones, 2012; Nieh et al., 2015; Nieh et al., 2016; Petrovich, 2018; Schiffino et al., 2019; Sharpe, Marchant, et al., 2017; Stuber & Wise, 2016; Tyree & de Lecea, 2017). Work from our lab has also suggested a role for this pathway in regulating Pavlovian learning through LH_{GABA}-mediated disinhibition of VTA dopamine neurons [VTA_{DA}; (Nieh et al., 2016)] to modulate learning (Sharpe, Marchant, et al., 2017). Thus, it is plausible that LH receive dopamine prediction errors coming from VTA to update learned associations. Therefore, we were also interested in revealing a potential role for the $VTA_{DA}\rightarrow LH$ pathway in supporting learning. To do this, we will first verify the anatomical existence of this pathway. Subsequently, we will probe how this pathway contributes to associative learning using optogenetic inhibition (to test necessity) and excitation (to test sufficiency) combined with outcome devaluation to characterize the specific nature of this learning. Thus, the overarching goals for this chapter and its experiments are two-fold: 1) to reveal the reinforcement learning mechanism that drives learning in LH_{GABA} neurons and 2) to determine whether VTA_{DA} neurons send prediction error signals to LH to facilitate learning occurring in this structure.

Materials and Methods

Surgeries

63 adult, Long Evans rats were used across all behavioral experiments in this study. For LH_{GABA} inhibition (**Experiment 1**), 39 transgenic rats total (18 female, 21 male) expressing Cre-recombinase under the control of the glutamate decarboxylase-1 (GAD) promoter (Sharpe, Marchant, et al., 2017) were used (RRRC#751; Rat Resource and Research Center, MO). Pathway validation using immunohistochemical techniques (**Experiment 2**) used 4 non-transgenic, wild-type male rats (Charles River, MA). Optogenetic manipulations of the $VTA_{DA}\rightarrow LH$

pathway (**Experiment 3-4**) used 36 different transgenic rats (14 female, 24 male) expressing Cre-recombinase under the control of tyrosine hydroxylase (TH) promoter (RRRC#659; Rat Resource and Research Center, MO).

Virus infusions and optic fiber implantation

General surgical procedures have been described elsewhere (Sharpe et al., 2017; Sharpe, Marchant, et al., 2017). All surgical coordinates are relative to bregma. Rats were given 4-6 weeks to recover from surgical procedures and to allow for sufficient time for the virus to incubate in cell bodies and axonal projections. To optogenetically inhibit LH_{GABA} neurons, GAD-Cre rats were bilaterally infused with 1.0 µL of Cre-dependent adenoassociated virus carrying either inhibitory halorhodopsin (AAV5-Ef1a-DIO-eNpHR3.0-eYFP; Addgene: #26966) or control virus without opsin (AAV5-Ef1a-DIO-eYFP; Addgene: #27056) into LH [AP: -2.4 mm; ML: ±3.5 mm; DV: -9.0 (males) or -8.4 (females); angled at 10° towards midline]. Optic fibers were also bilaterally implanted into LH [AP: -2.4 mm; ML: ±3.5 mm; DV: -8.5 (males) or -7.9 (females); angled at 10° towards midline]. To optogenetically inhibit VTA dopamine terminals in LH, TH-Cre rats received bilateral infusions of 2.0 µL of either Cre-dependent halorhodopsin or control AAV into VTA [AP: -5.3 mm; ML: ±0.7 mm; DV: -7.0 and -8.2 mm (males) or -6.5 and -7.7 mm (females)]. Optic fibers were placed bilaterally over LH. Similar virus and fiber approaches were used for stimulation of the VTA_{DA}→LH pathway, with the exception that TH-Cre rats were infused with 2.0 µL per hemisphere of Cre-dependent, excitatory channelrhodopsin [AAV5-Ef1a-DIO-hChR2(E123T/T159C)-eYFP; Addgene: #35509], and fiber placed bilaterally over the LH.

Retrograde tracing

Rats were bilaterally infused with 0.6 µL per hemisphere of retrograde tracer, *Cholera Toxin Subunit B* (Thermo Fisher Scientific, MA), fluorescing at 555nm, into the LH using the same viral

coordinates for this region as described earlier. Rats were allotted 6 days for tracer incubation before they were perfused for whole brain collection and immunohistochemical validation.

Behavioral procedures

Experiment 1

CS+/CS- Pavlovian Conditioning

Sessions took place in operant behavior chambers encased in a sound-attenuating box that were controlled by MED-PC V software (Med Associates, Inc., Fairfax, VT). Each rat was assigned to its own chamber. Sessions consisted of one 30-s auditory cue (click or white noise, counterbalanced) followed by two 45-mg sucrose pellets (Test Diet, MA; CS+) and the alternative 30-s auditory cue not resulting in pellets (CS-). Cues were presented in pseudorandom order. The cohort in **Experiment 1A** received 8 conditioning sessions consisting of 12 trials (6 trials per cue) separated by a variable 6-min intertrial interval (ITI). The cohort in **Experiment 1B** received 20 conditioning sessions consisting of 16 trials per session (8 trials per cue) separated by a variable 4-min ITI. Behavioral responding was measured either as the percent of time spent in the food port or the number of entries made to the food port during presentation of CS+ relative to CS- across conditioning sessions.

Lithium Chloride-induced Devaluation

Rats in **Experiment 1A** were first habituated to the devaluation context by placing them each individually in empty cages in a separate behavioral room from conditioning. Rats received two 30-minute sessions of habituation before being returned to their home cages. Following the last day of habituation, rats were then given 3 daily pairings of the pellets and lithium chloride (LiCl; Sigma-Aldrich, MA), which consisted of 30-minute access to consume 10 grams of pellets immediately followed by intraperitoneal injections of LiCl (0.15M, 10 mL/kg). Six hours after injection, rats were given their normal home chow to avoid any pairing of their normal diet with

LiCl-induced sickness. Consumption of pellets was measured across days. Rats were allowed to recover from immediate LiCl effects across 24 hours before being administered conditioned reinforcement tests.

Conditioned Reinforcement

Four 30-minute sessions of conditioned reinforcement were conducted in which two levers, never experienced before, were inserted into the behavior chamber. Pressing on one lever (left or right, counterbalanced) produced a 2-s presentation of CS+ while pressing the other produced the CS-cue. Green laser light (532 nm; 16-18mW) was delivered for 3-s across cue duration, beginning 0.5-s before cue onset and terminating 0.5-s after cue offset. Number of lever presses made for either CS+ or CS- delivery averaged across the session was used as the behavioral measure. Rats that did not press the lever during these tests were removed from all analyses.

Statistical Analyses

Data were analyzed using repeated measures Analysis of Variance (ANOVA) statistical tests. For Pavlovian conditioning and conditioned reinforcement tests, within-subjects factors of CS (CS+ vs. CS-) and session (average of 2 consecutive sessions per session block for conditioning; all 4 sessions for tests) were included. Only within-subjects factor of day was used to analyze consumption behavior during devaluation procedures. Between-subjects factor of virus group (eYFP vs. NpHR) was included in all analyses. Follow-up simple main effects were conducted for detection of significant interactions. Data were tested for normality using Mauchly's test of sphericity and when sphericity could not be assumed in repeated measures ANOVAs, the Greenhouse Geiser adjustment was reported. Analyses used an alpha level of 0.05. Logarithmic transformation of lever press data averaged across sessions to normalize for individual variability for **Experiment 1A** were used for data analysis. Lever press data were analyzed across session for **Experiment 1B**.

Experiment 3

CS+/CS- Pavlovian Conditioning

Animals received 14 sessions of the same CS+/CS- conditioning protocol described above. For VTA$_{DA}$→LH inhibition during conditioning, represented in **Figure 4D**, green laser light (532 nm; 16-18mW) was delivered at the time of pellet delivery on CS+ trials, beginning 0.5-s prior to cue offset and terminating 2-s after cue offset. Behavioral responding was measured as the percent of time spent in the food port during presentation of CS+ relative to CS- across conditioning sessions.

Lithium Chloride-induced Devaluation

Following Pavlovian conditioning sessions, rats received the same devaluation protocol described above.

Probe Test

A probe test session was conducted following the 24-hour recovery period from devaluation procedures. 6 presentations of CS+ and CS- cues each were presented throughout the session in pseudorandom order. Behavioral responding was measured as the percent of time spent in the food port during presentation of CS+ relative to CS- across conditioning sessions. To account for response differences between groups at the end of Pavlovian conditioning, changes in responding after devaluation were calculated as the difference in percent of time spent during cue presentation in the probe test (after devaluation) and in a conditioning session without laser inhibition conducted prior to devaluation procedures (before devaluation).

Statistical Analyses

Data were analyzed using repeated measures ANOVA statistical tests with follow-up simple main effect analyses conducted following detection of a significant interaction. A one-tailed t-test was

used with a directional *a priori* hypotheses. For Pavlovian conditioning, within-subjects factor of CS (CS+ vs. CS-) and session (average of 2 consecutive sessions per session block for conditioning) were included in the analysis. For probe tests, only within-subjects factor of CS was included to analyze the change in responding before and after devaluation. Within-subjects factor of day was used to analyze consumption behavior during devaluation procedures. Between-subjects factor of virus group (eYFP vs. NpHR) was included in all analyses. Data were tested for normality using Mauchly's test of sphericity and when sphericity could not be assumed in repeated measures ANOVAs, the Greenhouse Geiser adjustment was reported. Analyses used an alpha level of 0.05.

Experiment 4

Blocking Procedure

Rats first received 8 sessions of Pavlovian conditioning to acquire two distinct visual cue-pellet associations. Cues (flashing cue lights or steady house light, counterbalanced) were presented for 30-s followed by a 1-s gap before one pellet [45-mg sucrose or 45-mg grain (Test Diet, MA), counterbalanced] was delivered into the food port. Trials were separated by a variable 3-min ITI. The subsequent 4 sessions of conditioning introduced novel auditory cues (click or white noise, counterbalanced) each to be presented concurrently with each of the visual cues, followed by the same reward deliveries as before. For one of the cue-pellet pairings, blue light [473 nm; 14-16mW; 1-s, 20Hz; 5-ms pulse duration, 45-ms interval (Millard et al., 2022; Sharpe et al., 2017; Sharpe et al., 2020)] was delivered into the brain at the time of pellet reward, represented in **Figure 5E**. To test whether learning was facilitated by optogenetic stimulation, rats received a probe test, each consisting of 8 presentations of auditory cues alone without rewards or laser stimulation (variable 2.5-min ITI, interleaved cue order). Behavioral responding was measured as entries made to the food port during cue presentation.

Lithium Chloride-induced Devaluation

Following the probe tests, rats underwent a between-subjects design of the devaluation procedure previously described for the pellet outcome (already counterbalanced across subjects from the previous phase of the study) associated with the unblocked cue for each rat. Here, subjects were split into two groups: devalued and non-devalued control. The devalued group followed the same procedures described above. For the non-devalued control group, rats received LiCl injections and were given access to the pellets in their home cages six hours later. All rats were given at least 24 hours to recover from acute effects of lithium chloride injections. A probe test was then administered to test the effects of devaluation, consisting of 8 presentations of just the unblocked cue for each rat without reward delivery (variable 2.5-min ITI). To ensure conditioned aversion to the pellet was present at the time of test, rats received a consumption test conducted immediately after their probe test such that all rats had 10 minutes of free access to pellets in the devaluation cages without subsequent injections.

Statistical Analyses

Data were analyzed using repeated measures ANOVA statistical tests and paired/independent samples t-tests. For acquisition and unblocking phases, CS (blocked, unblocked, and baseline) and session (average of 2 sessions per session block) were included as within-subjects factors in the repeated measures ANOVA. The laser-free probe test following acquisition and unblocking was analyzed with a one-tailed, paired samples t-test comparing the average responses to either blocked or unblocked cues. Consumption across devaluation pairings was analyzed with a repeated measures ANOVA that included day as a within-subjects factor and group (devalued vs. non-devalued) as a between-subjects factor. Because of *a priori* directional hypotheses based on results from the previous experiment and work from others (Keiflin et al., 2019; Millard et al., 2022; Sharpe et al., 2017; Sharpe et al., 2020; Steinberg et al., 2013), the probe test and the immediate consumption test following devaluation were analyzed with one-tailed, independent samples t-

tests comparing the average responses to presentation of the unblocked cue between devalued and non-devalued groups. Data were tested for normality and homogeneity using Mauchly's test of sphericity (repeated measures ANOVA) and Levene's test for equality of variances (independent t-tests). When normality could not be assumed, the Greenhouse Geiser adjustment (ANOVA) was reported. A Welch's t-test was conducted in the event equal variances were not assumed and the output for this was reported. Analyses used an alpha level of 0.05.

Histology

At the conclusion of experiments, animals were induced with carbon dioxide and then transcardially perfused with 1X phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA) in 1X PBS solution. Brains were extracted and placed in 4% PFA overnight before being changed to 30% sucrose solution made up in 1X PBS for at least 48 hours. Brain tissue was then sectioned into 30 µm coronal sections using a cryostat and collected in well-plates containing 1X PBS. Sections were mounted onto microscope slides and sealed with glass coverslips using ProLong™ gold antifade reagent with DAPI (Thermo Fisher Scientific, Waltham, MA; P36930). CTb tracer co-expression (Alexa Fluor™ 555) with tyrosine-hydroxylase (TH+; Alexa Fluor™ 488) was used to quantify anatomical projections in **Experiment 2**. eYFP fluorescence was used to confirm TH+ expression in VTA cell bodies in **Experiment 4**. Floating 30 µm coronal sections were first washed 3 times in 1X PBS for 30 minutes before being blocked in a solution consisting of 3% normal donkey serum, 0.3% Triton X-100, and 1X PBS for 2 hours. Sections were washed with 1X PBS another 3 times for 15 minutes before incubating in blocking solution containing rabbit anti-TH antibody (1:1000; Millipore Sigma, Burlington, MA; AB152) for 48 hours at 4°C. After primary incubation, sections were washed with 1X PBS another 3 times for 30 minutes before secondary incubation in blocking solution containing goat anti-rabbit IgG (H+L), Alexa Fluor™ 488 conjugate (1:500, Thermo Fisher Scientific; A11008) for 2 hours at room temperature. Goat anti-rabbit IgG (H+L), Alexa Fluor™ 594 conjugate (1:500; Thermo Fisher

Scientific; A11012) was used as the secondary antibody for staining sections for **Experiment 4**. Sections were washed another 3 times with 1X PBS before being incubated in DAPI (4',6'-Diamidino-2-Phenylindole, Dihydrochloride; 1:10000; Thermo Fisher Scientific; D1306) made up in di H_2O to stain for nuclei for 30 minutes. Following one final set of 3 washes with 1X PBS, sections were mounted onto microscope slides and sealed with glass coverslips using ProLong™ gold antifade reagent. Slides were viewed with a Confocal Microscope (Zeiss) for virus and fiber placement verification and images were taken using a 10x objective and tiled together or a 20x objective for Z-stacked layers.

Quantification of neurons

Tissue from rats used for retrograde tracing (*n*=4) was imaged following immunohistochemical processing under a 20x fluorescence microscopic objective (Carl Zeiss Confocal Microscopy). Quantified images comprised of 20 different focal layers merged together. Unilateral cell counts of TH and CTb tracer expression were analyzed in VTA spanning four levels across the anterior-posterior plane (AP: -4.92, -5.04, -5.28, -5.40) by one observer.

Results

Experiment 1A: LH$_{GABA}$ neurons are necessary for behavior governed by model-based associations between cues and rewards

We have previously shown that LH$_{GABA}$ neurons are necessary for both the acquisition and expression of cue-reward associations (Sharpe et al., 2021; Sharpe, Marchant, et al., 2017). However, it is unclear whether these cue-reward associations reflect the general, scalar value of the reward (i.e., model-free) or the unique, sensory-specific properties of the reward (i.e., model-based). To answer this question, we probed the content of cue-reward information in LH$_{GABA}$ neurons (**Figure 1A**). We first infused GAD-Cre rats bilaterally with a Cre-dependent adeno-

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associated virus (AAV) carrying either the inhibitory halorhodopsin (AAV5-Ef1a-DIO-eNpHR3.0-eYFP; NpHR; n=9) or a control vector (AAV5-Ef1a-DIO-eYFP; eYFP; n=14) into LH and implanted optic fibers bilaterally above LH (**Figure 1B-C**). This would allow us to optogenetically inhibit LH_{GABA} neurons (Sharpe et al., 2021; Sharpe, Marchant, et al., 2017). Four weeks after surgery, rats were food restricted and began Pavlovian conditioning procedures. Here, two distinct, 30-s auditory cues (click and white noise; 8 sessions; 12 presentations/session) were presented with one stimulus leading to delivery of two 45-mg sucrose pellets (CS+) and the other without consequence (CS-; counterbalanced). Across conditioning, eYFP and NpHR groups increased time spent in the food port during the CS+ relative to CS- presentation with no between-group differences (**Figure 1D**; CS+ vs. CS-: $F_{(1,21)}$ = 63.483, p<0.001; session: $F_{(3,63)}$ = 1.056, p=0.374; group: $F_{(1,21)}$ = 0.728, p=0.403; CS x group: $F_{(1,21)}$ = 0.891, p=0.356; session x group: $F_{(3,63)}$ = 0.106, p=0.956; CS x session: $F_{(3,63)}$ = 5.813, p=0.001; CS x session x group: $F_{(3,63)}$ = 0.109, p=0.954).

After learning about the CS+ and CS-, rats underwent a devaluation procedure, where the reward associated with CS+ was paired with injection of lithium chloride (LiCl; 0.15M, 10 mL/kg; 3 days). This procedure produced a taste aversion to the reward, reflected in a reduction in consumption of the reward across injection days (**Figure 1E**; day: $F_{(2,42)}$ = 40.758, p<0.001; group: $F_{(1,21)}$ = 0.038, p=0.846; day x group: $F_{(2,42)}$ = 0.048, p=0.953). The devaluation procedure allows us to test the associative information contained in the cue-reward associations in LH. That is, if LH harbors model-based information, this association will be sensitive to reward devaluation. This is because the cue will evoke a representation of the reward, and the reward will evoke a feeling a sickness, which will lead the rat to reduce responding to the CS (Balleine & Dickinson, 1998; Balleine et al., 2005; Rescorla, 1987). However, if the information harbored in LH is model-free and based on a general value that has transferred to the CS across learning, the CS will not evoke a representation of the reward and responding will be insensitive to devaluation (Clark et al., 2012; Colwill & Motzkin, 1994; Galarce et al., 2007; Rescorla, 1987). As inhibition of LH_{GABA} neurons

during a cue will simply reduce the appetitive response (Sharpe et al., 2021; Sharpe, Marchant, et al., 2017), we need to circumvent this issue by arranging a situation where we assess the content of information in LH$_{GABA}$ without requiring a response during inhibition of LH$_{GABA}$ neurons. Thus, instead of presenting the CS and measuring appetitive responding, we gave rats a test where they could press one lever to get presentation of the CS+ and another for the CS-. During this test, we delivered green light (532 nm; 16-18mW) into the brain to inhibit LH$_{GABA}$ neurons during CS+ and CS- presentation. This allowed us to selectively inhibit LH$_{GABA}$ neurons during the CS *after* the response was made. As rats had no prior experience with the levers, continued lever-pressing would indicate that the CSs were capable of supporting development of instrumental response and were valuable in some way [known as the well-established phenomenon, conditioned reinforcement (Burke et al., 2007; Hyde, 1976; Shahan, 2010; Thrailkill & Shahan, 2014; Williams, 1994)]. However, given we had devalued the reward paired with the CS+, if rats are using model-based associative information to direct behavior, then this CS should not be capable of supporting conditioned reinforcement because the CS would be associated with the now devalued reward (Burke et al., 2007, 2008). Indeed, we found that the eYFP group did not demonstrate the conditioned reinforcement effect after devaluation (**Figure 1F**). That is, there was no difference in the ability of the CS+ or CS- to drive conditioned reinforcement, reflecting sensitivity of this effect to devaluation (Burke et al., 2007, 2008). In contrast, inhibition of LH$_{GABA}$ neurons prevented the ability of rats in the NpHR group to use model-based information to drive behavior, illustrated by the ability of the CS+ to support conditioned reinforcement despite devaluation of the associated reward (**Figure 1F**). This was supported by statistical analyses which revealed no main effect of CS or group (CS+ vs. CS-: $F_{(1,21)}$ = 2.615, p=0.121; group: $F_{(1,21)}$ = 0.001, p=0.970), but a significant CS by group interaction (CS x group: $F_{(1,21)}$ = 7.061, p=0.015). Simple-effect analyses following the interaction revealed no difference in lever-press responding for the CS+ and CS- in the eYFP group ($F_{(1,21)}$ = 0.691; p=0.415). However, there was a significant difference in lever-press responding for the CS+ and CS- in the NpHR group ($F_{(1,21)}$ = 7.504;

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p=0.012). Furthermore, no significant simple main effect of group was detected for responding to either cue (CS+: $F_{(1,21)} = 0.578$, *p*=0.456; CS-: $F_{(1,21)} = 0.965$, *p*=0.337). These results demonstrate that LH$_{GABA}$ neurons encode model-based associations that entail representations of the cue and the sensory-specific features of the predicted reward and that inactivating these neurons prevented the ability to use model-based associative information to govern responding.
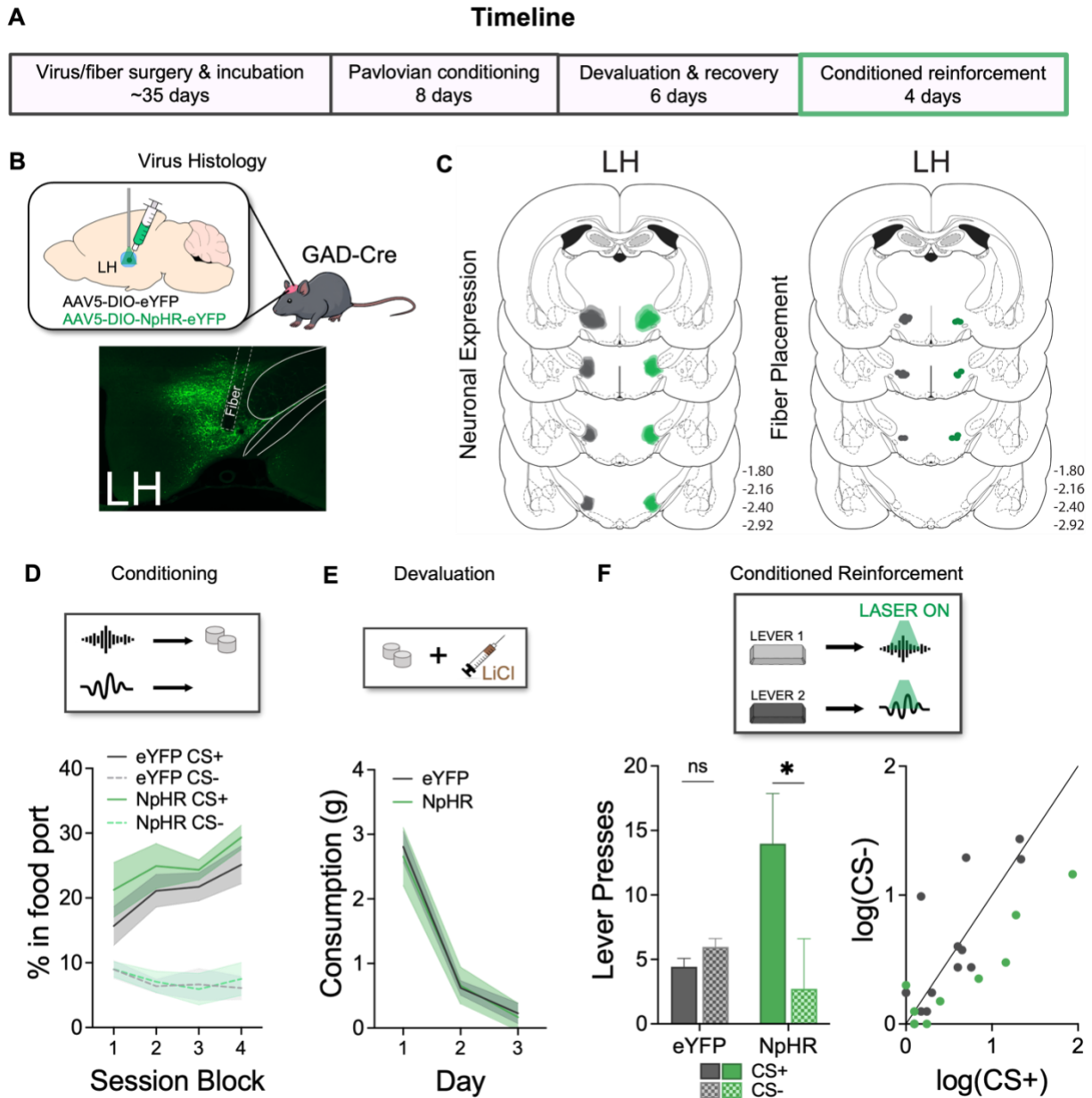

Figure 1. Inhibition of LH$_{GABA}$ neurons prevents the use of model-based associations to guide behavior.
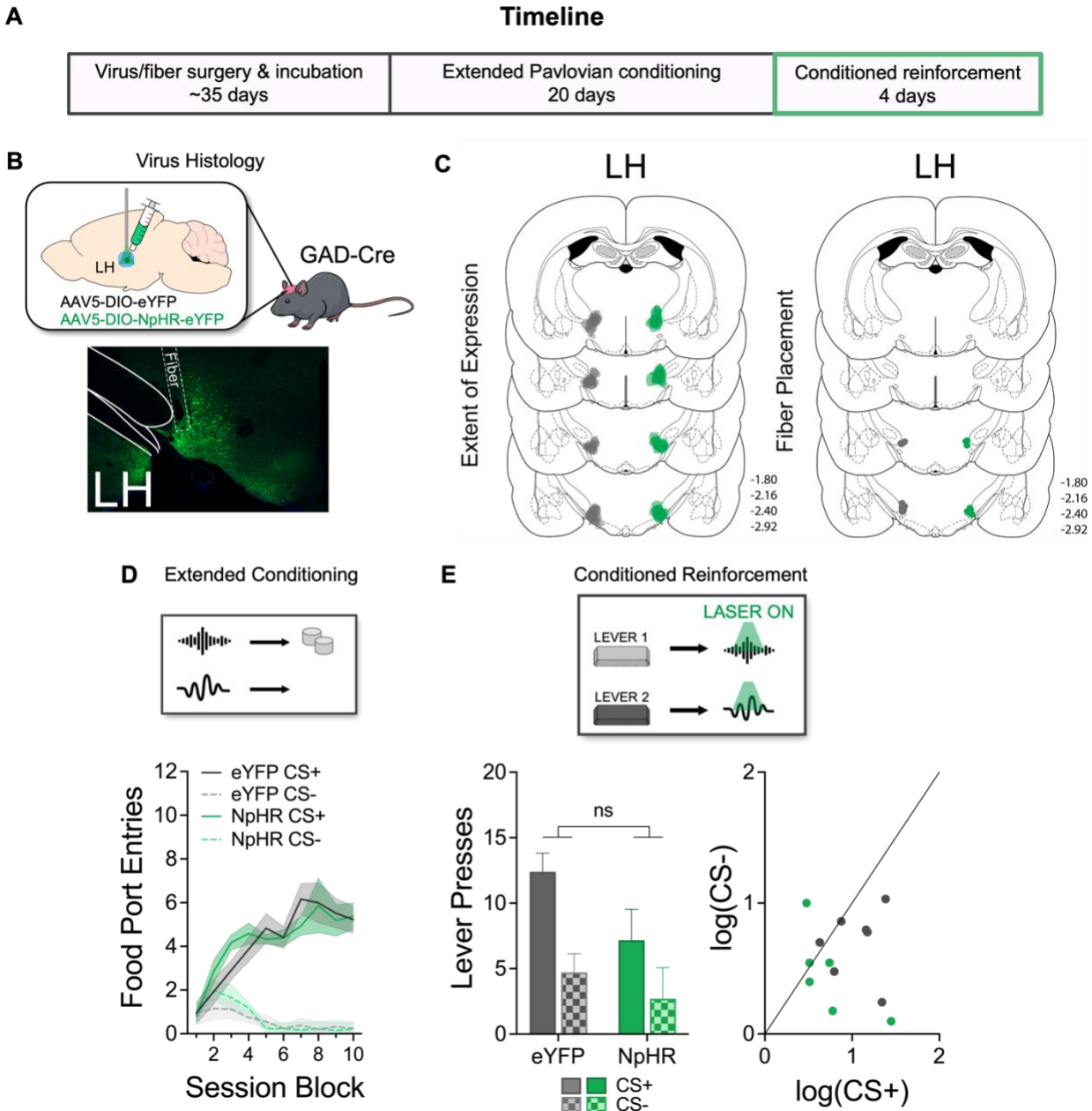
(A) Experimental timeline. **(B)** Optogenetic approach: GAD-Cre rats were bilaterally infused with a Cre-dependent AAV with inhibitory halorhodopsin (NpHR; *n*=9), or without (eYFP; *n*=14), in LH and implanted with optic fibers in LH. Below shows a unilateral example of bilateral virus expression in the cell bodies of GABAergic neurons in LH. **(C)** *Left*: Unilateral representation of bilateral virus expression in LH for the eYFP group (grey) and the NpHR group (*green). Right*: Dots indicate approximate location of fiber tips in LH. **(D)** Rats learned that one auditory cue led to food pellets (CS+), and another was without consequence (CS-). Responding is represented as the percent of time spent in the food port (mean ± SEM). Rats in both eYFP and NpHR groups increased responding to the CS+ relative to the CS- across 4 session blocks (2 sessions/block). **(E)** Rats received pairings of LiCl with the reward and all rats reduced consumption of the food reward. **(F)** Next, rats were allowed to press two levers to earn presentations of either CS+ or CS-. Here, we inhibited LH$_{GABA}$ neurons during presentation of CS+ and CS-. Rats in the eYFP group do not demonstrate conditioned reinforcement for the CS+ predicting the devalued reward. However, rats in the NpHR group showed robust conditioned reinforcement for the CS+. Rates of responding are represented as number of lever presses made for presentation of either cue (mean ± SEM). Individual data points reflect responding of each rat as a normalized value (logarithmic transformation) for eYFP (grey) and NpHR (green) groups. To the extent that responding for the CS+ and CS- is equal, dots should congregate on the diagonal. Rats in the eYFP group showed low levels of lever-pressing for both CS+ and CS-. However, NpHR rats showed high responding for the CS+ and not CS-. *$p \leq 0.05$, mean (± SEM).

Experiment 1B: LH$_{GABA}$ neurons are not required for expression of model-free value

The findings from **Experiment 1A** suggested that inhibition of LH$_{GABA}$ neurons prevented

the use of model-based associations to reduce conditioned reinforcement following devaluation.

If this is the case, then inhibition of LH$_{GABA}$ neurons should not impact conditioned reinforcement

when a model-based representation is not necessary to influence behavior. Accordingly, we

conducted a follow-up study to investigate whether inhibition of LH$_{GABA}$ neurons in rats that were

overtrained on the cue-reward association continue to show conditioned reinforcement (**Figure**

2A). Typically, overtraining renders behavior less sensitive to devaluation, making a model-based

association unnecessary to direct responding for a reward-predictive cue (Burke et al., 2007,

2008; Holland, 2004). Thus, inhibition of LH$_{GABA}$ neurons should not disrupt conditioned

reinforcement following overtraining. A separate group of GAD-Cre rats received the same virus

and fiber surgeries as the prior cohort (**Figure 2B-C**; eYFP, *n*=7; NpHR, *n*=6). After surgical

recovery, rats received the same CS+/CS- conditioning procedure with 10-s auditory cues (click

or white noise; 16 presentations/session) where one cue was reinforced with sucrose pellets

(CS+) and another cue was not (CS-; counterbalanced). Here, rats were given 20 sessions of

conditioning as overtraining has been shown to reduce goal-directed behavior (adjacent to using model-based information) and increase habitual behavior (more akin to model-free value) (Coutureau & Killcross, 2003; Dayan & Berridge, 2014; Dezfouli & Balleine, 2012; Keefer et al., 2020). Both eYFP and NpHR groups showed greater responding to CS+ relative to CS- without group differences (**Figure 2D**; CS+ vs. CS-: $F_{(1,11)}$ = 70.626, $p<0.001$; group: $F_{(1,11)}$ = 0.040, p=0.845; CS x group: $F_{(1,11)}$ = 0.002, p=0.969). Further, responding for CS+ grew while responding for CS- remained low across conditioning sessions without between-groups differences (session: $F_{(9,99)}$ = 8.288, $p<0.001$; CS x session: $F_{(9,99)}$ = 21.937, $p<0.001$; session x group: $F_{(9,99)}$ = 1.560, p=0.221; CS x session x group: $F_{(9,99)}$ = 0.270, p=0.818).

Following extended conditioning, we allowed rats to earn presentations of either CS+ or CS- by pressing on individual levers associated with each cue. Similar to **Experiment 1A**, inhibition of LH$_{GABA}$ neurons took place during cue presentation after a lever-press response was made. We found that both eYFP and NpHR groups showed greater responding for the lever associated with CS+ relative to the CS- lever, but without differences between groups (**Figure 2E**; CS+ vs. CS-: $F_{(1,11)}$ = 5.183, p=0.044; group: $F_{(1,11)}$ = 2.093, p=0.176; CS x group: $F_{(1,11)}$ = 0.365, p=0.558). This response pattern was consistent across sessions and the magnitude of this effect did not vary between groups, such that eYFP and NpHR showed comparable levels of responding for each lever (session: $F_{(3,33)}$ = 1.007, p=0.402; CS x session: $F_{(3,33)}$ = 1.034, p=0.390; session x group: $F_{(3,33)}$ = 1.070, p=0.375; CS x session x group: $F_{(3,33)}$ = 0.467, p=0.708). These results suggest that inhibition of LH$_{GABA}$ neurons does not affect the expression of conditioned reinforcement in a setting where model-based associations are unlikely to govern behavior.

Figure 2. Inhibition of LH_GABA neurons does not disrupt the expression of conditioned reinforcement after overtraining.

(A) Experimental timeline. **(B)** Optogenetic approach: GAD-Cre rats were bilaterally infused with a Cre-dependent AAV with inhibitory halorhodopsin (NpHR; *n*=6), or without (eYFP; *n*=7), in LH and implanted with optic fibers in LH. Below shows a unilateral example of bilateral virus expression in the cell bodies of GABAergic neurons in LH. **(C)** *Left*: Unilateral representation of bilateral virus expression in LH for the eYFP group (grey) and the NpHR group (*green*). *Right*: Dots indicate approximate location of fiber tips in LH. **(D)** A separate group of rats from the first procedure were given CS+/CS- conditioning, extended to 10 session blocks (2 sessions/block). Responding is represented as the number of entries in the food port during cue presentations (mean ± SEM). Rats in both eYFP and NpHR groups increased responding to the CS+ relative to

the CS-. **(E)** Following extended conditioning, rats received sessions where they were allowed to press on levers that produced presentations of either CS+ or CS-. LH$_{GABA}$ neurons were inhibited during cue presentation. Rats in both eYFP and NpHR groups showed conditioned reinforcement for the CS+. Rates of responding are represented as number of lever presses made for presentation of either cue averaged across 4 sessions (mean ± SEM). Individual data points reflect responding of each rat as a normalized value (logarithmic transformation) for eYFP (grey) and NpHR (green) groups. To the extent that responding for the CS+ and CS- is equal, dots should congregate on the diagonal. Rats in both virus groups biased their lever-pressing responses for CS+ over CS-. *$p \leq 0.05$, mean (± SEM).

Experiment 2: Determining the presence of a novel dopaminergic projection from VTA to LH

Our first experiment provides evidence to suggest that the LH contains model-based information that can be called upon to influence adaptive behavior. This begs the question of which neural substrates facilitate this function. One candidate mechanism is input from dopamine neurons in the VTA (Bubser et al., 2005; Yonemochi et al., 2019). Historically, dopamine prediction errors have been thought to contribute to cue-reward learning by endowing cues with a model-free, scalar value (Bayer & Glimcher, 2005; Chang et al., 2016; Glimcher, 2011; Maes et al., 2020; Schultz et al., 1997; Steinberg et al., 2013; Tsai et al., 2009). However, recent studies have revealed that these phasic signals can also support the development of model-based associations (Chang et al., 2017; Engelhard et al., 2019; Gardner et al., 2018; Jeong et al., 2022; Keiflin et al., 2019; Langdon et al., 2018; Nasser et al., 2017; Seitz et al., 2022; Sharpe et al., 2017; Sharpe et al., 2020; Stalnaker et al., 2019; Takahashi et al., 2017). For example, VTA$_{DA}$ neurons are necessary for the development of associations between cues and sensory-specific representations of rewards (Howard & Kahnt, 2018; Keiflin et al., 2019; Seitz et al., 2022; Stalnaker et al., 2019). Though there is a body of literature showing dopamine activity regulates LH function, this is largely based on local pharmacological manipulations through dopamine receptor agonists and antagonists and focused on the canonical role of LH in regulating feeding (Amador et al., 2014; Ikeda et al., 2018; Parada et al., 1988; Parada et al., 1990; Parada et al., 1991; Sato et al., 2001; Touzani et al., 2009; Yonemochi et al., 2019). Thus, while the VTA is well-

situated anatomically and functionally to contribute to model-based encoded in LH, the role of this circuit in learning is unknown.

Given there is sparse anatomical evidence for the existence of VTA projections to LH and few showing they are dopaminergic in nature (Aransay et al., 2015; Beckstead et al., 1979; Bubser et al., 2005; Taylor et al., 2014; Yonemochi et al., 2019), we first verified the existence of VTA dopamine (VTA$_{DA}$) input to LH by injecting retrograde tracer cholera toxin subunit B (CTb-555; Alexa Fluor^{TM} 555 conjugate) into LH (**Figure 3A**). Any neuronal projections terminating in LH take up the retrograde tracer and subsequently express in the originating cell bodies (**Figure 3C**). We then used an antibody to stain tyrosine hydroxylase (TH), an enzyme that converts tyrosine into dopamine, and imaged the VTA (**Figure 3D**). We found that injection of the CTb in LH resulted in considerable double labeling (~64% of 488 TH+ neurons) of TH and CTb in the VTA, demonstrating the projection from VTA$_{DA}$ neurons to the LH (**Figure 3B**).
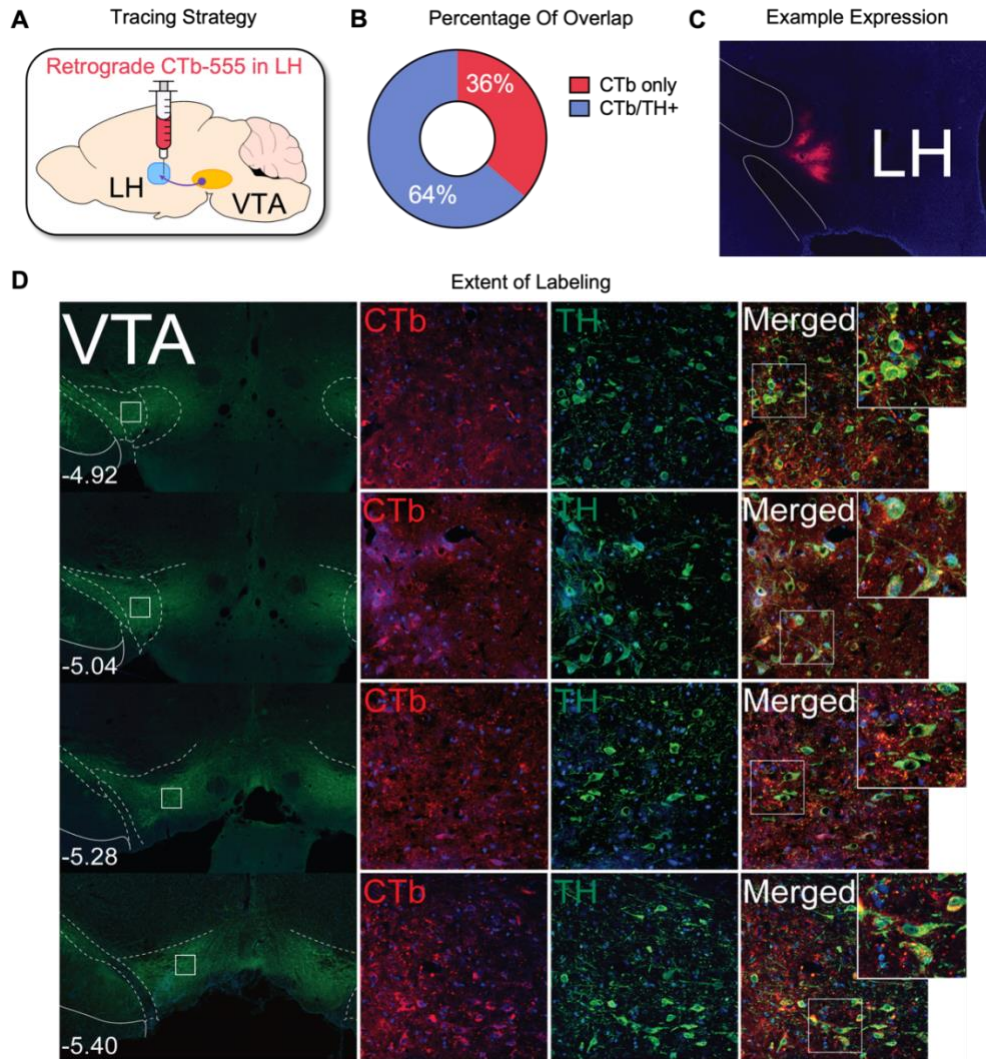
Figure 3. Revealing a novel dopaminergic projection to LH from VTA.

(A) Schematic of retrograde tracing approach: rats were injected with cholera toxin subunit B (CTb-555) into LH. **(B)** Colocalization of CTb tracer and TH expression reveals ~64% overlap in LH-projecting VTA cell bodies (n=377/488). **(C)** Example of CTb expression in LH. **(D)** Extent of CTb expression in VTA across the anterior/posterior plane relative to bregma, stained for CTb (red), TH+ (green), and overlap (merge) at 10x (left) and 20x (right, inset) magnification.

Experiment 3: VTA$_{DA}$ projections to LH are necessary for model-based associations between cues and rewards

After verifying the existence of a VTA$_{DA}$ projection to LH, we asked whether this circuit was necessary for the development of model-based associations in LH. We first bilaterally infused TH-Cre rats with a Cre-dependent NpHR virus (n=12) or eYFP control vector (n=10) into VTA and

placed our optic fibers in LH (**Figure 4A-C**). This would allow us to selectively inhibit VTA$_{DA}$ terminals in LH during learning when this pathway would be active under normal circumstances if it is receiving a prediction error signal (Schultz et al., 1997). Following virus incubation, rats were food restricted and received Pavlovian conditioning (14 sessions; 12 presentations/session), where one 10-s auditory cue leads to food reward (CS+), and another was without consequence (CS-; click or white noise; counterbalanced). During learning, green laser light was delivered at the time of reward following CS+ presentation (2.5-s; 532 nm; 16-18mW) using parameters that have been shown to suppress dopamine firing without causing a negative prediction error (Chang et al., 2018; Sharpe et al., 2017). Specifically, these parameters do not produce extinction learning, which is seen with shorter bursts of inhibition that better mimic a negative prediction error (Chang et al., 2016; Chang et al., 2018). We found that inhibition of VTA$_{DA}$ terminals in LH reduced learning about the CS+ and not the CS-. This was supported by statistical analyses, revealing that both eYFP and NpHR groups elevated responding to CS+ relative to the CS- (**Figure 4D**; CS+ vs. CS-: $F_{(1,20)}$ = 46.083, $p<0.001$; session: $F_{(6,120)}$ = 5.582, $p<0.001$; group: $F_{(1,20)}$ = 2.017, $p=0.171$; CS x session: $F_{(6,120)}$ = 20.323, $p<0.001$; CS x group: $F_{(1,20)}$ = 0.903, $p=0.353$; session x group: $F_{(6,120)}$ = 1.571, $p=0.161$). However, there was a significant interaction between the groups and the rate at which they elevated their responding to the CS+ (CS x session x group: $F_{(6,120)}$ = 3.788, $p=0.002$), which was most pronounced in the final session (simple main effect of group, CS+: $F_{(1,20)}$: 10.629, $p=0.004$), and without between-groups differences in CS- responding (simple main effect of group, CS-: $F_{(1,20)}$ = 1.444 $p=0.244$). These results show that inhibition of VTA$_{DA}$ terminals in LH impaired the ability of rats to learn about reward-predictive cues.

Although learning about the CS+ was reduced in the NpHR group of rats, responding was not completely abolished by VTA$_{DA}$→LH inhibition. This afforded the opportunity to probe the nature of the learning that remained in these rats. To do so, we devalued the reward paired with CS+ by pairing the reward with injection of LiCl. Both eYFP and NpHR groups reduced their consumption of the reward with consecutive pairings of LiCl injections (**Figure 4E**; day: $F_{(2,40)}$ =

55.124, $p<0.001$; group: $F_{(1,20)}$ = 0.297, $p=0.592$; day x group: $F_{(2,40)}$ = 0.394, $p=0.677$). Finally, rats were given a probe test to examine the effects of reinforcer devaluation on responding to the CS+. Given CS+ responding was reduced in the NpHR group relative to the eYFP group during learning, we compared the change in responding to the CSs before and after the devaluation procedure. Here, we found that the eYFP group reduced responding to the CS+ after devaluation, indicated by a negative change in responding to the CS+ but not the CS-. However, the NpHR group failed to show any change in responding to the CS+ (**Figure 4F**). This was supported by statistical analyses, which revealed no main effect of CS (CS+ vs. CS-: $F_{(1,20)}$ = 0.595, $p=0.449$), but a significant main effect of group (eYFP vs. NpHR: $F_{(1,20)}$ = 6.671, $p=0.018$) and a significant CS x group interaction ($F_{(1,20)}$ = 3.827, $p=0.033$) owed to a significant difference in responding to the CS+ between the eYFP and NpHR groups ($F_{(1,20)}$ = 7.646, $p=0.012$), and not the CS- ($F_{(1,20)}$ = 0.914, $p=0.350$). Importantly, there was no effect of trial ($F_{(5,100)}$ = 1.077, $p=0.378$) or any interaction between trial and CS ($F_{(5,100)}$ = 0.321, $p=0.899$), confirming an effect of devaluation and not extinction. This demonstrates that the *residual* learning to the CS+ was insensitive to reward devaluation, indicating what is learned in the VTA$_{DA}$→LH circuit is model-based.
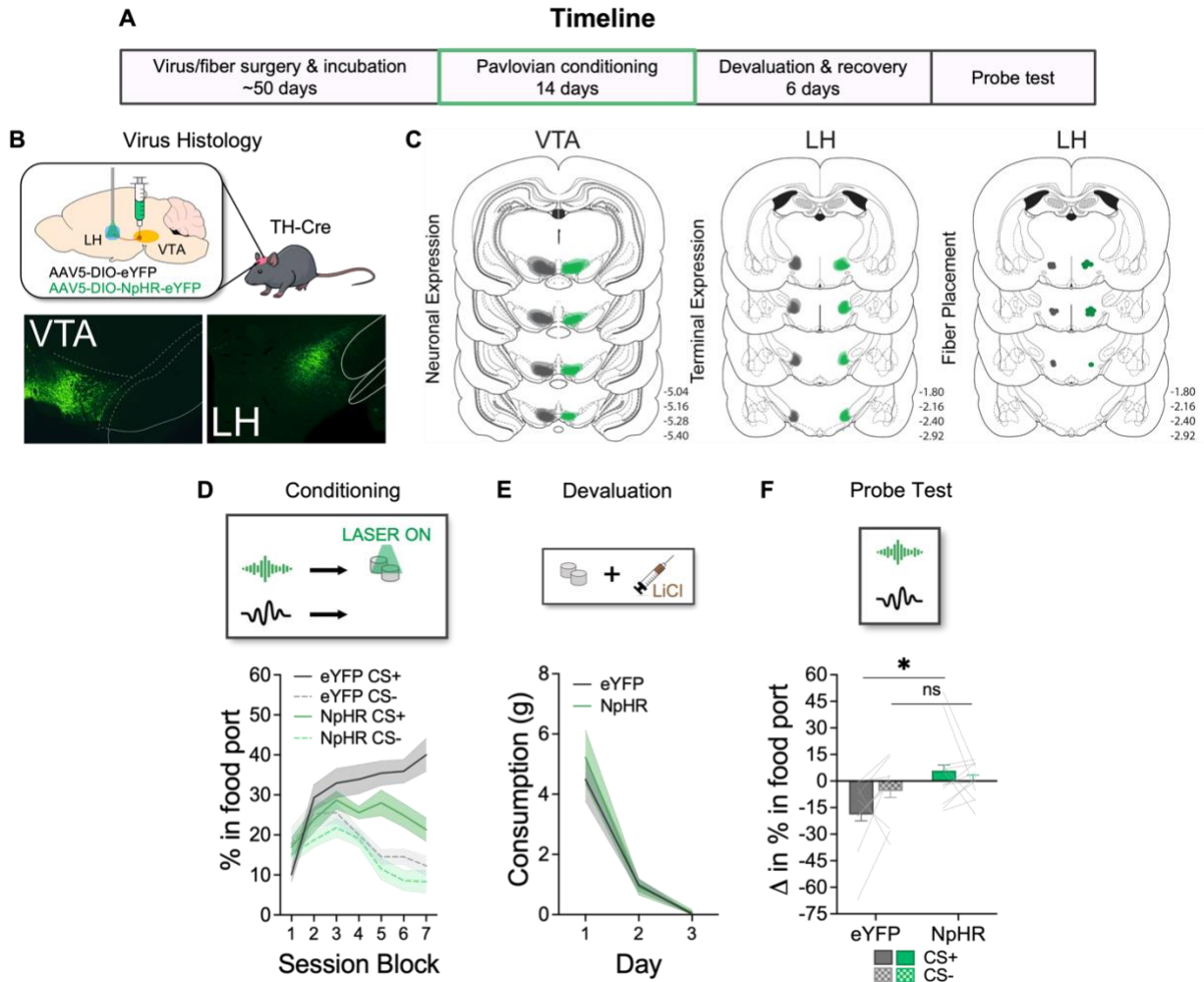
Figure 4. Inhibition of VTA_DA projections to LH reduces model-based learning about cues and rewards.

(A) Experimental timeline. **(B)** Optogenetic approach: TH-Cre transgenic rats were bilaterally infused with a Cre-dependent AAV with inhibitory halorhodopsin (NpHR; *n*=12), or a control vector (eYFP; *n*=10) in VTA and implanted with optic fibers in LH to allow for the inhibition of VTA_DA terminals in LH. Below shows unilateral examples of bilateral virus expression in VTA_DA neurons (*left*) and axonal terminals in LH (*right*). **(C)** *Left*: Unilateral representation of bilateral cell body virus expression in VTA for the eYFP group (grey) and the NpHR group (green). *Middle:* Unilateral representation of bilateral axonal terminal expression in LH. *Right*: Dots indicate approximate location of fiber tips in LH. **(D)** Rats learned that a CS+ leads to reward and a CS- has no consequence. VTA_DA terminals in LH were inhibited during food delivery across learning, when a prediction error would occur. Inhibition of VTA_DA terminals in LH significantly reduced learning about the CS+. **(E)** Reward was then paired with injections of LiCl and both eYFP and NpHR groups reduced their consumption across LiCl pairings. **(F)** Rats received a probe test where the CS+ and CS- were presented without reward. Here, rats in the eYFP group reduced responding to CS+ after devaluation, while the NpHR group showed no difference. *$p \leq 0.05$, mean (± SEM).

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Experiment 4: Phasic stimulation of VTA_DA projections to LH is sufficient to drive model-based learning between cues and rewards

We found that inhibition of VTA_DA projections to LH attenuates model-based learning about cues and their specific rewards, suggesting that this pathway is necessary for acquiring model-based cue-reward associations. However, given there was reduced responding in the NpHR experimental group, it was difficult to definitively say that there was a change in devaluation sensitivity. In order to address this, we next asked if stimulation of the VTA_DA→LH pathway would be sufficient to drive model-based learning between cues and rewards (**Figure 5A**). To test this, we utilized the blocking procedure (Kamin, 1967; Keiflin et al., 2019; Millard et al., 2022; Sharpe et al., 2017; Sharpe et al., 2020; Steinberg et al., 2013), which allows us to test if we can biologically rescue associative learning by stimulation of the VTA_DA to LH pathway. TH-Cre rats were bilaterally infused with a Cre-dependent, excitatory channelrhodopsin (AAV5-Ef1a-DIO-hChR2(E123T/T159C)-eYFP; ChR2; *n*=14) in VTA and had an optic fiber placed over LH (**Figure 5B-C**). This allowed us to stimulate VTA_DA terminals in LH. After virus incubation, rats were food restricted and began Pavlovian conditioning, where two 30-s visual cues were paired with two distinct rewards (flash and steady lights, counterbalanced; 8 sessions; 8 presentations/session). We then introduced two novel 30-s auditory cues (click and white noise, counterbalanced) presented in compound with the visual cues and followed by the same distinct rewards (4 sessions; 8 presentations/session). Normally, rats will not learn about the novel auditory cues because no new information can be attributed to them as there is no change in the reward contingency (i.e., blocking) (Kamin, 1967). However, during one of the rewards, we stimulated VTA_DA terminals in LH as a prediction error by delivering blue light into LH (1-s, 20Hz; 473 nm; 14-16mW) (Millard et al., 2022; Sharpe et al., 2017; Sharpe et al., 2020), to examine whether we could facilitate learning about one of the auditory cues ("unblocked") while the other cue, would serve as a control, is not learned about ("blocked"). As we and others have previously shown that light alone in eYFP controls does not unblock learning using these parameters (Chang et al.,

2016; Millard et al., 2022; Sharpe et al., 2017; Sharpe et al., 2020), we opted for a within-subjects blocking design where all rats had ChR2 infused into VTA$_{DA}$ neurons which allowed us to compare responding to unblocked and blocked cues in each rat. All rats elevated responding above baseline to the visual stimuli in initial conditioning sessions, and this was unaffected by introduction of the auditory cues and stimulation of the VTA$_{DA}$ to LH pathway (**Figure 5D-E**; CS (blocked vs. unblocked vs. baseline): $F_{(2,26)}$ = 18.892, $p<0.001$; session: $F_{(5,65)}$ = 12.463, $p<0.001$; CS x session: $F_{(10,130)}$ = 17.342, $p<0.001$). Follow-up simple main effects analyses revealed greater responding during presentation of either cue relative to baseline (blocked vs. baseline: $F_{(10,130)}$ = 25.918, $p<0.001$; unblocked vs. baseline: $F_{(10,130)}$ = 26.450, $p<0.001$; blocked vs. unblocked: $F_{(10,130)}$ = 0.158, $p=0.698$). Rats then received a probe test in which each of the auditory cues were presented without rewards or stimulation. We found that rats made greater responses to the unblocked cue than the blocked cue (**Figure 5F**; $F_{(1,13)}$ = 4.28, $p=0.030$) demonstrating that phasic stimulation of the VTA$_{DA}$ terminals in LH successfully facilitated learning about a reward-paired cue. Following the conclusion of all behavioral procedures, we later assessed the reinforcing effects of phasic stimulation of this VTA$_{DA}$→LH pathway (for further discussion and supplementary information, see **Appendix A**, pg. 110-111).

Finally, we probed the content of learning supported by stimulation of the VTA$_{DA}$→LH pathway. To investigate this, we employed a devaluation procedure in which half of the rats received pairings of LiCl injections with consumption of the reward associated with the unblocked cue ("devalued") and the other half would experience LiCl injections separate from reward consumption ("non-devalued"). The non-devalued group maintained consumption of the reward across devaluation days, while the devalued group reduced consumption, demonstrating development of a conditioned taste aversion (**Figure 5G**; day: $F_{(2,24)}$ = 6.064, $p=0.007$; group: $F_{(1,12)}$ = 22.561, $p<0.001$; day x group: $F_{(2,24)}$ = 11.412, $p<0.001$). Rats then received a probe test where the unblocked cue was presented alone without reward. Here, the devalued group exhibited a reduced level of responding to the cue relative to the non-devalued group (**Figure 5H**;

Welch's test: $F_{(1,13)} = 3.404$, $p=0.050$). This suggested that learning about the unblocked cue was model-based. Further, a consumption test for the devalued reward was conducted immediately following the probe test, which confirmed devaluation procedures were successful (**Figure 5I**; Welch's test; $F_{(1,13)} = 17.247$, $p=0.003$). In accordance with the data from inhibition of this circuit (**Figure 4**), these data suggest that the VTA$_{DA}$→LH pathway functions to support learning of model-based cue-reward associations.
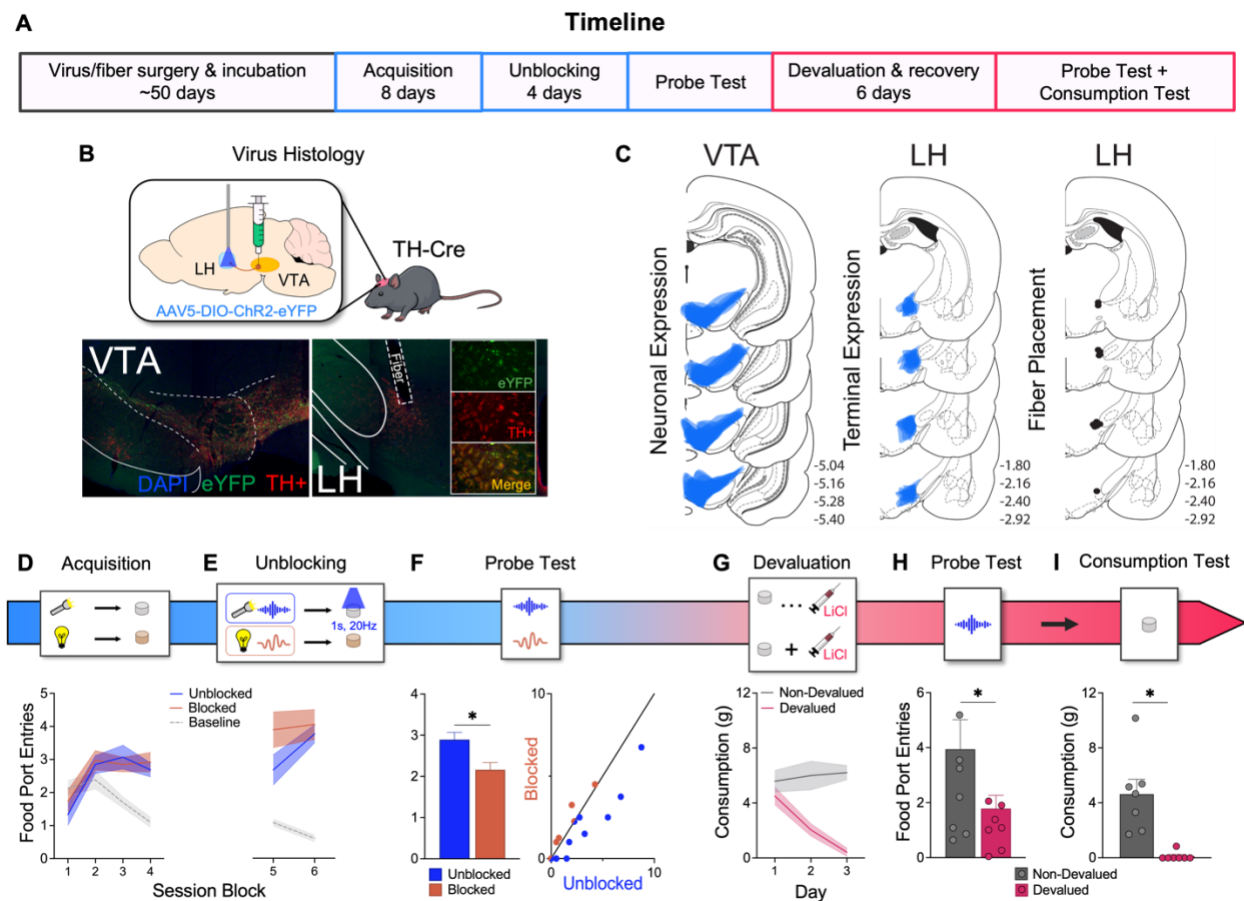
Figure 5. Phasic stimulation of the VTA$_{DA}$→LH pathway facilitates model-based learning for cues and rewards.

(A) Experimental timeline. **(B)** Optogenetic approach: TH-Cre transgenic rats were bilaterally infused with a Cre-dependent AAV with channelrhodopsin (*n*=14) in VTA and implanted with optic fibers in LH. *Middle:* unilateral examples of bilateral virus expression in the cell bodies of dopamine neurons in VTA and *Bottom*: in axonal terminals in LH. Slices were stained with DAPI (blue) and TH (red) for immunohistochemical verification of co-localization of virus expression (tagged with eYFP, green) and TH. **(C)** *Left*: Unilateral representation of bilateral virus expression

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in VTA. *Middle:* Unilateral representation of bilateral terminal expression in LH. *Right:* Black dots indicate approximate location of fiber tips in LH. **(D)** Rats first learned an association between two visual cues and two distinct rewards. Responding is represented as the number of entries made into the food port during cue presentation across session blocks (2 daily sessions per block). **(E)** Novel auditory cues were presented in compound with the visual cues and led to the same rewards. Blue light (20Hz; 473nm; 14-16mW) was delivered concurrently with one of the rewards. **(F)** Rats were then given a probe test for the auditory cues. Here, rats showed significantly more responding to the cue paired with stimulation of VTA_{DA} terminals in LH (i.e., unblocked cue). Individual responding is represented as dots on the scatterplot, with color indicating a preference for blocked (orange) or unblocked (blue) cue. To the extent that responding is equivalent to these cues, dots will congregate on the diagonal. **(G)** Rats underwent a reward devaluation procedure where the reward associated with the unblocked cue was paired with injections of LiCl. The devalued group, but not the non-devalued group, reduced consumption of the reward across LiCl injections. **(H)** Rats received a final probe test to examine the devaluation-sensitivity of responding to the unblocked cue. Here, the devalued group made significantly fewer responses to the cue. **(I)** Rats also underwent a consumption test to confirm that devaluation procedures were effective. The devalued group consumed less of the reward than the non-devalued group. *$p \leq 0.05$, mean (± SEM).

Discussion

In the present study, we set out to understand the nature of learning that underlies LH function and the wider circuitry that supports this function. Our first study revealed that LH_{GABA} neurons are necessary for the use of model-based associations to guide behavior. Specifically, we found that rats without LH_{GABA} neuronal activity did not adjust their behavior with outcome devaluation. This extends our previous work demonstrating that LH_{GABA} neurons are needed to acquire and express learning about cues and rewards (Sharpe, Marchant, et al., 2017), revealing that the nature of this learning involves the development of model-based associations comprised of representations between cues and their specific reward predictions.

Importantly, the finding for LH_{GABA} neurons in accumulating model-based information was confirmed in a follow-up study showing that inhibition of LH_{GABA} neurons does not impact on conditioned reinforcement after extended training in the absence of devaluation when there was likely to be little influence of model-based learning. However, it is worth noting that there appeared to be a non-significant reduction in the magnitude of conditioned reinforcement in the NpHR inhibition group compared to the eYFP control group. While we attempted to reduce the model-based component present in conditioned reinforcement by overtraining the Pavlovian

contingencies, we cannot rule out that there was a model-based component of conditioned reinforcement that remained after extended conditioning. Thus, the presence of a model-based component during conditioned reinforcement could underlie reduced responding in our NpHR inhibition group. Indeed, previous work suggests that conditioned reinforcement is driven by a balance of general, model-free value inherent in a reward-predictive cue and the sensory-specific, model-based aspects of the cue (Burke et al., 2007, 2008). Thus, overtraining tilted this balance to rely more on model-free value than on model-based associations. Therefore, inhibition of LH$_{GABA}$ neurons was still impacting conditioned reinforcement, just to a lesser extent than the dramatic difference seen between our NpHR and eYFP groups in **Experiment 1A** because there was less model-based information necessary to express conditioned reinforcement in **Experiment 1B**.

Next, we identified a novel projection from VTA dopamine neurons to the LH. Previous anatomical studies had suggested that a projection from VTA dopamine neurons to LH existed, but none had explicitly quantified this projection (Aransay et al., 2015; Beckstead et al., 1979; Bubser et al., 2005; Yonemochi et al., 2019). Here, we used a retrograde tracing strategy to confirm and quantify this dopaminergic projection from VTA to the LH. We found that a majority (~64%) of VTA projections to the LH were dopaminergic. Importantly, we focused our quantification on lateral VTA as this region has been shown to contain more dopamine neurons that exclusively release dopamine, whereas populations of dopamine neurons in medial VTA have been found to co-release dopamine with other neurotransmitters (Ma et al., 2023; Morales & Margolis, 2017). However, the remaining proportion of projections from VTA to LH that were non-dopaminergic should not be overlooked. Others have shown that the LH also receives a substantial number of GABAergic projections from VTA, albeit this was found in mice (Taylor et al., 2014). However, this study infused anterograde tracers into both medial and lateral portions of VTA, making it difficult to discern from which area of the VTA these GABAergic projections identified in LH are coming from. Thus, this warrants future work to more closely delineate efferent

projections of dopamine neurons from other populations in VTA, as well as their spatial organization in this region.

We then probed the function of this VTA$_{DA}$→LH pathway to determine if this circuit was necessary to support learning in LH. Given our hypothesis that VTA$_{DA}$ neurons send out a prediction error signal to LH to facilitate learning in this region, we optogenetically inhibited this pathway during Pavlovian cue-reward conditioning and restricted the duration of inhibition to suppress prediction errors evoked by initially unexpected rewards in a manner that does not produce a negative prediction error (Chang et al., 2018; Sharpe et al., 2017). We found that inhibition of the VTA$_{DA}$→LH pathway at the time of reward delivery attenuated, but not abolished, learning for the reward-predictive cue. Devaluation procedures then revealed that the remainder of learning for the reward cue was insensitive to devaluation (i.e., model-free), suggesting that inhibition of the VTA$_{DA}$→LH pathway suppressed the model-based component.

To complement our test for necessity, our final experiment assessed whether the VTA$_{DA}$→LH pathway was sufficient for supporting learning in the LH. Using optogenetics, we phasically stimulated terminal projections in this pathway to mimic the firing patterns of endogenous prediction errors seen in VTA dopamine neurons. Importantly, our manipulations were precisely timed to closely reflect the endogenous firing patterns of VTA dopamine neurons (Bayer & Glimcher, 2005; Cohen et al., 2012; Schultz et al., 1997). We found that this facilitated new learning in the LH. Furthermore, consistent with the results from the inhibition experiment, devaluation procedures here revealed the learning driven by VTA$_{DA}$→LH stimulation was model-based in nature.

Though our optogenetic manipulations should have approached physiological conditions of this circuit, it should be noted that the laser parameters used to stimulate and inhibit VTA$_{DA}$ terminals in LH were borrowed from previous work examining dopamine *cell bodies* in the VTA in the context of learning (Chang et al., 2016; Chang et al., 2018; Millard et al., 2022; Sharpe et al., 2017; Sharpe et al., 2020). Here, we applied those same parameters, but to axonal terminals of

dopamine neurons, which could produce different effects post-synaptically than if the cell bodies were manipulated. Additionally, stimulating the dopamine terminals in LH could have impacted the fibers of passage on route to nucleus accumbens (Bielajew & Shizgal, 1986). While this is possible, we do not think it could drive our effects because the same result was found with inhibition of these terminals, which is unlikely to impact processes distant from the terminals (Babl et al., 2019). Separately, we confirmed a large proportion of VTA dopamine neurons project directly to LH. Nonetheless, the work of future studies can help to confirm our behavioral effects. One direction would be to validate these parameters using ex vivo slice electrophysiology in VTA dopamine neurons and recording post-synaptic responses in the LH. Another direction would be replication of this study using an alternative intersectional viral strategy targeting the cell bodies of VTA$_{DA}$ neurons projecting to the LH (Kakava-Georgiadou et al., 2019; Poulin et al., 2018; Weinholtz & Castle, 2021). For example, infusion of a retrograde Cre-dependent AAV carrying Flp-recombinase into the LH of TH-Cre transgenic animals combined with an anterograde Flp-dependent opsin virus and optic fiber in the VTA would allow for selective expression of opsin and optogenetic manipulation of LH-projecting dopamine neurons in the VTA.

Collectively, these data are in line with modern accounts of dopamine prediction errors in supporting associative learning beyond scalar, model-free value (Chang et al., 2017; Engelhard et al., 2019; Gardner et al., 2018; Howard & Kahnt, 2018; Jeong et al., 2022; Keiflin et al., 2019; Langdon et al., 2018; Nasser et al., 2017; Seitz et al., 2022; Sharpe et al., 2017; Sharpe et al., 2020; Stalnaker et al., 2019; Takahashi et al., 2017) and reveal a novel hypothalamic-midbrain circuit that underlies model-based learning about cues and rewards. This is notable because it is one of the first implications of a specific and direct circuit comprising dopamine neurons that contributes to model-based learning (Howard & Kahnt, 2018; Sias et al., 2024). The findings from this set of studies have now laid the foundational groundwork for future research integrating this novel circuit for the LH and VTA into reinforcement learning frameworks that can be used to study the neural underpinnings for learning-related behaviors.

Chapter 3: Impacts of methamphetamine on hypothalamic-midbrain circuit regulation of model-based decision-making

Our day-to-day decisions are often influenced by assessment of prospective outcomes that are evoked by their associated cues. Though normally an adaptive process, decision-making can become pathological such as for individuals with a substance use disorder (Everitt et al., 2001; Hogarth et al., 2013; Keiflin & Janak, 2015; Lamb et al., 2016; Pickens et al., 2011; Volkow et al., 2010; Voon et al., 2017). A hallmark of addiction is the vulnerability of these individuals to succumb to the influence of drug- and other reward-related cues in controlling their behavior (Carter & Tiffany, 1999a, 1999b; Volkow et al., 2010). This susceptibility can be detrimental as it makes abstaining from drug use difficult and directly contributes to increases in relapse rates (Liu et al., 2023; Vafaie & Kober, 2022). Exactly how this sensitivity to reward-paired cues arises in these individuals remains unclear.

A body of literature dedicated to the canonical habit theory of addiction, which posits that drug-taking behaviors in the presence of environmental cues is repeatedly reinforced by the experience of the powerful drug high, has been used to explain this phenomenon (Belin et al., 2013; Everitt & Robbins, 2005, 2016; Sebold et al., 2017; Vandaele & Ahmed, 2021). In other words, persistent drug use in individuals with a substance use disorder is thought to be inflexible and driven by model-free habits that individuals engage in despite negative consequences. Critical to the definition of habits is the assumption that these behaviors do not contain an assessment of future outcomes. However, the bulk of the literature that actually tests this hypothesis examines instrumental responses that are insensitive to changes in their contingent outcomes (Furlong et al., 2018; Furlong et al., 2017; LeBlanc et al., 2013; Leong et al., 2016; Nelson & Killcross, 2006; Schoenberg et al., 2022; Wassum et al., 2009; Zapata et al., 2010). Few have explored whether the Pavlovian component entails a representation of associations

between cues and outcomes and if it is disrupted by prior drug use (Pickens et al., 2024; Schoenbaum & Setlow, 2005).

Studies investigating the influence reward-predictive cues have in governing decisions have consistently demonstrated that exposure to drugs of abuse amplifies cue-potentiated behavior, argued as a heightening of general invigoration of behavior by reward-related cues (Corbit & Janak, 2007, 2016; LeBlanc et al., 2013; Ostlund et al., 2014; Pecina & Berridge, 2013; Pecina et al., 2006; Saddoris et al., 2011; Shields & Gremel, 2021; Wyvell & Berridge, 2000, 2001). Yet again, few have investigated whether such heightened behaviors are elicited by detailed reward predictions contained in the cues (i.e., specific cue-reward associations). Those that have either compared sucrose with ethanol, which is confounded by the fact that ethanol is caloric, or fail to show generally heightened responding from drug exposure (Alarcon & Delamater, 2019; Corbit & Janak, 2007, 2016; Shiflett, 2012). Thus, the following study set out to determine the specific nature of the heightened ability of reward-predictive cues to control decision-making after a history of drug self-administration by employing the outcome specific Pavlovian-to-Instrumental Transfer (PIT) paradigm (Cartoni et al., 2016). In this task, subjects first independently acquire two distinct Pavlovian associations and two distinct instrumental associations. Then, to examine how these cues are able to selectively invigorate behavior, subjects receive a transfer test in which action selections during each individual cue presentation are measured. Specific PIT is demonstrated when subjects perform an action directed to the same outcome as the current cue being presented more than the opposite action that led to a different outcome. Because cue-reward and action-reward associations are separately learned about in this procedure, subjects must rely on model-based reward predictions generated by Pavlovian stimuli in directing behavior towards responses associated with those same predictions.

Further, it is also of interest to investigate the neural underpinnings for drug-induced heightening of specific cue-guided behavior. We have shown that the LH encodes model-based associations and that this is facilitated by input from VTA_{DA} neurons (see Chapter 2, pg. 17-45).

Thus, perhaps this neural circuit adapts following exposure to drugs of abuse to potentiate learning about reward-predictive information (Ahmed et al., 2005; Glimcher, 2011; Kauer & Malenka, 2007). Interestingly, prior research has implicated a strengthening of hypothalamic circuits in the neural changes that occur with exposure to drugs of abuse (Ahmed et al., 2005; Aston-Jones et al., 2009; Cornish et al., 2012; Harris et al., 2005; James et al., 2019; Mahler & Aston-Jones, 2012; Marchant et al., 2012). Specifically, it has been shown that the LH undergoes robust gene expression changes for pre- and postsynaptic proteins involved in neurotransmission following cocaine self-administration (Ahmed et al., 2005) and that increased Fos activity of neurons in this region after exposure to drugs of abuse is associated with addiction-like phenotypes (Cornish et al., 2012; Harris et al., 2005; James et al., 2019). LH neurons are also activated during context-induced relapse of drug- and alcohol-seeking (Blacktop & Sorg, 2019; Hamlin et al., 2008; Hamlin et al., 2007; Harris et al., 2005; Marchant et al., 2009; Marchant et al., 2014). Further, exposure to substances such as cocaine or morphine increase Fos expression of LH neurons projecting to VTA in response to drug-predictive cues and contexts (Aston-Jones et al., 2009; Mahler & Aston-Jones, 2012). Separately, it has also been shown that dopamine signaling is dysregulated following drug exposure (Bhimani et al., 2021; Everitt et al., 2001; Howard et al., 2013; Johansen & McFadden, 2017; Keiflin & Janak, 2015; Koob & Volkow, 2016; Lin et al., 2016; McCann et al., 2008; McCann et al., 1998; Segal et al., 2005; Y. K. Takahashi et al., 2019; Volkow, Chang, Wang, Fowler, Franceschi, et al., 2001; Volkow, Chang, Wang, Fowler, Leonido-Yee, et al., 2001; Volkow et al., 2010; Yorgason et al., 2020), leading to increases in extracellular dopamine which can induce changes in phasic amplitude and frequency of transients (Bhimani et al., 2021; Lin et al., 2016; Yorgason et al., 2020). Thus, if the neural changes that occur in this circuit are relevant to changes in learning and behavior that occur with drugs of abuse, this would lead to the prediction that drug exposure would enhance the use of model-based associations between cues and rewards to influence decision-making.

To interrogate whether LH-VTA circuits become strengthened after drug exposure, we assessed the changes in patterns of optical self-stimulation for these pathways as a proxy for measuring sensitization in animals with previous drug experience. If this pathway is strengthened in drug exposed animals, greater rates of intracranial self-stimulation will be exhibited, suggesting that drug-induced neural plasticity of LH-VTA circuits took place. Thus, the overall goals for this chapter and its experiments are to determine how methamphetamine experience affects model-based decision-making and whether prior exposure to this drug induces strengthening of LH-VTA circuits.

Materials and Methods

Surgeries

30 experimentally-naïve, adult Long Evans rats were used in this study (16 female, 14 male; Charles River, MA). Of this total, half were used for drug self-administration (**Experiment 5**) and the other half were used experimenter-administration procedures (**Experiment 6**), while a subset of these animals in each experiment (*n*=14 total) was used for intracranial self-stimulation sessions. Animals were housed in a 12-hour reverse light cycle room for the entirety of experimental procedures. Unless otherwise stated, rats were given ad libitum home chow and water.

Intravenous catheterization

All rats in the self-administration experiment (**Experiment 5**) first received surgery to implant a homemade intravenous (I.V.) catheter into its jugular vein. Catheters were flushed daily with 0.1 mL of saline and 0.2 mL heparinized saline containing enrofloxacin antibiotic (Baytril). Following catheterization, animals were randomly placed into either Methamphetamine or Control groups, allocated by sex and weight. Rats were given 1 week to recover from surgery before beginning

self-administration procedures. Twice daily flushing with the above solutions for all animals before and after self-administration sessions also served as our catheter patency check in place of intravenous injection of an anesthetic. This was to avoid potential impacts on behavior and confounded changes to neural circuits in future learning tasks.

Virus infusions and optic fiber implantation

All surgical coordinates are relative to bregma. Rats were given 6 weeks to recover from surgical procedures and to allow for sufficient time for the virus to incubate in cell bodies and axonal projections. To establish intracranial self-stimulation of the LH→VTA pathway, rats received 1.2 μL bilateral infusions of CaMKIIa-driven channelrhodopsin [AAV9-CaMKIIa-hChR2(H134R)-eYFP; Addgene: #26969] into LH (AP: -2.4 mm; ML: ±3.5 mm; DV: -9.0 mm; angled at 10° towards midline) and optic fiber implants over VTA (AP: -5.3 mm; ML: ±2.61 mm; DV: -7.55 mm; angled at 15° towards midline). To allow for stimulation of the VTA→LH pathway, rats received 2.0 μL bilateral infusions of CaMKIIa-driven channelrhodopsin [AAV9-CaMKIIa-hChR2(H134R)-eYFP] into VTA [AP: -5.3 mm; ML: ±2.61 mm; DV: -7.0 and -8.2 mm (males) or -6.5 and -7.7 mm (females)] with fibers placed bilaterally over LH [AP: -2.4 mm; ML: ±3.5 mm; DV: -8.5 mm (males) or -7.9 mm (females); angled at 10° towards midline].

Drugs

Methamphetamine HCl (#M8750, Sigma-Aldrich, MA) was dissolved in 0.9% saline (Hospira, Lake Forest, IL, USA) and self-administered intravenously at a dose of 0.1 mg/kg/infusion. Experimenter-administered injections (i.p., 1 mL/kg) followed an escalating dose regimen based on the average intake curve of rats in the drug group of the self-administration experiment ranging from 0.5 – 1.7 mg/kg.

Behavioral procedures

Experiment 5

Self-administration

Rats were singly-housed in a 12-hour reverse light-dark cycle vivarium. Training and testing were conducted during the early portion of the dark cycle. Following 1-week recovery from catheterization, rats were food restricted and maintained at 85% of their free-feeding weight for the entirety of experimental procedures except during the 3-week abstinence period prior to Pavlovian-to-Instrumental Transfer training. Sessions took place in operant behavior chambers encased in a sound-attenuating box that were controlled by MED-PC V software (Med Associates, Inc., Fairfax, VT). Each rat was assigned to its own chamber. Rats received 14 daily self-administration sessions using an adapted procedure (Mueller et al., 2021; Y. K. Takahashi et al., 2019; Wied et al., 2013). Animals were trained on increasing fixed-ratio (FR) reinforcement schedules (6x FR-1, 4x FR-3, 4x FR-5) (Hart et al., 2018). One lever ("active") would result in reward while the other ("inactive") would have no programmed consequences (lever designation counterbalanced). The Methamphetamine group could earn a 4-s infusion of methamphetamine (0.1 mg/kg/infusion) per reward while the Control group could earn two 45-mg grain pellets (Test Diet, MA). Following conclusion of self-administration training, all rats were subjected to a 3-week abstinence period where they would remain in their home cages and given *ad libitum* home chow.

Outcome-specific Pavlovian-to-Instrumental Transfer

All procedures for PIT training and tests were adapted from previous work and conducted in separate contexts from self-administration (Bradfield et al., 2018). Briefly, rats received Pavlovian conditioning sessions in which two distinct auditory cues (click or white noise, counterbalanced) were pseudo-randomly presented, each paired with one of two outcomes (sucrose pellets or maltodextrin, counterbalanced). Performance was measured by entries made into the food port during CS presentation relative to pre-CS baseline of equal duration. Rats then received instrumental training across increasing random-ratio reinforcement schedules (2x FR-1, 3x RR-

5, 3x RR-10) in which lever-pressing could earn them delivery of the two outcomes (lever-outcome pairings counterbalanced). Finally, the PIT test was administered in which both levers were available throughout the session, but no outcomes were delivered. Each of the auditory cues were individually presented and PIT performance was measured based on responses made on the lever corresponding to the same outcome as the current cue presented ("Same") relative to the opposite lever ("Diff") during CS presentation relative to pre-CS baseline. PIT procedures took place in a different context than self-administration and intracranial self-stimulation procedures.

Intracranial self-stimulation

Rats received six intracranial self-stimulation sessions defaulted to an FR-1 training schedule unless otherwise stated. Pressing on one lever ("active") delivered optogenetic stimulation of the respective terminal projections and pressing on the other ("inactive") had no programed consequences (counterbalanced). Stimulation consisted of 2-second trains of blue light [473 nm; 14-16mW; 20Hz: 10-ms pulse duration, 40-ms interval (Barbano et al., 2016)]. Intracranial self-stimulation took place in a different context in different behavioral chambers from self-administration and PIT procedures.

Experiment 6

Experimenter-administered injections

Rats were singly-housed in a 12-hour reverse light cycle vivarium room without other cohorts of rats. Rats were divided into two groups based on sex and weight. One group (Methamphetamine) would receive i.p. injections (1 mL/kg) of methamphetamine prepared in 0.9% saline on an escalating dose regimen (0.5-1.7 mg/kg). The other group (Control) would receive i.p. injections of saline vehicle (1 mL/kg). Injections were administered 3 hours into the animals' dark cycle daily for 2 weeks in the vivarium where they were housed. Animals were weighed immediately before injections to determine appropriate volume to administer. Group order for injections was

interleaved and subject order for injections was randomized each day. The same experimenter injected animals every day but was blind to injectable solution for each subject, while a second experimenter was aware of group assignments and arranged the subject order and solution preparation. Animals were given a 3-week abstinence period upon completion of the 2-week injection protocol as previously described for self-administration.

Outcome-specific Pavlovian-to-Instrumental Transfer

Following abstinence, all animals received PIT training and tests as described above in **Experiment 5**.

Intracranial self-stimulation

A subset of rats received intracranial self-stimulation sessions similar to those described above. Training began with three sessions of an FR-1 reinforcement schedule, followed by three sessions shifted up to an RR-5 schedule. Animals started with 20Hz stimulation on this training course and then received the same training course with 50Hz stimulation. Stimulation consisted of 1-second trains of blue light [473 nm; 14-16mW; 20Hz: 5-ms pulse duration, 45-ms interval (Millard et al., 2022); 50Hz: 5-ms pulse duration, 15-ms interval (Millard et al., 2022; Sharpe et al., 2017; Sharpe et al., 2020)]. Data for **Experiment 6** are visually presented as the difference in lever presses between active and inactive levers averaged across sessions for each frequency and reinforcement schedule (**Figure 7**). Intracranial self-stimulation took place in a different context in different behavioral chambers from PIT procedures.

General Statistical Analyses

Data were analyzed using repeated measures Analysis of Variance (ANOVA) and one-way ANOVA statistical tests. For self-administration procedures, within-subjects factors of lever (active vs. inactive) and session (across 14 sessions) were included to analyze operant behavior. Only

within-subjects factor of session was included to analyze respective reward intake. Separate repeated measures ANOVA analyses were conducted for self-administration data (lever press data and intake data) for Control and Methamphetamine groups. For PIT procedures, only session (average of 2 sessions per block for Pavlovian conditioning; all 8 sessions for instrumental training) was included as a within-subjects factor in repeated measures ANOVAs. To analyze data from the transfer test, a repeated measures ANOVA was conducted including response (same vs. different) as a within-subjects factor. Follow-up simple main effects were analyzed for significant interactions. A one-way ANOVA was conducted to analyze baseline responses during the transfer test between groups. Because we also include a within-subjects factor of time across cue presentation (four 30-second bins) for **Experiment 6**, we subtracted baseline responding averaged between same and different responses from responding during cue presentation to reduce risk of a type 1 error. Data for **Experiment 6** were analyzed with a one-tailed t test based on an *a priori* directional hypothesis. For intracranial self-stimulation, lever presses made on active vs. inactive lever were compared across session blocks. Data in **Experiment 6** also included stimulation frequency (20Hz vs. 50Hz) and training schedule (FR-1 vs. RR-5) in the repeated measures ANOVA. Between-subjects factor of treatment group (Control vs. Methamphetamine) was included in all analyses except for self-administration data. Data were tested for normality using Mauchly's test of sphericity and when sphericity could not be assumed in repeated measures ANOVAs, the Greenhouse Geiser adjustment was reported. Separate Pearson's correlations for **Experiment 5** were conducted for Control and Methamphetamine groups on the magnitude of the specific PIT effect [ratio of Same and Different responses during the transfer test (i.e., Same/Diff)], and self-administration performance [the change in ratio of active lever presses relative to total number of presses (i.e., active / active + inactive) averaged across the last 3 sessions of self-administration compared to the first 3 sessions]. Analyses used an alpha level of 0.05.

Histology

At the conclusion of experiments, animals were induced with carbon dioxide and then transcardially perfused with 1X phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA) in 1X PBS solution. Brains were extracted and placed in 4% PFA overnight before being changed to 30% sucrose solution made up in 1X PBS for at least 48 hours. Brain tissue was then sectioned into 30 μm coronal sections using a cryostat and collected in well-plates containing 1X PBS. Sections were mounted onto microscope slides and sealed with glass coverslips using ProLong™ gold antifade reagent with DAPI (Thermo Fisher Scientific, Waltham, MA; P36930). eYFP fluorescence was used to virus expression in LH and VTA cell bodies and terminals.

Results

Experiment 5: Methamphetamine self-administration enhances use of model-based associations to drive behavior and strengthens the LH→VTA pathway

Prior research in humans and rodent models of substance use disorders have shown increased susceptibility to the control that reward cues exert over behavior (Corbit & Janak, 2007, 2016; LeBlanc et al., 2013; Ostlund et al., 2014; Pecina & Berridge, 2013; Pecina et al., 2006; Saddoris et al., 2011; Shields & Gremel, 2021; Wyvell & Berridge, 2000, 2001). However, it remains undetermined whether this influence arises from changes in model-based or model-free strategies. Furthermore, others have demonstrated that LH inputs to VTA are sensitized following exposure to drugs of abuse (Ahmed et al., 2005; Aston-Jones et al., 2009; Cornish et al., 2012; Harris et al., 2005; James et al., 2019; Mahler & Aston-Jones, 2012; Marchant et al., 2012). Given our results demonstrating a role for LH in model-based behavior (see Chapter 2, pg. 17-45), we predicted that a history of drug self-administration would enhance the LH→VTA circuit and produce enhanced control of reward-predictive cues over behavior in a model-based manner.

Experimentally-naïve rats first underwent surgery for the implantation of intravenous catheters in their jugular vein (**Figure 6A**). Rats were then food restricted and trained to self-administer either grain pellets (Control, *n*=8) or methamphetamine infusions (Methamphetamine, *n*=7). We opted to have our drug-naïve control group self-administer grain pellets instead of infusions of saline as many other I.V. drug self-administration protocols would use to account for the inherently rewarding experience of the act of earning drug rewards. Self-administration sessions comprised of 3-hour sessions across a 14-day protocol, beginning with rats pressing the active lever once for rewards (fixed-ratio 1; FR-1) and escalating to FR-3 and then FR-5 reinforcement schedules (Hart et al., 2018; Y. K. Takahashi et al., 2019; Wied et al., 2013). In the Control group, pressing the active lever resulted in delivery of two 45-mg grain pellets (Test Diet, MA). In the Methamphetamine group, pressing the active lever resulted in a 0.1 mg/kg intravenous methamphetamine infusion. For both groups, pressing the inactive lever had no programmed consequences. The Control group increased lever-pressing on the active lever across time relative to the inactive lever (**Figure 6B (left)**; lever: $F_{(1,7)} = 256.163$, $p<0.001$; session: $F_{(13,91)} = 54.412$, $p<0.001$; lever x session: $F_{(13,91)} = 65.966$, $p<0.001$), which was also reflected in them earning more pellets (g/kg) across time (**Figure 6B (right)**; session: $F_{(13,91)} = 7.699$, $p<0.001$). Similarly, the Methamphetamine group also increased responding on the active lever relative to the inactive lever (**Figure 6C (left)**; lever: $F_{(1,6)} = 16.641$, $p=0.007$; session: $F_{(13,78)} = 23.628$, $p<0.001$; lever x session: $F_{(13,78)} = 9.241$, $p<0.001$). This was also reflected in escalation of their methamphetamine intake across time (mg/kg) (**Figure 6C (right)**; session: $F_{(13,78)} = 6.119$, $p<0.001$).

Three weeks after self-administration, both groups were trained on a specific Pavlovian-to-Instrumental Transfer (PIT) paradigm in a new context. First, rats learned two Pavlovian contingencies (Bradfield et al., 2018). Here, a 2-min auditory cue (click and white noise, counterbalanced; 8 sessions, 8 presentations/session) predicted one of two distinct rewards [45-mg sucrose pellets (Test Diet, MA) and 15% maltodextrin solution (Earthborn Elements, OR);

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counterbalanced]. Both Control and Methamphetamine groups readily acquired the conditioned entry response into the food port during the cues (**Figure 6D**; CS vs. pre-CS: $F_{(1,13)}$ = 137.677, $p<0.001$; session: $F_{(3,39)}$ = 4.406, $p=0.009$; CS vs. pre-CS x session: $F_{(3,39)}$ = 17.374, $p<0.001$). Statistical analyses also confirmed that there were no between-groups differences in responding (group: $F_{(1,13)}$ = 0.053, $p=0.822$; CS vs. pre-CS x group: $F_{(1,13)}$ = 0.195, $p=0.666$; session x group: $F_{(3,39)}$ = 0.244, $p=0.865$; CS vs. pre-CS x session x group: $F_{(3,39)}$ = 0.778, $p=0.513$). Rats were then trained to press two different levers to receive the two outcomes (e.g., left lever→sucrose, right lever→maltodextrin; counterbalanced) across increasing random-ratio (RR) reinforcement schedules (FR-1, RR-5, RR-10; 8 sessions). Both groups readily acquired instrumental contingencies without between-group differences (**Figure 6E**; session: $F_{(7,91)}$ = 477.738, $p<0.001$; group: $F_{(1,13)}$ = 0.053, $p=0.821$; session x group: $F_{(7,91)}$ = 0.386, $p=0.908$).

Finally, rats were given the PIT test in which both levers were available and each of the auditory cues were presented. Importantly, no rewards were delivered so that we could test for the representation evoked by the cues without reward feedback. Specific PIT is observed when greater responses are made on the lever predicting the same outcome as the presented cue ("Same"), relative to the lever leading to the alternative outcome ("Diff"). This illustrates the use of model-based associative information to drive decision-making (Aitken et al., 2016; Bradfield et al., 2015; Lichtenberg et al., 2017; Lichtenberg et al., 2021; Sias et al., 2021). We found that while both groups responded more on the same lever relative to the different lever prior to cue onset (**Figure 6F**; response: $F_{(1,13)}$ = 17.981, $p<0.001$), the Methamphetamine group showed overall heightened responding compared to Controls (group: $F_{(1,13)}$ = 6.133, $p=0.028$), driven by a marked increase in responding on the same relative to different levers (response x group: $F_{(1,13)}$ = 5.050, $p=0.043$). This was specifically due to a significant group difference in responding on the same lever ($F_{(1,13)}$ = 6.509, $p=0.024$) without differences in responding on the different lever ($F_{(1,13)}$ = 0.012, $p=0.913$), indicating an enhancement of the specific PIT effect. Indeed, while the Methamphetamine group showed a significant difference in responding on the same vs. different

levers when analyzed independently (simple main effect of response, Methamphetamine: $F_{(1,13)}$ = 19.730, $p<0.001$), our Control group did not (simple main effect, Control: $F_{(1,13)}$ = 2.128, $p=0.168$). A separate one-way ANOVA found no between-groups differences in baseline responding [Methamphetamine: 7.21 ± 1.49 (mean ± SEM); Control: 5.23 ± 0.67; $F_{(1,13)}$ = 1.590, $p=0.229$]. Moreover, we found a strong positive correlation between self-administration and the magnitude of specific PIT in the Methamphetamine group (**Figure 6G (right)**; R^2 = 0.908, $p<0.001$) but not the Control group (**Figure 6G (left)**; R^2 = 0.008, $p=0.837$). These data show that methamphetamine self-administration produced an increase in the influence of model-based cue-reward associations over decision-making.

Next, we asked whether changes in our novel LH-VTA circuit could result from methamphetamine self-administration that may be related to enhancements in specific PIT. To test this, we compared how much our Control and Methamphetamine groups would show intracranial self-stimulation for the LH→VTA pathway, where intracranial self-stimulation is driven by GABAergic input from LH to VTA (Nieh et al., 2016). A subset of rats from our Control and drug groups underwent surgeries to bilaterally infuse channelrhodopsin (AAV9-CaMKIIa-hChR2(H134R)-eYFP) in LH and implant an optic fiber placed over VTA, allowing for stimulation of LH terminal projections in VTA (**Figure 6I**). Rats were then given intracranial self-stimulation sessions in a new context (**Figure 6H**; 30-min sessions, 6 sessions). Here, rats could press an active lever that delivered light-mediated stimulation of LH terminals in VTA (2-s, 20Hz; 473nm; 14-16mW) or an inactive lever which had no programmed consequences. We found that the Methamphetamine group showed significantly faster acquisition of self-stimulation relative to the Control group, illustrated by steeper increases in active lever presses across time (session x lever x group: $F_{(2,8)}$ = 6.806, $p=0.019$; simple effect of lever during the 2nd block: $F_{(2,8)}$ = 8.275, $p=0.042$). This demonstrates that the input from LH to VTA is enhanced in our rats experiencing methamphetamine self-administration. Altogether, these data implicate a strengthening of the LH-

VTA circuits following a history of methamphetamine self-administration that may be contribute to

the enhancements in LH-dependent model-based processes.
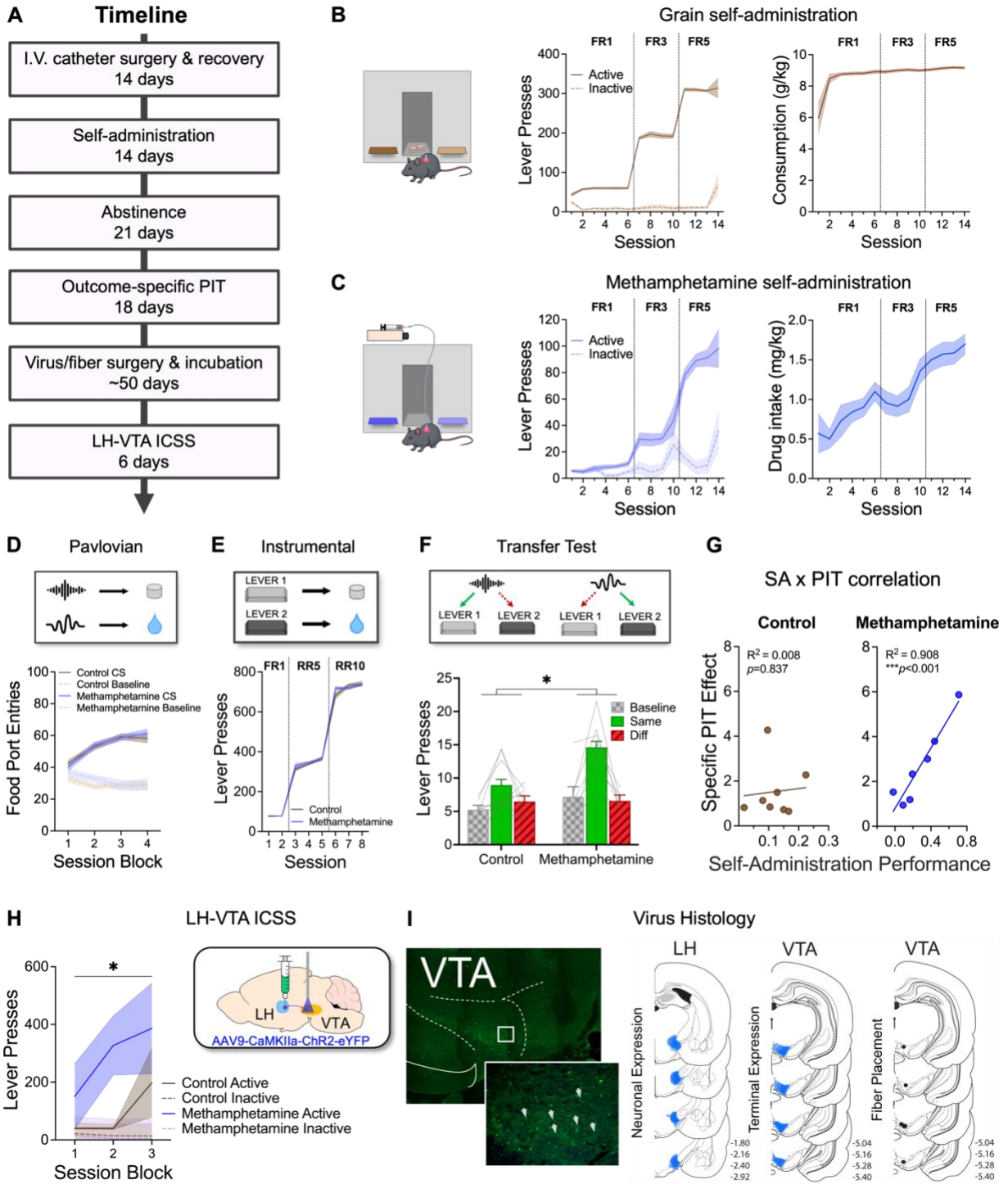
Figure 6. A history of methamphetamine self-administration enhances specific PIT and strengthens the LH→VTA pathway.

(A) Experimental timeline. **(B)** Rats in the Control group first learned to press a lever for grain. These rats increased responding on the active lever (*left*), resulting in an increase in pellets earned (*right*). **(C)** Rats in the Methamphetamine group learned to press a lever for 0.1 mg/kg infusions of methamphetamine. Lever-pressing for drug escalated across time (*left*) as well as

their methamphetamine intake across time (*right*). **(D)** Following abstinence, all rats then received Pavlovian conditioning, increasing food port entries across sessions. **(E)** Next, rats learned the instrumental contingencies and increased responding for the two distinct rewards across time. **(F)** Finally, rats underwent the critical PIT test. Here, the Methamphetamine group showed an enhanced specific PIT effect. **(G)** There was no correlation between grain self-administration and the magnitude of specific PIT (*left*), however, a strong positive correlation was present between methamphetamine self-administration and the outcome-specific PIT effect (*right*). **(H)** We tested how much rats were willing to earn stimulation of the LH→VTA pathway (intracranial self-stimulation; ICSS). Here, the Methamphetamine group showed significantly greater ICSS, implicating sensitization of the LH-VTA pathway. **(I)** Unilateral example of bilateral virus expression in the axonal terminals in VTA (*left*), and schematics of virus expression in the LH and VTA with fiber placements in VTA (*right*). *$p \leq 0.05$, mean (± SEM).

Experiment 6: Experimenter-administered methamphetamine produces enhancements in use of model-based predictions to guide behavior and strengthens the VTA→LH pathway

The previous experiment found that rats with a history of methamphetamine self-administration exhibited heightened use of model-based reward information to guide their decision-making. Though these results are suggestive of methamphetamine-induced enhancements in specific PIT, an alternative interpretation could be that animals in the drug-naïve control group showed less specific PIT due to their prior experience with lever-pressing for a food reward. That is, the drug naïve control group might be showing a reduced specific PIT effect, rather than the methamphetamine-experienced group showing an enhancement of specific PIT. Thus, we conducted a follow-up study to determine whether methamphetamine treatment alone by route of experimenter administration would achieve the same enhancements in specific PIT as self-administered methamphetamine.

Experimentally-naïve rats were first counterbalanced by sex and weight and then randomly assigned to the Methamphetamine drug group (*n*=7; 3 male, 4 female) or the Saline control group (*n*=8; 4 male, 4 female). Rats in the drug group received experimenter-administered injections of methamphetamine that followed an escalating dose regimen (0.5 – 1.7 mg/kg; 1 mL/kg) mirroring the intake curve of the drug group in the self-administration experiment (**Figure 6C**). The control group received equivalent injection volumes (1 mL/kg) of saline vehicle. During this time, rats were food restricted and maintained at ~85% of their free-feeding weight. After 2-

weeks of injections, rats were allowed to rest and recover from withdrawal effects during a 3-week abstinence period in which food was given *ad libitum*. Both groups then began training on the same specific PIT procedure as rats did in **Experiment 5** (**Figure 7A**).

Starting with Pavlovian conditioning, both Methamphetamine and Saline groups increased conditioned responding to both cues above baseline across time (**Figure 7B**; CS vs. pre-CS: $F_{(1,13)}$ = 182.768, $p<0.001$; session: $F_{(3,39)}$ = 10.862, $p<0.001$; CS vs. pre-CS x session: $F_{(3,39)}$ = 10.473, $p<0.001$). Statistical analyses also confirmed no between-groups differences throughout conditioning (group: $F_{(1,13)}$ < 0.001, $p=0.996$; CS vs. pre-CS x group: $F_{(1,13)}$ = 2.187, $p=0.163$; session x group: $F_{(3,39)}$ = 1.294, $p=0.290$; CS vs. pre-CS x session x group: $F_{(3,39)}$ = 1.582, $p=0.209$). Next, rats received instrumental training across increasing reinforcement schedules. Both groups acquired instrumental responses for each of the rewards without between-groups differences (**Figure 7C**; session: $F_{(7,91)}$ = 300.031, $p<0.001$; group: $F_{(1,13)}$ = 1.466, $p=0.247$; session x group: $F_{(7,91)}$ = 1.296, $p=0.261$). Finally, the critical PIT test was administered in which each cue was presented individually, and rats could elect to press on either lever without reward deliveries. More responses made on the lever previously associated with the same outcome as the current cue being played ("Same") relative to responses made on the opposite ("Diff") lever demonstrates the specific PIT effect. Here, we found that rats in both the Saline and Methamphetamine groups showed greater responding on the same lever compared to the different lever, demonstrating the specific PIT effect (**Figure 7D**; response: $F_{(1,13)}$ = 14.935, $p=0.002$). Further, both Saline and Methamphetamine groups showed an increase in overall responding over the course of cue presentation, which was found to be driven by increases in the same response (**Figure 7D**; time: $F_{(3,39)}$ = 6.694, $p=0.004$; time x response: $F_{(3,39)}$ = 2.747, $p=0.028$). Although no group differences were detected generally (group: $F_{(1,13)}$ = 0.241, $p=0.632$), by overall responding across time (time x group: $F_{(3,39)}$ = 0.924, $p=0.219$), or overall response pattern (response x group: $F_{(1,13)}$ = 1.130, $p=0.154$), rats in the Methamphetamine group showed an enhanced specific PIT effect that was most apparent later in cue presentation (time x response

x group: $F_{(3,39)}$ = 2.497, p=0.037; simple main effect of response, Methamphetamine: $F_{(1,13)}$ = 50.678, p<0.001; simple main effect, Saline: $F_{(1,13)}$ = 2.827, p=0.117), mirroring our effects seen with self-administration of methamphetamine. This confirmed that it was not the *lever pressing* for methamphetamine (or grain pellets) that produced the changes in the specific PIT effect. Rather, methamphetamine itself was sufficient to drive the changes in reward learning.

In **Experiment 5**, we also found that rats previously exposed to methamphetamine show sensitization of the LH→VTA pathway, indicated by faster rates of self-stimulation acquisition relative to the drug naïve control group (**Figure 7E**). Given we have evidence that supports a role for dopaminergic input from VTA to the LH in mediating learning (see Chapter 2, pg. 17-45), we were interested to see if the opposite direction of the LH-VTA circuit also becomes sensitized following methamphetamine exposure. Specifically, we used the same optogenetic viral approach as in **Experiment 5** except that channelrhodopsin was infused into VTA and optic fibers were placed into the LH of rats that received experimenter-administered methamphetamine or saline. Rats then underwent the intracranial self-stimulation procedure for stimulation of the VTA→LH pathway. Compared to Saline controls, rats in the Methamphetamine group showed greater self-stimulation (**Figure 7F**), represented as the difference in presses between active and inactive levers (i.e., active – inactive), for VTA terminals in LH when the stimulation condition increased to a higher frequency (lever x stimulation x schedule x group: $F_{(1,6)}$ = 4.226, p=0.043; simple main effect of stimulation on FR-1, Methamphetamine: $F_{(1,6)}$ = 6.301, p=0.046; simple main effect of stimulation on FR-1, Saline: $F_{(1,6)}$ = 0.305, p=0.601). This suggested that Methamphetamine produced a hypersensitivity to VTA→LH stimulation when stimulation of this pathway shifted to higher frequencies (Corbett & Wise, 1980; Fibiger et al., 1987; Garris et al., 1999).

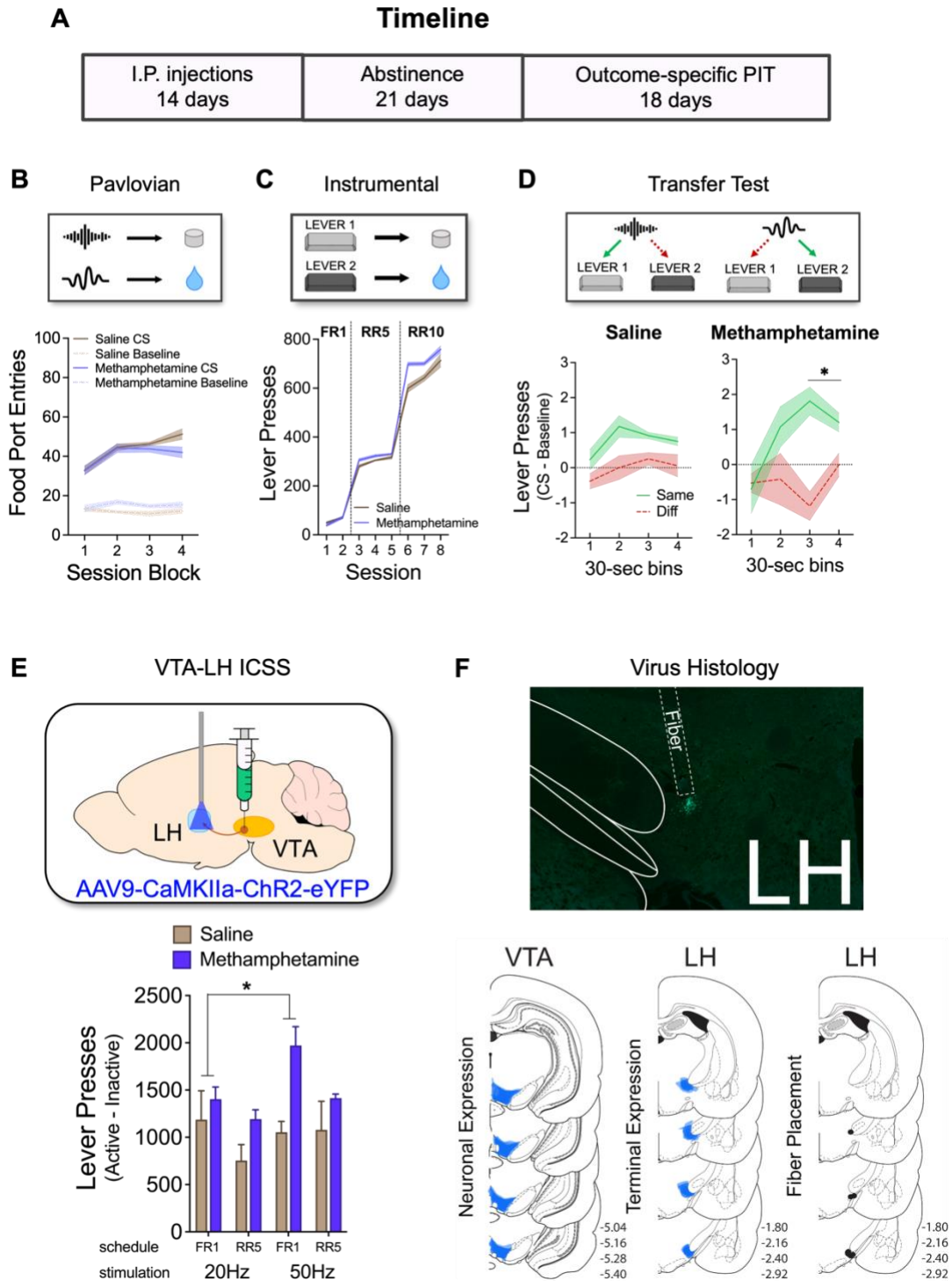**Figure 7. Experimenter-administered methamphetamine enhances specific PIT and sensitizes the VTA→LH pathway.**

(A) Experimental timeline. Rats were divided into two treatment groups, with one receiving methamphetamine injections across an escalating dose regimen (Methamphetamine, *n*=7; 1 mL/kg, i.p.) and the other receiving equivalent volumes of saline (Saline, *n*=8; 1 mL/kg, i.p.). **(B)**

Following 3 weeks of abstinence, all rats received Pavlovian conditioning sessions where two auditory cues were paired with two distinct rewards. Both groups increased conditioned responding during CS presentation relative to baseline responding. **(C)** Rats were then given instrumental training where two lever presses led to the two distinct rewards in which both groups readily acquired instrumental contingencies. **(D)** Finally, rats received the critical PIT test where the auditory cues were presented. Mirroring our effects when rats voluntarily self-administered methamphetamine (or grain pellets), rats that received experimenter-administered methamphetamine injections exhibited enhancements in the specific PIT effect, most prevalent towards the end of the CS, relative to Saline controls. **(E)** Rats underwent surgeries to infuse an excitatory channelrhodopsin virus into the VTA with optic fibers implanted over LH. Rats then received sessions of intracranial self-stimulation (ICSS) under low (20 Hz) and high (50 Hz) frequency optogenetic stimulation of the VTA→LH pathway across increasing schedules of reinforcement. Prior exposure to methamphetamine appears to also strengthen this pathway as stimulation shifted to higher frequencies. **(F)** Unilateral example of bilateral virus expression in the axonal terminals in LH (*top*), and schematics of virus expression in VTA and LH with fiber placements in LH (*bottom*). *$p \leq 0.05$, mean (± SEM).

Discussion

The present study sought to uncover the specific nature of drug-induced sensitivity to reward-paired cues in controlling behavior. Using a specific PIT paradigm, we trained rats to independently acquire unique cue-reward and action-reward associations, and then tested the influence of these cues to guide behavior towards instrumental actions that shared (or did not share) reward predictions with the cue. We revealed that animals who previously self-administered methamphetamine showed enhancements in the ability of reward-paired cues to promote instrumental actions predictive of the same rewards. Prior research has shown that drugs of abuse increase the impact of cues in invigorating responding directed to rewards in both rats and humans without having determined the specific nature of this enhancement (Corbit & Janak, 2007, 2016; Glasner et al., 2005; Hogarth & Chase, 2012; LeBlanc et al., 2013; LeBlanc et al., 2012; Manglani et al., 2017; Ostlund et al., 2014; Pecina & Berridge, 2013; Pecina et al., 2006; Saddoris et al., 2011; Shields & Gremel, 2021; Wyvell & Berridge, 2000, 2001). Here, we found that the cues exerted heightened control over behavior in a manner that reflected behavior directed towards specific rewards after drug exposure, indicating a model-based process (Bradfield et al., 2015; Daw et al., 2005). Further, prior drug self-administration performance was positively correlated with the specific PIT effect (a relationship not found with our control group

for food intake). This is in line with other work that shows positive correlations between the general ability of a cue to excite actions and self-administration of cocaine (T. T. Takahashi et al., 2019). Our work expands on this by characterizing the specific nature of heightened cue-guided behavior by previous drug experiences and showing that the degree of this enhancement scales with greater drug use.

Importantly, we also found that the increases in specific cue-guided behavior in methamphetamine-experienced animals were not due to a reduced PIT effect in our drug-naïve control group. That is, animals who received experimenter-administered methamphetamine also showed similar enhancements in specific PIT as animals who self-administered the drug, affirming our previous findings and demonstrating that methamphetamine increases the specific PIT effect. Interestingly, the results found with experimenter-administered methamphetamine were less robust than with self-administered methamphetamine, such that enhancements in specific PIT only became more apparent later in cue presentation. This may be indicative of how treatment with methamphetamine and earning methamphetamine differentially impacts future reward learning. Indeed, this raises an outstanding question that remains in the addiction literature, which is whether it is the act of drug taking that alters reward circuits in the brain (Chen et al., 2008; Stefański et al., 1999; Stefański et al., 2007) or whether it is the drug itself that produces these neural changes, rendering individuals with a substance use disorder sensitive to the influence of reward-paired cues (Bocklisch et al., 2013; Francis et al., 2022; Furlong et al., 2018; Furlong et al., 2017; Nelson & Killcross, 2006). Our results suggest that both the act of taking the drug and the effects of the drug itself produce the same behavioral enhancements from a qualitative perspective, but differ in magnitude, such that earning drug rewards produces stronger effects than drug treatment alone. Previous research efforts have attempted to disentangle the neural changes induced by passive, non-contingent drug administration (e.g., methamphetamine, cocaine, etc.) from those induced by active drug self-administration (Jacobs et al., 2003). On the other hand, others have found that drugs of abuse administered involuntarily produce similar

structural changes in neural circuits to those induced by reward experiences [for an in-depth review, see: Robinson and Kolb (2004)]. Based on our data, a mix of both factors produces the changes in reinforcement learning seen following drug exposure.

In addition to examining behavioral changes following methamphetamine experience, we also assessed the neural changes induced by a history of methamphetamine. Given the knowledge that the LH and its inputs to the VTA are strengthened following psychostimulant exposure (Ahmed et al., 2005; Aston-Jones et al., 2009; Cornish et al., 2012; Espana et al., 2010; Harris et al., 2005; James et al., 2019; Mahler & Aston-Jones, 2012; Marchant et al., 2012; McPherson et al., 2007), we were interested in testing whether a history of methamphetamine enhanced the use of model-based reward associations by strengthening hypothalamic-midbrain circuits, which we have shown is critical for model-based learning (see Chapter 2, pg. 17-45). In these same rats, we showed behavioral evidence that suggests prior exposure to methamphetamine strengthens LH-VTA circuits. When given the opportunity to self-stimulate LH-VTA pathways months after their last encounter with drug, methamphetamine-experienced rats demonstrated greater self-stimulation than drug-naïve rats, pointing to long-lasting changes in these neural circuits that now supported greater reinforcement. These findings are in line with other accounts showing neuroplasticity and increased activation of hypothalamic circuits following exposure to drugs of abuse (Ahmed et al., 2005; Aston-Jones et al., 2009; Cornish et al., 2012; Espana et al., 2010; Harris et al., 2005; James et al., 2019; Mahler & Aston-Jones, 2012; Marchant et al., 2012; McPherson et al., 2007), and suggest for the first time that this novel input from VTA neurons to the LH is also strengthened following psychostimulant exposure. Given our results in the previous chapter show LH-VTA circuits to be important for acquiring model-based associations (see Chapter 2, pg. 17-45), these data implicate drug-induced sensitization of this neural circuit that may underlie the heightened use of specific reward representations evoked by cues to guide behavior.

It is important to note that the AAV used for intracranial self-stimulation experiments was CaMKIIa-driven, which preferentially binds to excitatory, glutamatergic neurons. Thus, our self-stimulation findings may not be specific to GABAergic neurons in the LH, which we have implicated in model-based learning (see Chapter 2, pg. 17-45). For example, prior methamphetamine exposure for animals in the first experiment could have strengthened LH glutamatergic inputs to the VTA. However, Nieh et al. (2015) showed with ex vivo electrophysiology using this same viral strategy targeting the LH→VTA pathway that this virus also infects GABAergic neuronal projections. The rewarding nature of self-stimulation for this LH→VTA pathway was later revealed to be attributed to GABAergic projections from LH to VTA to disinhibit VTA dopamine neurons (Nieh et al., 2015), whereas the glutamatergic projection actually induced aversion (Nieh et al., 2016). Work from others have separately verified that the LH$_{GABA}$→VTA pathway is appetitive (Barbano et al., 2016; Schiffino et al., 2019). Separately, repeated cocaine exposure has been shown to disinhibit VTA$_{DA}$ neuronal firing by reducing the activity of GABAergic neurons in VTA, which synapse onto VTA$_{DA}$ neurons (Bocklisch et al., 2013).Thus, it is likely that our data showing enhanced self-stimulation in methamphetamine-experienced animals is due to changes in the GABAergic neurons projecting to the VTA, rather than glutamatergic input.

Nevertheless, although our findings for sensitization in LH-VTA circuits after a history of methamphetamine do not specify the particular pathways that are changed, what can be determined is that this circuit undergoes enduring changes that may likely support the behavioral enhancements observed. Of course, future research is necessary to determine the way in which these circuits are changed following drug exposure. For example, it could be the case that the depletion of dopamine transporters following methamphetamine use (McCann et al., 2008; McCann et al., 1998; Segal et al., 2005; Volkow, Chang, Wang, Fowler, Franceschi, et al., 2001) means drug-exposed subjects require more stimulation to experience the same level of reinforcement as in drug-naïve subjects. While this would be inconsistent with other research

showing increased activity in these circuits (Ahmed et al., 2005; Aston-Jones et al., 2009; Cornish et al., 2012; Harris et al., 2005; James et al., 2019; Mahler & Aston-Jones, 2012; Marchant et al., 2012), it warrants further consideration.

Finally, one important technical caveat to consider with these data is the use of similar instrumental responses conserved throughout the experiments. That is, the experimental designs for self-administration, specific PIT, and intracranial self-stimulation all incorporate a lever-press response to deliver its respective contingency. Thus, we acknowledge the possibility that our findings may be confounded by this shared action across the experiments despite changing the contexts and position of levers for each paradigm and counterbalancing the contingencies of the levers across experiments for each animal. However, we found that the enhancements in specific PIT were conserved in experimenter-administered methamphetamine animals, indicating that it was not the result of having lever-pressing in common between self-administration and instrumental training during PIT procedures. In any case, future studies replicating this work with alternative instrumental actions (e.g. nosepoke or chain pull) may help to resolve these technical issues in experimental design.

The findings from this chapter showing enhancements in model-based decision-making as a result of prior drug exposure are surprising as it counters the canonical habit, or model-free, theory of addiction (Belin et al., 2013; Everitt & Robbins, 2005, 2016; Furlong et al., 2018; Furlong et al., 2017; LeBlanc et al., 2013; Nelson & Killcross, 2006; Sebold et al., 2014; Sebold et al., 2017; Vandaele & Ahmed, 2021; Voon et al., 2017; Wassum et al., 2009). That is, substance use disorder is often conceptualized as promoting habitual behavior directed towards drug rewards, which explicitly lacks a representation of the specific reward. Indeed, this could be in part because persistent drug seeking alters the perception of the instrumental cost to obtain rewards following drug exposure (Hart et al., 2018; Thompson et al., 2017). Our data suggest that this development of habits following exposure to psychostimulants like amphetamine (Furlong et al., 2018; Furlong et al., 2017; Nelson & Killcross, 2006) may be specific to instrumental responding, and that the

influence of Pavlovian cues over drug seeking and drug taking is in fact model-based. Thus, a more encompassing perspective on the reinforcement learning mechanisms underlying substance use disorder could integrate the model-based influence that drug-paired cues have over habitual instrumental behaviors directed towards drug taking. This would facilitate a better understanding of the complexities of drug taking in naturalistic environments that comprise both instrumental and Pavlovian components. In line with this hypothesis, recent work from Deserno et al. (2021) suggests that increases in dopamine enhances the use of model-based inferences to guide model-free value assignment. Thus, such a perspective could reconcile contradictions in the literature as to whether drug seeking is the result of habitual or goal-directed mechanisms (Everitt & Robbins, 2005; Hogarth, 2020; Hogarth et al., 2019), demonstrating that it depends on whether the influence is based on instrumental or Pavlovian processes. Nevertheless, our results demonstrate, for the first time, evidence to suggest enhancements in the model-based control that cues have over behavior induced by prior drug experiences and that this is accompanied by changes in a novel, bidirectional LH-VTA circuit.

Chapter 4: Monitoring hypothalamic dopamine release across Pavlovian learning and how this changes following methamphetamine exposure

In the previous chapters, we provided causal evidence that supports a role for VTA_{DA} input to LH in contributing to Pavlovian learning. Specifically, we found that inhibition of the $VTA_{DA}\rightarrow LH$ pathway attenuates model-based learning (see Chapter 2; **Figure 4**, pg. 38) while phasic stimulation of this pathway can facilitate learning for model-based associations (see Chapter 2; **Figure 5**, pg. 41). Further, prior methamphetamine exposure led to sensitization for self-stimulation of this pathway at higher frequencies, suggesting that methamphetamine may have strengthened hypothalamic circuits (see Chapter 3; **Figure 7**, pg. 64). In this chapter, we were interested in exploring the endogenous activity of dopamine within the LH during learning. Previous work exploring hypothalamic dopamine signaling have focused on its canonical role in feeding behaviors (Fetissov et al., 2000; Ikeda et al., 2018; Legrand et al., 2015; Meguid et al., 2000; Meguid et al., 1995; Parada et al., 1988; Parada et al., 1990; Yonemochi et al., 2019). A consistent finding from these studies, which used *in vivo* microdialysis techniques to measure extracellular dopamine concentrations, show that dopamine levels in the LH increase with food consumption (Fetissov et al., 2000; Ikeda et al., 2018; Legrand et al., 2015; Meguid et al., 2000; Meguid et al., 1995; Yonemochi et al., 2019). While microdialysis allows for measurement across time, its low sampling rate misses out on sub-second nuances in neural activity changes. With modern neuroscience techniques, we can now elaborate on these measurements with higher temporal resolution to elucidate the activity profile of dopamine release in the LH. Specifically, we can analyze the temporal profile of dopamine release during time-locked events, such as those occurring during cue-reward learning procedures.

This final chapter will focus on determining dopamine release dynamics in LH across learning and will test how methamphetamine self-administration might influence these dynamics. To do this, we will measure bulk dopamine release activity in LH across Pavlovian learning using *in vivo* fiber photometry (Li et al., 2019; Sun et al., 2018; Sun et al., 2020) and examine the temporal characteristics of hypothalamic dopamine signaling. Traditionally, dopamine is thought to contribute to learning as phasic prediction error signals (Bayer & Glimcher, 2005; Schultz et al., 1997). During Pavlovian cue-reward learning, dopamine neurons initially fire to the reward, but this signal backpropagates to the preceding cue after subjects come to reliably predict reward delivery (Maes et al., 2020; Schultz et al., 1997). Research examining "error-like" dopamine transmission to target regions from the midbrain in the context of learning has focused primarily on the nucleus accumbens (NAc) where phasic dopamine release activity resembles this prediction error signaling (Day et al., 2007). Given we have shown that stimulating VTA_{DA} terminals in the LH to mimic phasic prediction errors supports learning in this region, we reasoned that dopamine release activity in the LH would resemble the dopamine release activity observed in NAc. To our knowledge, there is currently no published work examining the dynamic profile of dopamine release in the LH using fiber photometry or related recording techniques. Thus, the data from this study provide one of the first and earliest demonstrations of hypothalamic dopamine release activity patterns in response to rewards and reward-predictive stimuli.

In this chapter, we hypothesized that dopamine release activity measured in the LH will follow conventional reward prediction error dynamics, such that dopamine will first increase to rewards early on in conditioning but then this increase in dopamine will shift to the antecedent cue by late conditioning when the cue comes to reliably predict the reward. Furthermore, as the previous chapter demonstrated a strengthening of LH-VTA circuits in animals with methamphetamine experience, which could be underlying their enhancements in cue-guided behavior, we also hypothesized that this would be reflected in the dopamine dynamics in LH and methamphetamine would boost dopamine prediction error signaling in LH across learning for a

reward-predictive cue. The results from these studies will reveal the patterns of dopamine signaling in LH during cue-reward learning and examine how neural activity in this circuit is changed with drug exposure, which could be underpinning maladaptive behaviors seen in substance use disorders.

Materials and Methods

Surgeries

A total of 17 adult Long Evans rats (10 females, 7 males) were included in the studies for this chapter. Animals were taken from litters bred in-house (UCLA) and their genotypes were confirmed to be non-transgenic, or wild-type. All rats first underwent intracranial surgeries for virus infusions and optic fiber implantations to measure dopamine release in the LH. 7 of the rats received solely the intracranial surgeries (**Experiment 7**), while the remaining 10 rats also received jugular vein catheterization for intravenous (I.V.) drug self-administration in the same surgery (**Experiment 8**). Rats were bilaterally infused with 0.7 μL of hSyn-promoter-driven GRAB$_{DA}$ dopamine biosensor (Sun et al., 2018; Sun et al., 2020) [AAV9-hSyn-DA2h (Addgene: #140554) or AAV9-hSyn-DA2m (Addgene: #140553)] into LH (AP: -2.4; ML: ±3.5 mm; DV: -9.0 mm (males) or -8.4 mm (females); angled at 10° towards midline). Similar to calcium indicators developed for photometry, the GRAB$_{DA}$ biosensor attaches fluorophores to the C-terminus of dopamine G-protein coupled receptors (GPCR), which fluoresce when the GPCR undergoes a conformational change upon receptor activation (Sun et al., 2018). Optic fibers (8mm metal ferrule, O.D.: 1.25mm, 200μm/NA 0.37 core; *Doric Lenses*, Québec, Canada) were also placed over LH (AP: -2.4 mm; ML: ±3.5 mm; DV: -8.5 (males) or -7.9 (females); angled at 10° towards midline). This viral strategy allowed for measurement of bulk dopamine release into the LH through activity-dependent fluorescent changes. Initially, we had animals in **Experiment 7** infused with one of two variants of the GRAB sensor, high-affinity (-DA2h) and medium-affinity (-DA2m) to screen for which variant provided the most reliable and robust signal. For animals in

Experiment 8, we used the medium-affinity GRAB virus as it resulted in more reliable signals from **Experiment 7**. Rats were given at least 4 weeks to allow for sufficient virus expression before beginning *in vivo* photometry recordings.

Drugs

Methamphetamine HCl (#M8750, Sigma-Aldrich) was dissolved in 0.9% saline (Hospira, Lake Forest, IL, USA) and self-administered intravenously at a dose of 0.1 mg/kg/infusion.

Behavioral procedures

CS+/CS- Pavlovian Conditioning

All rats were food-restricted to and maintained at ~85% of their free-feeding weight for the duration of the experiment following surgical recovery. When food restriction began, rats were pre-exposed to 5g of sucrose pellets in their home cage to reduce neophobia to the pellets when delivered during their behavioral sessions. Sessions took place in operant behavior chambers encased in a sound-attenuating box that were controlled by MED-PC V software (Med Associates, Inc., Fairfax, VT). Each rat was assigned to its own chamber. Before starting conditioning, rats received one magazine training session [30 trials, 1-min variable inter-trial interval (ITI)] consisting of trials where two 45-mg sucrose pellets were randomly delivered each trial to habituate animals to the behavioral chamber. Rats were then given a CS+/CS- Pavlovian conditioning procedure spanning 8 conditioning sessions. In this procedure, each trial consisted of a cue (click or white noise; counterbalanced) that was presented for 10-s where one cue was immediately followed by delivery of two sucrose pellets upon cue offset (CS+) while the other was not (CS-). A 3-min variable ITI was used. To track the development of dopamine release activity across learning, photometry recordings took place during the 1st (*early*), 4th (*middle*), and 8th (*late*) conditioning sessions. This selective recording scheme helps to minimize exposure to photobleaching which can lead to cell death and produce complications in signal collection (Li et al., 2019). During

magazine training and all conditioning sessions, a mono fiberoptic patch cord covered with black shrink tubing (fiber core diameter: 200μm, *Doric Lenses Inc.*) connected to the photometry system (*Neurophotometrics LLC*) was unilaterally attached to the rat's ferrule with a black zirconia split sleeve. Unless it was a recording day, the photometry system was switched off. In **Experiment 8**, subjects underwent 2 weeks of self-administration [grain (Control group) or drug infusions (0.1 mg/kg/infusion; Methamphetamine group)] followed by 3 weeks of abstinence as previously described (see Chapter 3 Materials/Methods, pg. 49-55) prior to photometry recordings across CS+/CS- conditioning. Self-administration procedures took place in a different set of behavioral chambers and context from Pavlovian conditioning.

Sessions with Unexpected Rewards

Following conditioning procedures, all subjects were recorded across 4 sessions of unexpected rewards. During these sessions, two sucrose pellets were randomly delivered every trial for 30 trials on a variable 1.5-min ITI. As dopamine signaling in the LH has previously been implicated for its role in feeding (Ikeda et al., 2018; Meguid et al., 2000; Meguid et al., 1995; Yonemochi et al., 2019), we reasoned that the dynamics of reward-evoked dopamine in this region might vary depending on the motivational state for a food reward. Thus, for two of these unexpected reward sessions, animals remained hungry (*restricted*); for the other two sessions, animals were first sated prior to the session (*sated*). All animals were first given a *restricted* session, followed by a *sated* session. Animals were given a second set of these sessions but conducted in reverse order.

Subjects were first habituated to the satiation context twice leading up to the first *sated* session. Habituation sessions had subjects placed in a bedding-less home cage with an empty ramekin located in a different room from photometry recordings for 30 minutes. On the day of *sated* sessions, animals were placed in the satiation cages with pre-weighed ramekins containing ~20g of their home chow for 1 hour before recording. Post-consumption weight of the ramekin was measured to determine amount of food consumed. We sated animals with home chow as

opposed to the sucrose pellets delivered during their behavior sessions. This method was intended to leave behavioral responding for sucrose pellets intact, which would allow us to record dopamine release during delivery of the unexpected rewards. Immediately after their recording session, animals were placed back in their satiation cages with ~20g of home chow for a 10-minute consumption test to ensure satiety.

Behavioral data analysis

To assess performance during conditioning used the percent of time spent in the food port as the primary behavioral measure. Baseline responding was defined as responding during 10 seconds prior to cue onset (pre-CS) and was compared to conditioned responding during cue presentation (CS) in order to establish a CS-preCS score. Behavioral responding during conditioning was analyzed using a repeated measures ANOVA, including session (across time), cue (CS+ vs. CS-), and period (in the case of comparing responding during the CS and pre-CS baseline) as within-subjects factors.

During unexpected rewards sessions, we used latency to enter the food port after pellet delivery as the primary behavioral measure. To analyze these data, we performed a repeated measures ANOVA on the average post-pellet latency to food port across trials that included motivational state (*restricted* vs. *sated*) and session order (first vs. second) as within-subjects factors. For **Experiment 8**, a between-subjects factor of group was included in the repeated measures ANOVA analyses to compare responding between treatment groups (Control vs. Methamphetamine). Separate repeated measures ANOVA analyses were conducted for self-administration data (lever press data and intake data) for Control and Methamphetamine groups. Analyses use an alpha level of 0.05.

In vivo **photometry collection and analysis**

Dopamine release-dependent fluorescence was collected using the Neurophotometrics Fiber Photometry System (FP3002) and monitored using a custom Bonsai workflow that simultaneously tracked photometry traces and event-triggered TTL signals. Recordings used two wavelength channels (excitation channel, 470nm; isosbestic control channel, 415nm; ~30-100 µW) that would alternate with a 20Hz frame rate. The signal from both hemispheres of each rat was first assessed on the first day of conditioning prior to initializing photometry recordings for the session. The side that displayed the strongest fluorescence signal in the excitation channel was recorded from thereon for subsequent recording sessions. Photometry and TTL signals were processed through a custom MATLAB script. The isosbestic channel was first fit and aligned to the signal channel using a linear regression model. Next, a change in fluorescence measure was obtained by subtracting out the isosbestic trace from the signal trace and normalized to the isosbestic ($\Delta F/F$). This measure was then converted to a z-score to offer a more meaningful index relative to the mean trace. The trace was then analyzed by trial across the session around behavioral events such as cue onset/offset, reward delivery, and reward retrieval. Traces aligned to each of these events were compiled for each trial across a 10-s window post-event and a 5-s window pre-event treated as baseline fluorescence.

To examine the development of dopamine signaling to LH across learning, we conducted waveform confidence interval analyses and permutation tests on the collected traces aligned to time-locked events from conditioning (Jean-Richard-Dit-Bressel et al., 2020). This method was selected in order to characterize the temporal dynamics of dopamine release activity in the LH that summary statistics cannot reveal (Jean-Richard-Dit-Bressel et al., 2020). Here, bootstrapped 95% confidence intervals were calculated to determine significant differences within the normalized signal from baseline (z-score = 0). Additionally, permutation tests were performed to detect signal differences between events and treatment groups. For unexpected reward sessions, we analyzed the trace after the first food port entry was made following pellet delivery. Unlike cue-reward conditioning, no discrete predictor of pellets was present in these unexpected reward

sessions, elongating the delay for the animal to reach the food port post-pellet delivery. Thus, we examined dopamine release after animals reached the food port to retrieve and consume the pellet.

To determine if dopamine signaling in the LH evolves with reward learning, we correlated dopamine release activity following CS+ onset with the amount of behavioral responding to CS+ across learning. Post-cue area under the curve (AUC) was taken across 10-s for cue-evoked responses and across 4-s for reward-evoked responses to be used as a summary measure of dopamine release activity for correlations. Pearson's correlation coefficients were calculated for correlations between the post-cue AUC on CS+ trials and the animal's CS-preCS scores for CS+ during conditioning. Analyses use an alpha level of 0.05.

Histology

At the conclusion of experiments, animals were induced with carbon dioxide and then transcardially perfused with 1X phosphate buffered saline (PBS), followed by 4% paraformaldehyde (PFA) in 1X PBS solution. Brains were extracted and placed in 4% PFA overnight before being changed to 30% sucrose solution made up in 1X PBS for at least 48 hours. Brain tissue was then sectioned into 30 μm coronal sections using a cryostat and collected in well-plates containing 1X PBS. The signal for GRAB$_{DA}$ expression in the LH was immunohistochemically amplified with the following procedures. Floating 30 μm coronal sections were first washed 3 times in 1X PBS for 30 minutes before being blocked in a solution consisting of 3% normal donkey serum, 0.3% Triton X-100, and 1X PBS for 2 hours. Sections were washed with 1X PBS another 3 times for 15 minutes before incubating in blocking solution containing chicken anti-GFP polyclonal antibody (1:1000; Abcam, Cambridge, MA; ab13970) for 48 hours at 4°C. After primary incubation, sections were washed with 1X PBS another 3 times for 30 minutes before secondary incubation in blocking solution containing goat anti-chicken IgY, Alexa Fluor^{TM} 488 conjugate (1:500, Abcam; ab150169) for 2 hours at room temperature. Following one final

set of 3 washes with 1X PBS, sections were mounted onto microscope slides and sealed with glass coverslips using ProLong™ gold antifade reagent with DAPI. Slides were viewed with a Confocal Microscope (Zeiss) for virus and fiber placement verification and images were taken using a 10x objective and tiled together.

Results

Experiment 7: Dopamine release in the LH increases to cues and rewards across Pavlovian conditioning

We have found that phasic stimulation of VTA dopamine terminals in LH using parameters that mimic endogenous prediction errors is capable of driving cue-reward learning (see Chapter 2; **Figure 5**, pg. 41). This result led us to hypothesize that LH receives phasic dopamine signals that resemble and follow conventional characteristics of prediction error firing to support learning in this region for a reward-predictive cue. To test this, we employed *in vivo* fiber photometry using a green-fluorescing dopamine biosensor, GRAB$_{DA}$, to monitor bulk dopamine release activity in the LH as rats underwent Pavlovian conditioning (**Figure 8A**). Rats (n=8; 4 male, 4 female) were first bilaterally infused with either one of two variants of the GRAB$_{DA}$ sensor (AAV9-hSyn-DA2h; DA2h; n=4 or AAV9-hSyn-DA2m; DA2m; n=4; counterbalanced across sexes) into the LH and had optic fibers placed over this region (**Figure 8B-C**). To minimize attrition of subjects due to misplacement of virus or fiber, we chose to inject the virus and place a fiber in both hemispheres of each animal even though photometry recordings occurred unilaterally. Ultimately, one female rat infused with DA2h was excluded from analyses for lack of signal detection throughout experimental procedures. While not fully powered to detect sex differences, we did include both sexes in the experiment and parsed out the data by sex to examine any trends in dopamine release in the LH between males and females (for further discussion and supplementary information, see **Appendix B**, pg. 111-115).

After waiting 4 weeks for virus incubation, rats were food restricted and began a differential Pavlovian conditioning procedure. Here, two 10-s auditory cues (click and white noise, counterbalanced; 8 sessions, 12 presentations/session) were presented individually on each trial pseudorandomly with a variable 3-min ITI. One cue terminated with the delivery of two 45-mg sucrose pellets (CS+) and the other cue was not reinforced (CS-). All rats spent greater time in the food port during CS+ presentations relative to baseline responding (pre-CS), which increased across conditioning (**Figure 8D**). This was confirmed with statistical analyses which revealed significant main effects of CS and session [CS (CS+ vs. CS- vs. baseline): $F_{(2,12)} = 9.841$, $p=0.019$; session: $F_{(7,42)} = 5.587$, $p<0.001$], and a significant interaction between CS and session (CS x session: $F_{(14,84)} = 5.925$, $p<0.001$). Follow-up simple main effect analyses for CS demonstrated that CS+ responding was above baseline (CS+ vs. baseline: $F_{(14,84)} = 10.498$, $p=0.018$). Responding to CS- presentations did not differ from baseline (CS- vs. baseline: $F_{(14,84)} = 5.669$, $p=0.055$). Further, animals showed greater CS+ responding compared to CS- responding that developed across sessions (CS+ vs. CS-: $F_{(14,84)} = 7.212$, $p=0.036$). This demonstrated that subjects were able to distinguish which cue predicted reward and which cue did not.

During conditioning procedures, we were interested in monitoring dopamine release activity in the LH across cue-reward learning. We selected the first, the fourth, and the final eighth session to record this activity to capture how dopamine dynamics change at *early*, *middle*, and *late* points of learning, respectively. Unsurprisingly, given dopamine signaling to LH has been implicated in contributing to its role for feeding (Amador et al., 2014; Caulliez et al., 1996; Fetissov et al., 2000; Fetissov et al., 2002; Ikeda et al., 2018; Legrand et al., 2015; Meguid et al., 2000; Meguid et al., 1995; Parada et al., 1988; Parada et al., 1990; Parada et al., 1991; Sato et al., 2001; Touzani et al., 2009; Yonemochi et al., 2019), the most prominent dopamine response occurred following the delivery of pellets upon CS+ offset [**Figure 8E**; orange gradient bars below trace post-pellet; session (*early, middle,* and *late*) vs. baseline (z=0)]. This post-pellet response appeared to increase across learning phases with waveform analyses detecting significant

differences in this response between *early* and *late* learning, and *middle* and *late* learning (**Figure 8E**; blue (*early* vs. *late*) and green (*middle* vs. *late*) bars). Additionally, while there was no significant dopamine release to the CS+ cue initially, dopamine signaling gradually emerged by *late* learning [**Figure 8E**; orange bar below trace post-CS+ onset; *late* vs. baseline (z=0)]. Unlike the post-pellet dopamine response where the trace is immediate and spans several seconds, the response to the CS+ cue ramped up in the second half of cue presentation as expected reward delivery approaches (**Supplementary Figure 1**; "Naïve" panel). Lastly, no changes in dopamine release activity to CS- were detected over the course of learning (**Figure 8E**). While the *early* learning trace to CS- shows an apparent increase in dopamine release towards the end of cue presentation, this was likely owed to an occurrence on a single trial (**Supplementary Figure 1**; "Naïve" panel). Further, waveform analyses across the trace window for CS- did not detect significant differences between any of the learning phases nor elevation above baseline. Finally, to determine if dopamine release activity to the CS+ cue is related to reward learning, we took the 10s post-cue AUC for each CS+ trial across learning and correlated this with the CS-preCS score in amount of time spent in the food port for CS+ trials as a measure of acquisition. We found that CS+ AUC and acquisition of the CS+ association were positively correlated (**Figure 8F**; R^2 = 0.09613, *p*<0.001). When separated out by learning phase, this positive correlation emerges during *middle* learning and strengthens by *late* learning (**Supplementary Figure 2**).

Given the robust dopamine release response to pellet delivery seen during conditioning, we next examined dopamine release activity in the LH in response to delivery of rewards that were not signaled by a preceding cue. Rats received sessions where two sucrose pellets were randomly delivered throughout the session. As the LH is traditionally known for a homeostatic role in regulating hunger and the motivation to consume, we reasoned that dopamine release into this region in response to pellet rewards may be modulated by the degree of hunger. Thus, to determine how motivational state may affect dopamine release in the LH in response to pellet delivery, we had sessions where rats remained food restricted and others where rats were sated

prior (session order counterbalanced). For *sated* sessions, subjects were sated with their home chow as opposed to the sucrose pellets given during the behavioral sessions. We predicted that this method would produce no differences in latency to retrieve the delivered pellet rewards between motivational states, but that there would still be a change in the subjects' level of hunger. Indeed, animals ate significantly more home chow prior to their *sated* sessions compared to afterwards [**Figure 8G**; time (pre-session vs. post-session): $F_{(1,6)}$ = 126.819, $p<0.001$]. While overall consumption differed between the two *sated* sessions, statistical analyses reveal a significant interaction in which the simple main effect of session was exclusive to consumption prior to the session relative to post-session [session: $F_{(1,6)}$ = 11.914, $p=0.014$; session x time: $F_{(1,6)}$ = 13.302, $p=0.011$; pre-session (session 1 vs. session 2): $F_{(1,6)}$ = 13.432, $p=0.011$; post-session (session 1 vs. session 2): $F_{(1,6)}$ = 0.945, $p=0.369$]. Importantly, the simple main effect of time was significant for both sessions [session 1 (pre-session vs. post-session): $F_{(1,6)}$ = 178.089, $p<0.001$; session 2 (pre-session vs. post-session): $F_{(1,6)}$ = 36.072, $p<0.001$]. This suggested that animals required more food to reach satiety in the first *sated* session compared to the second. Further, we found that retrieval latency did not differ between *restricted* (mean ± SEM, 2.03 ± 0.29s) and *sated* conditions (2.58 ± 0.47s; condition: $F_{(1,6)}$ = 1.694, $p=0.241$) and this also did not vary between sessions generally or by condition (session: $F_{(1,6)}$ = 0.614, $p=0.463$; condition x session: $F_{(1,6)}$ = 1.286, $p=0.300$).

Unlike conditioning sessions where animals were able to use the presentation of a cue to anticipate when rewards would arrive, there were no explicit predictors of reward delivery during these unexpected reward sessions. Thus, to appropriately analyze the dopamine release response to unexpected rewards, we examined the collected photometry traces aligned to the animals' first visit to the food port following pellet delivery. Interestingly, we found that dopamine release in the LH to unpredictable rewards was virtually the same in both *restricted* and *sated* states (**Figure 8G**; **Supplementary Figure 4**; "Naïve" panel). That is, an elevation in signal above baseline after pellets were delivered was detected for both *restricted* and *sated* conditions (**Figure**

8G; orange [*restricted* vs. baseline (z=0)] and yellow [*sated* vs. baseline (z=0)] bars below trace post-retrieval). When comparing the two motivational states, waveform permutation tests detected only brief differences in the dopamine release response between *restricted* and *sated* conditions, such that the response in the *sated* condition was subtly higher around the peak than in the *restricted* condition [**Figure 8G**; red bar (*restricted* vs. *sated*)]. Taken together, these data suggest that reward-evoked dopamine released into the LH is not substantially impacted by motivational state.

Lastly, we examined whether the variant of the $GRAB_{DA}$ sensor produced any differences in the magnitude of dopamine release recorded. After separating out the collected traces by virus variant, we found that the animals infused with the medium-affinity $GRAB_{DA}$ sensor showed a more pronounced dopamine release response than those infused with the high-affinity sensor during unexpected reward sessions [**Supplementary Figure 3**; green (DA2m) and red (DA2h) gradient bars; session *(restricted* and *sated*) from z=0]. Importantly, the reward-evoked responses observed were not driven by one virus variant, as waveform analyses detected the same temporal pattern in responses above baseline that were exhibited by both viruses. We interpret this difference as the DA2m sensor being more robust and enduring in signal detection than the DA2h sensor for dopamine release into the LH. Thus, we selected to use the DA2m variant moving forward.
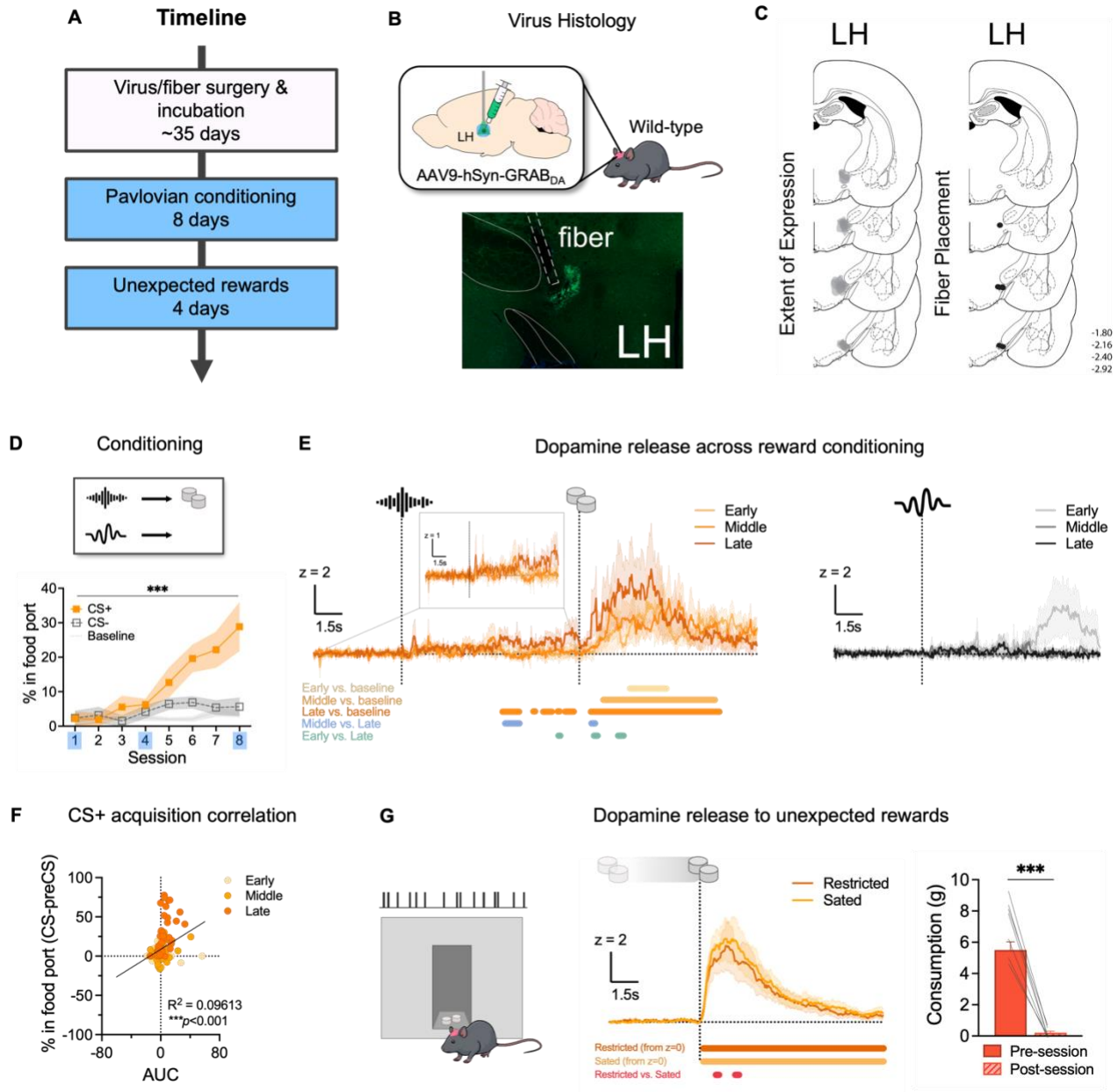
Figure 8. Dopamine release in the LH increases to cues and rewards across learning.

(A) Experimental timeline. **(B)** Fiber photometry approach: rats were bilaterally injected with a dopamine biosensor, GRAB$_{DA}$, and implanted with optic fibers in the LH. Unilateral example of bilateral GRAB$_{DA}$ expression and fiber placement. **(C)** *Left:* Unilateral representation of bilateral extent of expression. *Right:* Black dots indicate approximate location of fiber tips in LH. **(D)** Rats underwent differential Pavlovian conditioning: one cue led to sucrose pellets (CS+) and the other was without consequences (CS-). Responding is represented as the percent of time spent in the food port during presentation of each cue and during a baseline period of the same duration prior to cue onset, averaged across cues. Blue highlighted session numbers indicate a recording session. **(E)** Normalized ∆F/F traces represented as z-scores aligned to cue onset, CS+ (*left*) and CS- (*right*), across each learning phase (mean, solid line; SEM, shaded area). Vertical dotted lines mark the onset of behavioral events. Significance bars below the traces comparing within-

event signal (*early*, *middle*, and *late*) relative to baseline represent bootstrapped 95% confidence intervals(Jean-Richard-Dit-Bressel et al., 2020). Significance bars comparing between-event differences represent permutation tests. Inset shows the trace aligned to CS+ on a tighter-ranged scale for clarity (scale bar: z=1). **(F)** Correlation between post-CS+ AUC and CS+ acquisition, represented as the difference in the amount of time spent in the food port during CS+ presentation and pre-CS+ onset. **(G)** Dopamine response to unexpected pellet deliveries under either *restricted* or *sated* conditions. Average traces are aligned to the first entry made to the food port to retrieve the pellet rewards. Orange and yellow significance bars below the trace indicate bootstrapped 95% confidence intervals where the signal was significantly different from baseline (z=0) (Jean-Richard-Dit-Bressel et al., 2020). Red significance bar indicates permutation tests comparing the traces under *restricted* and *sated* conditions. The bar graph shows the consumption of home chow before and after *sated* recording sessions. Consumption across the 1-hour sate period (pre-session) was greater than the amount consumed during the consumption test conducted immediately after recording (post-session). ***$p \leq 0.001$, mean (± SEM).

Experiment 8: Prior experience with methamphetamine amplifies cue-evoked dopamine release to the LH

Having characterized the temporal profile of dopamine release activity in the LH during cue-reward learning, we were now interested in interrogating how previous methamphetamine experience affects these dynamics across learning (**Figure 9A**). Rats (*n*=18; 9 male, 9 female) first received dual surgeries within the same procedure in which they were implanted with intravenous catheters in their right jugular vein and also received bilateral infusions of GRAB dopamine biosensor (AAV9-hSyn-DA2m) along with an optic fiber in the LH (**Figure 9B-C**). After concluding experimental data collection, a total of 10 subjects were used for data analyses as a number of rats were excluded from analyses due to either fiber misplacement or lack of fluorescent signal during recording. Animals were given at least one week to recover from surgery before they began our 2-week self-administration protocol (see Chapter 3 Materials/Methods, pg. 49-55). Subjects were split into two groups: drug-naïve control group (Control, *n*=5; 2 male, 3 female) and methamphetamine-experienced group (Methamphetamine, *n*=5; 1 male, 4 female). During self-administration, subjects in the Methamphetamine group could earn intravenous infusions of methamphetamine (0.1 mg/kg/infusion) while the Control group earned grain pellets instead as a drug-naïve, reward experience control. Indeed, both groups increased responding

for the active lever that earned them their respective reward relative to the inactive lever across sessions and across increasing reinforcement schedules (**Figure 9D-E**). Statistical analyses using repeated measures ANOVAs confirmed this for both Control and Methamphetamine groups by finding significant main effects of session and lever and a significant interaction for session and lever [Control: session ($F_{(13,52)}$ = 158.743, $p<0.001$), lever ($F_{(1,4)}$ = 370.852, $p<0.001$), session x lever ($F_{(13,52)}$ = 227.814, $p<0.001$); Methamphetamine: session ($F_{(13,52)}$ = 10.482, $p<0.001$), lever ($F_{(1,4)}$ = 13.557, $p=0.021$), session x lever (F$_{(13,52)}$ = 18.546, $p<0.001$)]. Follow-up analyses detected simple main effects of lever such that both groups clearly distinguished responding for the active lever from the inactive lever (Control: $F_{(13,52)}$ = 1389.053, $p<0.001$; Methamphetamine: $F_{(13,52)}$ = 374.229, $p<0.001$). Furthermore, lever-press behavior was reflected in the increase of each group's respective reward intake across time [Control: session ($F_{(13,52)}$ = 2.907, $p=0.003$); Methamphetamine: session ($F_{(13,52)}$ = 9.778, $p<0.001$)]. After completing the self-administration phase, all rats were subject to a 3-week abstinence period, as previously described (see Chapter 3 Materials/Methods, pg. 49-55), to allow for the Methamphetamine group to recover from any drug withdrawal effects that could impact their subsequent performance during conditioning and recording.

Rats then began the same differential conditioning procedures described in **Experiment 7**. Both groups increased their time spent in the food port during CS+ presentation but not to CS- presentation over the course of conditioning (**Figure 9F**; cue: $F_{(2,16)}$ = 43.638, $p<0.001$; session: $F_{(7,56)}$ = 4.912, $p<0.001$; cue x session: $F_{(14,112)}$ = 7.540, $p<0.001$). Simple main effects of cue support these results, such that by the final session, only CS+ responding was elevated above baseline (CS+ vs. baseline: $p<0.001$; CS- vs. baseline: $p=0.091$) and there was a significant difference in responding for CS+ relative to CS- (CS+ vs. CS-: $p=0.002$). Statistical analyses also determined there were no between-group differences in responding for either cue nor were there between-group differences across sessions (group: $F_{(1,8)}$ = 0.758, $p=0.409$; cue x group: $F_{(2,16)}$ = 0.730, $p=0.423$; session x group: $F_{(7,56)}$ = 0.848, $p=0.553$; cue x session x group: $F_{(14,112)}$ = 0.660,

p=0.809). Thus, past methamphetamine experience does not appear to affect the rate of cue-reward learning, in line with the data from the previous chapter showing no between-group differences in performance during the Pavlovian conditioning phase of the PIT procedure (see Chapter 3, **Figure 6**, pg. 60).

We next examined the photometry traces collected across conditioning for both groups. Similar to subjects in **Experiment 7**, both groups showed a prominent increase in dopamine release following pellet delivery that was elevated above baseline in each phase of learning [**Figure 9G-H**; brown (Control) and purple (Methamphetamine) gradient bars below trace post-pellet; session (*early, middle,* and *late*) vs. baseline (z=0)]. Additionally, waveform permutation tests comparing responses between learning phases detected significant differences in the post-pellet responses for both groups, such that there was greater dopamine release during *middle* and *late* learning compared to *early* learning (**Figure 9G-H**; red (*early* vs. *middle*) and green (*early* vs. *late*) bars). However, there were several more time points across the trace where significant increases in post-pellet dopamine release were detected in the Methamphetamine group (**Figure 9H**; red and green bars) compared to the Control group (**Figure 9G**; red and green bars). Thus, the dopamine response was prolonged in the Methamphetamine group relative to the Control group (**Figure 9H**; purple gradient bars below trace post-pellet).

In **Experiment 7**, we again found that LH dopamine ramped up as the end of the reward-predictive cue approaches. Here, however, the increase in dopamine release evoked by the CS+ cue appeared earlier in learning for animals in the Control group, such that dopamine release was found to be above baseline during *early* and *middle* learning [**Figure 9G**; brown gradient bars below trace post-CS+ onset; session (*early* and *middle*) vs. baseline (z=0)]. Additionally, no significant differences in dopamine release during CS+ presentation was detected between learning phases in the Control group, suggesting dopamine release emerged early and stayed elevated across learning (**Figure 9G**). Animals in the Methamphetamine group showed elevated dopamine release activity above baseline to the CS+ cue early on starting in *middle* learning

[**Figure 9H**; purple gradient bar below trace post-CS+ onset; *middle* vs. baseline (z=0)]. By *late* learning, this response spanned a lengthy component of the cue [*late* vs. baseline (z=0)]. Permutation tests found brief increases in the CS+ signal between each of the three phases of learning for the Methamphetamine group (**Figure 9H**; red (*early* vs. *middle*) and blue (*middle* vs. *late*) bars below trace post-CS+), but this was most pronounced by *late* learning compared to *early* learning [green bar (*early* vs. *late*)]. Waveform analyses conducted on traces aligned to CS- onset revealed a significant increase above baseline that emerged in the last 2 seconds of cue presentation during *middle* learning for the Control group, but this disappeared by *late* learning (**Figure 9G**; brown (*middle* vs. baseline) and red (*early* vs. *middle*) bars below trace post-CS- onset). No significant changes in dopamine release activity during CS- presentation were detected at any point across conditioning for the Methamphetamine group (**Figure 9H**). Finally, we found that the behavioral response and the dopamine response to the CS+ cue were positively correlated for both Control and Methamphetamine groups (**Figure 9I**; **Supplementary Figure 2**). Collectively, these data suggest that dopamine release evoked by reward-predictive cues into the LH is heightened in animals with prior methamphetamine experience.

Lastly, we monitored dopamine release activity in the LH to unexpected rewards under *restricted* and *sated* conditions for both groups. For the Methamphetamine group, one female rat was excluded from analyses due to loss of signal (i.e., no reliable photometry responses were observed following pellet retrieval). Both groups showed reward-evoked dopamine responses above baseline for both *restricted* and *sated* conditions [**Figure 9K-L**; brown (Control) and purple (Methamphetamine) gradient bars below trace post-retrieval; condition (*restricted* and *sated*) vs. baseline (z=0); **Supplementary Figure 4**; "Control" and "Methamphetamine" panels]. Waveform permutation tests detected a brief difference in the traces between conditions for the Control group, like in **Experiment 7**, but this difference was opposite in direction (i.e., the *restricted* was greater than the *sated* trace) and appeared as activity was returning to baseline [**Figure 9K**; red bar (*restricted* vs. *sated*)]. No differences in the traces between *restricted* and *sated* conditions

were identified for the Methamphetamine group (**Figure 9L**). We observed that greater home chow was consumed before *sated* sessions compared to after the session by both groups consistently across both sessions [**Figure 9M**; time (pre-session vs. post-session): $F_{(1,7)}$ = 107.009, p<0.001; session (Session 1 vs. Session 2): $F_{(1,7)}$ = 0.376, p=0.559; time x session: $F_{(1,7)}$ = 0.331, p=0.583]. However, the total amount consumed by the Methamphetamine group exceeded that of the Control group for pre- and post-session consumption of both *sated* sessions (**Figure 9M**; group: $F_{(1,7)}$ = 10.503, p=0.014; time x group: $F_{(1,7)}$ = 3.384, p=0.108; session x group: $F_{(1,7)}$ = 1.299, p=0.292; time x session x group: $F_{(1,7)}$ = 0.061, p=0.812). Despite this, latency to retrieve pellet rewards did not differ between groups for *sated* states (mean ± SEM; Control: 3.28 ± 0.29s; Methamphetamine: 3.31 ± 1.35s), nor did it differ under *restricted* conditions (Control: 1.49 ± 1.51s; Methamphetamine: 1.54 ± 0.25s). This was supported by statistical analyses, which found no significant effects of condition or group nor a significant interaction (condition (*restricted* vs. *sated*): $F_{(1,7)}$ = 0.016, p=0.903; group (Control vs. Methamphetamine): $F_{(1,7)}$ = 0.031, p=0.864; condition x group: $F_{(1,7)}$ = 2.225, p=0.179). Moreover, these null effects were consistent across sessions for each condition (session: $F_{(1,7)}$ = 0.026, p=0.877; session x group: $F_{(1,7)}$ = 1.284, p=0.294; condition x session: $F_{(1,7)}$ = 2.700, p=0.144; condition x session x group: $F_{(1,7)}$ = 0.021, p=0.889). Altogether, these findings show that dopamine release to food rewards does not dramatically change with motivational state, and that prior methamphetamine experience did not meaningfully impact this response.
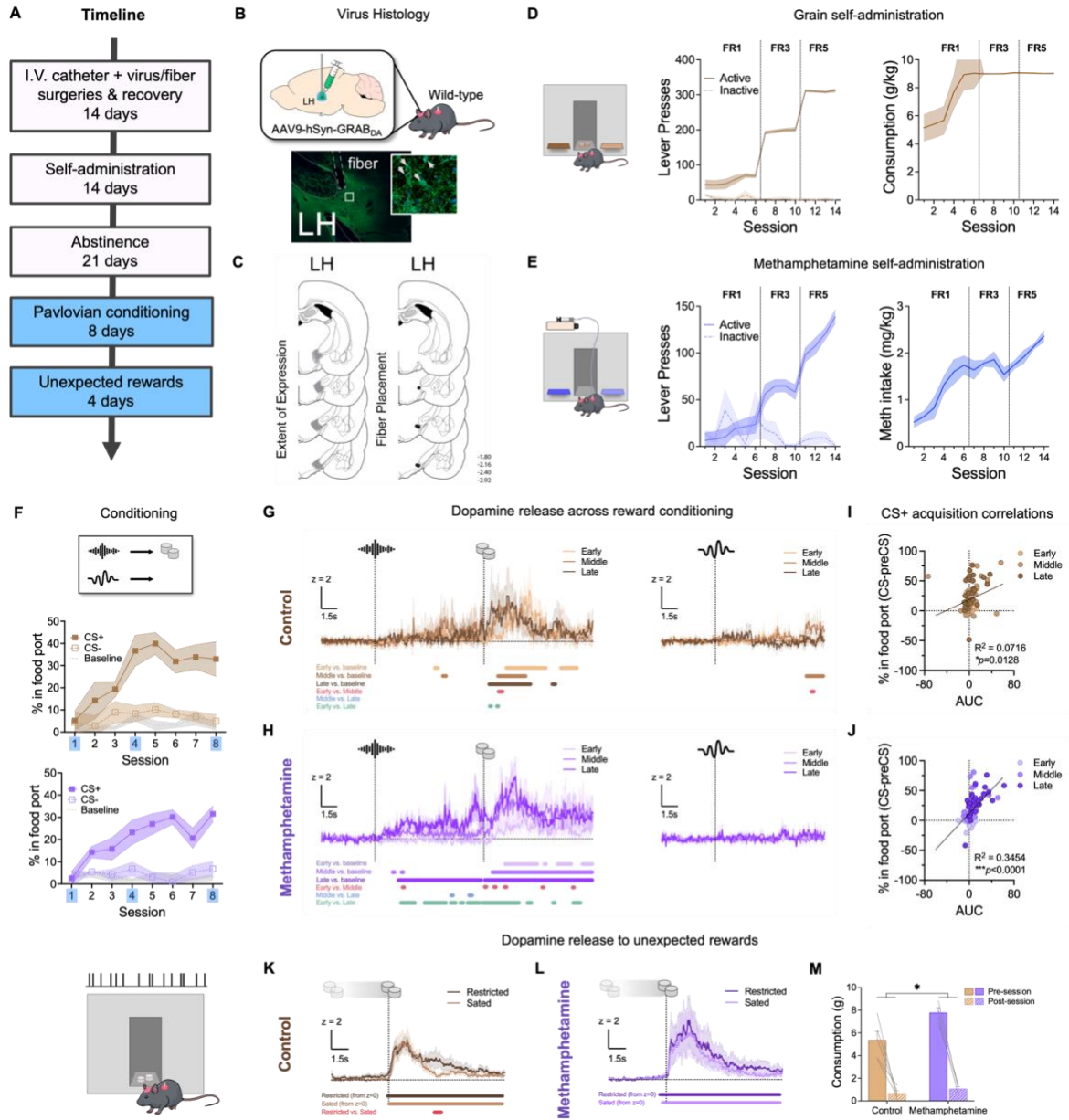
Figure 9. A history of methamphetamine enhances dopamine released in the LH to reward cues.

(A) Experimental timeline. **(B)** Dual surgery approach: all rats received intravenous catheterization and were bilaterally injected with the dopamine biosensor, GRAB$_{DA}$, and implanted with optic fibers in the LH. Unilateral example of bilateral virus expression and fiber placement. **(C)** *Left:* Unilateral representation of bilateral extent of expression. *Right:* Black dots indicate approximate location of fiber tips in LH. **(D-E)** After surgeries, rats were divided into two groups, Control and Methamphetamine, and underwent a 2-week self-administration protocol, either earning grain pellets **(D)** or infusions of methamphetamine **(E)**. *Left* panels show lever-pressing responses for respective rewards across increasing reinforcement schedules. *Right* panels reflect rewards earned across sessions as a measure of consumption (Control, grain pellets) or intake (Methamphetamine, drug infusions). **(F)** After undergoing an abstinence period, rats received

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differential Pavlovian conditioning: one cue led to sucrose pellets (CS+) and the other was without consequences (CS-). Responding is represented as the percent of time spent in the food port during presentation of each cue and during a baseline period of the same duration prior to cue onset, averaged across cues for the Control group (*above*) and the Methamphetamine group (*below*). Blue highlighted session numbers indicate a recording session. **(G-H)** Dopamine release in the LH across conditioning plotted as normalized ∆F/F traces represented as z-scores aligned to cue onset, CS+ (*left*) and CS- (*right*), across each learning phase for the Control group **(G)** and the Methamphetamine group **(H)** (mean, solid line; SEM, shaded area). Vertical dotted lines mark the onset of behavioral events. Significance bars below the traces comparing within-event signal (*early*, *middle*, and *late*) relative to baseline represent bootstrapped 95% confidence intervals for each group. Significance bars comparing between-event signal differences represent permutation tests on the waveform. **(I-J)** Correlations between post-CS+ AUC and CS+ acquisition for the Control group **(I)** and the Methamphetamine group **(J)** across trials for all learning phases. **(K-L)** Dopamine release activity in the LH in response to unpredictable rewards for the Control group **(K)** and the Methamphetamine group **(L)** aligned to the first visit made to the food port post-pellet delivery. Significance bars below the traces comparing within-event signal (*restricted* and *sated* conditions) relative to baseline represent bootstrapped 95% confidence intervals. Between-event signal differences revealed with waveform permutation test analyses are shown as significance bars in red. **(M)** Consumption of home chow before and after *sated* sessions. Lines represent individual subject data. *$p \leq 0.05$, ***$p \leq 0.001$, mean (± SEM).

While dopamine release dynamics in the Control group generally followed the same patterns during conditioning as those observed in the reward-naïve animals from **Experiment 7**, event-related dopamine release activity in the Control group was overall less robust. Thus, to ensure a conservative comparison of dopamine release in the Methamphetamine group relative to controls, we compared waveforms between all three cohorts to determine exactly how methamphetamine experience impacts dopamine release activity in the LH across learning. To disambiguate group labels as we report our findings from cross-cohort analyses, we define each group as follows: Naïve (drug and food reward-naïve), Control (drug-naïve, food reward-experienced), and Methamphetamine (drug-experienced, food reward-naïve).

Differences between groups emerged when comparing the dopamine response across learning. Permutation tests revealed that there was greater dopamine release towards the end of the CS+ cue for the Control group relative to the Naïve group during *middle* learning [**Figure 10**; red bar (Naïve vs. Control)]. This difference between groups was not apparent during *late* learning (**Figure 10**). These findings support the interpretation that increases in dopamine release to CS+ emerge earlier in learning for the Control group, but that by *late* learning, both groups showed

equivalent ramping of dopamine release activity to the reward cue. When comparing dopamine release across learning between subjects in the Control group and subjects in the Methamphetamine group, differences were detected at several points across CS+ during *late* learning, such that methamphetamine-experienced animals displayed significantly greater dopamine release than animals in the Control group [**Figure 10**; green bar (Control vs. Methamphetamine)]. Furthermore, dopamine release across CS+ presentation in the Methamphetamine group was found to also be increased above the Naïve group in a similar manner during *late* learning [**Figure 10**; blue bar (Naïve vs. Methamphetamine)]. Waveform permutation tests detected greater, though subtle, dopamine release toward the end of CS- presentation for the Control group compared to the Naïve group during *middle* learning, though this did not persist through *late* learning [**Figure 10**; red bar (Naïve vs. Control)]. A brief difference in responses to CS- was found between the Control and Methamphetamine groups during *late* learning, but the traces otherwise fluctuated close to z=0 [**Figure 10**; green bar (Control vs. Methamphetamine)]. Lastly, no group differences were found for dopamine release responses to CS- between the Naïve and Methamphetamine groups (**Figure 10**). Therefore, these findings suggest that a history of methamphetamine intake amplifies cue-evoked dopamine release to the reward-predictive cue.

Generally, the amplitude of dopamine release responses following pellet delivery across conditioning was comparable for all three cohorts. A brief difference between the Naïve and Control groups was detected in the rise of the response during *middle* learning, but this was not maintained in *late* learning [**Figure 10**; red bar (Naïve vs. Control)]. The post-pellet dopamine release response appeared prolonged in methamphetamine-experienced animals, suggesting that methamphetamine experience produces more sustained dopamine responses after reward receipt without necessarily heightening the response magnitude (**Figure 9H**). This idea was further supported by waveform permutation test analyses on post-pellet traces showing significantly greater dopamine release several seconds out from pellet delivery in the

Methamphetamine group compared to the Control group during both *middle* and *late* learning phases [**Figure 10**; green bar (Control vs. Methamphetamine)]. However, follow-up permutation tests comparing the Methamphetamine group to the Naïve group did not find these same differences in prolonged dopamine release across both *middle* and *late* phases [**Figure 10**; blue bar (Naïve vs. Methamphetamine)]. Thus, prior methamphetamine experience does not appear to clearly impact dopamine release in the LH following reward delivery during conditioning.
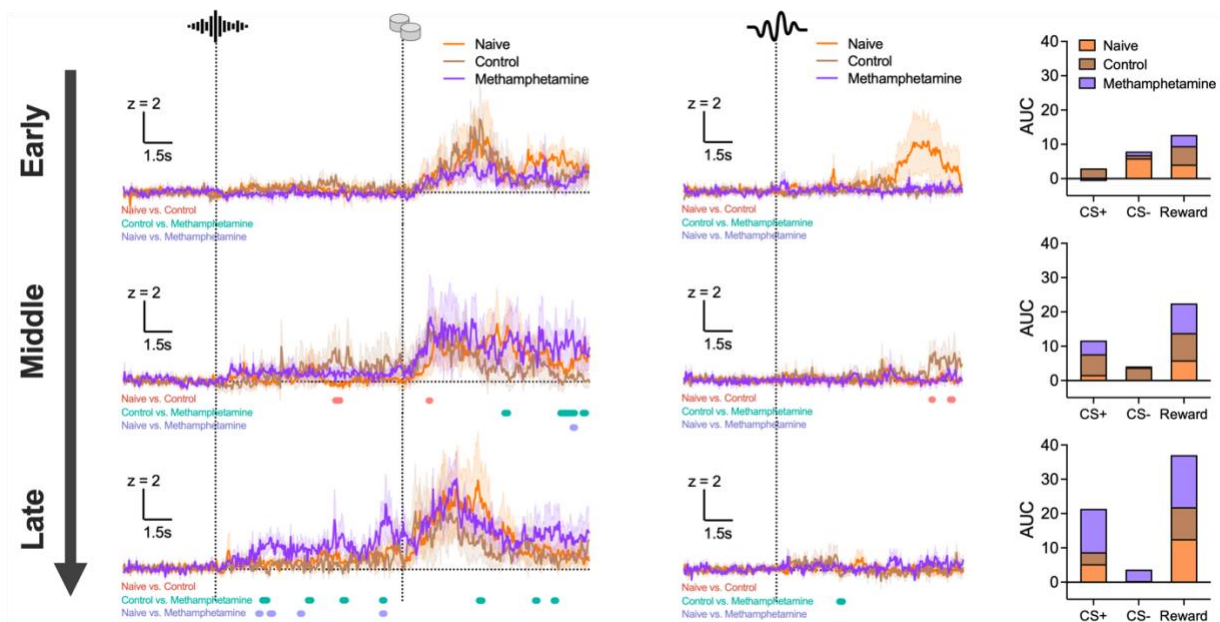

Figure 10. Methamphetamine self-administration enhances dopamine release in the LH to reward cues, regardless of the control group used.

Reward-naïve animals (Naïve), drug-naïve, food reward-experienced animals (Control), and food-naïve, drug reward-experienced animals (Methamphetamine) show different patterns of dopamine release activity for behavioral events over the course of learning [mean (solid lines) ± SEM (shaded area)]. *Left* and *Middle* panels show photometry traces aligned to the CS+ followed by pellet delivery and CS-, respectively. *Right* column panel displays AUC magnitude for each group by event. Rows divide the data by the phase of learning for *early (top)*, *middle (center)*, and *late (bottom)*. Significance bars below the traces represent waveform permutation test analyses conducted to compare groups [Naïve vs. Control (red), Control vs. Methamphetamine (green), and Naïve vs. Methamphetamine (blue)] that detected temporally specific differences between signal traces.

Discussion

Having characterized a role for the LH in acquiring reward outcome expectations with evidence for midbrain dopaminergic inputs to this region to support this function, we sought to uncover the profile of hypothalamic dopamine signaling during cue-reward learning. To do this, we employed fiber photometry techniques in freely moving animals to measure dopamine release as they underwent a differential Pavlovian conditioning procedure. Here, rats infused with a dopamine biosensor in the LH learned about a cue that led to sucrose pellet rewards and another cue that was not reinforced. Given we have shown that stimulating VTA_{DA} terminals in the LH to mimic prediction errors supports cue-reward learning, we hypothesized that endogenous dopamine release in the LH would resemble this pattern of prediction error signaling previously seen in other structures (e.g., NAc) over the course of learning. That is, we expected to see dopamine release to unexpected rewards early in learning, which would shift to the preceding cue later in learning. Rather than a *phasic* signal as one would expect for a prediction error, we found that while dopamine release activity in the LH to the reward-predictive cue increased across learning, dopamine release was sustained during cue presentation and ramped up as reward delivery approached. Further, we observed that dopamine release in the LH was robust following reward delivery, characterized by sustained elevations above baseline, and that this response grew across learning instead of reducing once the reward was expected as indicated by conditioned responding. Importantly, the magnitude of cue-evoked dopamine was positively correlated with behavioral responding to the CS+ across conditioning, demonstrating that dopamine release activity in the LH is learning-related. That there was no difference in the magnitude of conditioned responding between our Methamphetamine and Control groups further indicates that the increase in dopamine release to the CS+ seen in our Methamphetamine group is an effect of learning and not movement *per se*. Thus, dopamine signaling in the LH reflects a unique pattern of dopamine release that is dissociable from phasic prediction errors. This is a novel finding in and of itself as

there have been no published demonstrations examining dopamine release in this region as it relates to physiologically-relevant rewards.

This temporal pattern of dopamine release activity in LH is uncharacteristic of dopaminergic prediction error signals typically associated with temporal difference reinforcement learning models (Suri & Schultz, 2001). Instead, the dynamics observed here are more reminiscent of anticipatory signals previously identified in striatal and cortical neurons in which the expectation of reward during CS+ cue presentation gradually increases as the arrival of reward nears (Khamassi et al., 2008; Suri & Schultz, 2001). We found that dopamine ramps up in the LH to a reward-predictive cue and the magnitude of the ramp scales up across learning. Similar dopamine ramps have been reported in rat striatum as animals traversed through mazes and distant rewards became more proximal (Howe et al., 2013). There are at least two possibilities of how this signal originates in the LH. Firstly, it is possible that the LH receives prediction error signals from VTA dopamine neurons that is then locally transformed within the LH by terminal regulation to produce the sustained release we see across learning (Burdakov & Karnani, 2020; Mohebi et al., 2019; Noritake & Nakamura, 2019). Secondly, it could be that a specific subpopulation of dopamine neurons in the VTA relay a more sustained signal across the cue to the LH, consistent with the physiological heterogeneity of neurons in this structure (Lerner et al., 2021; Morales & Margolis, 2017). Indeed, work recording the activity of dopamine neuron ensembles in more lateral portions of mouse VTA with two-photon calcium imaging found that a subset of neurons shows ramping activity when the subject's spatial distance from reward reduces (Engelhard et al., 2019). More recently, others have shown with fiber photometry that population neural activity of medial VTA dopamine neurons in mice ramps up when the subject's distance from a *predictor* of reward reduces (Guru et al., 2020). Thus, while our previous investigations of VTA dopaminergic projections to the LH have focused on this input as coming from lateral VTA (see Chapter 2, pg. 17-45), it is possible that the sustained patterns in dopamine release observed in the present study come from both lateral and medial portions of the VTA.

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The dopamine dynamics outlined in this study are especially interesting considering the LH has been shown to bias learning towards proximal predictors and oppose learning for distal predictors of reward (Hoang & Sharpe, 2021; Sharpe et al., 2021). In line with this facet of learning by LH, we found that greater dopamine was released to the expected reward than to its preceding cue. While reinforcers like sucrose pellets are typically thought of as unconditioned stimuli, they could also be considered conditioned stimuli for themselves as the sensory features that make up the reinforcer can act as direct predictors. Thus, perhaps endogenous dopamine release activity in the LH facilitates learning for the most relevant cues associated with reward in a manner that does not follow the conventional profile of dopaminergic prediction errors.

In the previous chapter, we found that past methamphetamine experience sensitizes LH-VTA circuits and implicate this neural underpinning for the drug-induced enhancements in behavior guided by outcome expectations. Thus, in the present study, we examined whether a history of methamphetamine heightens dopamine signaling in the LH during cue-reward learning. We found that with prior methamphetamine experience, dopamine release to reward-predictive cues, but not rewards, was amplified compared to drug-naïve animals. Strikingly, cross-cohort comparisons revealed that this enhancement in dopamine release exhibited by the Methamphetamine group compared to drug-naïve animals was consistently evident regardless of whether or not drug-naïve animals had prior experience with food rewards. Furthermore, the heightened dopamine release observed in animals with methamphetamine experience did not appear to be the result of better performance in responding to the reward-predictive cue compared to drug-naïve animals in the Control group, underscoring a distinction between learning and performance. This was an exciting finding as it could help to explain the heightened sensitivity to reward-related cues seen in individuals with a substance use disorder.

One important caveat to these results is that dopamine signaling in our Control group was less robust than in the Naïve cohort from the first study. This could have occurred for a variety of reasons. For example, prior experience with a similar food reward may have induced plasticity in

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the LH that influenced how this region participates in future learning (Kolb & Whishaw, 1998). This is consistent with our finding that dopamine release responses emerged earlier in the Control group relative to the Naïve group. Another explanation could be that the Control group had a smaller sample size compared to the Naïve group. Perhaps the addition of more subjects would have produced dopamine dynamics that more closely resemble the magnitude and pattern of dopamine release in animals in the Naïve group. Nonetheless, our cross-cohort analyses did not detect any major differences between groups, suggesting that these two cohorts were generally comparable.

Lastly, we found that dopamine is also released in the LH in response to unexpected rewards. This was consistent with previous research measuring dopamine levels in the LH using microdialysis, which showed increases in dopamine when animals consume food (Fetissov et al., 2000; Ikeda et al., 2018; Legrand et al., 2015; Meguid et al., 2000; Meguid et al., 1995; Yonemochi et al., 2019). Our data extend this finding and reveal substantial increases in dopamine release to the retrieval of food rewards on a finer timescale. We also determined that varying the motivational state for food rewards does not dramatically impact reward-evoked dopamine signaling in this region. Broadly, these effects were consistent across all cohorts. This was surprising considering sated animals generally show higher basal dopamine levels in LH compared to fasting animals (Fetissov et al., 2000). Thus, we expected to see a greater dopamine response to food rewards in sated conditions compared to food restricted conditions. Though our data did detect subtle increases in dopamine release around the peak of the response when animals were sated with home chow beforehand in the Naïve group, we observed the opposite effect in the Control group such that satiety subtly *reduced* reward-evoked dopamine release. Further, no significant differences between motivational states were found for methamphetamine-experienced animals. Importantly, for our methods, we selected to sate animals with home chow to not disturb behavioral responding for sucrose pellets during unexpected reward sessions in order to measure dopamine release activity in response to rewards. However, we must consider

how these results might differ had we opted to sate animals with the same sucrose pellets they earn during these sessions instead. Perhaps future work using a dual-outcome design paired with sensory-specific satiety (i.e., animals are sated on one of the two outcomes prior to sessions) can be used to disentangle how exactly motivational state may be modulating reward-evoked dopamine release in the LH.

Importantly, the methods used in this study measure bulk, population-level dopamine release into the LH. Thus, it is unclear whether the observed dopamine release activity was a result of VTA dopaminergic inputs or from elsewhere containing dopaminergic neurons with projections to the LH (e.g., substantia nigra, SN) (Yang et al., 2019). Furthermore, the fluorescent biosensor used to record photometry signals does not distinguish between subtypes of dopamine receptors, which means the dopamine release activity collected is pooled receptor activation in the LH. As this region contains both D_1 and D_2 receptors (Fetissov et al., 2002; Meguid et al., 2000; Sato et al., 2001), it remains to be determined how endogenous activation of these receptor subtypes allows for the LH to contribute to learning and reward-motivated behaviors. Previous work using immunofluorescence labeling found that D_1 receptor expression density in the LH is significantly decreased when dopamine neurons in the SN are obliterated, suggesting that dopamine release activity in the LH is likely D_1 receptor-mediated (Yang et al., 2019). This would also align with previous findings implicating a more prominent role for D_1 receptors than D_2 receptors in LH for the acquisition of conditioned taste preference and aversion (Amador et al., 2014; Caulliez et al., 1996; Sclafani et al., 2011; Touzani et al., 2009). Moreover, given the LH is a hub for neurons that express a vast variety of neuromodulatory peptides, it is of interest to identify which neuronal populations within this region receive dopaminergic inputs to facilitate ongoing learning in this region (Bonnavion et al., 2016; Bubser et al., 2005; Fadel & Deutch, 2002; Noritake & Nakamura, 2019; Petrovich, 2018; Sharpe et al., 2021; Sharpe, Marchant, et al., 2017; Touzani et al., 2010). Nevertheless, these data set the stage for future endeavors to further tease apart the complexities of the LH as a region that contributes to cognitive processes.

Chapter 5: General Discussion

The studies included in this dissertation have characterized a novel hypothalamic-midbrain circuit for model-based learning and revealed how these neural and behavioral underpinnings are changed with drug experience to influence decision-making. In Chapter 2, we first determined that GABAergic neurons residing in LH harbor model-based reward expectations used to guide behavior. We also showed that inhibition of LH_{GABA} neurons does not disrupt the expression of conditioned reinforcement unless model-based associations were necessary to drive the effect, suggesting this population of cells is involved with the acquisition of model-based associations. Furthermore, we identified VTA dopamine projections as an input circuit mechanism that likely sends prediction error signals to support learning in this region. Inhibition of the $VTA_{DA} \rightarrow LH$ pathway at the time of reward prediction errors resulted in attenuated model-based learning, while stimulation designed to mimic a prediction error in this pathway facilitated model-based learning. Next, we found that rats with a history of methamphetamine, whether self- or experimenter-administered, enhanced cue control over instrumental behavior in a highly specific manner. In the specific PIT procedure used to demonstrate this effect, model-based outcome representations evoked by the cue were used to guide decision-making. In these same methamphetamine-experienced animals, we found evidence to suggest that LH-VTA circuits became sensitized with prior drug exposure. This implicated that a strengthening of model-based LH-VTA learning circuits was associated with methamphetamine-induced enhancements in cue control. Finally, using fiber photometry, we uncovered the temporal profile of dopamine release in LH during cue-reward learning and showed that methamphetamine experience augments these dopamine dynamics across learning. We found that dopamine release in LH does not resemble classic reward prediction error signals – in this region, dopamine release is sustained, increases to rewards across learning, and ramps up during the antecedent cue as reward delivery approaches. Altogether, these results demonstrate how LH-VTA neural circuits uniquely

contribute to model-based learning processes and how exposure to methamphetamine potentiates the use of model-based associations to direct behavior, likely driven by drug-induced sensitization of these neural pathways.

The LH as a learning arbitrator

Our reported findings shed new light on how the LH contributes to learning. Optogenetic inhibition of LH$_{GABA}$ neurons at the time when reward predictions are retrieved showed that these predictions contain model-based associative information. In the context of previous work demonstrating that LH$_{GABA}$ neurons bias learning toward cues proximal to the reward and away from distal cues (Sharpe et al., 2021), we can now view this bias toward proximal cues exhibited by the LH as a model-based phenomenon. This has interesting implications for the theoretical frameworks of model-based learning (Daw et al., 2005), which do not distinguish between neural substrates that are responsible for the distal and proximal steps in the model-based cognitive map. Within these frameworks, the neural substrates that govern proximal or distal model-based learning are the same. Thus, we would argue that LH$_{GABA}$ neurons are preferentially involved in biasing learning towards cues proximal to rewards in a model-based manner, revealing a neural dissociation in the proximal and distal features of model-based associations.

In carving a path forward, these data provide impetus to next study how LH and its GABAergic neuronal populations process this learned information. That is, what is the pattern of neural activity in LH when learning about cues that predict reward? This can be accomplished using one of the many sophisticated calcium imaging tools available, such as fiber photometry for population level monitoring or GRIN lens calcium imaging to track the development of activity in individual neurons across learning. Given we proposed this region to be working in tandem with the VTA to regulate learning by providing reward expectation signals through its GABAergic projections (Hoang & Sharpe, 2021; Sharpe, Marchant, et al., 2017), the prediction would be that

the activity of LH$_{GABA}$ neurons during learning would show a sustained increase in response that ramps up across a reward-predictive cue, similar to our recordings of dopamine release in LH, which ramped up as the expectation of rewards neared. This would be consistent with previous in vivo electrophysiology recordings that distinguished phasic reward prediction error-like responses of VTA$_{DA}$ neurons from the persistent excitation responses of GABAergic neurons in VTA to reward cues, which are thought to be reflective of reward expectations (Cohen et al., 2012). Indeed, recent work from Alonso-Lozares et al. (2024) has begun to embark on this endeavor by revealing a sustained increase in LH$_{GABA}$ activity to alcohol-paired cues using fiber photometry. Interestingly, the authors showed that LH$_{GABA}$ neuronal activity initially increases to both the alcohol-predictive CS+ cue and to the inhibitory CS- cue at the start of Pavlovian conditioning, with responses to both cues spanning the entire duration of cue presentation. However, only activity evoked by the alcohol cue grows over the course of learning while activity to the CS- cue reduces. The fact that LH$_{GABA}$ neurons also respond to a cue that predicts no outcome, but then only increases its activity to a predictor of reward could suggest that this region learns to distinguish reward-relevant cues over time.

Indeed, we found a similar feature in the dopamine release profile in LH, such that there was an increase in dopamine to the CS- cue during earlier phases of learning that disappeared over the course of conditioning. Although this was non-significant in our initial characterization of dopamine release activity in LH during cue-reward learning in naïve animals, this pattern emerged again in a second cohort of rats that was found to be statistically significant during middle learning, but disappeared by late learning. This could suggest that dopamine release in LH may also be contributing to the determination of reward-relevant cues. Astoundingly, this dopamine release pattern to the CS- cue was absent in methamphetamine-experienced animals, which could allude to the idea that methamphetamine explicitly enhances the ability of the LH to divert learning towards cues that are proximal to reward. This has exciting implications when taken together with

data from Sharpe et al. (2021) showing that inhibition of LH$_{GABA}$ facilitates learning for cues distal to reward, providing causal evidence to support the notion that this region actively opposes learning for cues that are not directly relevant for predicting rewards.

New insights into hypothalamic interactions with midbrain dopamine

Upon uncovering the necessity of GABAergic neurons in the LH in the expression of model-based associations, we proposed that VTA$_{DA}$ inputs to LH act as a route for prediction error signals to reach the LH in support of its role in acquiring model-based associations. Previous research with conditioned taste paradigms using dopamine receptor antagonists in the LH provided the first lines of evidence to support the role of dopamine in LH for Pavlovian learning (Amador et al., 2014; Caulliez et al., 1996; Sclafani et al., 2011; Touzani et al., 2009). We found that inhibition of VTA$_{DA}$ terminals in LH attenuates acquisition of Pavlovian cue-reward associations and stimulation of this pathway facilitates acquisition, mirroring earlier pharmacological findings with selective blockade of D$_1$ dopamine receptors. Paired with our own findings, this may suggest cue-reward learning mediated by the VTA$_{DA}$→LH pathway is due to D$_1$ dopamine receptor activity. Collectively, however, these data still lack cell-type specificity to determine which neuronal populations within the LH are in receipt of this dopaminergic input. Given we showed that LH$_{GABA}$ neurons acquire and use model-based information, our data could be extended to implicate that this dopaminergic input from the VTA targets GABAergic neuronal populations in the LH. Indeed, there is some evidence to suggest that a set of LH neurons do encode reward prediction errors (Nieh et al., 2015), but their genetic identity has yet to be determined. Further research investigating the projection sites of VTA$_{DA}$ to the LH could serve as support for this proposed neural circuit. For example, if VTA$_{DA}$ inputs were disconnected from LH$_{GABA}$ neurons during learning, the expectation would be that you disrupt acquisition of model-based associations, on the basis of GABAergic populations in LH being responsible for the model-

based component of learning. Importantly, this should leave background model-free learning undisturbed and in fact, our causal data support this argument.

If we suspect that GABAergic neurons are in receipt of dopamine prediction errors to support its learning, then this should be reflected in the dopamine dynamics in this region. Thus, we investigated the endogenous patterns of dopamine release in LH across reward learning. Curiously, dopamine in the LH does not seem to follow canonical prediction error signaling. Dopamine release increases to both rewards and their cues across learning, rather than a shift in dopamine signaling from the reward to the cue over the course of learning as temporal difference reinforcement learning models for dopamine would predict (Suri & Schultz, 2001). Further, the dopamine response to cues and rewards is not phasic but, rather, a sustained dopamine response that ramps up as reward delivery approaches. In conjunction with recordings of LH neural activity (Alonso-Lozares et al., 2024; Harada et al., 2023; Nieh et al., 2015), our data supports the notion that the LH transforms prediction error signals from the VTA to facilitate learning in this region.

Unpublished work from Harada et al. (2023) found somewhat conflicting results with ours in the pattern of dopamine release in LH across sessions of a Pavlovian task in which the onset of a cue is followed by optogenetic VTA$_{DA}$ stimulation. Here, the authors argue that dopamine dynamics in LH during learning do follow conventional reward prediction error signaling. Specifically, they found the development of a phasic dopamine response to a cue predictive of VTA$_{DA}$ stimulation that emerged across learning to support this claim. However, the dopamine response to the stimulation reward did not reduce over time, which is a prominent feature of reward prediction errors (Schultz et al., 1997). This could have been the result of selecting to use optical stimulation of VTA$_{DA}$ neurons as the reinforcer in their Pavlovian task, as well as having it overlap with cue presentation. Using sucrose pellets, our data also found that dopamine responses to reward do not reduce over time, while the dopamine responses to the reward cue increase. Thus, it seems that dopamine release in LH might be following prediction error signaling

to some extent but is unique in its expression based on the reinforcer used. In other words, a natural reward like food elicits a gradual ramp in dopamine release to its predictive cue whereas an artificial reward like optical stimulation elicits a phasic response to the cue. Because of these discrepancies in the dopamine response to reward-predictive cues, this warrants further investigation into the varying patterns of dopamine release in LH when learning about cues that predict different types of rewards and how these differing patterns relate to cue-reward learning. For instance, a cue that predicts a psychostimulant drug may evoke phasic dopamine responses in LH while a cue that predicts alcohol might evoke a gradual dopamine ramp because of its caloric and gastric nature.

The dynamics of dopamine release in LH are intriguing in the context of our optogenetic manipulations of the $VTA_{DA} \rightarrow LH$ pathway. To show that learning is supported by VTA_{DA} prediction error signals sent to the LH, we used laser parameters that follow endogenous VTA_{DA} transients, which are brief increases or reductions in activity (Chang et al., 2018; Cohen et al., 2012; Schultz et al., 1997; Sharpe et al., 2017; Sharpe et al., 2020). However, we observed that the timescale of dopamine release in LH to rewards and cues during learning did not match the temporal strategy used for optogenetic studies, which was instead characterized by sustained increases across the cue and reward. If dopamine release in LH is exclusively from VTA_{DA} inputs, then this would mean our optogenetic manipulations may not have been entirely suppressing or recapitulating the dopamine release arising from VTA_{DA} projections to the LH. Therefore, future work is needed to determine exactly how dopamine release in LH is altered by stimulation or inhibition of VTA_{DA} neurons, which would reveal how exactly VTA_{DA} neurons modulate dopamine release in LH.

Methamphetamine disrupts LH-VTA circuits that give rise to maladaptive decisions

Heightened control of drug-paired cues is commonly seen in individuals with substance use disorder and has often been attributed to the reinforcing value inherent in a drug reward (LeBlanc et al., 2013; Robinson & Berridge, 1993; Wyvell & Berridge, 2001). This has laid much of the groundwork for habit theories of addiction, which suggest that persistent drug taking is the result of habitual responding (Belin et al., 2013; Everitt & Robbins, 2005, 2016; Sebold et al., 2017; Vandaele & Ahmed, 2021). Critically, habits are defined as responding elicited by cues that are devoid of an outcome representation. Thus, the crux of these theories in explaining the maladaptive drug use behaviors in recovering individuals is that these decisions are governed by processes that do not consider the adverse consequences of their actions (e.g., health risks, negative social impacts, financial loss, etc.). However, we have shown that this drug-induced enhancement in cue-directed behavior does contain a sensory-specific outcome representation. That is, the cues that were paired with specific outcomes were more likely to drive actions towards those same outcomes than actions towards different outcomes in animals with previous methamphetamine experience. This effect employs a model-based strategy, such that it requires inference between cues, actions, and outcomes, to appropriately select a response in the presence of a cue. This comes at odds with the aforementioned habit theories of addiction, as habitual responding is typically associated with a model-free cached value strategy.

Indeed, many computational theories for addiction have proposed behavioral mechanisms such as an accelerated shift from model-based to model-free strategies in substance use disorders (Furlong et al., 2018; Nelson & Killcross, 2006), or a reduction in model-based mechanisms, which can be taken as a heightening of model-free mechanisms (Gillan et al., 2016; Groman et al., 2019; Voon et al., 2015). Our findings, which characterized the specific nature of cue control following drug exposure, seemingly contradict the model-free habitual account model of addiction as we showed that model-based strategies were strengthened after drug use. Thus,

these data may help to reconceptualize the driving forces underlying substance use disorders by showing that individuals with this disorder use detailed cue-drug associations to influence their instrumental decisions. This is particularly important for improving relapse models of substance use disorder as drug-predictive cues in the environment can exert excessive control over behavior in abstaining individuals, which based on our data, is facilitated by the use of a cognitive representation of drug rewards and not simply aberrant habitual responding.

Our study used methamphetamine to model the effect of prior drug experience on model-based cue control over behavior, but whether these effects can be extended to other classes of addictive substances is an outstanding question. Previous work in rodents examining other substances (e.g., amphetamine, cocaine, alcohol) has only replicated the general effect that exposure to drugs of abuse increases the ability of cues to invigorate actions (Glasner et al., 2005; LeBlanc et al., 2013; LeBlanc et al., 2012; Ostlund et al., 2014; Pecina & Berridge, 2013; Pecina et al., 2006; Saddoris et al., 2011; Shields & Gremel, 2021; T. T. Takahashi et al., 2019; Wyvell & Berridge, 2000, 2001). While there are similarities in the clinical profiles of all substance use disorders, they are not all driven by the same mechanisms. For example, we reasoned that enhancements in model-based processes in our effects are due in large part to neural changes in dopamine-based reward circuits as psychostimulants, like methamphetamine and cocaine, directly impact dopamine and vesicle monoamine transporters. However, opioid and alcohol use disorders, for instance, are more associated with disruptions in opioid receptor systems, which in turn, can drive a different phenotype of addiction (e.g., avoidance of withdrawal-induced negative affect) (Evans & Cahill, 2016; Koob & Volkow, 2016). Thus, further studies distinguishing the impacts of different drugs of abuse can determine whether enhancements in model-based decision-making is a common feature to all substance use disorders. If such is the case, this would greatly aid in the development of an all-encompassing therapeutic approach to treating this neuropsychiatric disorder.

Conclusion and final remarks

Combined with prior data (Sharpe et al., 2021; Sharpe, Marchant, et al., 2017), we now understand that the hypothalamic-midbrain circuit contributes to model-based learning about cues and rewards in a manner that biases learning about cues most proximal to reward. Thus, the strengthening of the LH-VTA circuit following drug exposure would enhance the bias in learning and behavior directed towards reward-paired cues, which increases the control that these cues have over decision-making relative to other information in the environment that may not be directly reward relevant. This mirrors the pattern of reinforcement learning changes seen in humans with drug addiction, and rodent models of the disorder (Corbit & Janak, 2007, 2016; Glasner et al., 2005; Hogarth & Chase, 2012; Lamb et al., 2016; LeBlanc et al., 2013; LeBlanc et al., 2012; Manglani et al., 2017; Ostlund et al., 2014; Pecina & Berridge, 2013; Pecina et al., 2006; Saddoris et al., 2011; Shields & Gremel, 2021; Wied et al., 2013; Wyvell & Berridge, 2000, 2001). This reveals the LH-VTA circuit as a critical node in the reinforcement learning changes seen with drug addiction, consistent with data implicating the LH in cue-induced reinstatement (Aston-Jones et al., 2009; Blacktop & Sorg, 2019; Cornish et al., 2012; Hamlin et al., 2008; Hamlin et al., 2007; Harris et al., 2005; James et al., 2019; Mahler & Aston-Jones, 2012; Marchant et al., 2009; Marchant et al., 2012; Marchant et al., 2014). Beyond this, these data could suggest that targeting LH circuits would not only reduce the impact of drug cues on behavior, but also re-establish an appropriate balance in learning about other information in the environment. However, an important limitation to the conclusions drawn from the studies in this dissertation comes from a lack of causality. While we showed that prior drug experience heightens the use of model-based associations to guide behavior and that this strengthens the LH-VTA circuit, we did not causally link a role for the LH-VTA circuit in producing enhanced cue control over behavior. Thus, future work that directly interrogates the how drug-induced changes in the LH-VTA circuit produces enhancements in model-based learning would strengthen this idea.

Future research is needed to understand how the LH-VTA circuit integrates with the wider dopaminergic network. For example, we have previously hypothesized that the LH-VTA circuit forms a wider circuit with the basolateral amygdala (Hoang & Sharpe, 2021). Here, we argue that the basolateral amygdala provides the LH with sensory-specific information about motivationally significant events relevant to the current circumstance (Hoang & Sharpe, 2021). This then allows LH to influence VTA dopamine signaling and bias learning towards information most relevant to current motivational states and goals. In contrast, given evidence that LH actively opposes learning about model-based information not directly related to rewards (Sharpe et al., 2021), it is also likely that the LH-VTA circuit acts to reduce the impact of other dopamine circuits in achieving their learning goals. For example, work has shown that inhibition of orbitofrontal circuit produces a dissociable effect from the LH on learning about distal model-based associations (Hart et al., 2020). This reveals a tension between the LH-VTA circuit and those comprising orbitofrontal cortex, which likely also involve input from VTA dopamine neurons (Howard & Kahnt, 2018). Furthermore, the LH has canonically been implicated in the indirect pathway extending from the nucleus accumbens (NAc) to the VTA in inhibiting behavior (Castro et al., 2015). Thus, our findings may have greater implications for how this may moderate the coordination between LH and VTA in reward learning and addiction (Castellanos-Ryan et al., 2011; Chambers et al., 2009; Keiflin & Janak, 2015; Morein-Zamir & Robbins, 2015). For example, perhaps response inhibition via the indirect pathway from the NAc could be supporting LH function in arbitrating responding between reward-relevant and irrelevant cues (e.g., suppression of responding for cues proximal to rewards) (O'Connor et al., 2015; Sheng et al., 2023). That is, NAc inputs to the LH could be providing the inhibitory brakes for LH to not aberrantly respond to proximal predictors of reward. Given that drug addiction is associated with deficits in response inhibition that give rise to persistent drug taking (Castellanos-Ryan et al., 2011), this could be the result of hypoactivity of a NAc→LH pathway with ongoing drug use in which response suppression to proximal drug-predictive cues is reduced and thus, potentiates maladaptive drug use behaviors to perpetuate

the cycle of addiction (Sheng et al., 2023). Nevertheless, this is just the beginning to our understanding of how such a complex and dynamic dopamine system contributes to our navigation of the world.

Supplementary Materials

Appendix A: Phasic stimulation of VTA$_{DA}$ terminals in LH is not robustly reinforcing

Following the conclusion of Pavlovian procedures, we gave rats 30-minute sessions where they could optogenetically self-stimulate the VTA$_{DA}$→LH pathway by pressing on an active lever that produced a 1-s train of 20Hz stimulation (**Figure 11A**). Another inactive lever was available, but responses on it had no programmed consequences. Rats were first tested under a continuously reinforced, or fixed ratio-1, schedule (i.e., every lever press earned a stimulation reward; FR-1) for 3 sessions before upshifting the reinforcement schedule to random ratio-5 (RR-5; on average 5 lever presses needed to earn stimulation). We found that subjects would consistently lever-press for VTA$_{DA}$→LH pathway stimulation across all FR-1 sessions as well as when the lever-press requirement increased to RR-5. This was supported by statistical analyses, which found a significant main effect of lever, but no main effect of session nor an interaction between lever and session (**Figure 11B**; lever (active vs. inactive): $F_{(1,9)}$ = 8.969, p=0.015; session: $F_{(3,27)}$ = 0.603, p=0.618; lever x session: $F_{(3,27)}$ = 1.403, p=0.272). However, on this leaner reinforcement schedule, animals earned significantly less stimulation rewards despite continued lever-pressing behavior (**Figure 11B**). A repeated measures ANOVA conducted on the number of rewards earned across sessions revealed a significant main effect of session ($F_{(3,27)}$ = 6.347, p=0.002). Post-hoc pairwise comparisons between sessions found that the number of rewards earned when the reinforcement schedule shifted up was significantly less than the amount earned under a continuously reinforced schedule (FR-1 session 1 vs. RR-5: $t_{(9)}$ = -3.121, p=0.012; FR-1 session 2 vs. RR-5: $t_{(9)}$ = -3.446, p=0.007; FR-1 session 3 vs. RR-5: $t_{(9)}$ = -3.590, p=0.006). Furthermore, the degree of intracranial self-stimulation was far below what has previously been shown with stimulation of VTA$_{DA}$ cell bodies at this same frequency (Millard et al., 2022). Thus, because phasic stimulation of the VTA$_{DA}$→LH pathway was not reinforcing enough to support robust self-

stimulation, these parameters likely did not facilitate learning by virtue of being paired with the stimulation *per se*.
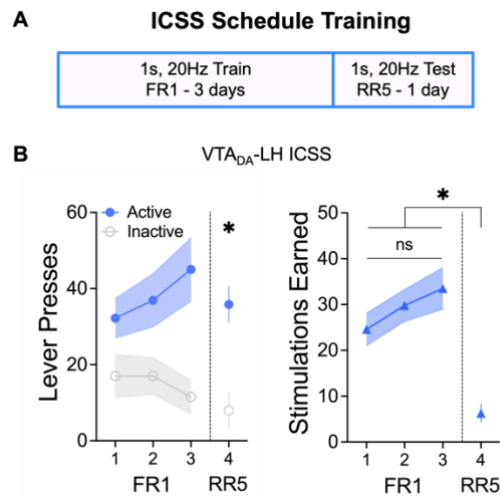

Figure 11. Self-stimulation of the VTA$_{DA}$→LH pathway does not maintain reinforcement.

(A) Training schedule for intracranial self-stimulation at 20Hz frequency in animals from **Experiment 4** of Chapter 2 (see pg. 39-42). **(B)** *Left:* Lever-press responses for an active lever that delivered a 1-s train of 20Hz stimulation and an inactive lever that had no programmed consequences, first across 3 sessions under a continuously reinforced schedule (FR-1) followed by a session on a leaner reinforcement schedule (RR-5). Greater responses were made for the active lever compared to the inactive lever under both reinforcement schedules. *Right:* Number of stimulation rewards earned across sessions under each schedule of reinforcement. Less stimulation rewards were earned when the lever-press requirement shifted up. *p ≤ 0.05, mean ± SEM.

Appendix B: Sex-dependent trends in dopamine release activity in the LH

While not fully powered to detect sex differences, we examined the data collected for conditioning and unexpected reward sessions from **Experiment 7** in Chapter 4 for any sex-dependent trends (see pg. 79-84). Here, we conducted waveform analyses on the photometry data collected during these sessions for females and males, separately. Typically, neural activity data from studies using techniques like *in vivo* electrophysiology have historically used smaller subject sample sizes but gather data from hundreds of individual neurons through single- or multi-unit recordings. Thus, we performed statistical analyses on photometry traces split by sex, but not on the behavioral data to be as conservative as possible when discussing our observations.

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The results from these analyses were intended primarily for illustration purposes and although informative, should be considered with caution solely as *trends* in the data at this time. Replication of this study and continuing to include both sexes in the future could eventually be included in a meta-analysis study to expand on sex differences in hypothalamic dopamine signaling across reward learning.

Generally, females (*n*=3) showed more robust dopamine signaling in the LH during conditioning than males (*n*=4). In males, waveform analyses detected significant increases in dopamine release above baseline to reward delivery across all phases of learning, which was most evident during *middle* learning [**Figure 12B**; blue gradient bars below trace post-pellet delivery; session (*early*, *middle*, and *late*) vs. baseline (z=0)]. However, these differences were sparse compared to females, in which longer spans of dopamine release above baseline following reward delivery were detected during *middle* and *late* learning [**Figure 12A**; red gradient bars below trace post-pellet delivery; session (*early*, *middle*, and *late*) vs. baseline (z=0)]. Furthermore, dopamine release activity after reward delivery appeared to grow across learning in females, indicated by waveform analyses comparing traces between learning phases (**Figure 12A**; blue (*middle* vs. *late*) and green (*early* vs. *late*) bars below trace post-pellet delivery). This was not the case for males, which did not find any significant differences in dopamine release activity across learning (**Figure 12B**).

Similar patterns in dopamine signaling to those found following reward delivery in males and females were also found to the antecedent CS+ cue. Here, dopamine release ramps were less apparent in males, with waveform analyses only detecting a brief increase in dopamine release towards the end of the cue in *late* learning [**Figure 12B**; blue gradient bars below trace post-CS+ onset; *late* session vs. baseline (z=0)]. Ramping up of dopamine release to the CS+ cue was more obvious in females, showing significant elevations in dopamine release above baseline during *early* and *late* learning [**Figure 12A**; red gradient bars below trace post-CS+ onset; session (*early* and *late*) vs. baseline (z=0)]. In addition, waveform permutation tests

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detected significantly greater dopamine release to the CS+ cue during *late* learning compared to *early* or *middle* phases in females (**Figure 12A**; blue (*middle* vs. *late*) and green (*early* vs. *late*) bars below trace post-CS+). Both males and females showed no significant increases in dopamine release to the CS- cue (**Figure 12A-B**).

We next examined if these trends persisted during unexpected reward sessions. Waveform analyses found that reward-evoked dopamine release responses did not significantly differ between motivational states for both males and females (**Figure 12C-D**; black bar (*restricted* vs. *sated*) below trace post-retrieval). Interestingly, dopamine release responses in both *restricted* and *sated* conditions were elevated above baseline for both sexes, though dopamine release returned to baseline faster for males than females and appeared smaller in magnitude in males compared to females [**Figure 12C-D**; red (females) and blue (males) gradient bars below trace; condition (*restricted* and *sated*) vs. baseline (z=0)]. These findings were surprising considering previous work examining dopamine levels in the LH showed the opposite effect in which food-restricted males had higher levels of dopamine compared to females (Meguid et al., 2000). Importantly, males appeared to consume more food than females to sate themselves prior to recording (**Figure 12E**; mean ± SEM; males: 6.53 ± 0.45g; females: 4.13 ± 0.83g). For the consumption test conducted after the sessions, males and females seemed to consume comparable amounts (**Figure 12E**; males: 0.33 ± 0.14g; females: 0.05 ± 0.05g). As it has been shown that LH dopamine levels rise in fasting animals that were recently fed (Fetissov et al., 2000; Ikeda et al., 2018; Meguid et al., 2000; Meguid et al., 1995; Yonemochi et al., 2019), we would have expected that if the degree of consumption was correlated with concentration levels of dopamine (Meguid et al., 1995), then males during *sated* sessions might display heightened dopamine release than females given they consumed more food prior to the session. However, there did not appear to be differences in the pattern of the response between sexes across *restricted* and *sated* sessions.

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One possibility to explain this trend is an underlying metabolic process that differs between sexes to produce the magnitude difference in reward-evoked dopamine release into the LH (Fukushima et al., 2015). Indeed, work from others has explored whether activity of peptidergic neuronal populations within the LH associated with feeding behaviors could be underpinning metabolic differences between sexes (Funabashi et al., 2009; Messina et al., 2006; Mogi et al., 2005). Interestingly, it was found that food restriction increased orexin neuronal activation in female rats, but not male rats (Funabashi et al., 2009). Other research using pharmacological agonism and antagonism of dopamine receptors in the LH has shown that the activity of orexinergic neurons in this region is, at least in part, regulated by dopamine (Bubser et al., 2005; Yonemochi et al., 2019). Thus, perhaps females showed greater dopamine release responses both to conditioned rewards and to unexpected rewards than males due to fasting-induced activation of LH orexin-expressing neurons, which have been shown to be involved with reward learning. While there appears to be conflicting evidence showing elevated levels of dopamine in food-restricted males compared to females (Meguid et al., 2000), this may suggest that there is more dopamine-receptor occupation in females than in males. In other words, more dopamine is bound to its receptors than unbound in females compared to males, which then microdialysis measurements of extracellular dopamine may detect as different dopamine levels between sexes.

In line with the previous point, another possibility could be inherent differences in the expression of dopamine receptors in the LH or the number of dopaminergic inputs that led to more robust detection of dopamine release activity in this region for females compared to males. Though not powered for sex differences, one recent study quantifying and mapping the distribution of neurotransmitter-defined cell types within the midbrain suggest that a greater percentage of cells in females than in males are exclusively monaminergic (likely dopaminergic) (Conrad et al., 2024). Given other work has identified differences in the ratio of dopamine receptor expression between males and females in NAc, it would not be farfetched to consider that a similar sex difference could be found in the LH (Williams et al., 2021). Future work is needed to more

closely examine sex differences in dopamine release activity in the LH as it may help to explain behavioral and cognitive phenomena.
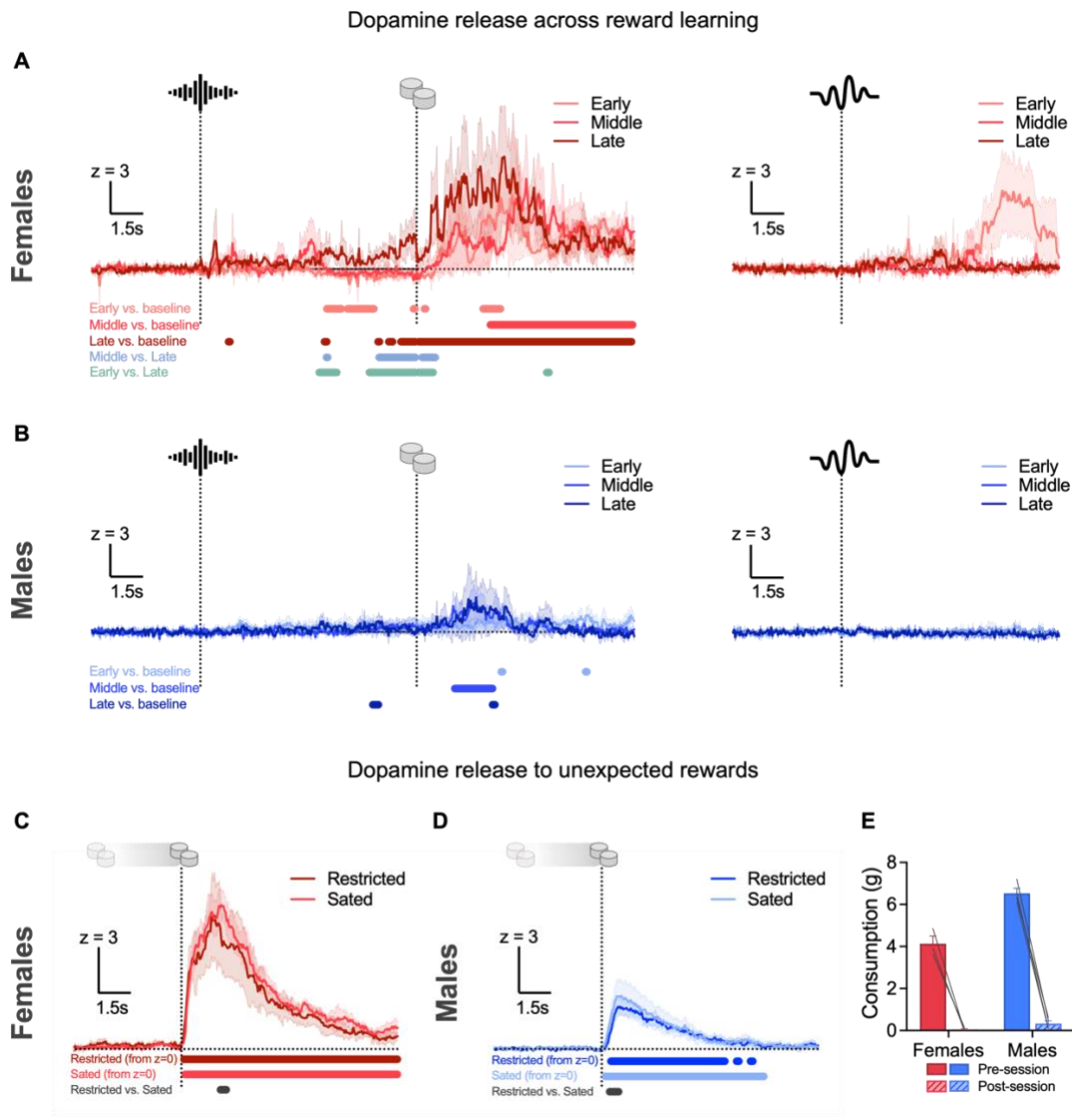

Figure 12. Females exhibit greater dopamine release activity in the LH across Pavlovian conditioning and to unexpected rewards than males.

Data collected in **Experiment 7** were separated by sex. Although not powered to detect sex differences, females (n=3) tended to show a stronger reward-evoked dopamine response than males (n=4). **(A-B)** Dopamine release activity in females **(A)** and males **(B)** aligned to CS+ followed by reward delivery (*left*) and CS- (*right*) across learning phases [mean (solid lines) ± SEM (shaded area)]. Colored significance bars below traces represent bootstrapped 95% confidence intervals detecting significant differences from baseline (z=0). Black significance bar represents permutation tests detecting differences in the traces between conditions. **(C-D)** Dopamine release activity in females **(C)** and males **(D)** aligned to retrieval of unexpected rewards. **(E)** Average consumption of home chow for each sex before and after *sated* sessions (mean ± SEM).
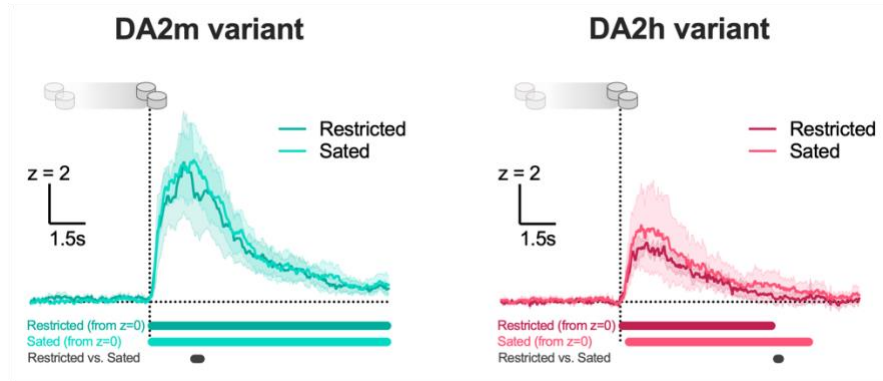
Supplementary Figures
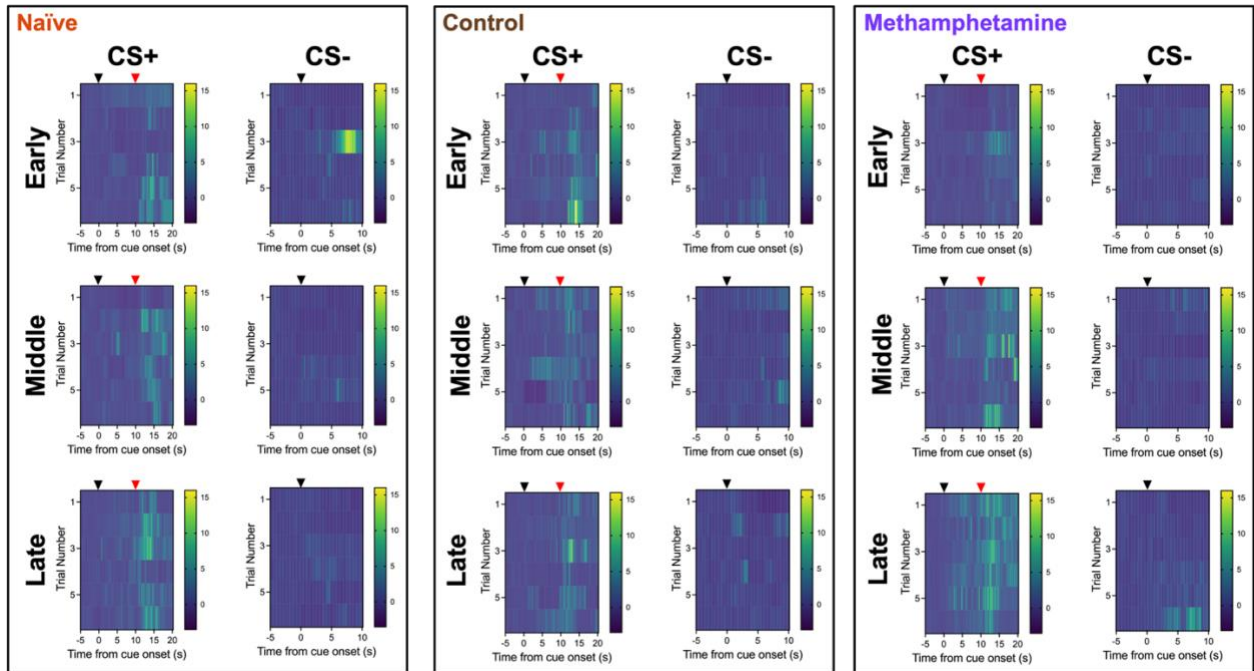
Supplementary Figure 1. Dopamine release activity in the LH across trials during cue-reward learning.

Data from Pavlovian conditioning sessions in Chapter 4 were examined on a trial-by-trial basis. Heat maps display normalized ΔF/F z-scored data aligned to cue onset for all CS+ and CS- trials split by each learning phase for each cohort of animals [Naïve (**Experiment 7**); Control & Methamphetamine (**Experiment 8**)]. Trial-by-trial data are averaged across animals within that group. Black triangles indicate cue onset and red triangles indicate reward delivery.
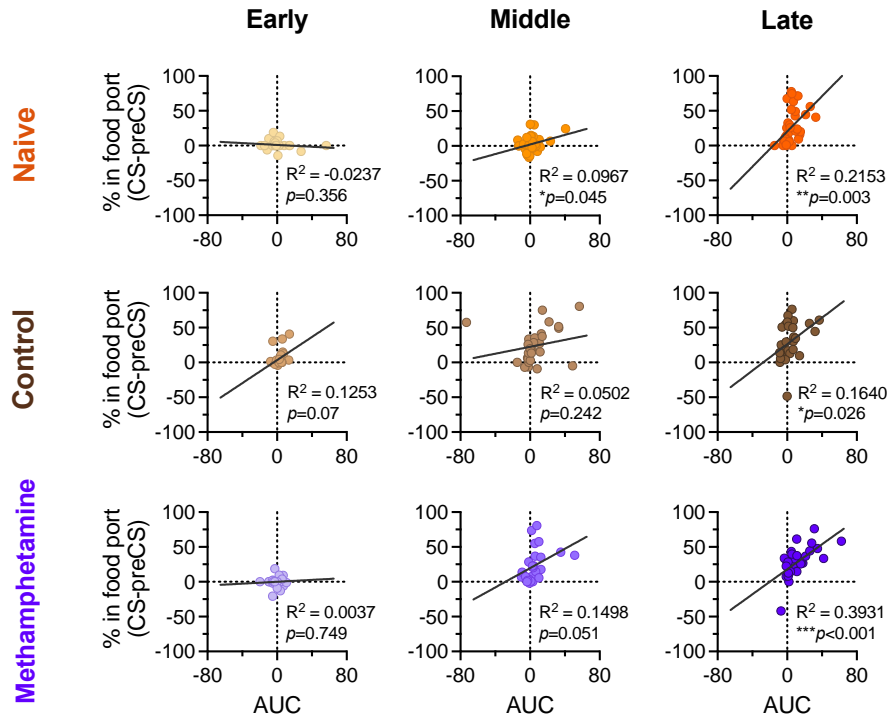
Supplementary Figure 2. Correlations between cue-evoked dopamine release and acquisition of the reward-predictive cue across learning phases.

Data from **Figures 8F, 9I, and 9J** split up by learning phase for each cohort of animals. The coefficient of determination (R^2) and corresponding p-value is listed for each correlation. *$p \leq$ 0.05, **$p \leq$ 0.01, ***$p \leq$ 0.001.

Supplementary Figure 3. Reward-evoked dopamine responses to unexpected rewards is more robust with the medium-affinity GRAB$_{DA}$ virus.

Data from unexpected reward sessions in **Experiment 7** were separated by medium-affinity (n=4) and high-affinity virus variants (n=3). Colored significance bars below traces represent bootstrapped 95% confidence intervals detecting significant differences from baseline (z=0). Black significance bar represents permutation tests detecting differences in the traces between conditions.
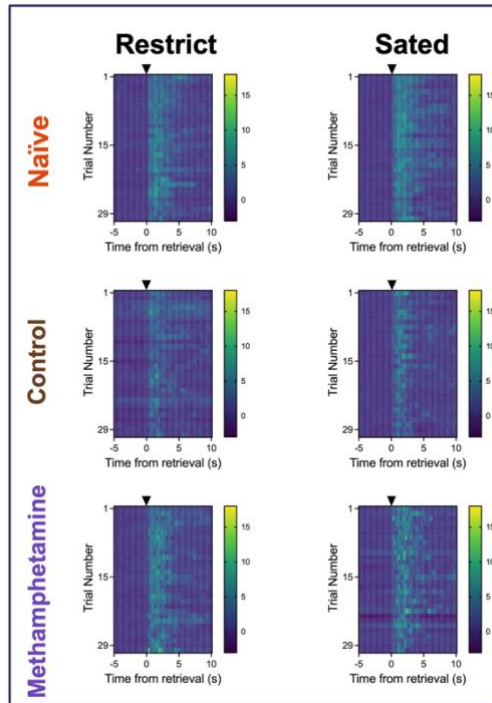
Supplementary Figure 4. Dopamine release activity in the LH across trials during unexpected reward sessions.

Data from unexpected reward sessions in Chapter 4 were examined on a trial-by-trial basis. Heat maps display normalized ∆F/F z-scored data aligned to pellet retrieval split by *restricted* and *sated* conditions across all trials for each cohort of animals [Naïve (**Experiment 7**); Control & Methamphetamine (**Experiment 8**)]. Trial-by-trial data are averaged across animals within that group. Black triangles indicate retrieval onset.

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