SETTING THE OCCASION FOR REWARD-SEEKING IN BRAIN AND BEHAVIOR

by

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Abstract

The ability to resolve uncertainty surrounding reward-associated cues is essential for the proper organization and generation of reward-seeking. Traditional approaches have modelled this dynamic process with distinct physical settings which preclude a collective neural and behavioral assessment of both how such contextual or higher-order cues are encoded and how they subsequently act on conditioned stimuli to in turn effect behavior. Here, I investigated the ability of rats to use higher-order cues to resolve the likelihood of reinforcement to an ambiguous conditioned stimulus, a process termed occasion setting. In Chapter 2, I characterized a novel approach to observing the hierarchical control of reward-seeking and discover unique motivational characteristics of higher-order contextual stimuli. In Chapter 3, I use optogenetic methods in combination with genetically modified rats to probe the contribution of dopamine neurons in the ventral tegmental area to occasion setting. These findings present a real-time contribution of the degree of dopamine neuron activity to the amount of reward-seeking which is consistent with reports of dopamine neurons encoding the expected utility of conditioned stimuli. In Chapter 4, I show that neural activity and dopamine signaling with the nucleus accumbens is essential for occasion setting. I further characterized the activity of nucleus accumbens neurons using *in vivo* electrophysiology and, in collaboration with colleagues at the University of Minnesota, monitored dopamine release in the nucleus accumbens to provide a mechanistic account of the role of the nucleus accumbens in the organization of reward-seeking. In Chapter 5, I find that basolateral amygdala and orbitofrontal cortex, but not dorsal hippocampus, are necessary for occasion setting. In Chapter 6, I described the encoding of occasion

setters in the basolateral amygdala and the necessity of activity in the basolateral amygdala for this higher-order cue to influence conditioned reward-seeking. These results are contrasted with recordings and optogenetic manipulations in the orbitofrontal cortex. Collectively these results detail neural and behavioral mechanisms for the generation of flexible cue-triggered reward-seeking which have implications for our understanding of aberrant motivation in psychiatric illness.

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In loving memory of Kathleen Marie Lowry

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Chapter 1

Introduction: Hierarchical regulation of reward-seeking, reinforcement, and relapse

Introduction

We exhibit extreme flexibility in our behavior. For instance, we may have a goal in mind to run to the store after work and pick up some fresh salmon for dinner to fit with our new diet, but on the way speak to a colleague who just returned from Japan and instead coordinate dinner plans for izakaya that evening. This brief conversation overcame your previous goal to enjoy a more sustainable meal and save on costs, instead leading you to opt for a costlier, less healthy, but much more satisfying dinner experience. While the conversation itself did not involve a direct relation to the outcome, chicken karaage, it was able to exert control over your future behavior by altering the motivational value of the izakaya restaurant's appeal. Systems neuroscience has long been fascinated with such concepts, yet the models of behavior used to probe and detail neural substrates responsible have not been sufficiently complex to capture these influences. These overly reduced approaches to cue-guided behavior have resulted from influential models of error-based learning that relate changes in behavior to changes directly related to the presence or absence of an outcome (Rescorla and Wagner, 1972; Pearce and Hall, 1980). Behavior, though, is sculpted by the presence of cues and occurs prior to the appearance of an outcome and in many cases can become immune to the influence of the presence or absence of reward so it is not apparent that trial-and-error learning best captures dynamical behavior. Thus, it is necessary to ask how motivation can dynamically alter our behavior and how neural systems could

exhibit exquisite scaling of responses to an identical cue according to the appropriate circumstance in which it was encountered.

In the laboratory, it is difficult to model such complex influences on goal-directed reward-seeking and pair these approaches with careful neurobiological investigations in animals. One way in which to model flexibility is to make use of cues that have distinct meanings in differing settings. In this approach, animals must make use of a multitude of stimuli that are long-lasting and compose what is colloquially referred to as a context to disambiguate whether the cue will predict a given outcome or not on that day. While context-based models of reward-seeking are very popular and have been useful for understanding phenomenon like renewal of responding, relating the influence of a context in the lab back to potential contextual influences on real-world reward-seeking is difficult (Bouton, 1988, 2002, 2004; Zironi et al., 2006; Chaudhri et al., 2008; Maren et al., 2013; Remedios et al., 2014; Liberzon and Abelson, 2016; Trask et al., 2017; Valyear et al., 2017). Parsing the precise actions of an arrangement of stimuli like a context on a cue-outcome relationship is hard to disentangle given their undefined temporal structure, the typical requirement for both predictive learning and extinction learning to occur to observe context-based effects, and that they only provide a readout of contextual influences at a final test and not in the ongoing organization and generation of reward-seeking.

One way in which our reward-seeking can be influenced is through the actions of stimuli that act to set the occasion for when cues are important. This occasion setting allows for the resolution of ambiguity about cues without the occasion setter itself generating a specific prediction about the likelihood of an outcome (Holland, 1992;

Schmajuk and Holland, 1998; Fraser and Holland, 2019). The actions of physical contexts and settings, both in the laboratory and in real life, likely sculpt behavior as a result of their occasion setting properties, but they can also become directly linked to reward and so it is unclear if the occasion setting or direct predictive properties of these contexts explains their actions (Bouton and Swartzentruber, 1986; Grahame et al., 1990). Fortunately, occasion setting can be modeled in a number of tractable ways in the laboratory that allows for a mechanistic account of the generation of flexible cue-generated behavior at both a psychological and neurobiological level (Fraser and Holland, 2019). In this review, I will expand on the ability of contexts and cues to act as occasion setters and highlight the utility of this overarching framework to advancing our understanding of the hierarchical control of reward-seeking. In particular, I hope to make apparent the potential pitfalls with traditional context-oriented approaches and avenues to overcome these issues by modeling the ongoing resolution of uncertainty with occasion setting. As a result, I provide predictions for the function of dopamine neurons in the ventral tegmental area and dopamine release in the nucleus accumbens in guiding such hierarchical cue-triggered motivations to hopefully guide future investigations in a vastly underexplored area of behavioral and systems neuroscience.

Cue-triggered motivations

One of the primary triggers for reward-seeking and relapse is the presence of cues associated with reward (Bolles, 1972; Bindra, 1974; Stewart et al., 1984; Childress et al., 1993; Robinson and Berridge, 1993; Toates, 1998; Berridge et al., 2009). Through associative learning processes, cues that are present during encounters with rewards, like drugs of abuse, come to predict them and can serve to generate

expectations of reward receipt. Cues can have simple or highly complex relationships with rewards and other cues which determine a number of factors in their influence on our behavior. In general, much research has focused on the actions and consequences of learning simple cue-reward relationships, where either a single cue is paired with reward, or one cue is reward-paired and a different cue is never paired with reward (Rescorla, 1988; Fanselow and Wassum, 2015). Even in these extremely simple cases, cues can come to predict a wide number of properties about the reward. For instance, the sound of an ice cream truck might elicit the taste of chocolate soft-serve, including its creamy texture and its sweetness that promotes reward-seeking. Cues may also elicit a general prediction about how good some outcome is, absent any mental representation of that quality or sensory properties of that reward, and this general value is used to shape behavior (Holland, 1990; Dayan and Berridge, 2014; Sutton and Barto, 2018). These mechanisms can be dissociated in the laboratory.

Take for example a rat that has learned that white noise predicts a banana flavored sugar pellet and that a beeping tone predicts grape flavored sucrose syrup. As a result of the pairings of each cue with reward, the rat sculpts its checking of the port where the rewards are delivered to be highest when the cues come on, getting ready and exhibiting anticipatory appetitive behavior. If the rat was attracted to check for the rewards by the cues electing a rich and detailed representation of the crunchy, banana pellet or the sticky-sweet syrup, then manipulating the value of a reward should have a specific effect on the likelihood the rat will exhibit port-checking. There are two common ways to do this, either by letting the animal eat either reward freely until they are sated on it or by pairing consumption of one of the rewards with nausea produced by injection

of lithium chloride (Garcia et al., 1955; Rolls et al., 1981; Loy and Hall, 2002). If the rats used a rich representation of the sensory properties of the reward, they should respond less to the pre-fed or nausea-paired reward-predictive cue, but if the rats instead are generally motivated by the value of the cues resulting from their repeated pairings with a good outcome, then alterations of the reward's value will be without effect (Holland, 1990; Pickens and Holland, 2004). If responding to the cue is sensitive to making the reward less desirable, then we call this behavior model-based as it requires a link between the sound of the cue and the sensory properties of its associated outcome, but if the port-checking behavior is insensitive to this manipulation this behavior is called model-free, as there is not a need for a representation of reward, merely an abstract representation of the value of the cue (Figure 1) (McDannald et al., 2011; Dayan and Berridge, 2014; Sutton and Barto, 2018). It is important to note model-free and model-based strategies that guide reward-seeking to either cue currently are not easily disentangled without a manipulation of the reward's value (McDannald et al., 2011). These strategies have implications for computational models of reinforcement learning and much is unknown about what factors tip the scales between one strategy or the other (Gläscher et al., 2010; McDannald et al., 2011; Dayan and Berridge, 2014; Lee et al., 2014; Langdon et al., 2018; Groman et al., 2019a). Contextual influences and the scenarios that tip the favor towards model-free or model-based representations and strategies are largely unexplored, and evidence suggests the weighting of each strategy is affected by drug history and that the potential for arbitration between two strategies leaves much to be investigated (Groman et al., 2019b).

Which of these strategies is more dynamic and thus more able to rapidly adapt? If an agent makes use of model-free representations it should be readily apparent that it will require repeated trials to update changes in its behavior based on the value of the reward associated with the cue (Dayan and Berridge, 2014; Sutton and Barto, 2018). Thus, learning to respond to a cue differently when one is hungry versus sated will be a gradual and require many trials. In contrast, model-based representations do not necessarily dictate such a rigorous trial and error-based approach to updating. As model-based agents maintain rich representations of the sensory, motivational, and emotional components of cues and rewards they are inherently flexible and dynamic (Dayan and Berridge, 2014; Langdon et al., 2018). One of the most striking demonstrations of the extremely rapid updating afforded by a model-based system is in studies of salt appetite, an experimenter-induced physiologic need for salt that will never naturally be experienced by laboratory animals (Berridge et al., 1984; Berridge and Schulkin, 1989). If rats first learn in a normal state absent a need for salt that a cue predicts an extremely disgusting salty solution they are repulsed and reproach from the cue on its appearance (Robinson and Berridge, 2013). However, after the induction of salt appetite, on the very first presentation of this cue, and critically before the experience of the now rewarding salty solution, the rats are attracted, energized, and approach the cue before they can experience the now desired and pleasant salt (Robinson and Berridge, 2013). These model-based systems thus allow for a rapid reshaping of cue-triggered motivations and permit extreme flexibility in Pavlovian-based learning beyond what can be captured by predictions from Rescorla-Wagner based models where the value of a reward requires updating cue-value generated from the

experience and receipt of the actual reward (Rescorla and Wagner, 1972; Domjan, 2005; Dayan and Berridge, 2014; Sutton and Barto, 2018). Cue-triggered behaviors, thus, depart in meaningful ways from predictions generated from popular theories of learning when factors such as internal state are incorporated as regulating factors.

Apart from providing a way of predicting the likelihood of an outcome, cues can also become imbued with incentive motivational value, which allows their presentation to generate states of motivation that elicit reward-seeking and produce desire (Bindra, 1978; Cardinal et al., 2002; Berridge and Robinson, 2003; Berridge et al., 2009; Robinson et al., 2014b). The incentive motivational and predictive properties of cues can be dissociated, in other words, just because a cue predicts a reward does not mean that the cue is motivationally relevant and acts to produce behavior or craving for reward (Flagel et al., 2009; Robinson and Flagel, 2009; Flagel et al., 2011; Robinson et al., 2014b). The attribution of incentive salience, or incentive motivational value, to reward-paired cues is essential for those cues to generate and provoke behavior (Berridge, 2004). Incentive motivational value renders reward-paired cues attractive, desirable, and able to invigorate reward-seeking actions (Bindra, 1978; Cardinal et al., 2002; Flagel et al., 2009). This process is distinct from reward-learning in that rats can learn a cue-reward relationship yet fail to show characteristic responding to that cue that indicates the cue is motivationally relevant (Robinson and Flagel, 2009; Fraser et al., 2016).

For example, if a group of rats learn that the insertion of a lever predicts a food reward there is individual variation in the conditioned responses rats exhibit upon lever insertion. Some rats rapidly approach, press, nibble, and sniff at the lever when it is

inserted, a response called sign-tracking, whereas other rats rapidly approach and enter the adjacent food cup where reward will be delivered when the lever ultimately retracts. Rats who are attracted and interact with the lever, sign-trackers, and those that approach the food cup, goal-trackers, both learn the lever-reward relationship at an equal rate and respond equivalently during training. However, to probe the motivational value of the lever rats can be asked to learn a new response to earn the lever itself in a test of conditioned reinforcement. Only sign-trackers will learn to work for the lever in the absence of food, goal-trackers typically do not persist in earning the reward-associated lever (Figure 1) (Robinson and Flagel, 2009; Fraser et al., 2016). Conditioned reinforcement is thus a useful readout that clarifies if incentive motivational processes contribute to conditioned reward-seeking (Robinson et al., 2014b). What is less clear is how incentive motivational processes relate to the distinctions between model-based and model-free strategies (Dayan and Berridge, 2014). For instance, does sign-tracking indicate a model-free strategy (Huys et al., 2014; Lesaint et al., 2014)? Is conditioned reinforcement a readout of model-based or model-free processes (Parkinson et al., 2005; Burke et al., 2007, 2008; Sharpe et al., 2017a; Langdon et al., 2018)? Is the attribution of incentive motivational value a requirement for model-based or model-free strategies (Morrison et al., 2015; Nasser et al., 2015)? Thus, even learning a simple single cue and reward relationship results in numerous possibilities for the manner in which that cue is represented and the underlying systems guiding responding to that cue. Yet, even still these single cue-reward relationships significantly oversimplify reduce the complexity of real-world interactions. In particular, in the real world the predictability of a cue and the subsequent response to it are largely shaped by

uncertainty and its resolution that can be guided by a number of internal and external factors.

Uncertainty about a given cue can arise from a number of features including its temporal relation to reward, its predictability of reward delivery, or its perceptual ambiguity in discriminating it from related sensory stimuli. It is well appreciated that uncertainty can enhance and magnify cue-directed motivation, in particular for cues that unreliably predict a given reward (Anselme et al., 2013; Singer et al., 2020). In the real world this is best captured by slot machines and gambling situations in which the cues received and your interactions with have no influence on the outcome yet they become powerful generators of motivation to seek reward. Most of what is known about cue-generated uncertainty has arisen from similar gambling-like cues in the laboratory, where a given cue has an absolute probability of predicting reward (Anselme et al., 2013; Robinson et al., 2014a, 2019, 2020; Anselme and Robinson, 2019). These instances of relatively known uncertainty mirror our expectations of gambling situations but likely fail to capture instances in which expectations are dynamically shaped during normal reward-seeking. In particular, these deterministic cases are unable to model the dynamic changing of ambiguity about the instantaneous importance and certainty of reward receipt of an uncertain cue as in these instances the probabilities are fixed or slowly drifting and must be resolved by numerous encounters with that cue and its given probability of reward to execute behavior according to a running average of expected value. In contrast, reinforcement uncertainty is able to resolved by means of exploiting other available external stimuli, recent history-based outcomes, or internal states and in turn this information can be used to compute a more nuanced expectation of reward on

a given instance, or even totally resolve uncertainty and render a given cue absolutely predictive of reward receipt.

Together, cue-triggered motivations occur in many forms and learning a cue-reward association can result in a number of complex representations, even in the simplest cases. Despite this, these deterministic, all-or-nothing, expectations are rigid and limit flexibility in behavior. It is critical to appreciate the dynamic control of behavior by cues, and their ever-changing motivational significance. In this dissertation I seek to capture dynamic cue-triggered behavior, which ultimately builds off of these fundamental properties of these reduced cue-reward relationships. Cue-triggered motivation is inherently flexible and our approaches to capture these dynamics should be sufficiently complex to model the factors influencing this flexibility.

Occasion setting

Capturing the complexity of dynamical reward-seeking requires an understanding of the mechanisms generating cue-triggered behavior. The ability of behavior to come under the control of complex mechanisms dates back to early musings by the famous behaviorist Skinner in which he became fascinated by the idea that a rat's lever pressing could come to be shaped to occur only during a light, given the responding was only reinforced accordingly (Skinner, 1935, 1937). For Skinner, though this was a simple arrangement of stimuli and actions it was difficult to model how the light would act to control responding. In modern times, these stimuli are commonly known as discriminative stimuli - a term reserved exclusively for those stimuli that shape instrumental actions. However, discriminative stimuli do not necessarily capture the nuance needed for a true hierarchical relationship as they critically lack the ambiguity

needed to promote such a representation. For instance, in most cases a given discriminative stimulus is always associated with reward and always presented alongside the ability to perform the associated action, so there is not any cue-generated ambiguity or hierarchy. Discriminative stimuli instead directly gate the performance of actions and in turn can also become direct predictors of outcomes, which complicates their actions as true hierarchical mediators of behavior (Holland, 1991; Trask et al., 2017).

In contrast to discriminative stimuli, investigations into the determinants of Pavlovian conditioning gave rise to the study of the modulation of cue-driven responding. These investigations were primarily led by Peter Holland and colleagues into the mechanisms of such feature-based - a term for a cue that is not a typical conditioned stimulus - mechanisms of modulation (Ross and Holland, 1981; Holland, 1989, 1992). Alongside Holland's investigations into the control of conditioned responding by such modulatory cues in rodents were efforts made in pigeons by Rescorla, presumably motivated to expand upon the prevailing Rescorla-Wagner theory that was incapable of capturing such modulation (Rescorla, 1985, 1987). Prevailing views at the time on such modulatory actions suggested the ability of animals to modulate responding resulted from a configural account. Configural accounts of learning presume unique representations of the combination of two or more stimuli that allow for behavioral discrimination (Pearce, 1987, 1994). However, Holland and colleagues argued for a more nuanced and hierarchical representation wherein modulatory cues, like physical settings, internal states, and external stimuli instead act to modulate the strength of the association between a given conditioned stimulus and

the outcome it predicts (Holland, 1992; Delamater, 2012; Bonardi et al., 2017). This modulatory action was termed occasion setting as a given event *sets the occasion* for whether a conditioned stimulus will or will not be motivationally significant (Ross and Holland, 1982). While much interest developed around occasion setting following a number of critical papers, interest in these mechanisms waned in the 1990s, perhaps owing the advent of new techniques for investigation in the neurobiology of learning and motivation that lacked appropriate specificity to identify such modulatory processes (Fraser and Holland, 2019).

There are a number of important distinctions that separate occasion setting, and the events that perform such modulatory actions called occasion setters, from simple Pavlovian conditioning, discriminative stimuli, and configural learning (Holland, 1992; Fraser and Holland, 2019). I will present here a brief discussion of the findings in Chapter 2 to provide additional context for the motivation to exploit occasion setting as a model of hierarchical control. One of the primary obstacles to identifying occasion setting is that it can reflect summation between the probabilities of the occasion setter and conditioned stimulus. In a novel preparation we recently developed, modeled after positive patterning designs (Woodbury, 1943), the occasion setter and conditioned stimulus presented alone never predict reward, but their serial presentation indicates a conditioned stimulus will predict reward, with the probability of any given cue being presented on a trial being equal (Figure 2) (Fraser and Janak, 2019). As expected, rats trained in this preparation respond more to the conditioned stimulus when its occasion is set, than alone (Figure 3). However, we reliably observed responding at the time of expected conditioned stimulus presentation on trials when only the occasion setter was

presented. To rule out such summation from explaining results, we explicitly extinguished responding to the occasion setter, making obvious that this cue has no relationship with reward. If rats summated the probabilities of reinforcement this manipulation would eliminate differences in responding to the conditioned stimulus when it was or was not occasion set. In contrast, rats exhibited identical levels of modulation to the conditioned stimulus absent any responding to the occasion setter ruling out summation.

The ability of occasion setters to modulate the relationships between cues and rewards are not well captured by popular theories of reinforcement learning (Fraser and Holland, 2019). For instance, the famous Rescorla-Wagner model only allows for a cue to be updated on a trial by trial basis on its ability to have earned reward or not (Rescorla and Wagner, 1972). In the model we present in Chapter 2 for occasion setting, both the probability of the occasion setter and the conditioned stimulus of predicting reward are equivalent, so an agent making use of reinforcement learning algorithms should always rely on the average reinforcement rate to guide their behavior. This is clearly not the case as animals are able to exploit occasion setters to modulate their response to the same conditioned stimulus on different instances, reflecting a process not well captured by models like Rescorla-Wagner and Pearce-Hall that require local updating of value by the most recent instance of reinforcement (Figure 3) (Rescorla and Wagner, 1972; Pearce and Hall, 1980; Sutton and Barto, 2018). Configural accounts may overcome this difficulty as they treat the combination of the occasion setter and its conditioned stimulus as a single cue distinct from either occasion setter or conditioned stimulus alone (Pearce, 1987, 1994). However, data from

experimental studies, including data from our preparation, indicate that occasion setting is a distinct process from configural learning. For instance, configuration of cues should be resistant to the order (occasion setter->conditioned stimulus is the same as conditioned stimulus->occasion setter) as they are combined into a unique stimulus, yet in occasion setting the order is necessary and generally requires the occasion setter to be presented before the conditioned stimulus it modifies (Figure 3) (Ross and Holland, 1982; Holland, 1992). Moreover, the trace between the occasion setter and conditioned stimulus is often quite long, and the trace between the occasion setter and potential reinforcement is even longer, presumably making it difficult for backpropagation of reinforcement signals to the occasion setter from the conditioned stimulus and the rare instance of reinforcement to occur. Together these support a view that occasion setting is the result of a hierarchical representation between modulatory events and the conditioned stimuli they act on (Fraser and Janak, 2019).

The nature of the representation modulated by occasion setters has lacked investigation and remained unclear. Occasion setting has primarily been described as an alteration in the value of the conditioned stimulus, in accordance with model-free theories of reinforcement learning (Delamater, 2012; Bonardi et al., 2017; Fraser and Holland, 2019). In contrast, occasion setting appears to engage hierarchical representations, and in turn it is possible that occasion setters actually act to shape a rich representation of the reinforcer predicted by a conditioned stimulus, and this anticipation of the sensory qualities of the reinforcer are what guide behavior (Dayan and Berridge, 2014). We sought to provide evidence to bear light on this discrepancy and made use of sensory-specific satiety to investigate if occasion setting involves

model-free or model-based representations. In our preparation of occasion setting, devaluation of the normally received reward by pre-feeding resulted in a reduction in reward-seeking on trials in which the occasion setter indicated the conditioned stimulus would be reinforced, but in contrast when rats were pre-fed on a novel reward that was just as preferred and consumed in equal amounts there was no effect on behavior (Figure 3). These hierarchical occasion setting cues thus appear to actually alter the nature of the representation evoked by conditioned stimuli and the ability of these occasion setting cues to modulate reward-seeking is in part due to the sensory features of the expected reward.

Much focus on occasion setting and hierarchical control has focused on the ability of these events to modulate the predictability of a conditioned stimulus. However, the predictive and motivational properties of cues can be dissociated (Berridge and Robinson, 2003; Robinson and Flagel, 2009). In Chapter 2, we directly tested if occasion setters can not only modulate the predictive relationship between a conditioned stimulus and reward, but if these hierarchical cues can shape the motivational significance of these cues (Fraser and Janak, 2019). In tests of conditioned reinforcement, where animals report the value of a given cue by executing a novel response, we found that occasion setters indeed enhance the motivational value of conditioned stimuli, and that these conditioned stimuli absent their occasion setter lack any inherent motivational value (Figure 3). Interestingly, occasion setters themselves had motivational value and we found that this motivational value, evidenced by conditioned reinforcement, persisted even in rats for whom the occasion setter was extinguished and responding to the occasion setter alone was absent (Figure 3). Taking

these behavioral findings together, 1) occasion setting is a model of hierarchical control that involves modulation of an association between a cue and reward, 2) acts to modify the nature of that representation that involves the sensory properties of the predicted outcome, is resistant to extinction of the hierarchical cue, 3) involves the generation of a motivational state triggered by the hierarchical cue, and 4) can modify the motivational value of a conditioned stimulus.

Occasion setting lacks a plausible theory-driven model of how such a representation can be structured and enacted, unlike the case for the simplest of conditions that are explained by error-driven models like the Rescorla-Wagner model (Bonardi et al., 2017; Fraser and Holland, 2019). One possibility that is alluring is that occasion setters act as some scalar to influence the strength of an association. In a model of reinforcement learning put forth originally by Berridge and colleagues to describe the influence of an internal motivational state on behavior (e.g, appetite or stimulant-induced sensitization), they proposed a scalar factor, kappa, that allowed for dynamic updating without new learning (Zhang et al., 2009). However, the exact manner that these states are constructed and represented remains difficult to ascertain as this model merely introduces a factor into computations designed after the Rescorla-Wagner model. Moreover, it lacks a way for the scalar factor to be incorporated during learning or a means by which this factor is computationally updated to allow its use apart from demonstrations of it capturing the influence of drastic changes in physiological state. It is also possible that occasion setting could be described by state-based learning models such as a Markov-based decision model (Daw et al., 2006; Dayan and Daw, 2008; Langdon et al., 2018). These approaches could prove useful in

capturing the state-based transitions inherent in occasion setting and hierarchical control, yet the application of these models in capturing the many behavioral characteristics of occasion setting beyond modeling the basic features of occasion setters to enhance or reduce responding to an ambiguous conditioned stimulus is unclear, especially given the unique transfer and extinction functions of these hierarchical cues (Fraser and Holland, 2019). As such, more theory-driven computational approaches are needed to capture hierarchical processes in reinforcement learning and will be an important direction for research outside the scope of the present work.

Contexts and contextual control – potential failures of models of context-influenced behavior

While we have focused on occasion setting as the prototypical model for the hierarchical control of reward-seeking, other approaches have attempted to model the modulation of reward-seeking. The most popular of which is to use essentially a very distinct room, referred to as a context, to indicate that during the length of a behavioral session a cue will or will not be rewarded. In rodents, a popular model is one created to mimic the effects of rehabilitation therapy in humans seeking treatment for a variety of psychiatric disorders like PTSD and addiction (Crombag et al., 2008; Maren et al., 2013; Liberzon and Abelson, 2016). In essence, the animals learn in one physically distinct box composed of unique smells, lights, and tactile properties that a cue predicts a given outcome, then without warning are placed into a new box with a new arrangement of lights, smells, etc. and the same cue as before is presented but without its outcome. The animal learns over a number of days being in this new context that they should stop

seeking reward when the cue comes on. Interestingly, if you place the animal back into the original context and play the cue they respond exactly as if they had never had the extinction treatment (Figure 4). This model has been adapted for a variety of approaches including with drugs of abuse and has been commonly described as a model of context-induced relapse.

In this context-induced relapse models be it for drugs of abuse, aversive footshocks, or food reward, the primary learned relation is that of a conditioned inhibitory association between the extinction context and reward (Holland and Bouton, 1999; Bouton, 2002, 2004; Bouton et al., 2006; Crombag et al., 2008; Janak and Chaudhri, 2010; Maren et al., 2013; Liberzon and Abelson, 2016; Valyear et al., 2017). There is a lack of evidence that the original context itself has a hierarchical relation with either the cue or the outcome, it may merely just be a background stimulus of little importance. It remains unclear under which conditions this initial context will or will not be encoded in a manner relevant for it to actually modulate behavior (e.g. (Zinn et al., 2020)). In contrast, the presence or absence of the extinction context is the sole determinant of behavior. Depending on the arrangement of actions, cues, and outcomes the extinction context can directly inhibit the production of actions or act to inhibit representations of the outcomes (Holland and Bouton, 1999; Bouton, 2002; Trask et al., 2017). While this may appear a semantic point, these differences ultimately shape different neural substrates involved in the modulation of behavior and are essential to our understanding of the underlying neural circuits. The use of the term context-induced has thus been a filler term that lacks an appropriate psychological mechanism to describe the actions of the stimuli that result in reinstated responding in these scenarios

(Krakauer et al., 2017). In many of these cases, the use context-induced implies that it is the action of the original drug-, fear-, or reward-paired context that uniquely triggers relapse and renewal, yet this is perhaps better envisioned as a loss of contextually-controlled extinction.

Beyond a difference in psychological mechanisms triggering active renewal or relapse of responding as opposed to a failure of inhibitory control, there exist a number of confounding effects not always accounted for in these approaches. One primary concern is that the probability of reward in each context is not equal, and solely based on the reward rates there is a direct association between the original context and reward. Direct associations between the initial context and reward make difficult claims that the action of the context is to modulate responding. Even if there is evidence for the initial context to modulate responding, it is not apparent how to distinguish between contextually-modulated responding from a summation of cue-reward and context-reward associations at test, particularly as it is not apparent how to measure or observe extinction of context-reward associations. Moreover, it is not evident that the original context itself enters into a hierarchical relationship with the reward-predictive cue. If the animal were returned not to the initial training context, but instead a novel or familiar context where no training had occurred at test they would frequently respond to the cue as if it still predicted its outcome (Holland and Bouton, 1999; Bouton, 2002; Bouton et al., 2006; Remedios et al., 2014; Millan et al., 2015; Sciascia et al., 2015). This observation most strongly challenges the idea that the original context hierarchically controls the strength of the initial cue-outcome association and results in relapse. Ultimately, this model of contextually-mediated extinction is best viewed as a model of

the failure of rehabilitation to transfer to novel scenarios. Extinction learning itself is a unique phenomena that is prone to failure as it is well-documented that the strength of extinction training wanes with time and the initial cue-outcome memory eventually outcompetes the extinction memory (Bouton, 2002, 2004; Millan et al., 2011; Dunsmoor et al., 2015; Trask et al., 2017).

Two prominent psychiatric disorders, PTSD and addiction, are characterized by their frequent relapsing into either extreme fear and anxiety or drug use, respectively. It is critical then for our models to capture the dynamic and profound influence of cues and contexts in resulting in relapse. It has long been noted that rehabilitation treatments are not extremely effective in reducing the risk of relapse for these disorders (Bouton, 2002; Maren et al., 2013; Dunsmoor et al., 2015). That relapse can occur long-after treatment and the return to contexts associated with distress or drug use implies more complex psychological interactions underlie the ability of these cues to suddenly become motivational magnets and generate intense craving, for example in substance abuse. To better understand these influences we need our models to have tractable and fluctuating control over the motivational impact of cues and allow for dynamic reward-seeking that is accompanied by a precise understanding of the psychological mechanisms at play. Many of these issues are achieved by utilizing an occasion setting framework in an effort to capture the rapid, dynamic, and experimenter-controlled utilization of hierarchical information to guide reward-seeking in the face of ambiguity. A shift from a view of contexts as the main experimental method to observe modulation of responding to embracing occasion setting in its many forms will ultimately better guide behavioral and neurobiological interventions.

Neural substrates of hierarchical control

Given the complexity of occasion setting it is critical to identify potential neural circuits and systems could underlie the ability of hierarchical cues to rapidly and dynamically modulate the associative strength between a conditioned stimulus and reward. In contrast to the decades of work on the neural substrates underlying classical one cue – one outcome Pavlovian conditioning, there is little experimental work in comparison on the neurobiology of occasion setting. What evidence exists was recently reviewed and suggested a role for orbitofrontal and striatal circuits being involved in negative occasion setting (Meyer and Bucci, 2016a; Fraser and Holland, 2019). However, the mechanisms and manner in which these systems participate remains unclear based on these early studies as they employed manipulations that make it difficult to distinguish effects on simple conditioning from occasion setting. I wish here to highlight experimental approaches in simple Pavlovian conditioning that share features captured by occasion setting which may make clear routes for future investigations to bear light on the neural substrates of hierarchical control. In particular, I emphasize the potential contribution of mesolimbic dopamine and its striatal targets as critical avenues for future research.

The most well-characterized system in Pavlovian conditioning is the mesolimbic dopamine system arising from the ventral tegmental area. Dopaminergic neurons arising from the ventral tegmental area project throughout the limbic system primarily targeting the ventral striatum, prefrontal cortex, and amygdala (Fields et al., 2007; Breton et al., 2019). The activity of these neurons supports reinforcement and their activity has been well-characterized in simple conditioning tasks where they appear to

track the learning of a cue-reward association in accordance with error-based theories of learning, like the Rescorla-Wagner model (Olds and Milner, 1954; Schultz and Dickinson, 2000; Stuber et al., 2008; Steinberg et al., 2014; Keiflin and Janak, 2015; Coddington and Dudman, 2019). In contrast, manipulations of dopaminergic function in their terminal regions, in particular the ventral striatum, suggest a role for dopamine in invigorating reward-seeking and tracking the motivational value of cues to spur responding and not necessarily in learning the association between cues and outcomes (Berridge and Robinson, 1998; Berridge, 2007; Flagel et al., 2011; Clark et al., 2012, 2013; Berke, 2018). This contrast remains an active area of debate that is difficult to disentangle given multiple levels of complexity in differentiating the contributions of dopamine neuron firing and synaptic control dopamine neuron release in terminal fields (Berke, 2018; Collins and Saunders, 2020). To make accurate predictions for the function of the dopamine system in hierarchical control more generally we will focus separately on properties of mesolimbic dopaminergic neurons that make them exquisitely tuned for encoding occasion setting and potential mechanisms for dopamine release in the ventral striatum to contribute to behavioral flexibility.

The initial report of dopaminergic neurons firing in accordance with a Rescorla-Wagner reward prediction error ignited research into the computational properties of dopamine neurons (Schultz et al., 1997). The bulk of this focus has remained on the conditions under which dopamine neuron firing is consistent or inconsistent with predictions from popular learning theories (Schultz and Dickinson, 2000; Keiflin and Janak, 2015; Watabe-Uchida et al., 2017; Langdon et al., 2018). While these theories have influenced and inspired much work, here we wish to focus on properties of

dopamine neurons revealed by these theories that may indicate potential for the coding of hierarchical cues. Particularly, hierarchical regulation requires to some extent a neural substrate to be sensitive to information about probability and uncertainty. In foundational experiments probing the influence of probabilities on prediction error-style signaling, dopamine neurons were recorded while animals learned about cues that had a fixed probability with predicting reward and the magnitude of reward was stable (Fiorillo et al., 2003). Interestingly, dopamine neuron firing was highest to cues that always predicted reward, but the increase in firing resulting from reward receipt was highest to the most uncertain cues, predicting reward with 0 probability (Fiorillo et al., 2003). Probabilities that are stable as these can be combined with information about the amount of reward to generate an expected value of how good a given cue is on average. If the probability and reward magnitude are altered in a way that produces differences in expected value, then dopamine neuron firing is in accordance with peaks for the highest expected value (Tobler et al., 2005). Dopamine neurons have thus been ascribed with calculating the expected value of a cue based on the probability of reward delivery and amount of reward it predicts. Thus, sensitivity to probability in dopamine neurons is confounded by the probability coinciding with alterations in expected value of reward. Whether dopamine neurons encode exclusively the long-running expected value of a given cue or are capable of rapidly integrating contextual and state-relevant information to generate a current estimation of expected value remains to be determined.

Recent investigations in rodents, primarily, have focused on the ability of dopamine neurons to become sensitive to predictions about “states” in the world as an

extension of uncertainty shaping error-based models of learning (Gershman and Uchida, 2019). For instance, experiments have made use of tasks where rodents must infer whether the current block of trials indicates reward will follow a cue with 100% or 90% probability or if the current reward size in a block of trials is now large or small (Starkweather et al., 2017, 2017; Babayan et al., 2018). Dopamine neurons do indeed appear to report errors about the transitions into an unsignaled state change at the time of reward owing to the receipt or absence of the changing reward. This is true for both a single cue and experiments where two cues are used to bridge the gap between the first cue and reward, with second cue omission restoring dopamine firing to expected reward as a result of an error in temporal expectancy (Pan et al., 2005; Starkweather et al., 2017; Babayan et al., 2018). Dopamine neurons in these cases are primarily altered at the time of reward, not the time of the cue nor is it always clear that such cue-generated expectations are shaped by these changes in firing at the time of reward receipt. This transfer of error at the time of reward to the preceding cues is theorized to explain learning and the updating of behavior. In contrast there lacks clear evidence that these reports of prediction error at reward receipt are always causal in updating behavior on the next trial, in many cases reward related activity in dopamine neurons exist long after behavior has stabilized (Coddington and Dudman, 2018; Saunders et al., 2018; Mohebi et al., 2019). Regardless, these belief-based models require extensive experience and depend on the delivery of reward to shape these representations as they are modified variants of a Rescorla-Wagner model (Gershman and Uchida, 2019). As we have noted before, in hierarchical situations these models would erroneously attribute reward prediction solely to a hierarchical occasion setting cue, not well accounting for

behavioral findings explaining such control of cue-motivated behavior (Fraser and Holland, 2019). In hierarchical scenarios, the significance of a cue and its relationship with reward is the unit of modification, not merely the report of reward delivery. As a result, how well accounts of deterministic or stable uncertainty attributed to single cues account for the hierarchical control of cue-reward associations is not obvious. It remains less clear how the activity of dopamine neurons, which has primarily been investigated as reporting beliefs about uncertainty at the time of reward receipt, could account for hierarchy in cue-motivated behavior.

Perhaps motivational accounts of cue-triggered behavior are better explained by investigations into actual dopamine signaling within the striatum. Dissociations between reports of dopamine firing and dopamine release in accounting for simple Pavlovian behavior have been a topic of debate that are well described elsewhere (Berridge, 2012; Berke, 2018). There is much less evidence of dopamine release during uncertain or probabilistic tasks compared to dopamine neuron activity owing to the technical difficulty of previous techniques like fast-scan cyclic voltammetry to reliably detect dopamine release over many sessions. In one such study, dopamine release was recorded in the nucleus accumbens in rats learning that a cue predicted reward, with separate groups learning different probabilities of that cue predicting reward (Hart et al., 2015). In contrast to the finding that dopamine neurons respond highest to cues that fully predict reward, dopamine release was highest to the cue that had maximal uncertainty about reward delivery with a probability of 0.5 (Fiorillo et al., 2003; Hart et al., 2015). This finding is in accordance with behavioral and pharmacological data that uncertain cues are motivationally desirable, can attract approach more than certain

cues, and can sensitize the dopamine system (Anselme et al., 2013; Robinson et al., 2014a; Anselme and Robinson, 2019). Extrapolating from even this probabilistic scenario for predictions about dopamine release would suggest that there would be stable and equivalent dopamine release for both the occasion setting cue and the conditioned stimulus as the probability of these cues predicting reward is equal and set at 50%. In contrast, hierarchical accounts would predict more dynamic cue-triggered dopamine that would shift rapidly on a trial-to-trial basis.

In most preparations for observing occasion setting and hierarchical control there is a serial presentation of cues, requiring any neural substrate to be sensitive to the order of the cues and the instances in which either cue is relevant for motivation. A pioneering study by Pan and colleagues investigated potential ways in which dopamine neurons could generate a report of expected value. In their task, dopamine neuron activity was recorded while rats learned a task in which two cues presented in a sequence were followed by reward (Pan et al., 2005). In this task, every trial was composed of the ordered presentation of cues, neither was presented in isolation. Dopamine neurons responded to both cues equally, with some cells having a stronger response to the first cue, in accordance with expectations from Rescorla-Wagner based models where the earliest predictor of reward should be the only source of error after learning (Rescorla and Wagner, 1972; Schultz and Dickinson, 2000; Collins et al., 2016; Sutton and Barto, 2018). Interestingly, there was no report of error at the time of the second cue in the series if the presentation of the second cue was omitted, which suggests that the firing to this cue primarily was the result of its bridging the gap to reward delivery and not derived from a reward prediction *per se* (Schultz et al., 1993;

Pan et al., 2005). In a similar task in freely behaving rats, Smith and colleagues recorded neural activity in the ventral pallidum, a major output of the nucleus accumbens, when rats learned that an ordered presentation of two cues indicated the delivery of sucrose directly into their mouth (Smith et al., 2011). Neurons within the ventral pallidum, a region with direct inputs onto dopamine neurons, fired to each of these cues although intriguingly, firing to the first cue in the series was greater than for the second, in contrast with the pattern observed in dopamine neurons (Pan et al., 2005; Smith et al., 2011). If dopamine signaling was enhanced in the nucleus accumbens by infusing amphetamine, only neural responses to the second cue was enhanced (Smith et al., 2011). Similar findings were observed for more permanent alterations in dopaminergic function through the induction of psychomotor sensitization to amphetamine (Tindell et al., 2005). Although there lacks evidence of the influence of such serial cue presentation on dopamine release in the nucleus accumbens, it appears that both dopamine neurons and regions directly downstream of their striatal targets are sensitive to task structure. We hypothesize that nucleus accumbens neurons themselves can become sensitive to task structure in a way that can support hierarchical control and occasion setting. Moreover, on the basis of the influence of dopamine enhancing drugs selectively altering neural responses to reward-proximal cues, occasion setting may be observed by coincident alterations in dopamine release and striatal firing to a conditioned stimulus.

Discrepancies between dopamine firing and release to uncertain and probabilistic cues may result from interactions between dopamine release and terminal modulation from glutamatergic inputs to the striatum. In particular, glutamate inputs from the

thalamus and amygdala to the nucleus accumbens have been shown in anesthetized animals to enhance dopamine release (Floresco et al., 1998; Parsons et al., 2007). In awake, behaving animals it is possible for such interactions to shape release and in turn guide flexible behavior. For instance, basolateral amygdala input to the accumbens is necessary for the proper execution of a lever-press following an discriminative stimulus and inactivation of this amygdala input also reduces dopamine release to this discriminative stimulus (Ambroggi et al., 2008; Jones et al., 2010a, 2010b). Given that the basolateral amygdala is necessary for the updating of the significance of a cue given broad alterations in internal states, it is hypothesized that such input into the nucleus accumbens is critical for shaping not only the behavioral response to hierarchical cues, but does so as a result of shaping accumbal firing and dopamine release (Gallagher and Holland, 1994; Holland and Gallagher, 1999; Sharpe and Schoenbaum, 2016). However, most investigations into the interaction between striatal dopamine release, neural activity, and glutamatergic input have been conducted in instrumental tasks, so even such speculations of their contribution during cue-guided behavior requires clarification. Current accounts of glutamatergic input dynamically scaling dopamine release could provide a computational basis for the scalar kappa factor proposed by Berridge and colleagues, but we reiterate again that this model can not well capture occasion setting in its current form (Zhang et al., 2009). Such data for a contribution of basolateral amygdala input in shaping striatal responses and dopamine release is lacking and is an area requiring more extensive investigation, particularly as it comes to understanding dynamic reward-seeking.

Dopamine neurons and dopamine release in the nucleus accumbens are thus sensitive to the probability of reinforcement predicted by reward-associated cues. However, it remains to be shown if dopamine neurons can encode the relevance of a reward-associated cue in a hierarchical manner that would allow rapid updating of the probability of reward on a trial-to-trial basis. As dopamine neurons ultimately have been shown to encode the expected value of a single cue, presumably acquired as a result of the repeated pairing of that cue and both the probability and magnitude of reinforcement it predicts over extensive encounters, this raises questions about how such hierarchical coding could be implemented. Occasion setting acts to modulate the association between cues and rewards which dynamically scales the motivational value and expected value of a single conditioned stimulus. How dopamine neurons could signal such rapid changes is not obvious as the expected value of the cue remains stable and unchanging over long timescales, yet it is obviously apparent in behavior that the actual predictive and motivational value of this cue is rapidly scaled by occasion setters. We propose that dopamine neurons could inherent such a calculation from its striatal targets, as these are engaged in serial loops with their recipient midbrain dopaminergic sites (Haber et al., 2000; Haber and Knutson, 2010). Striatal dopamine signaling is under tighter control and has numerous potential mechanisms to allow the shaping of not only dopamine release but encoding within striatal projection neurons is likely gated by their glutamatergic input. This input may ultimately shape the striatal signals that in turn coordinate behavioral responding and dopamine signaling. Thus, the dopaminergic substrate of hierarchical control may be made more apparent from investigations into

striatal dopamine release in coordination with careful measurements and manipulations of defined glutamatergic input.

What sources of input, then, may be critical? The nucleus accumbens integrates diverse glutamatergic signals from prefrontal and orbitofrontal cortex, thalamus, hippocampus, and amygdala (Figure 5; Cardinal et al., 2002). While each of these structures is likely essential for some aspect of hierarchical control, we wish to highlight potential for the basolateral amygdala and orbitofrontal cortex to participate in this process. These structures both project densely into the ventral striatum but are also highly interconnected sharing reciprocal connections and projection targets, like the mediodorsal thalamus (Heilbronner et al., 2016). Given this anatomical basis, it is perhaps not surprising that lesions or manipulations of activity in basolateral amygdala and orbitofrontal cortex have similar impacts on behavior. For instance, lesions of these structures interfere with reinforcer devaluation, conditioned reinforcement, Pavlovian-to-instrumental transfer, and second-order conditioning (Everitt et al., 1989; Burns et al., 1993, 1999; Hatfield et al., 1996; Schoenbaum et al., 1998; Blundell et al., 2001; Holland et al., 2001; Setlow et al., 2002; Pickens et al., 2003, 2005; Corbit and Balleine, 2005; McDannald et al., 2005, 2011; Ostlund and Balleine, 2007; Johnson et al., 2009; Rudebeck and Murray, 2011; Parkes and Balleine, 2013; Stalnaker et al., 2015). Moreover, lesions of basolateral amygdala impairs reward- and cue-encoding in the orbitofrontal cortex and vice-versa (Saddoris et al., 2005; Takahashi et al., 2013; Lucantonio et al., 2015; Sharpe and Schoenbaum, 2016). Collectively it appears that these structures are essential to updating and behaving flexibly in the face of changing circumstances – suggesting a role in the hierarchical control of reward-seeking.

Indeed, early evidence has suggested that the orbitofrontal cortex and its interactions with the nucleus accumbens are critical for occasion setting. In a paradigm where occasion setters inform that a rat must withhold reward-seeking as the upcoming conditioned stimulus will not be rewarded alterations in the activity of orbitofrontal cortex and nucleus accumbens simultaneously resulted in behavioral impairments (Meyer and Bucci, 2016b). In a recent investigation, recordings of single neurons within the orbitofrontal cortex have found that these neurons can be modulated by occasion setters in a similar task adapted for head-fixed mice (Shobe et al., 2017). The orbitofrontal cortex is well studied with respect to its role in behavioral inhibition and control so whether there is a role for the orbitofrontal cortex in instances in which occasion setters facilitate reward-seeking remains unknown (Stalnaker et al., 2015). There is substantial evidence that basolateral amygdala and its interactions with the nucleus accumbens are essential when discriminative stimuli indicate the availability of actions to lead to reward (Ambroggi et al., 2008; Jones et al., 2010b, 2010a), yet whether there is any involvement of the basolateral amygdala in a purely Pavlovian occasion setting task is unclear. Despite a clear test of occasion setting, the basolateral amygdala is critical for the control of Pavlovian reward-seeking by distinct contexts, which may act as occasion setters (Bouton and Swartzentruber, 1986; Remedios et al., 2014; Valyear et al., 2017). Manipulations of activity and glutamatergic signaling in the basolateral amygdala interrupt the ability of rats to use a context to renew responding to previously extinguished cue, and this arises in part due to interactions with the nucleus accumbens and dopamine release in this structure (Fuchs et al., 2005; Chaudhri et al., 2013; Millan et al., 2015; Sciascia et al., 2015; Valyear et al., 2020). While evidence

suggests a role for both orbitofrontal cortex and basolateral amygdala in occasion setting their precise contributions and how neural activity in these regions could be dynamically shaped is not well known. Taking advantage of the ability to parse and separate the hierarchical stimulus from the target of its actions is an obvious boon to occasion setting that can provide novel mechanistic insight into the interactions of cortico-amygdalo-striatal loops in the control of behavior. The application of hierarchical control in neurobiological investigations of reward-seeking has the potential to highlight numerous diverse mechanisms relating to the organization and utilization of higher-order information in the ongoing resolution of uncertainty and production of dynamic reward-seeking that better mirrors real-world cue-motivated behaviors.

Main objectives

As I have summarized above there are numerous open questions concerning not only the behavioral features of hierarchical control but also the underlying neurobiological basis for such a process. The main goal of this dissertation is to provide a tractable model for the observation of occasion setting that is amenable to neurobiological investigation. The ability to exploit such a behavioral model would then make possible the ability to resolve outstanding questions concerning the contributions of mesolimbic dopamine neurons to cue-driven behavior, the functions and actions of nucleus accumbens in the resolution of uncertainty, and the potential interactions of basolateral amygdala and orbitofrontal cortex in the control of reward-seeking.

In Chapter 2 I provide a number of lines of behavioral evidence from a novel preparation to observe occasion setting in freely behaving rodents. This approach allowed the verification that reward-seeking in this task met characteristics to observe

occasion setting. Additionally, I took advantage of the ability to equate the probability of reward delivery among the occasion setting cue and conditioned stimulus to assess the motivational value of these cues. In a surprising finding it was the occasion setter, not conditioned stimulus, that acquired motivational value which is discussed with implications for our understanding of how hierarchical stimuli may act to influence reward-seeking. These findings provide a behavioral basis that is exploited in the rest of dissertation.

In Chapter 3 I turn to the function of midbrain dopamine neurons in the generation of cue-elicited reward-seeking. By taking advantage of the ability to selectively manipulate dopamine neurons by combining optogenetics with the use of transgenic rats I reveal that dopamine neuron activity is necessary for occasion setters to influence reward-seeking. I then attempt to rule out potential alternative hypotheses for this effect – mainly concerning the potential necessity of activity in midbrain dopamine neurons for responding to probabilistic cues. These findings provide evidence for the involvement of the mesolimbic dopamine system in the hierarchical organization of behavior.

For Chapter 4 I investigated the potential involvement of the nucleus accumbens, the primary target of midbrain dopamine neurons assayed in Chapter 3, in occasion setting. I reveal that manipulations of neural activity or dopamine signaling in this region interferes with the expression of occasion setting. To better understand these effects, I exploited the ability to record individual neurons and dopamine release in the nucleus accumbens that was made possible only by our behavioral design in Chapter 2. I find that occasion setters are strongly encoded within the nucleus accumbens, and that

dopamine release and neural responses to a conditioned stimulus are under the control of occasion setters. These findings are discussed with respect to the function of the nucleus accumbens in reward-seeking writ large.

In Chapter 5 I sought to provide answers to potential brain regions upstream of the nucleus accumbens that could be critical inputs for occasion setting and in turn future investigation. I find that activity within either basolateral amygdala or orbitofrontal cortex is essential for occasion setting.

In Chapter 6 I begin to detail potential mechanisms within basolateral amygdala and orbitofrontal cortex that make these structures necessary for occasion setting. I find more evidence of encoding and utilization of occasion setters in basolateral amygdala than in orbitofrontal cortex. In addition, optogenetic inhibition of the basolateral amygdala, but not orbitofrontal cortex, affected behavioral performance.

I provide a general discussion of the main findings in Chapter 7 while highlighting future directions and potential limitations with the approaches taken in this work. Ultimately, I provide evidence in numerous interconnected regions for the hierarchical control of reward-seeking and leave open the precise ways in which these regions interact for future investigations.

Conclusions

Cue-triggered behavior can appear simple to produce in the laboratory yet has appreciable complexity in the psychological mechanisms underlying behavior and the multitude of neural systems engaged. Appreciating this complexity is essential not only for a robust understanding of behavior, but to properly link an observed neural response

or manipulation to the psychological process supporting that behavior. It is apparent that dopamine neuron activity and release is critical for learning and responding to reward-predictive cues, but the ability of these neurons to be flexible in highly complex, rich, and dynamic cue-triggered behaviors is not well-defined. Moreover, animal models of psychiatric illness will benefit from increased complexity that can more closely capture the myriad cues, contexts, settings, states, and rewards that interact to spur our desires and choices. In particular, I hope that a greater appreciation of more rapid and dynamic reward-seeking in addiction can better capture the ability of hierarchical cues to spur motivation and trigger relapse.

The form of hierarchical control we have focused on is one that is precise and explicit in the time-course and mechanisms that allow the dynamic shaping of reward-seeking. Analogues of this control have been identified in certain models of animal drug self-administration and have promise for clarifying nuance in the triggers that spur relapse driven by discrete cues (Collins and Saunders, 2019). Hierarchical processes are likely at play in most behaviors as we integrate information about internal state and recent history to update our expectations and motivations. Such a framework is also useful in capturing the influence of drug-generated interoceptive states on influencing not just continued drug-seeking but also the factors and neural substrates that spur the co-abuse of drugs like alcohol and nicotine (Palmatier and Bevins, 2008; Bevins and Besheer, 2014; Jaramillo et al., 2016; Randall et al., 2016, 2019). Even the physiological response to drugs of abuse can be directly controlled in a hierarchical manner, determining tolerance or sensitization to a given dose of a drug, that have important implications for preventing overdose and drug-use related death (Badiani et

al., 1995; Anagnostaras and Robinson, 1996; Anagnostaras et al., 2002; Ramos et al., 2002). Hierarchical processes are evident at each step in substance abuse disorders, from the initial spur to take drug, the continued use of drug, the physiological response to drugs, and ultimately relapse and must be considered in our experimental approaches and investigations.

Here I have highlighted occasion setting as a model that allows for a precise understanding of the hierarchical control of behavior. Occasion setting allows for experimenter-based control of the nature of the hierarchy and in turn the modulation of behavior and allows not only for nuance in psychological investigations into such mechanisms but offers immense opportunities for neurobiological investigation. In contrast to approaches making use of physical settings such as a room, occasion setting allows for observations of immediate transformation of ambiguous cues into motivationally relevant triggers for reward-seeking. This precision is necessary to understand the ability of drug- and fear-related stimuli to become triggers for relapse in substance abuse disorders and PTSD. Ultimately, this approach offers numerous opportunities for a well-defined and tractable model for neurobiological investigations into hierarchical control of cue-motivated behavior that directly relate to a well-characterized psychological process. While simple cue-reward associations are standard in the laboratory, hierarchical control of cue-triggered behavior is the norm rather than the exception in guiding our decisions, actions, and desires. The time is ripe for behavioral and systems neuroscience to move beyond reduced models of learning and motivation and embrace hierarchy in cue-triggered behavior.

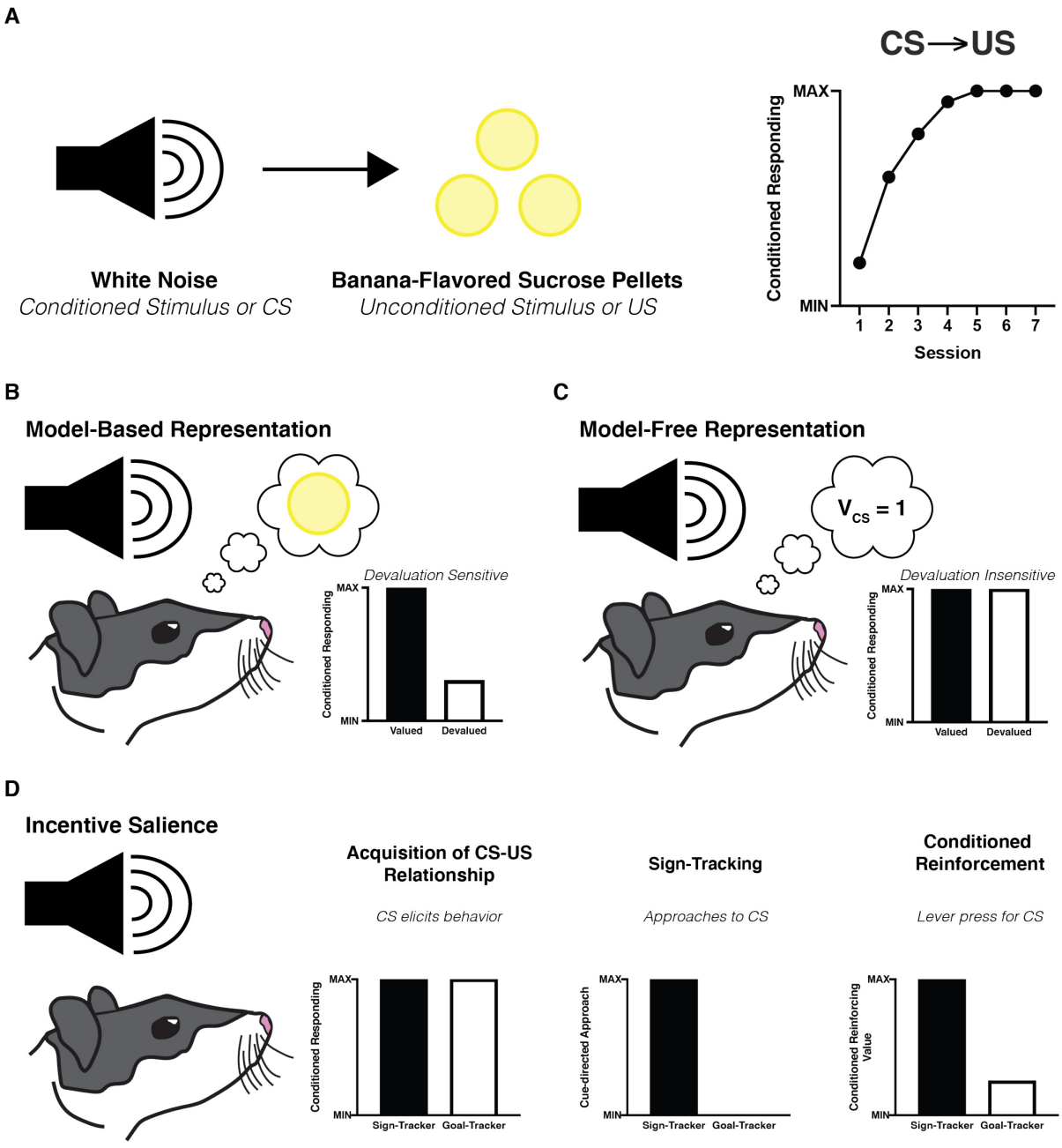

Figure 1.1. Representations and cue-triggered motivations arising as a result of the pairing of a cue with reward. The consequences of learning that a given cue predicts a given reward are perhaps not best captured by the evidence that a cue elicits responding. ***A,*** General example of Pavlovian conditioning where an auditory cue, in this case white noise, is paired with the delivery of a reward at its offset, in this case

banana-flavored sucrose pellets. As a result of the continued pairing of the white noise and sucrose pellets, rats begin to show behavior during the white noise, treating it as a CS. ***B,*** In some cases, rats can develop rich representations of the sensory features of the reward that are triggered when the CS is presented that spurs their responding. As a result, when the reward is devalued by either pre-feeding or pairing consumption of that food with nausea, responding to the cue decreases. ***C,*** In contrast, a CS can sometimes trigger a general representation of the abstract value of the cue and respond as a result of how much value has been ascribed to that CS over time. This representation is removed from the actual properties of the reward so responding to the CS is insensitive to devaluation of its associated outcome. ***D,*** A CS eliciting conditioned responding does not confer that CS with motivational value and there exists individual variation in the extent to which a given CS is likely to acquire this incentive salience. One way to assess this is to measure the amount of approaches and interactions with the CS during its presentation, termed sign-tracking, if the CS is localized and able to be easily contacted. Apart from these CS-directed approaches, the incentive salience attributed to a cue can be assessed by requiring the animal to acquire a novel response to earn just the CS. A CS that is imbued with incentive motivational value will support this conditioned reinforcement and is thus a useful measure in instances where the CS chosen is not able to be approached during initial CS-US pairings.

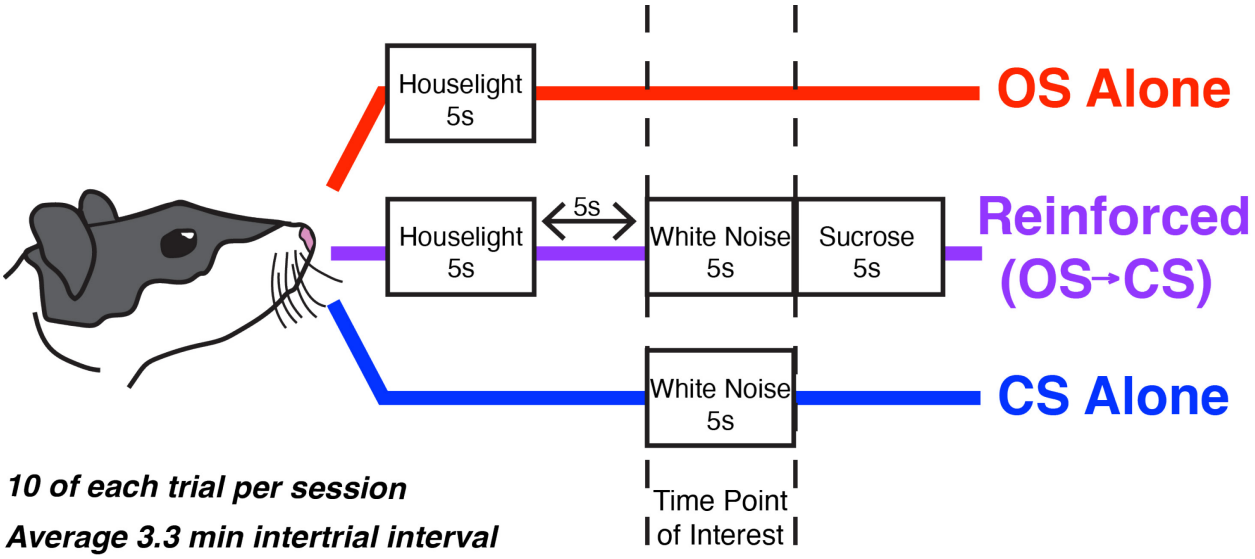

Figure 1.2. A model to observe hierarchical control of reward-seeking through occasion setting. Outline of a behavioral task designed by Fraser and Janak (2019) to observe occasion setting in freely moving rats. In a daily 2-hour session rats are presented with 10 each of three different trial types and their responding in a food cup is measured. On reinforced trials, a light and tone are serially presented with a gap between and sucrose reward is delivered at the offset of the white noise. The other trials are presentations of just the light or just the white noise alone. As a result of this arrangement of trials, rats use the light as an occasion setter to modulate their responses to the white noise conditioned stimulus. In contrast to previous models for occasion setting, the probability of reward predicted by either the light or tone are matched and there is a robust number of trials to support neurobiological investigation. OS, occasion setter; CS, conditioned stimulus.

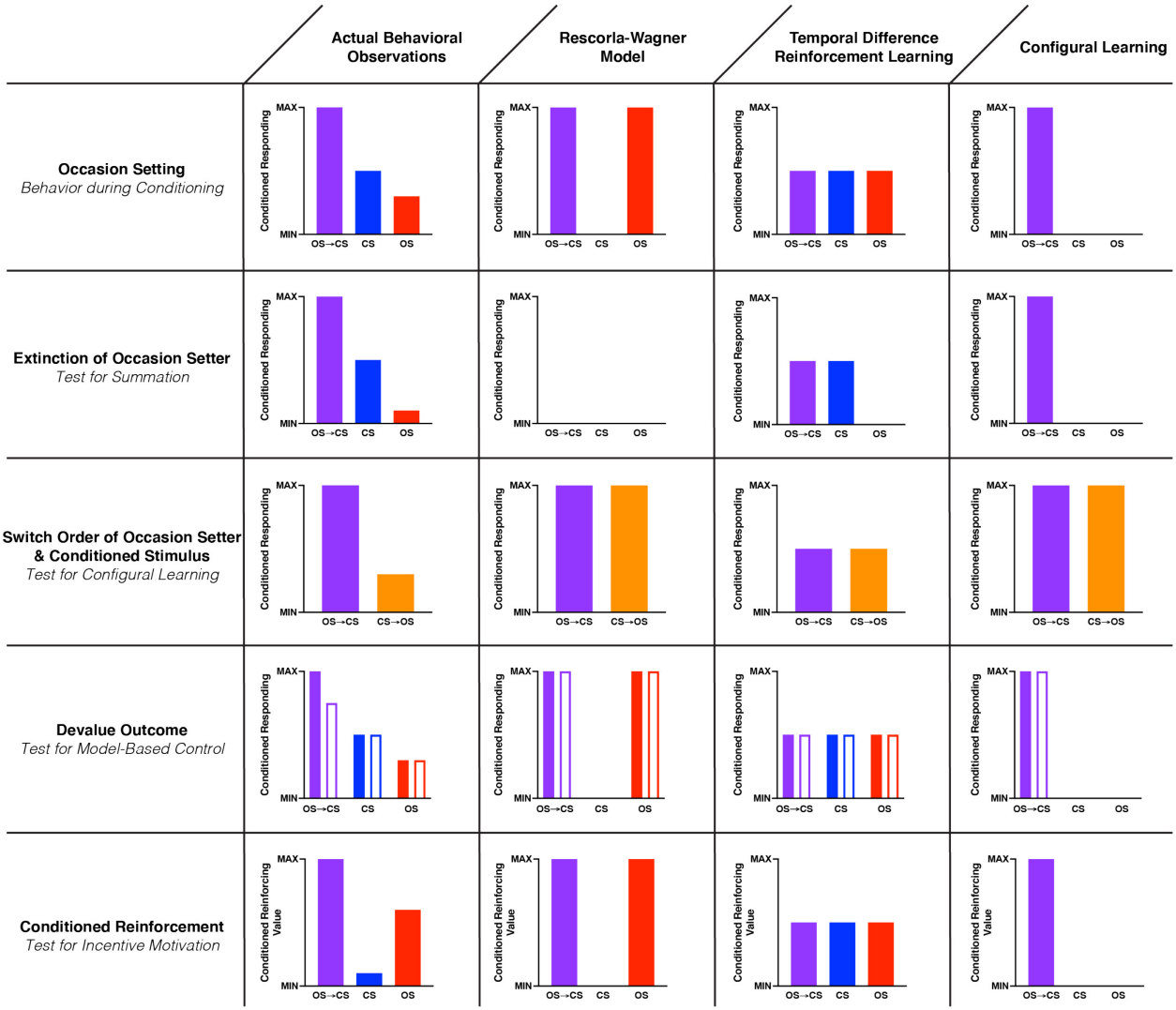

Figure 1.3. Occasion setting deviates from predictions derived from popular theories of learning. Observations from male Long-Evans rats trained in an occasion setting paradigm according to the procedures in Fraser and Janak (2019) are contrasted with predictions based on the Rescorla-Wagner model, Temporal Difference Reinforcement Learning, and configural learning. The data for the switched order of occasion setter and conditioned stimulus and the satiety-induced devaluation are unpublished data from a cohort of n=46 male Long-Evans rats. For switched order testing, 5 trials were added to a standard session in the style of Fraser and Janak

(2019) where the order of the occasion setter and conditioned stimulus were presented and the responding shown in orange is time in food cup during the occasion setter. For the devaluation, rats were pre-fed for one hour on the normally predicted outcome, 15% sucrose, or an alternative but equally consumed outcome, 15% maltodextrin, prior to a session in the style of Fraser and Janak (2019) conducted with no reward delivered. Open bars indicate the session where sucrose was devalued by pre-feeding, closed bars indicate the sucrose valued condition when maltodextrin was pre-fed. OS, occasion setter; CS, conditioned stimulus.

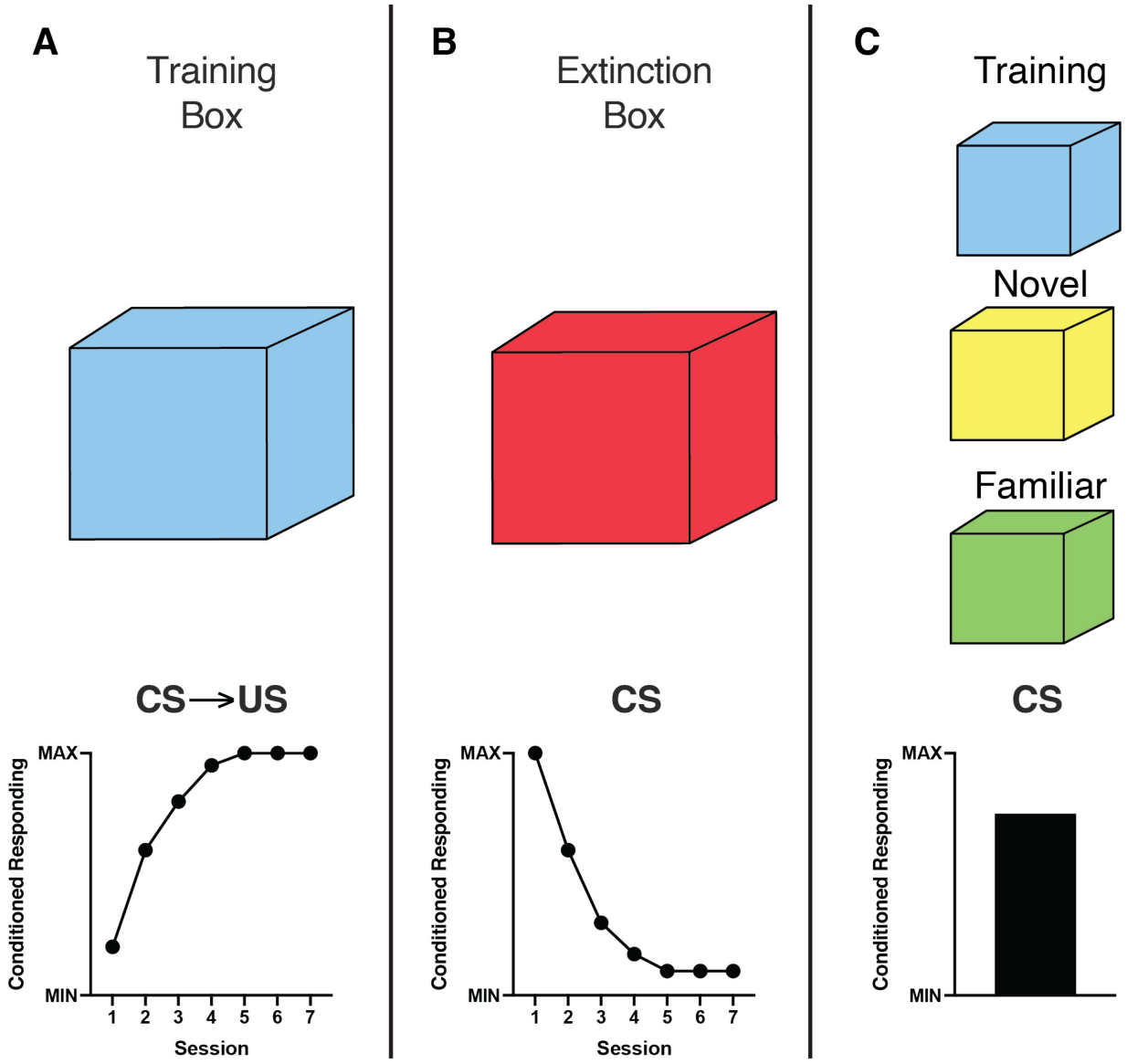

Figure 1.4. Loss of inhibitory control by an extinction-associated context, not the modulatory actions of the training context, explains renewal of responding. An example of the common use of different sights, smells, and textures in a conditioning chamber to produce a unique context during Pavlovian conditioning. ***A,*** In the original context a cue is paired with an outcome according to Pavlovian conditioning procedures and conditioned responding to the CS develops. ***B,*** Animals are then placed into a different context where the CS is presented but without its associated outcome, nor are

any unsignaled outcomes delivered. Conditioned responding to the CS decreases as a result of extinction learning. ***C,*** At test, placement of the animal back into the training context, a novel context, or a context that is familiar and equated in exposure to the training context, generally results in renewal of responding to the CS. Given that all of these contexts result in renewal, it is not apparent that the training context “induces” renewal by some modulatory action or by a direct association with reward. In contrast, this pattern is best captured by the loss of inhibitory modulatory control of the extinction context which signals that the CS is not rewarded, but only in the extinction context.

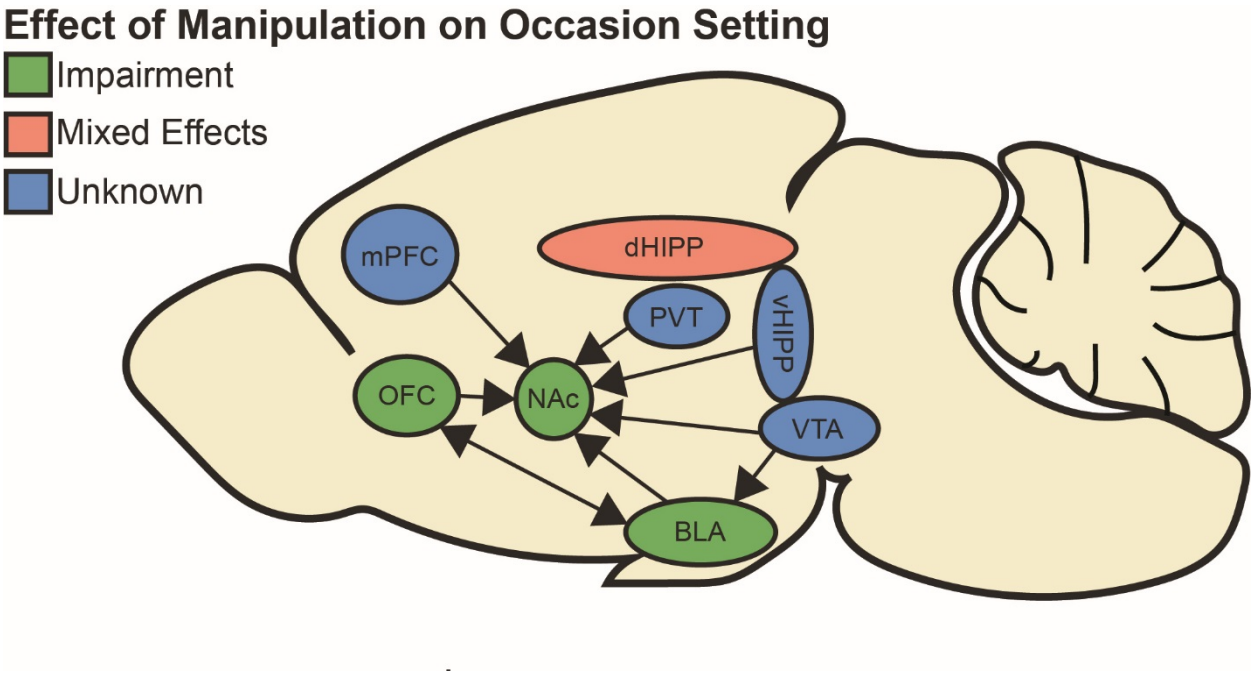

Figure 1.5. Neurobiological substrates of occasion setting. Sagittal section of a rat brain illustrating regions implicated in occasion setting and their relevant circuitry is indicated by the directional arrows. Green shading indicates regions for which evidence implicates activity within this region as being critical for the acquisition or expression of occasion setting. Other regions shaded in blue are implicated in simple Pavlovian conditioning but a role for these regions in occasion setting remains to be demonstrated. Evidence for the involvement of the dHIPP, shaded in red, in occasion setting is mixed and requires further study. BLA basolateral amygdala, dHIPP dorsal hippocampus, mPFC medial prefrontal cortex, NAc nucleus accumbens, OFC orbitofrontal cortex, PVT paraventricular nucleus of the thalamus, vHIPP ventral hippocampus, VTA ventral tegmental area.

Chapter 2

Occasion setters attain incentive motivational value: implications for contextual influences on reward-seeking

INTRODUCTION

Cues paired repeatedly with reward not only acquire a predictive relationship with reward but also attain incentive motivational properties, or incentive salience, that render Pavlovian reward-paired cues attractive and desirable. Indeed, the incentive motivational properties of cues are thought to be a primary trigger for food-seeking as well as relapse in drug-abstinent addicts (Stewart et al. 1984; Robinson and Berridge 1993; Flagel et al. 2009; Robinson et al. 2014b; Milton and Everitt 2010; Kelley 2004). Cue-based exposure therapy is employed clinically, sometimes alongside cognitive behavioral therapies, in an effort to extinguish the incentive motivational properties of drug-paired cues but is generally ineffective in producing lasting reductions in drug-seeking behavior and in preventing relapse (Carter and Tiffany, 1999a, 1999b; Conklin and Tiffany, 2002; Kavanagh et al., 2006; Martin et al., 2013; Mellentin et al., 2017). A possible explanation for these failures comes from viewing drug-seeking as controlled directly by a simple cue-reward relationship, which fails to capture the multitude of situations in which the cue may or may not be motivationally relevant (Bouton, 2002).

These issues are exemplified in the ability of a reward-associated context to renew responding to Pavlovian cues previously associated with reward despite their extinction in a separate, distinct setting. These findings indicate that the contexts in which reward-predictive cues are encountered can modulate the ability of those cues to trigger reward-seeking (Bouton et al., 2006; Chaudhri et al., 2008; Crombag et al., 2008). However, the exact underlying psychological process by which contexts act to

produce renewal of reward-seeking has remained unclear (Bouton, 1988; Holland and Bouton, 1999). For instance, a context may enter into a direct relationship with reward such that when a weakly predictive cue is presented it triggers responding as a result of summing the strength of the context-reward and cue-reward relationships. Others have proposed that contexts act in a more complex way as occasion setters which would instill them with the ability to resolve the ambiguity of reward-predictive cues (Grahame et al., 1990; Holland and Bouton, 1999; Valyear et al., 2017; Fraser and Holland, 2019). To date, however, there has been little investigation into the precise psychological mechanisms underlying context-induced effects on reward-seeking behavior.

To address these issues, we adapted an animal behavioral model of occasion setting to directly probe the underlying psychological mechanisms by which occasion setters, like a context, may act to modulate reward-seeking. In this model, a brief cue informs that in the near future a typical conditioned stimulus will be followed by reward. If either the occasion setting cue or conditioned stimulus are presented in isolation, they are nonreinforced. In essence, this model reduces a context to a brief, phasic, and localizable event in the environment. We investigated if occasion setting may act by magnifying the underlying incentive motivational value of ambiguous reward-predictive cues in tests of conditioned reinforcement. Indeed, occasion setters modulated both the predictive and motivational properties of their conditioned stimuli, but to our surprise we found that occasion setters acquired incentive motivational value in their own right and that the modulatory actions and motivational value of occasion setters were resistant to extinction. Together, these findings have important implications for our understanding of complex cue interactions in triggering reward-seeking and relapse.

MATERIALS AND METHODS

Subjects

Male Long-Evans rats (n=50) weighing 250 g were purchased from ENVIGO (Frederick, MD) and were single-housed in a temperature- and humidity-controlled colony (lights on at 07:00) with enrichment in their cages. Following one week of acclimation to the colony room, rats were food-restricted (95% of free-feeding weight). To acclimate them to the reinforcer used during training, rats were given 24 hour access to 15% sucrose (w/v in tap water) one day before behavioral procedures began. All behavioral training took place during the light cycle. *Sample sizes were determined based on pilot studies.* All procedures were approved by the Animal Care and Use Committee at Johns Hopkins University and are in accordance with the Guidelines for the Use and Care of Animals in Research, 8th Edition.

Apparatus

Behavioral training and testing took place in 10 MedAssociates chambers in individual sound- and light-attenuating cabinets and were controlled by a computer running MedPC IV software. Each chamber was equipped with a recessed port on the front wall of the chamber where liquids could be delivered via tubing attached to a 60 mL syringe placed in a motorized pump outside the cabinet. Port entries and exits were detected by infrared beams located within the recessed port. A white houselight (28 V) was located on the wall opposite the recessed port along with a white noise generator. Outside the behavioral chamber but within the cabinet was a red houselight (28 V) that provided background illumination during each behavioral session.

Occasion Setting

Rats were initially trained to drink reward freely from the port in a single session where the reward pump was randomly activated 80 times for 2 s (~0.08 mL per delivery) with a 60 s variable time schedule. Conditioning began the following day with 30 trials with a 200 s average (100-300 s range) inter-trial interval. We gradually introduced different trial types in this task as pilot studies indicated rats failed to learn if presented with all trial types from the onset of training. Initially, for each trial, the white houselight (occasion setter; OS) was illuminated for 5 s, followed by a 5 s gap with no stimuli, then the white noise generator (conditioned stimulus; CS) was active for 5 s, and, finally, upon CS termination, the reward pump was active for 5 s delivering ~0.2 mL of 15% sucrose reward. There was one session a day with each session lasting approximately 2 hours. Following 4 sessions, rats began discrimination training where 12 trials were reinforced as before, but the remaining 18 trials were nonreinforced presentations of the CS alone. After 6 more sessions, rats proceeded to the full occasion setting task where 10 trials were reinforced, 10 were nonreinforced presentations of the CS alone, and 10 were nonreinforced presentations of the OS alone, with trial type determined pseudorandomly, such that no trial type occurred more than 4 times in succession. Rats were trained for 8 sessions in the full task prior to either extinction or conditioned reinforcement tests.

The OS was a visual cue and the CS was an auditory cue as this arrangement has been previously found to promote the development of occasion setting and maximize differences in behavior (Holland, 1992). In particular, the use of an auditory cue for the CS encourages food cup approach (Holland, 1977), and given the cue is

only presented for 5 s, this encouraged an easily quantifiable and unbiased metric of conditioned reward-seeking.

Rats in the unpaired condition received an identical number of trial types in all phases with conserved timing of presentation of cues, but reward was delivered according to a separate ITI schedule that matched the rate of reward delivery in the paired condition.

For rats undergoing extinction of the OS, following the eighth day of training in the full occasion setting task, they were first tested in a session under extinction conditions where reward delivery was withheld. The OS was then extinguished across 4 sessions by presenting the OS alone for 30 unrewarded trials per session. The day after the last OS extinction session the rats were tested again in the final occasion setting task also without reward delivery. The following day rats proceeded to conditioned reinforcement testing without rewarded retraining in the occasion setting task.

Conditioned Reinforcement

Each conditioned reinforcement test lasted 40 minutes during which levers on either side of the recessed port were extended or nose pokes were available for responding. Each rat received two tests for different cues on one operant (e.g. CS alone and OS+CS) and then two tests on the other operant (e.g. OS+CS and OS alone), with a test to earn the OS+CS conducted on each. There was only cue available to be earned during each test. The order of testing, the identity (nosepoke vs lever), and the side (left vs right) of the active and inactive operant were counterbalanced and reversed between tests on the same operant. This required rats to acquire a unique operant response during each test. There was at least one day without testing between each

conditioned reinforcement test. In OS alone tests, responses on the active operant produced a 2 s presentation of the houselight OS. In CS alone tests, responses on the active operant produced a 2 s presentation of the white noise CS. In OS+CS tests active responses produced simultaneous 2 s presentation of the houselight OS and the white noise CS; we presented these cues simultaneously as brief cue presentations promote conditioned reinforcement (Taylor and Robbins, 1984; Robinson and Flagel, 2009; Fraser et al., 2016; Saunders et al., 2018) and because we surmised that a time gap in their presentation during free operant responding would make it difficult for the subject to link their responses to serial cue presentation. In each test, responses on the inactive operant were without consequence.

Statistical Analysis

Linear mixed-models were used to assess behavior across training using SPSS 24 (IBM) with session and trial type as repeated measures and group was a between subjects factor. Time in port was normalized by subtracting average time in port during a 10 s period prior to the onset of the first cue during a trial from time during the CS period. Repeated measures ANOVA were used to analyze the impact of extinction of the occasion setter on behavior. For extinction tests, we examined the microstructure of reward port approach by calculating the probability of observing a given rat in the reward port across all trials in 1 s bins, and then averaging these across all rats. Bias scores were calculated by subtracting responding during the CS period on either CS or OS alone trials from OS+CS trials and dividing by the sum of these values (e.g. ([OS+CS]-OS Alone) / ([OS+CS]+OS Alone)) giving a score between 1 and -1, with a value of 1 representing perfect discrimination in responding exclusively on OS+CS trials

relative to either CS alone or OS alone trials. One-way repeated measures ANOVA were used to analyze active to inactive responding, cues earned, and port entries in the conditioned reinforcement tests. There were 2 OS+CS tests for each rat, one on each operant, and we averaged responding across these tests. For conditioned reinforcement, active to inactive responding was analyzed two ways 1) using ANOVA to compare between tests and 2) using Wilcoxon signed-rank tests to assess if active ratios were significantly different than random responding (median value of 0). In cases where data were non-normally distributed, nonparametric tests were used. One rat was excluded from the paired group for the CS alone test due to his responding on all measures being an outlier as detected by Grubbs' test. Post hoc comparisons were conducted when significant main effects and interactions were observed. For all analyses, $\alpha=0.05$.

RESULTS

A novel procedure to observe occasion setting

Rats were trained in an occasion setting task in which a conditioned stimulus (CS) was reinforced only if its presentation had been preceded by the presentation of a separate occasion setter (OS) cue. If either the OS or the CS were presented in isolation they were not reinforced (Fig. 1A). This produced a situation in which rats had to constantly update their expectations of reinforcement based on the events surrounding encounters with the ambiguously predictive CS. To understand how the OS might affect responding produced by the CS we examined food cup activity when the CS was present, or in the corresponding time interval when the CS was withheld in the case of OS alone trials (the 'period of interest', depicted in Fig. 1A). The primary form of

the conditioned response was head jerk-related movements inside the food cup throughout the CS, in agreement with previous reports that in occasion setting the primary conditioned response resembles the form supported by the CS (Holland 1977, 1992). Rats in the paired condition responded maximally on OS+CS trials, i.e., when the OS preceded the CS, and this pattern of responding was identical for those rats who would undergo (n=20) or not undergo (n=20) extinction of the OS (interaction of session x group x trial type $F_{(40,106)}$=1.589; p=0.032; simple effect of trial type within each group p<0.0001; all within group Bonferroni post hoc comparisons between OS+CS versus CS alone and OS alone for sessions 11-18 p<0.001 for paired groups; all Bonferroni post hoc comparisons between paired groups for each session p>0.9; Fig. 1B). In contrast, the unpaired group (n=10) did not develop a noticeable degree of conditioned responding during the CS period (no simple effect of trial type within the unpaired group across sessions). Thus, this procedure, with a relatively large number of trials per session, and equal presentations of all trial types in a session, produces behavior akin to occasion setting with minimal training.

Occasion setters have incentive motivational value

We then assessed the motivational value of the OS, CS, and the combination of the OS+CS in a series of conditioned reinforcement tests for a subset of paired rats (n=20) and rats who received unpaired training (n=10). In this test, rats are asked to learn a novel response to earn presentation of one of the following stimuli in the absence of food reward: the OS alone, the CS alone, or a combination OS+CS stimulus. We found that paired rats earned the combination OS+CS stimulus and the OS alone more than unpaired rats (Main effect of cue $F_{(1.856, 51.03)}$=3.958, p=0.0289,

main effect of group $F_{(1,28)}$=8.095, p=0.0082, interaction $F_{(2,55)}$=3.28, p=0.0451; post hocs p<0.05; Fig. 2A). There was no effect of cue within unpaired rats suggesting that there was no contribution of differences in the degree of sensory reinforcement among tests to the pattern of responding we observed in paired rats (Meyer et al. 2014). Paired rats earned the combination of the OS+CS more than CS alone (p=0.0006), but also earned more OS alone presentations than CS alone (p=0.0403) while the number of cues earned did not differ between OS+CS and OS alone tests suggesting these cues had similar conditioned reinforcing value (p=0.285). This pattern was similar for the difference between active and inactive responses (planned comparisons in paired group OS+CS vs CS alone p=0.0089; OS alone vs CS alone p=0.0443; OS+CS vs OS alone p=0.4085; Fig. 2B). In addition, only in tests where rats could earn the OS+CS or OS alone did paired rats discriminate and respond significantly more on the active over inactive lever than expected by chance (Wilcoxon tests p<0.01). To better understand potential differences in the representations evoked by earning the OS+CS in combination and the OS alone we examined port entries made during the brief 2 s cue for each cue type. Although port entries during the 2 s cues were predictably low, paired rats made more port entries during each cue presentation when the cue they earned was the CS alone or the combination of OS+CS than for the OS alone (interaction of cue and group $F_{(2,83)}$=3.991, p=0.0221; post hoc comparisons p<0.001; Fig. 2C). This suggests that earning the OS alone in these tests did not evoke immediate reward-seeking behavior, but the addition of the OS to the CS increased both motivation to earn the CS and spurred reward-seeking. In contrast, rats did not work to earn the CS alone suggesting that in this preparation a reward-adjacent and ambiguous CS does not

support conditioned reinforcement. Collectively, this pattern of results indicates that an occasion setter, typically thought to be a cue that modulates the predictive significance of a cue-reward relationship, can increase the motivational value of an otherwise undesirable CS, as well as develop incentive motivational properties in its own right. Extinction of an occasion setter does not alter its ability to resolve predictive information of its conditioned stimulus

Behavior in the occasion setting task and the subsequent conditioned reinforcement tests could be explained by either of two hypotheses: 1) animals use the visual stimulus as an occasion setter to modulate the significance of the auditory CS and/or 2) the occasion setter and conditioned stimulus each are relatively weakly associated with reward and rats sum these two strengths to increase reward-seeking during combined OS+CS presentation, compared to OS or CS alone. The latter could explain why paired rats spent more time in the food cup following the OS alone than unpaired rats (Fig. 1B), and why paired rats work to earn just the occasion setter (Fig. 2), as the occasion setter itself could have become weakly associated with reward. To directly assess these possibilities, a separate group of rats (n=20; to be extinction group from Fig. 1B) were tested in the occasion setting procedure under extinction conditions where reward was withheld to examine the microstructure of their behavior across trial types. By quantifying the probability of being in the reward port on a second by second basis across the serial presentation of the OS and CS, it appeared that the OS alone evoked a small increase in the chance a rat would enter the reward port prior to CS onset (Fig. 3A). We then extinguished responding to the OS alone in a series of 4 sessions consisting only of OS presentations resulting in percent time in port on the final

day of OS extinction being 3.023 ± 0.9 % SEM. After this, we asked if this extinction of any direct links between the OS and reward would reduce its ability to serve as an occasion setter in a subsequent extinction test with all trial types. The effect of this manipulation was apparent in the microstructure of behavior across all three trial types, with rats no longer exhibiting any increase in reward port approach to the OS alone following OS extinction, yet still using the OS to increase their reward-seeking on trials where both the OS and CS were presented (Fig. 3B). Analyzing time in port during the CS period also revealed that, while responding in the second extinction test, after OS extinction, was lower overall (main effect of test $F_{(1,19)}$=113.4, p<0.0001), extinction of the OS did not produce a deficit in the ability of rats to use the OS as an occasion setter, as responding was still highest on the OS+CS trials (main effect of trial type $F_{(1,19)}$=87.3, p<0.0001; test x trial type interaction $F_{(2,38)}$=4.122, p=0.0240; post hoc comparisons between OS+CS and CS alone and OS alone post OS extinction, all p<0.01; Fig. 3C).

The preservation of occasion setting abilities of the OS after extinction was also readily apparent when looking at bias scores which are resistant to changes in the total amount of conditioned approach (see Methods). When analyzing their behavior during the CS period of interest, rats were better at discriminating between OS+CS versus OS alone trials than OS+CS versus CS alone trials (main effect of discrimination $F_{(1,19)}$=51.42, p<0.001; Fig. 3D). An interaction between extinction and discrimination ($F_{(1,19)}$=8.608, p=0.0085) revealed that after OS extinction rats responded even less during OS alone trials than before OS extinction (p=0.0169) but discrimination between CS alone and reinforced trials was unaffected by OS extinction (p>0.9999). These data

also confirm that the behavior observed during conditioning meet an important criterion for occasion setting, and is not simple summation of responding between the partially reinforced OS and CS. Thus, in the absence of possible direct predictive associations with reward, the OS cue still acts to set the occasion for reward-seeking.

The incentive motivational properties of an occasion setting cue are extinction resistant

We next asked whether direct extinction of the OS would affect the conditioned reinforcing properties of the OS by using the same conditioned reinforcement tests as before, immediately following the second extinction test. Rats who underwent OS extinction did not work for the CS alone but did work to earn combined presentations of the OS+CS, replicating our original finding (Friedman statistic for cue effect = 15.1, $p=0.0005$; post hoc $p=0.0008$; Fig. 4A). Critically, OS extinction did not alter the willingness of rats to earn the OS alone and rats earned significantly more presentations of the OS alone compared to the CS alone test ($p=0.008$; Fig. 4A). This pattern of responding was similarly reflected in the magnitude of active-inactive responses with rats working more on the active lever to earn the OS+CS and OS alone but not for the CS alone (Friedman statistic for cue effect = 6.3, $p=0.0429$; Wilcoxon tests for OS+CS and OS alone $p<0.05$; Fig. 4B). Interestingly, despite it being more than 2 weeks since these rats had received reward, they still made more port entries when they earned the combination of the OS+CS than the OS alone and CS alone (Friedman statistic for cue effect = 13.59, $p=0.001$; post hoc $p<0.05$; Fig. 4C). Taken together, these findings indicate that the incentive motivational value of an OS, and the ability of that OS to enhance the incentive motivational value of its CS, are both extinction resistant.

DISCUSSION

Cues repeatedly paired with reward become predictors of reward availability, but may also acquire incentive motivational properties that can render these cues desirable on their own and endow them with the ability to spur and energize action (Bindra, 1974, 1978; Stewart et al., 1984) . While much is known about the predictive and incentive properties of cues that have a deterministic, absolute relationship with reward availability (Flagel et al., 2009; Meyer et al., 2014; Ahrens et al., 2016), considerably less is known about ambiguous cues and the factors that regulate their predictive and motivational value. We assessed whether a special class of cues that regulate the strength of an ambiguous cue-reward relationship, called occasion setters (Holland, 1992; Meyer and Bucci, 2016a; Shobe et al., 2017; Fraser and Holland, 2019), could engender their own incentive motivational properties. We find that while cues trained as occasion setters do not obligatorily elicit reward seeking on their own, they acquire incentive salience and can act to enhance both the predictive and motivational value of a conditioned stimulus.

That occasion setters support conditioned reinforcement may suggest that this result is the consequence of second-order conditioning to the occasion setting cue (Rizley and Rescorla, 1972; Gewirtz and Davis, 2000), but a number of distinctions rule out that second-order conditioning could be responsible. First, second-order conditioning is most frequently observed when the introduction of the second-order cue follows the formation of a strong associative pairing of the first-order cue with reward, whereas training here proceeded from the outset with serial pairings of the cues. Second, the presence of reward generally discourages second-order conditioning,

requiring the introduction of the second-order cue in extinction. In our preparation the serial presentation of cues was always followed by reward, but either cue in isolation was never reinforced, making it unlikely that the motivational and predictive value of the conditioned stimulus is resulting in new learning about the occasion setting cue. Moreover, what is learned about the conditioned stimulus should be equivalent for the occasion setter if second-order conditioning is occurring, but we observed conditioned reinforcement for the occasion setter and not the conditioned stimulus. Third, we rarely observed behavior in the food cup during the occasion setter suggesting that the representations evoked by the occasion setter were distinct from those evoked by the conditioned stimulus. Finally, extinction of the occasion setter did not prevent conditioned reinforcement, whereas extinction of a first-order conditioned stimulus prevents the observation of conditioned reinforcement (Lindgren et al., 2003; Holland, 2016). Given second-order stimuli directly inherit their predictive and motivational properties from first-order stimuli, their conditioned reinforcing properties, if any, should also be sensitive to extinction (Sharpe et al., 2017a). While occasion setting and second-order conditioning may appear procedurally similar, there is little indication that second-order conditioning can explain the conditioned reinforcing properties of an occasion setting cue and the ability of this cue to enhance conditioned approach to an ambiguous conditioned stimulus. This suggests that the incentive and predictive properties of the occasion setting cue are not readily explained by existing associative models (Fraser and Holland, 2019).

A common model to assess the modulation of reward-seeking is the use of physical contexts to denote situations where cues will or will not lead to various

outcomes like food reward, aversive footshock, or drugs of abuse (Bouton and Bolles, 1979; Holland and Bouton, 1999; Bouton, 2002; Chaudhri et al., 2008; Crombag et al., 2008). However, it has remained difficult to isolate and mechanistically understand the underlying psychological processes that allow contexts to facilitate reward-seeking as contexts are multidimensional and have long-lasting temporal effects. To overcome this, we empirically assessed one of the proposed mechanisms of physical contexts, occasion setting, by substituting a brief and phasic event for a context. This overcame obstacles associated with using physical contexts and in particular this allowed us to extinguish behavior resulting from the occasion setter, which has been attempted but as contexts alone fail to evoke obvious observable behavior it has been unclear if context extinction occurs (Remedios et al., 2014). Surprisingly, extinction of an occasion setter did not impair its ability to resolve ambiguity about reward-paired cues, nor did extinction of the occasion setter affect its motivational value. Because we found that an occasion setter acquired incentive motivational value, as well as serving to disambiguate both the incentive motivational and predictive properties of conditioned stimuli, we suggest by extension that physical contexts may act in these ways. Taken together, occasion setting may be an essential and enduring process contributing to relapse as contexts, physiological states, and discrete cues can each function as occasion setters to generate states of motivation preceding encounters with cues directly associated with drug use that may ultimately overcome goal-directed attempts to maintain abstinence.

Our data suggest that occasion setters meet at least one of these criteria for an incentive stimulus, as the occasion setter on its own was able to reinforce the

acquisition of novel responses to earn its brief presentation. Incentive stimuli are learned cues that are able to evoke motivational and emotional states (Bolles, 1972; Bindra, 1978; Stewart et al., 1984; Toates, 1998). These stimuli can elicit conditioned approach upon their presentation, reinforce behavior in the absence of reward, and spur action (Berridge, 2001; Cardinal et al., 2002). In our procedure, we rarely if ever observed conditioned approach to the occasion setter, a localizable houselight, and it remains to be demonstrated if an occasion setter can act to invigorate reward-seeking actions in tests of Pavlovian-to-instrumental transfer. It was also rare to observe any approach to the food cup during the occasion setter's presentation, so in the absence of any overt behavioral response the occasion setter still became imbued with incentive motivational properties and was later able to support conditioned reinforcement. However, it is evident that the occasion setter regulated both the predictive and incentive motivational properties of reward-paired cues as evidenced by enhancing conditioned approach to the food cup during the conditioned stimulus and enhancing the conditioned stimulus's otherwise minimal or nonexistent conditioned reinforcing value. Despite both the occasion setter and the conditioned stimulus having an equal overall probability of reward, we only observed conditioned reinforcement for the occasion setter. This is counter to observations that uncertainty amplifies the incentive motivational value of Pavlovian cues (Anselme et al., 2013; Robinson et al., 2014a; Zack et al., 2014), but perhaps the hierarchical nature of occasion setting focuses the motivational enhancing aspects of uncertainty to the resolving, occasion setting cue. Together, these data suggest a dissociation between predictive and incentive motivational properties of occasion setters, in that an occasion setter can produce a

state of incentive motivation upon its presentation, but on its own it does not act as a predictor of reward to trigger reward-seeking. Instead, we argue that this occasion setter-evoked motivational state makes reward-associated cues desirable targets of motivation thereby facilitating reward-seeking for food, and potentially other rewards such as drugs of abuse.

Given that occasion setters support conditioned reinforcement, it is likely mesolimbic dopaminergic projections from the midbrain to the nucleus accumbens, which have been proposed to mediate both incentive motivation and reward prediction error, are involved in this behavioral process (Taylor and Robbins, 1984; Berridge, 2012; Saddoris et al., 2015; Saunders et al., 2018; Keiflin et al., 2019). Glutamatergic input to the nucleus accumbens from the basolateral amygdala may also be essential for the occasion setting studied here (Holland and Gallagher, 1999; Everitt et al., 2003; Wassum and Izquierdo, 2015). Lesions of the basolateral amygdala result in a profound deficit in updating the value attributed to a simple conditioned stimulus and adapting responding appropriately, suggesting that in the amygdala's absence the proper encoding, updating, and utilization of state value is lost (Hatfield et al. 1996; Sharpe and Schoenbaum 2016; Morrison and Salzman 2010). The occasion setting procedure utilized here could be especially helpful for facilitating investigations into neural circuitry underlying dynamic regulation of cue-triggered motivation in freely-moving rodents.

That occasion setters have conditioned reinforcing value may suggest that their actions are the result of a model-free representational system (Parkinson et al., 2005; Dayan and Berridge, 2014). Our evidence supports this, as manipulations of association between the occasion setter and reward, via direct extinction, failed to alter motivation

to work for the occasion setter in isolation. A strong test of model-free versus model-based systems is whether a given cue engages a representation of the outcome it predicts (Dayan and Berridge, 2014). In our conditioned reinforcement tests we left the food cup available despite the absence of reward availability in an effort to test this possibility. As rats earned the occasion setter in isolation they did not immediately proceed to check the food cup, suggesting that the occasion setter did not evoke a representation of the outcome that was sufficient to result in reward-seeking actions. However, when the occasion setter was paired with its conditioned stimulus in this test rats frequently made entries into the food cup during this brief cue. These data suggest that the representations evoked solely by an occasion setter in isolation may be model-free, that is, they are reflective of an averaged value that is independent of representation of reward. However, in conjunction with its conditioned stimulus the actions of the occasion setter to enhance both conditioned reinforcement may reflect the actions of a model-based system given that the combination of these cues trigger reward-seeking behaviors. These could suggest differing underlying neural substrates for the motivational states evoked by occasion setters versus their actions to guide appropriate reward-seeking triggered by ambiguous cues. In particular, conditioned reinforcement for Pavlovian cues can be sensitive to manipulations of the value of the outcome that they predict and that this sensitivity is dependent on the orbitofrontal cortex and basolateral amygdala (Burke et al., 2007, 2008). In contrast, conditioned reinforcement for well-trained, perhaps model-free, Pavlovian cues is dependent on dopamine and its actions within the nucleus accumbens core (Taylor and Robbins, 1984; Saunders et al., 2018). This suggests an observation of conditioned

reinforcement for a cue may not represent the sole contribution of either a model-free or model-based system and that there may be different neural substrates for model-based versus model-free conditioned reinforcement. It will be important to determine in future studies if direct manipulations of outcome value differentially affect responding for an occasion setter alone or in combination with its conditioned stimulus.

We have demonstrated that a unique class of cues that modulate the significance of a cue-reward relationship has the potential to generate states of motivation, even in the absence of direct associations with reward, which may energize and ultimately lead to pursuit of rewards like food and drugs. This property of occasion setters may explain the ability of physical contexts to renew food- and drug-seeking and, more broadly, the invigoration of reward-seeking brought about by the myriad cues encountered in the environment. Occasion setting need not solely be for regulating conditioned reward-seeking as other behavioral responses to a given dose of a drug of abuse, such as sensitization and tolerance, can also come under control of occasion setting mechanisms (Anagnostaras et al., 2002; Ramos et al., 2002). Further investigations into both the psychological and neurobiological processes underlying occasion setting may provide new avenues for future clinical interventions with lasting benefits for chronic relapsing disorders, like addiction and PTSD.

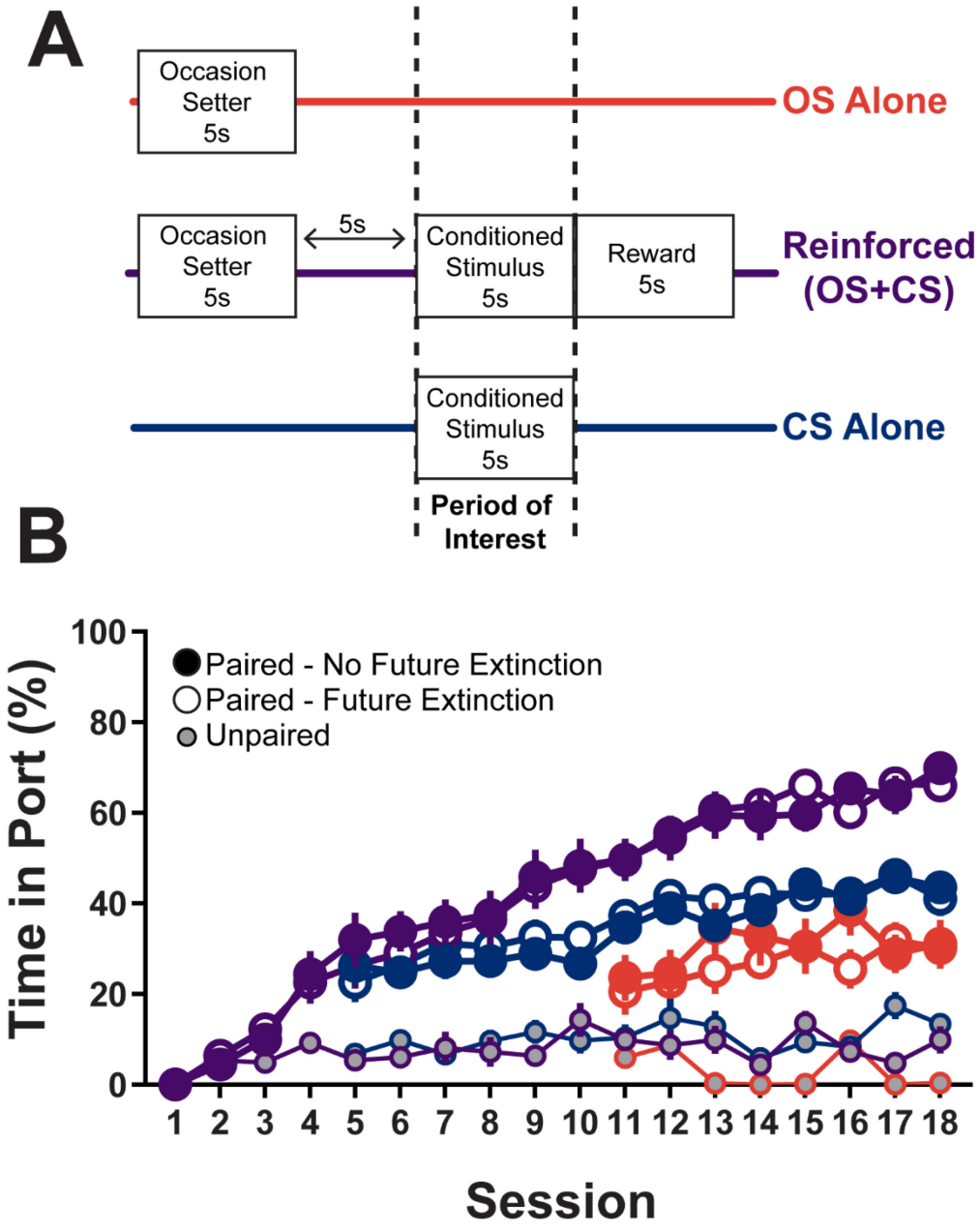

Figure 2.1. A novel model for occasion setting. ***A,*** Schematic of the final stage of the occasion setting task. In each session rats randomly receive one of three trials types, 10 of each per session. Only when the OS and CS are paired (with a 5 s gap between) is the termination of the CS followed by reward delivery. ***B,*** Normalized percent time in port during the CS period across training. Paired rats reinforced as in *A* acquire discriminatory responding in the task, and this is not true for Unpaired rats receiving

truly random delivery of reward (n=10; small gray circles). Paired rats who would (n=20; open symbols) or would not (n=20; solid symbols) undergo OS extinction following training did not differ from each other at any point during training. Data are presented as mean ± SEM. OS, occasion setter. CS, conditioned stimulus. Purple represents reinforced trials, blue represents CS alone trials, and red represents OS alone trials.

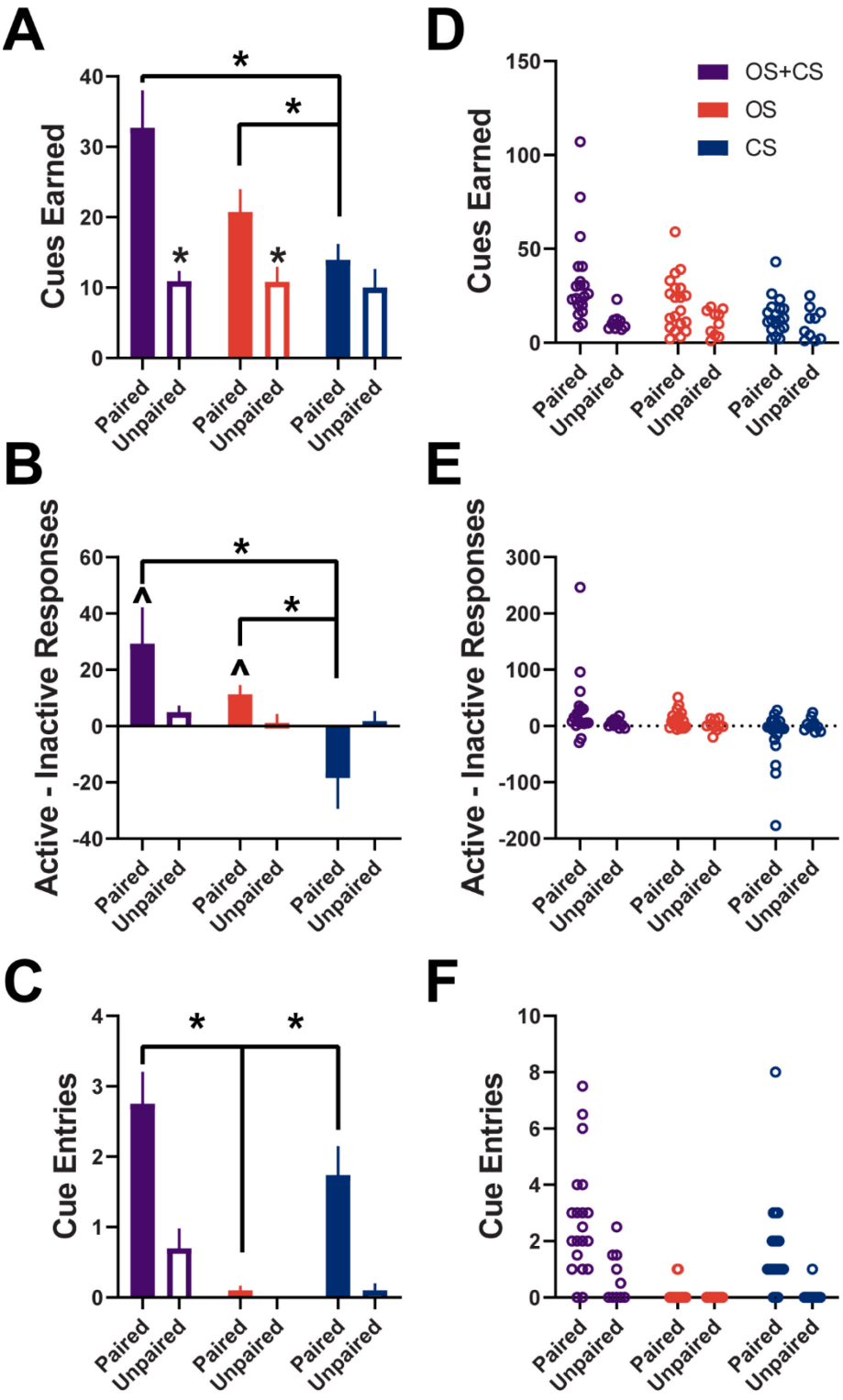

Figure 2.2. Occasion setters have incentive motivational value. ***A,*** Number of cues earned during the conditioned reinforcement test for each cue. ***B,*** Magnitude of

responses on the active minus inactive operant. ***C,*** Number of port entries made while the 2s cue was present following a response on the active operant. ***D-F,*** same as ***A-C*** but the individual data. For all figures bars indicate mean + SEM. Purple reflects the OS+CS test, red reflects the OS alone test, and blue reflects the CS alone test. OS, occasion setter. CS, conditioned stimulus. *p<0.05 for post hoc comparisons, ^p<0.05 for Wilcoxon signed rank test against null value of 0.

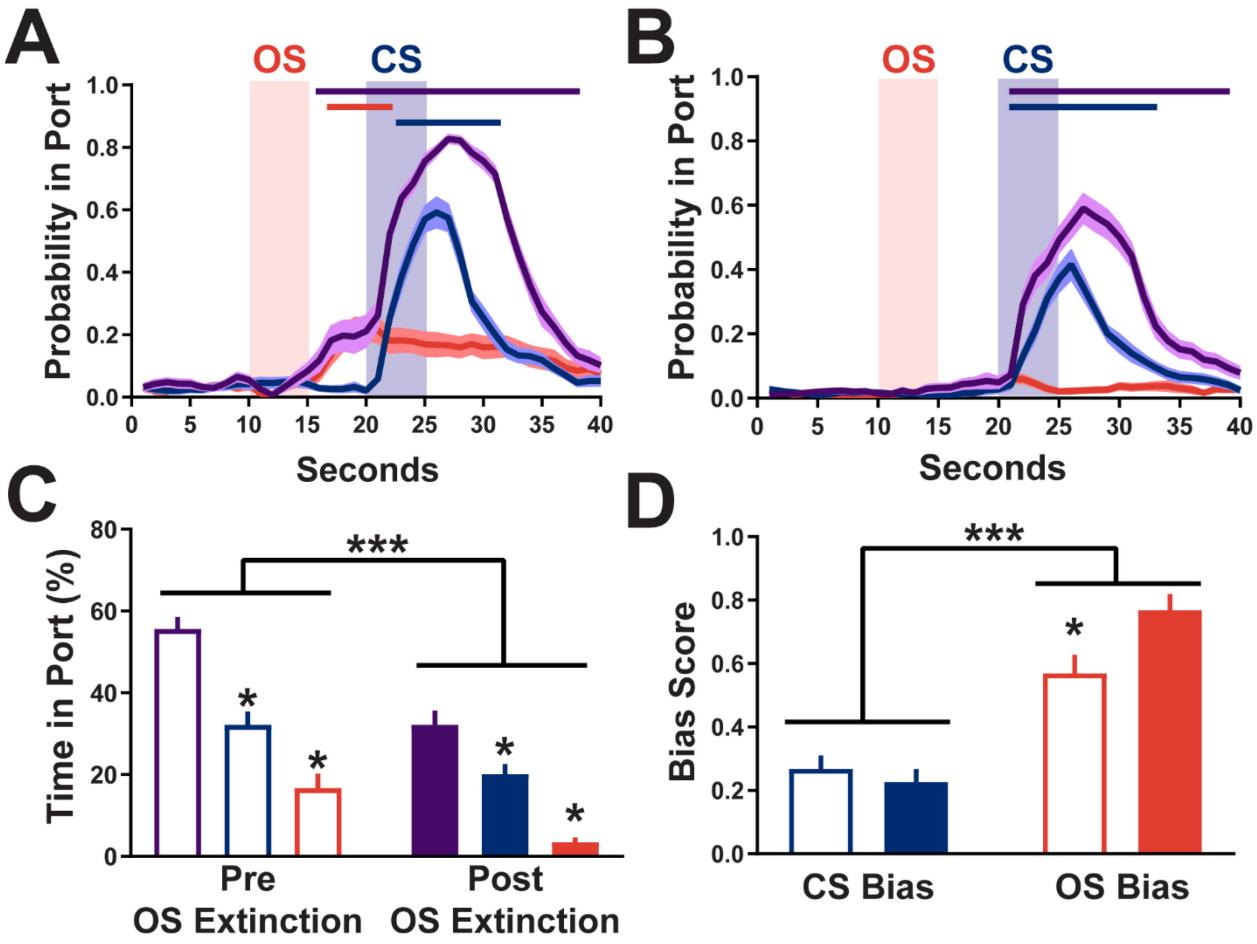

Figure 2.3. Extinction of an occasion setter does not impair its ability to enhance the predictive value of its conditioned stimulus. ***A,*** Probability of observing a rat in the reward port across each trial type during the initial extinction test (Interaction between seconds and trial type $F_{(78,1482)}$=42.69, p<0.0001). Lines indicate periods when OS+CS trials are significantly greater (Bonferroni post hoc p<0.05) than CS alone (purple) and CS alone is significantly greater than OS alone (blue), and OS alone is significantly greater than CS alone (red). ***B,*** Probability of observing a rat in the reward port across each trial type in the second extinction test following OS extinction (Interaction between seconds and trial type $F_{(78,1482)}$=40.52, p<0.0001). Lines indicate periods when OS+CS trials are significantly greater (Bonferroni post hoc p<0.05) than

CS alone (purple) and CS alone is significantly greater than OS alone (blue). ***C,*** Normalized percent time in port during the CS period for each extinction test. Purple represents reinforced trials, blue represents CS alone trials, and red represents OS alone trials. ***D,*** Discrimination scores for each extinction test. Empty bars represent data from the session prior to OS extinction and filled bars data from the session following OS extinction. For all figures symbols indicate mean ± SEM. OS, occasion setter. CS, conditioned stimulus. ***$p<0.05$ for main effects; *$p<0.05$ for post hoc comparisons.

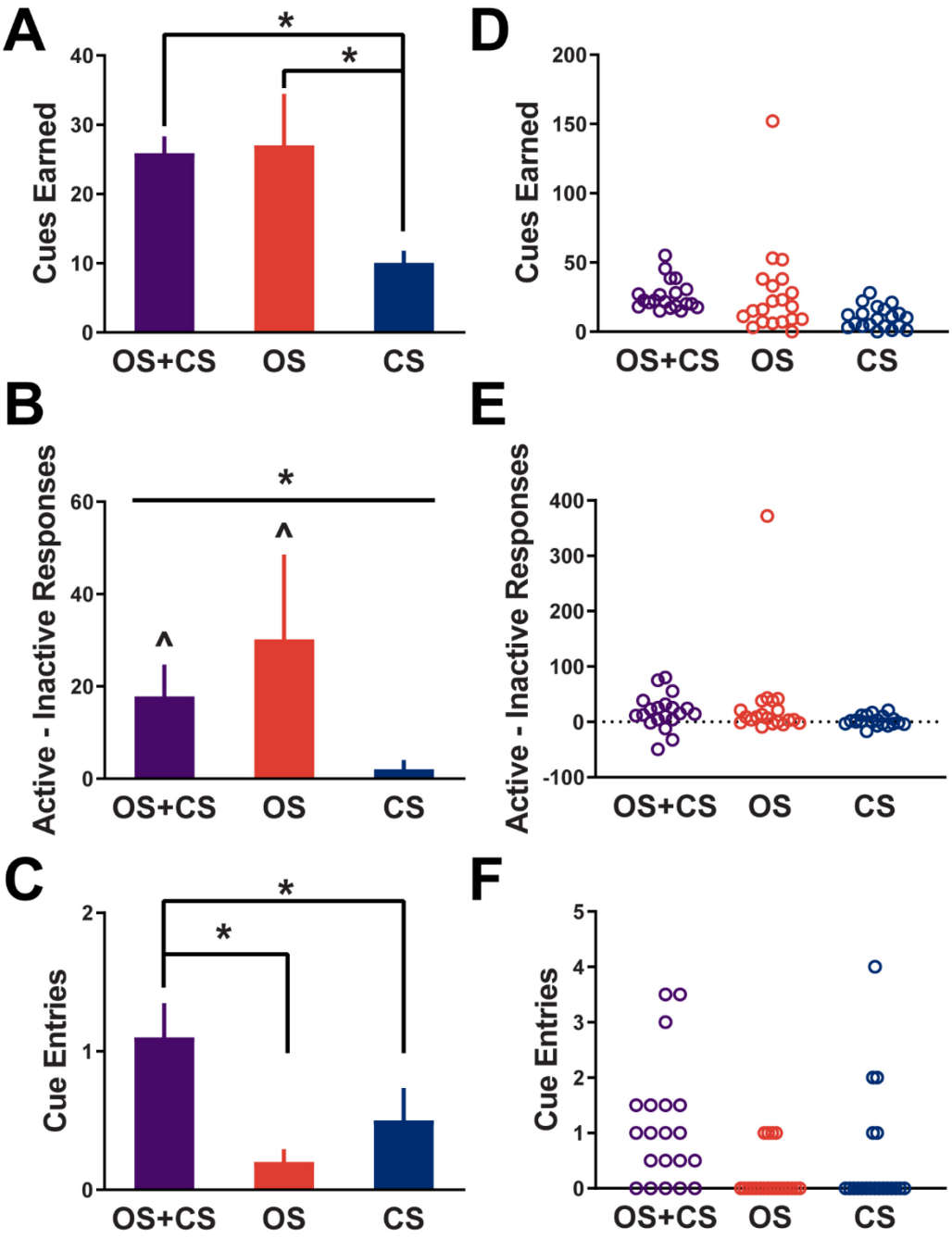

Figure 2.4. The incentive motivational properties of an occasion setter are extinction resistant. ***A,*** Number of cues earned during the conditioned reinforcement test for each cue. ***B,*** Magnitude of responses on the active minus inactive operant. ***C,*** Number of port entries made while the 2s cue was present following a response on the active operant. ***D-F,*** same as ***A-C*** but the individual data. For all figures bars indicate

mean + SEM. OS, occasion setter. CS, conditioned stimulus. *p<0.05 for post hoc comparisons, ^p<0.05 for Wilcoxon signed rank test against null value of 0.

Chapter 3

Dopamine neurons are necessary for the hierarchical organization of cue-triggered reward-seeking

INTRODUCTION

The ability to determine the importance of a given environmental stimulus and in turn generate the appropriate behavioral response is essential for survival. Dopamine neurons in the midbrain are suggested to be critically involved in one aspect of this evaluative process – determining the expected value of the outcome associated with a given stimulus (Rescorla and Wagner, 1972; Schultz et al., 1997; Schultz and Dickinson, 2000; Fiorillo et al., 2003; Pan et al., 2005; Tobler et al., 2005; Cohen et al., 2012; Watabe-Uchida et al., 2017). This foundational proposal is built on the finding that the activity of dopamine neurons at the time of a given environmental stimulus paired with reward is directly in relation to the amount and probability of reward predicted by that stimulus (Fiorillo et al., 2003; Tobler et al., 2005). This expected value encoding of dopamine neuron's is thought to be generated over the course of learning, where numerous pairings of that stimulus with its fixed probability and reward amount generates prediction errors that allow for the refinement of this value signal over numerous trials and, ultimately, the appropriate behavioral response (Fiorillo et al., 2003; Tobler et al., 2005; Hart et al., 2015; Keiflin and Janak, 2015).

Despite this, there exists little evidence that provides a link between alterations in the expected value of a conditioned stimulus and the utilization of such a signal in dopamine neurons to in turn generate behavior. In probabilistic situations where freely-behaving animals learn about numerous distinct stimuli with distinct expected values, conditioned responding commonly appears similar despite marked differences in the

response of dopamine neurons and in turn dopamine release in downstream striatal regions (Hart et al., 2015). As a result, whether such expected value encoding is a feature of dopamine systems that is critical for adaptive behavior remains unclear. Here, we took advantage of the ability to manipulate the activity of dopamine neurons in freely-behaving rats in a temporally precise manner to test their necessity in utilizing an expected value function to generate conditioned responding (Witten et al., 2011). We exploited occasion setting, an approach in which the long-running expected value of a conditioned stimulus is dissociated from its trial-to-trial value as the relation between this stimulus and reward is predicated on the prior presence of a separate, distinct stimulus (Holland, 1992; Fraser and Holland, 2019; Fraser and Janak, 2019). Ultimately, we reveal that dopamine neuron activity is necessary for the rapid utilization of an updated expected value to adjust behavioral responding, yet manipulations at the time of cues with stable expected values fail to alter behavior.

MATERIALS AND METHODS

Subjects

Subjects were 12 experimentally naïve male and female TH-Cre transgenic rats (on a Long-Evans background) and 12 male and female wildtype littermates bred in our laboratory. These rats express the bacterial recombinase Cre under the control of the tyrosine hydroxylase (TH) promoter, the rate-limiting enzyme for the synthesis of dopamine. Rats were single-housed in ventilated cages with *ad libitum* access to food and water in a temperature- and humidity-controlled room and maintained on 12:12 light/dark cycle (lights on at 07:00). After recovery from surgery, feeding was restricted to maintain weights at ~95% of *ad libitum* feeding weights. All behavioral procedures

took place between 13:00 and 20:00. All procedures were approved by the Animal Care and Use Committee at Johns Hopkins University and followed the recommended guidelines in the Guide for the Care and Use of Laboratory Animals: Eighth Edition, revised in 2011.

Surgery

Rats were anesthetized with isoflurane (5% induction, 1-2.5% maintenance) and standard stereotaxic procedures were used to infuse a Cre-dependent viral vector containing halorhodopsin (AAV5-Ef1a-DIO-eNpHR3.0-eYFP; titer 3.1 x 10^{12} viral particles/mL; University of North Carolina) for TH-Cre animals (n=12) or a control GFP virus (AAV5-hsyn-EGFP; titer 1 x 10^{13} viral particles/mL; University of North Carolina) for wildtype littermates (n=12) bilaterally into the VTA (AP: -5.8 ML: ±0.7 DV: -8.0 for males; DV -7.7 for females; 700 nL per site). A volume of 700 nL of virus was infused per site at a rate of 100 nL/min through a 31-G, gas-tight Hamilton syringe controlled by a Micro4 Ultra Microsyringe Pump 3 (World Precision Instruments). The needle was left in place for 10 minutes following the infusion to allow for diffusion of the virus from the injection site. In the same surgery, rats were bilaterally implanted with custom made optic fiber implants (300 μm glass diameter) targeted to the VTA (15° angle; AP: -5.8 ML: ±2.61 DV: -7.55 for males; DV -7.1 for females). Implants were secured to the skull with 4-5 screws placed in the skull and dental acrylic. After surgery, rats received injections of cefazolin (70 mg/kg, subcutaneous) to prevent infection and carprofen (5 mg/kg, subcutaneous) to relieve pain. Rats were allowed to recover for 10 days prior to the beginning of behavioral procedures, with at least 4 weeks passing before any optogenetic manipulations were performed.

Occasion Setting Task

Training in the occasion setting task was identical to procedures in (Fraser and Janak, 2019). In brief, in a single session rats there were 3 trial types, 10 of each trial, with a 3.3 minute average intertrial interval. On reinforced trials, the illumination of a white houselight for 5 s, a 5 s gap, and the presentation of white noise for 5 s (OS→CS trials) was followed by the activation of a reward pump containing 15% sucrose (w/v) for 5 s (~0.18 mL of sucrose delivered). The other trials consisted of either the sole illumination of the houselight for 5s (OS Alone trials) or the presentation of the white noise for 5s (CS Alone trials). Behavioral equipment and responses were controlled by a computer running MedPC IV software (MedAssociates). Rats had 18 total days of training before being habituated to being connected via a ceramic mating sleeve bilaterally to 200 μm core patch cords (Doric), which were connected to a fiber optic rotary join (Doric), connected to a separate 200 μm patch cord that interfaced with a 532 nm DPSS laser (Opto-Engine LLC). Laser delivery was controlled by transistor-transistor logic pulses from MedPC SmartCTRL cards that interfaced with a Master9 Stimulus Controller (AMPI), which dictated the duration of stimulation. During tests, constant laser light (15-20 mW) was delivered bilaterally for a constant duration of 5.5s, beginning either before a trial, beginning 0.5 s before either the houselight or white noise (or the time in which they would be presented in CS Alone and OS alone trials), beginning 4.75 s into the houselight and terminating 0.25 s into the white noise, or for one test in 3 1.5 s second pulses separated by 0.5 s intervals during the houselight. The order of tests were randomly determined with 1-2 days of performance in the task without light delivery, but still tethered to patch cables, to allow for the mitigation of any

possible observed carry-over effects at test. All tests were reinforced. The primary behavioral event of interest was the time rats spent in the port during the white noise stimulus.

Fictive Occasion Setting

After the tests described above, the presentation of the houselight was replaced by bilateral delivery of 532 nm light (15-20 mW) to attempt to create an optogenetic occasion setting stimulus. There were still 3 trial types, 10 of each type, with a 3.3 minute average intertrial interval. In these tests, 5 s delivery of light into the VTA was followed by a 5 s gap, the 5 s white noise but no reward was delivered for 10 trials. For 10 trials, the white noise was presented absent prior laser delivery and was followed by 15% sucrose reward. On the remaining 10 trials, light was delivered to the VTA for 5 s but no other events occurred. Rats were tested in this manner for 12 sessions.

Probabilistic Conditioning

Following a one week break after occasion setting tests, rats were trained in a probabilistic conditioning task. In this task, a 5 s 2900 Hz tone was followed by 15% sucrose reward on 50% of trials and a 5 s 4500 Hz tone was followed by 15% sucrose reward on 50% of trials. There were 40 trials total per session, with 20 presentations of each tone, separated by an average intertrial interval of 2.2 minutes. For each session in this task, 532 nm light (15-20 mW, constant) was delivered for 5.5 s bilaterally into the VTA beginning 0.5 seconds before one of the two tones (light-paired tone identity was counterbalanced). Rats were tested in this task for 6 sessions, with light delivery occurring in each session.

After these tests rats were trained to associate the flashing (5s, 0.5 Hz) of two stimulus lights to the right and left of the reward port, or on separate trials the darkening of the illuminated chamber for 5s with reward delivery. There were 40 trials each session, 20 for each cue, with an average intertrial interval of 2.2 minutes. Each cue, either the flashing lights or termination of chamber illumination was followed by 15% sucrose on 50% of trials. Constant, bilateral 532 nm light was delivered during the delivery of reward (5.5 s starting immediately at the end of the chosen cue if the rat was already in the port, or when rats first entered the port within 15 s following cue termination) for one of these two light cues (counterbalanced). Rats were tested in this manner for 10 sessions.

Histology

Following the conclusion of experiments, rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with 4% paraformaldehyde. Brains were post-fixed for 24 hours in 4% paraformaldehyde, cryoprotected in 25% sucrose in 0.1 M NaPB, and then sectioned on a freezing cryostat at -20° C in 50 um sections. Sections were processed for detection of GFP and TH using fluorescent immunohistochemistry. Sections were first washed in 0.1 M PBS containing 0.2% Triton-X (PBST) for 20 minutes and blocked for 30 minutes in 0.1M PBST containing 10% normal donkey serum. Primary antibody incubation (mouse anti-GFP, 1:1500, Invitrogen A11120, Lot#2180270; rabbit anti-TH, 1:500, Fisher AB152M1, Lot # 3510772) occurred overnight at 4C. The following day sections were washed in PBST for 15 minutes, blocked in PBS containing 2% normal donkey serum for 10 minutes, then secondary antibody incubation (Alexafluor 488 donkey anti-mouse, 1:200,

Invitrogen A21202, Lot# 2229195; Alexafluor 594 donkey anti-rabbit, 1:200, Invitrogen A21207, Lot# 2145022) occurred at room temperature for 2 hours. Following 15 minutes of washing in PBS sections were mounted onto Fisher SuperFrost Plus slides and coverslipped with Vectashield mounting medium containing DAPI. Brain sections were imaged with a Zeiss Axio 2 microscope for the reconstruction of final placements of optic fibers and virus expression with the VTA.

Statistics and Analysis

The primary behavioral metrics of interest for all tests was the time spent in port (indicated here as a percentage of the 5 s stimulus) during the white noise in the occasion setting task or the tones and lights in the probabilistic conditioning tasks. We normalized behavioral responding by subtracting average time in port measured in a period 10 s prior to the presentation of any stimuli. For the occasion setting task we also computed differences between responding on trials in which the white noise would be reinforced (OS→CS trials) and trials in which the white noise (CS Alone) was presented alone or the houselight presented alone (OS Alone) to allow for an assessment of each individual rat's representative ability to discriminate amongst the trial types. Repeated measure ANOVAs were used to compare time in port across trial types or difference scores between virus groups for each test separately. When applicable, post hoc comparisons were made with Sidak's correction. Statistical comparisons and graphics were made with Prism 9 (GraphPad). For all statistical tests $\alpha=0.05$.

RESULTS

VTA Dopamine Neuron Activity is Necessary for Occasion Setting

We trained male and female TH-cre rats and their wildtype littermates (n=12 per group; 6 males and 6 females per group) in an occasion setting task (Figure 1). In this task, rats must discriminate when an auditory conditioned stimulus (CS) is predictive of reward by exploiting the prior and non-overlapping presentation of a houselight occasion setter (OS). There were no differences in the behavior of TH-cre rats with halorhodopsin (Halo) or GFP expressed in the ventral tegmental area during training. At test we delivered 5.5s of 15-20 mW 532 nm light bilaterally into the VTA. Light delivery starting 10s before any trial was without effect on behavior (Figure 2A). Both rats with GFP and Halo exhibited higher reward-seeking on OS→CS trials than CS alone (Figure 2A; effect of trial type $F_{(1,34)}$=55.68, p<0.001; effect of group $F_{(1,22)}$=0.06043, p=0.8081; Halo p=0.0004; GFP p=0.0002) and OS alone trials (Halo p<0.0001; GFP p<0.0001), and the average discrimination for each group of rats in discerning when the CS would be rewarded was similar between groups (Figure 2B; effect of group $F_{(1,22)}$=3.760, p=0.5063; effect of difference $F_{(1,22)}$=3.299, p=0.0830). We next tested whether VTA dopamine neuron activity during the OS would be necessary for this cue to modulate reward-seeking. Light delivery during the OS disrupted reward-seeking for rats with Halo (Figure 2C; effect of group $F_{(1,22)}$=7.1, p=0.0142; effect of trial type $F_{(2,44)}$=33.02, p<0.0001; interaction $F_{(2,44)}$=5.457, p=0.0076), reducing their reward-seeking on OS→CS trials (p=0.0151) and OS Alone trials (p=0.0065) relative to GFP controls. In addition, rats with Halo no longer exhibited a significant elevation in reward-seeking on OS→CS trials relative to CS alone trials (p=0.0651). This reduction in reward-seeking resulted in a significant reduction in the ability of rats with Halo to discriminate between trials in which the CS was versus was not reinforced relative to GFP controls (Figure

2D; interaction $F_{(1,22)}$=5.080, p=0.0345; CS difference p=0.0025; OS difference p=0.9465). In contrast, when light delivery occurred during every CS, or the period in which it would be expected on OS Alone trials, reward-seeking for Halo rats was impaired (Figure 2E; effect of group $F_{(1,22)}$=7.375, p=0.0126; effect of trial type $F_{(1,37)}$=40.54, p<0.0001; interaction $F_{(2,44)}$=10.58, p=0.0002) although each group of rats still exhibited the expected behavioral pattern with no differences between GFP and Halo rats. Despite this, when examining the ability of rats to discriminate VTA dopamine neuron inhibition reduced reward-seeking selectively for rats with Halo in VTA dopamine neurons in telling apart trials in which the CS was reinforced versus not (Figure 2F; interaction $F_{(1,22)}$=16.25, p=0.0006; CS difference p=0.0129; OS difference p=0.0813).

We further asked what aspects of neural activity in VTA dopamine neurons underly the dramatic impairment produced by inhibition during the OS, and the reduction in discrimination with inhibition during the CS. It has been demonstrated that pulsed inhibition of VTA dopamine neurons for many trials can result in conditioned inhibition, a phenomenon related to occasion setting (Chang et al., 2018). We asked if delivery of 3, 1.5 s pulses of 532 nm light during the OS would recapitulate our behavioral effects. During this test there were no detectable differences between Halo and GFP rats in either reward-seeking (Figure 2G; effect of group $F_{(1,22)}$=3.414, p=0.0.781; effect of trial type $F_{(2,44)}$=51.27, p<0.0001; interaction $F_{(2,44)}$=4.032, p=0.0247; all p between GFP and Halo p>0.05, within group all p<0.001 relative to OS→CS trials) or discriminations (Figure 2H; effect of group $F_{(1,22)}$=0.3002, p=0.5893; effect of discrimination $F_{(1,22)}$=1.797, p=0.1937). VTA dopamine neuron activity has been implicated in working memory and performance in this task requires the retention

of the prior presentation of the OS to organize appropriate responding to the CS. To test a potential explanation of working memory, we delivered light for the final 250ms of the OS period through the first 250ms of the CS period (Cools and D'Esposito, 2011; Arnsten et al., 2012; Choi et al., 2020). Although we observed an overall effect of this manipulation on behavior for Halo rats relative to GFP control animals, there were no statistically significant differences between GFP and Halo rats for reward-seeking on any trial type, nor for did this affect within-group differences in total reward-seeking (Figure 2I; effect of group $F_{(1,22)}$=7.612, p=0.0115; effect of trial type $F_{(1,35)}$=57.41, p<0.0001; interaction $F_{(2,44)}$=0.7488, p=0.4788; all p between GFP and Halo p>0.05, no differences within group all p<0.001 relative to OS→CS) or discriminations (Figure 2J; effect of group $F_{(1,22)}$=0.01141, p=0.7387; effect of discrimination $F_{(1,22)}$=0.9352, p=0.3440; interaction $F_{(1,22)}$=0.9680, p=0.3359). Finally, we asked if light delivery selectively during reward delivery would alter behavior, given the prominent role of VTA dopamine neural activity at the time of reward in driving learning and updating behavior (Steinberg et al., 2013; Chang et al., 2016, 2017; Keiflin et al., 2019). Even with this manipulation, we observed no effect of light delivery on behavior between GFP and Halo groups on overall reward-seeking (Figure 2K; effect of group $F_{(1,22)}$=4.002, p=0.0579; effect of trial type $F_{(1,33)}$=34.5, p<0.0001; interaction $F_{(2,44)}$=2.598, p=0.0858) or in the ability of individual rats to discern between reinforced and non-reinforced CS presentations (Figure 2L; effect of group $F_{(1,22)}$=0.2549, p=0.6187; effect of discrimination $F_{(1,22)}$=1.327, p=0.2617). Collectively this pattern of effects suggest VTA dopamine neuron activity is necessary for the detection and use of hierarchical cues to flexibly respond to ambiguous reward-paired stimuli.

Dopamine neuron inhibition alone in insufficient for occasion setting

Our lab has demonstrated that under some circumstances, alterations in dopamine neuron activity itself is sufficient to act as a reward and drive Pavlovian learning (Saunders et al., 2018). We wondered if manipulating dopamine neuron activity alone would be sufficient in instructing rats that an upcoming ambiguous conditioned stimulus would not be rewarded, a behavioral pattern termed negative occasion setting (Holland, 1992; Meyer and Bucci, 2016a). We modified the timings of inhibitions in our task such that light delivery completely replaced the prior houselight OS, and now selectively delivered sucrose only when the CS was presented absent any prior OS (Figure 3A). We trained rats in this optogenetic occasion setting task to assess whether those rats with Halo in VTA dopamine neurons could use a brief inhibition in dopamine activity as a hierarchical cue to withhold reward-seeking. If rats with Halo were able to do so, reward-seeking for this group should be higher when the CS is presented alone relative to OS→CS trials, and as GFP rats express no functional virus and there is no real world OS stimulus, these rats should exhibit equal levels of reward-seeking the CS regardless of trial type – treating this cue as a probabilistic CS. We analyzed behavior in this optogenetic occasion setting task after 12 sessions of training. Contrary to our predictions, there was no evidence of rats with Halo in dopamine neurons of the VTA in being able to use a brief inhibition as an occasion setter. Rats in both the GFP and Halo exhibited a similar level of reward-seeking during the white noise CS (Figure 3B; effect of group $F_{(1,22)}=0.2407$, $p=0.6286$; effect of trial type $F_{(1,31)}=211.7$, $p<0.0001$; interaction $F_{(2,44)}=0.0414$, $p=0.9595$) that was similar irrespective of the prior delivery of light into the VTA for either group (within group comparison between OS→CS and CS alone

p's>0.05). We analyzed the discrimination amongst trials for each as before which confirmed the lack of effect of VTA dopamine neuron inhibition in acting as a negative occasion setter (Figure 3C; effect of group $F_{(1,22)}$=0.05737, p=0.8129; effect of discrimination $F_{(1,22)}$=404.2, p<0.0001). These findings support the notion that VTA dopamine neuron activity is necessary during a real-world hierarchical stimulus for that stimulus to act as an occasion setter.

Dopamine neuron inhibition is without effect on responding to probabilistic cues

Perhaps the effects of dopaminergic inhibition on occasion setting reflect not the rapid updating of expected value but instead can solely be attributed to altering the long running expected value of stimuli. If this were true, we would expect that VTA dopaminergic inhibition during one of two probabilistic conditioned stimuli, each with a 50% probability of reward delivery, should alter conditioned approach to the laser-paired CS selectively for Halo rats. We trained rats to learn that two distinct 5 s auditory stimuli (a high tone and a low tone) were associated each with the delivery of sucrose reward on 50% of trials. One of these probabilistic cues was paired with 5.5s of 15-20 mW 532 nm light delivery, starting 500 ms prior to its presentation and terminating with the tone. We trained rats in this manner for 6 sessions. Overall, while laser delivery altered reward-seeking, it did so in a manner consistent for both rats with GFP and Halo when we examined time in the food cup during each CS (Figure 4A; effect of group $F_{(1,22)}$=6.539, p=0.0180; effect of session $F_{(3,78)}$=9.652, p<0.001; effect of laser $F_{(1,22)}$=8.962, p=0.0067; no significant interaction of group and laser nor group by laser by session) and when analyzing CS-elicited food cup entries (Figure 4B; effect of group $F_{(1,22)}$=3.344, p=0.0810; effect of session $F_{(5,110)}$=4.313, p=0.0013; effect of laser

$F_{(1,22)}$=9.133, p=0.0063; no significant interaction of group and laser nor group by laser by session). We examined the difference in conditioned behavior between the laser-paired probabilistic CS and the non-laser paired probabilistic CS for each rat to determine if discriminations between these cues were not manifest in total levels of reward-seeking. Despite this, there was no difference between groups in the influence of laser-delivery impairing reward-seeking for both GFP and Halo rats for either the difference for time in the food cup between the two cues (Figure 4C; effect of session $F_{(2,46)}$=5.235, p=0.0079; effect of group $F_{(1,22)}$=2.241, p=0.1486) or when differences in food cup entries were analyzed (Figure 4D; effect of session $F_{(3,77)}$=2.958, p=0.0300; effect of group $F_{(1,22)}$=3.640, p=0.0695).

As a final test, we wondered if the lack of effect of inhibition during the CS itself could result from functional dopamine neuron activity during the time of reward consumption as being sufficient for learning the value of a probabilistic CS. To test this, we then trained rats to learn that two distinct visual stimuli (flashing cue lights or darkening of the operant box) were each associated with a 50% probability of sucrose reward. We delivered 5s of 15-20 mW 532 nm light as rats consumed the sucrose reward following one of these two visual stimuli (identity counterbalanced). We trained rats in this manner for 10 sessions and found that although both groups of rats learned the task (Figure 5A; port time; effect of session $F_{(3,76)}$=23.14, p<0.0001; Figure 5B; port entries; effect of session $F_{(9,198)}$=29.60, p<0.0001) there was a consistent lower level of overall reward seeking during each CS in for Halo rats relative to GFP rats (port time effect of group $F_{(1,22)}$=11.99, p=0.0022; port entries effect of group $F_{(1,22)}$=6.478, p=0.0164). Despite this, there was no significant effect of light delivery paired with

reward consumption selectively following one of the two CSs on behavior (port time effect of laser $F_{(1,22)}$=0.124, p=0.7281; port entries effect of laser $F_{(1,22)}$=0.006, p=0.9383). In agreement, when analyzing the difference in reward-seeking between the two CSs we found no difference between groups (Figure 5C; port time; effect of group $F_{(1,22)}$=0.5110, p=0.4822; Figure 5D; port entries; $F_{(1,22)}$=0.6414, p=0.4318). Together these data suggest that inhibition of VTA dopamine neuron activity is insufficient to alter learning or behavior for probabilistic CSs for whom there is no hierarchical control of its relationship with reward.

DISCUSSION

Here we made use of optogenetic inhibition in combination with transgenic TH-Cre rats to test the involvement of midbrain dopamine neurons in the hierarchical control of cue-triggered reward-seeking. We reveal that VTA dopamine neuron activity during hierarchical cues, namely occasion setters, is necessary for these cues to modulate reward-seeking to ambiguous conditioned stimuli. Inhibition of dopamine neurons alone was insufficient to substitute for an occasion setter, nor was dopamine neuron inhibition able to alter reward-seeking to probabilistic conditioned stimuli. Together, these results argue for an essential contribution of VTA dopamine neuron activity in exploiting hierarchical stimuli in the rapid updating and utilization of the value of reward-associated stimuli to guide reward-seeking.

Dopamine neurons in the midbrain are well-known for their ability to signal prediction errors – the difference between cue-elicited expectations and the outcome that was received (Schultz and Dickinson, 2000; Keiflin and Janak, 2015; Watabe-Uchida et al., 2017). Importantly, the degree of cue-elicited excitation in midbrain

dopamine neurons is sensitive to the probability and magnitude of reward predicted by a given stimulus leading to the notion that cue-elicited responses in VTA dopamine neurons reflect the expected value of a given reward-associated stimulus (Fiorillo et al., 2003; Tobler et al., 2005). Despite this, a link between expected value and cue-elicited behavior has remained unclear as freely-behaving animals exhibit similar levels of conditioned approach for cues of varying expected values (Hart et al., 2015). Exploiting occasion setting, we were able to dissociate long-running expected value from the current value of a conditioned stimulus and in turn observe a behavioral report that rats had an accurate estimation of the likelihood of reward receipt. Interestingly, we find that only in this preparation, where the current value of a conditioned stimulus is dynamically shaped, did VTA dopamine neuron inhibition alter behavior. These findings suggest the contribution of VTA dopamine neuron activity to behavior is not by signaling the long-running expected value of cues, but in utilizing a much more flexible and rapidly generated value signal to guide cue-triggered reward-seeking. This notion is particularly in agreement with the role of VTA dopamine neurons in a number of tasks that underscore a role for these in so-called model-based scenarios, where these neurons are proposed to integrate and have access to more computations than merely averaging value over long timescales (Chang et al., 2017; Sharpe et al., 2017b; Starkweather et al., 2017; Langdon et al., 2018; Gershman and Uchida, 2019; Keiflin et al., 2019).

Occasion setting is a process that captures the control of behavioral responding by contexts (Grahame et al., 1990; Holland, 1992; Holland and Bouton, 1999; Bouton et al., 2006; Fraser and Holland, 2019). Despite decades of evidence that dopamine

neurons and their striatal targets are essential for the contextual-control of reward-seeking it has been difficult to disentangle the contribution of dopamine neuron activity to hierarchical control by contexts or the generation of reward-seeking by cues as traditional context-based approaches are not able to parse these processes (Crombag et al., 2008; Floresco, 2015; Valyear et al., 2020). By reducing a context to a brief and discrete event, an occasion setter, we reveal a critical role for dopamine neuron activity during the presentation of this context-like occasion setting cue for that cue to then gate reward-seeking to an ambiguous conditioned stimulus that will now predict reward. Interestingly, despite a weaker effect of inhibition during the ambiguous conditioned stimulus on behavior, this manipulation also reduced discrimination in rats between to-be reinforced and non-reinforced conditioned stimuli. These results are generally consistent with the notion of a hierarchical control of reward-seeking by VTA dopamine neurons – activity is necessary at the time of a higher-order stimulus to update behavior and is also necessary at the time of the target of this higher-order cue to utilize such updating to guide reward-seeking. These individual processes, encoding the occasion setter versus its utilization during ambiguous conditioned stimuli, may be manifest by different striatal targets – the nucleus accumbens core and shell, respectively (Chaudhri et al., 2010; Floresco, 2015; Valyear et al., 2020). Future investigations should make use of optogenetic inhibition of VTA dopamine terminals in theses striatal subregions to test their potential contributions to the hierarchical control of reward-seeking.

Here we reveal a necessary role for VTA dopamine neuron activity for the hierarchical control of reward-seeking by discrete external stimuli. VTA dopamine neuron activity is also regulated by internal states such as hunger and thirst and their

associated hormones and neuropeptides (Cone et al., 2016; Fortin and Roitman, 2018; Hsu et al., 2020; Konanur et al., 2020). How VTA dopamine neuron and dopamine release, in turn, are affected by occasion setters remains an open question and may reveal similarities and differences between the control of reward-seeking by external versus internal stimuli. Interestingly, internal states are able to serve as occasion setters, and so by extension is likely that similar mechanisms are involved in the regulation of reward seeking by alterations in homeostasis and by external stimuli (Randall et al., 2019). Together, this implicates that the regulation, versus the generation, of conditioned reward-seeking is the norm rather than the exception when it comes to the contribution of VTA dopamine neuron activity in Pavlovian responding. Determining the circuits and computations that underlie this nuanced role of VTA dopamine neurons in the rapid regulation of behavior will be essential to understanding their dysfunction in psychiatric illness and substance abuse disorders.

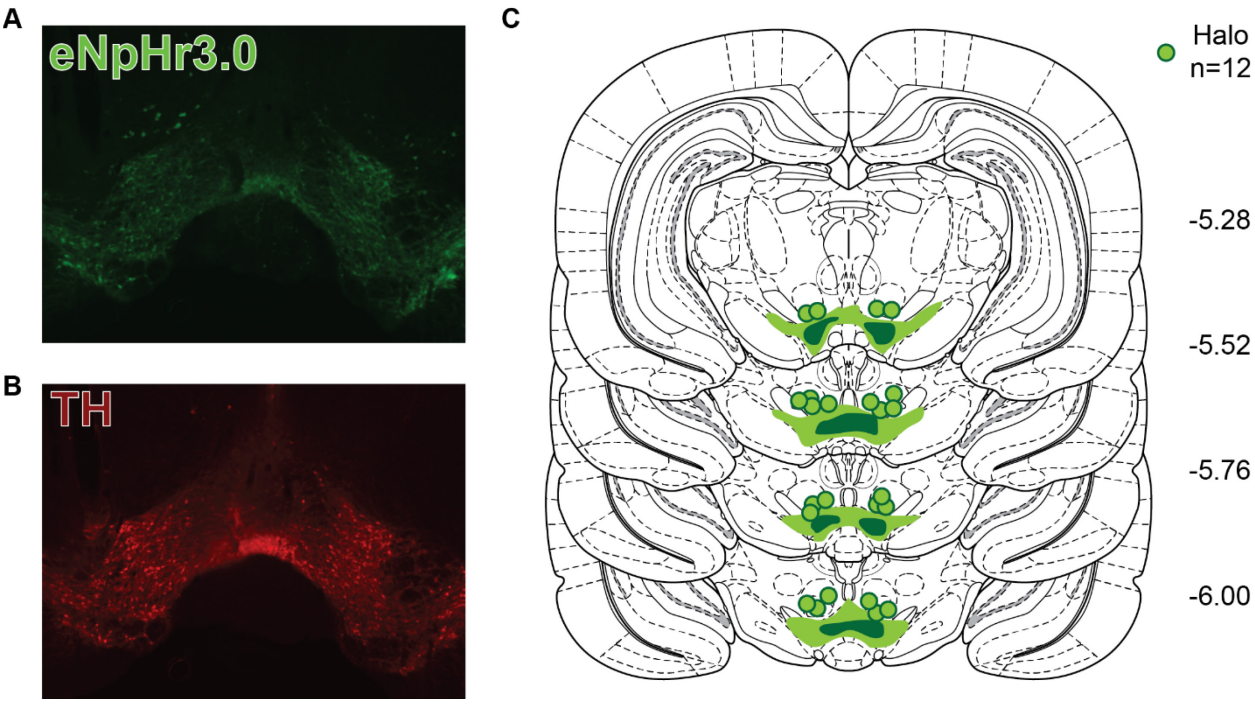

Figure 3.1. Histology and placement of optic fibers. ***A,*** Representative expression of YFP-tagged cre-dependent halorhodopsin the VTA of TH-Cre rats. ***B,*** Immunohistochemical staining for TH protein from the same slice in ***A***. ***C,*** Localization of optic fiber placements for Halorhodopsin-expresing rats and the largest, in light green, and smallest, in dark green, extent of cre-dependent halorhodopsin in the VTA. Numbers indicate distance from bregma in millimeters and the coronal sections were obtained from (Paxinos and Watson, 2007).

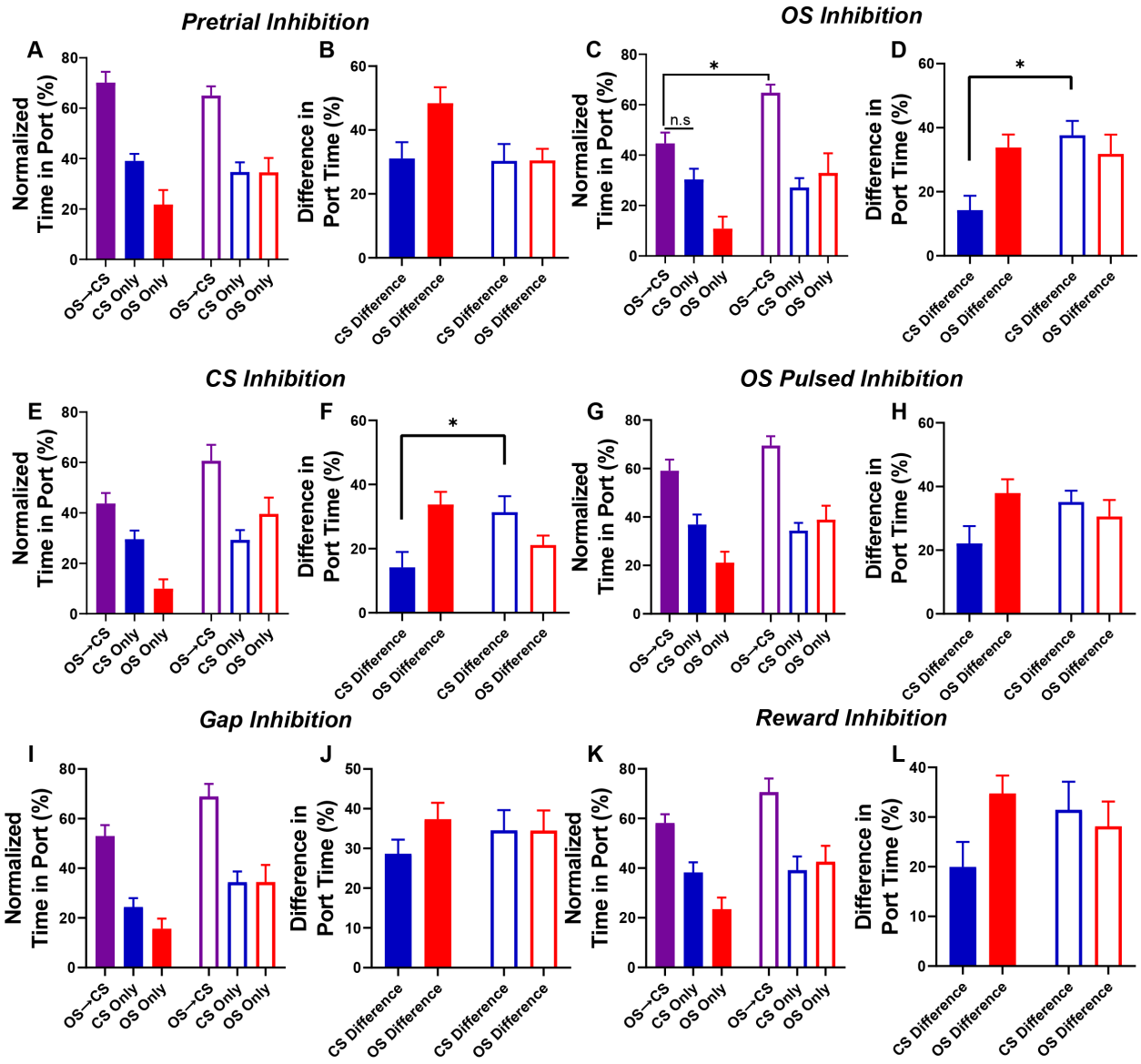

Figure 3.2. Optogenetic inhibition of VTA dopamine neurons during occasion setters disrupts the hierarchical control of cue-triggered reward-seeking. ***A,*** Average time in port during the conditioned stimulus period when light was delivered 10s before a trial. ***B,*** Individual differences in time in port during the conditioned stimulus period on OS→CS trials minus either conditioned stimulus alone or occasion setter alone trials when light was delivered 10s before a trial. ***C,D,*** same as ***A,B*** but when light was presented during the OS. ***E,F,*** same as ***A,B*** but when light was presented during the CS. ***G,H,*** same as ***A,B*** but when 3 1.5s pulses of light were

delivered during the OS. ***I,J,*** same as ***A,B*** but when light was presented for the last 500ms of the OS through the first 500ms of the CS. ***K,L, s***ame as ***A,B*** but when light was during reward delivery. For all figures bars indicate mean ± SEM. Empty bars represent data from the GFP animals and filled bars from rats with cre-dependent halorhodopsin. OS, occasion setter. CS, conditioned stimulus. *p<0.05.

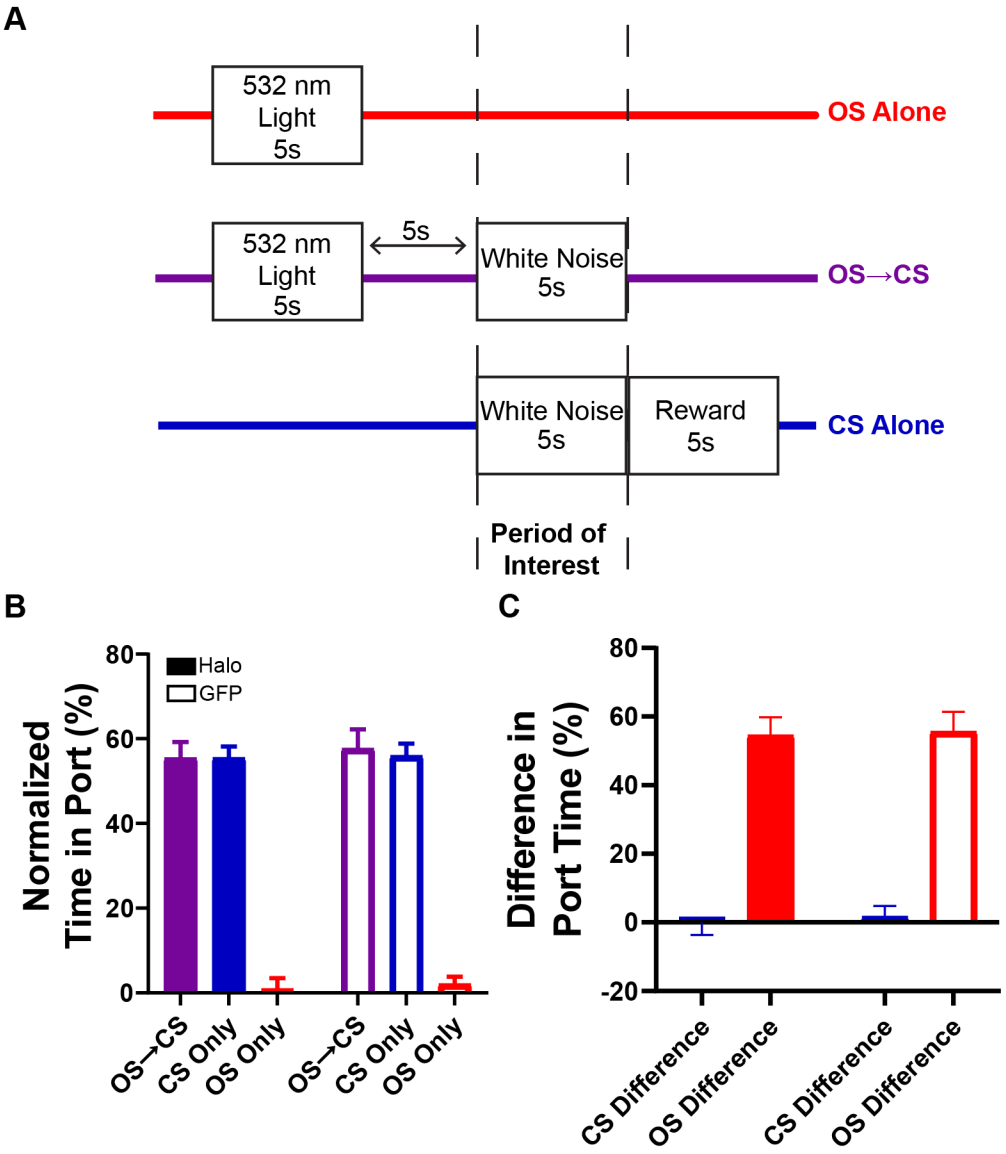

Figure 3.3. VTA dopamine inhibition alone is insufficient for the hierarchical control of reward-seeking. ***A,*** Task design for these tests where rats were trained for 12 sessions with light delivery acting as a negative occasion setter for 12 sessions. ***A,*** Average time in port during the conditioned stimulus period on the final session. ***B,*** Individual differences in time in port during the conditioned stimulus period on OS→CS trials minus either conditioned stimulus alone or occasion setter alone trials on the final

session. For all figures bars indicate mean ± SEM. Empty bars represent data from the GFP animals and filled bars from rats with cre-dependent halorhodopsin. OS, occasion setter. CS, conditioned stimulus. *$p<0.05$.

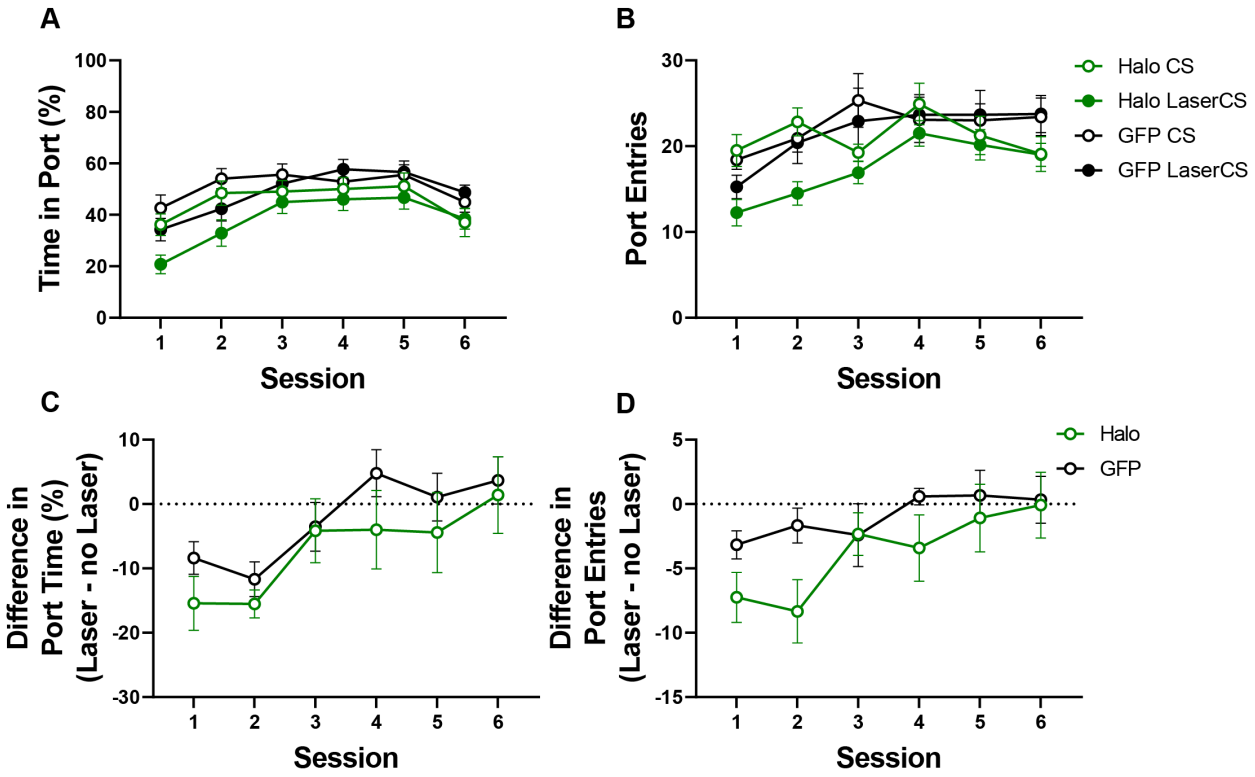

Figure 3.4. VTA dopamine inhibition paired with the presentation of a probabilistic CS does not alter learning nor conditioned approach. ***A,*** Time in port during the laser paired (solid circles) and non-laser paired (open circles) probabilistic CS. ***B,*** Number of port entries port during the laser paired (solid circles) and non-laser paired (open circles) probabilistic CS. ***C,*** Difference in the time in port during the laser paired CS minus the the non-laser paired CS. ***D,*** Difference in port entries made during the laser paired CS minus the the non-laser paired CS. For all figures data points indicate mean ± SEM. Black symbols represent data from the GFP animals and green symbols from rats with cre-dependent halorhodopsin. OS, occasion setter. CS, conditioned stimulus. *p<0.05.

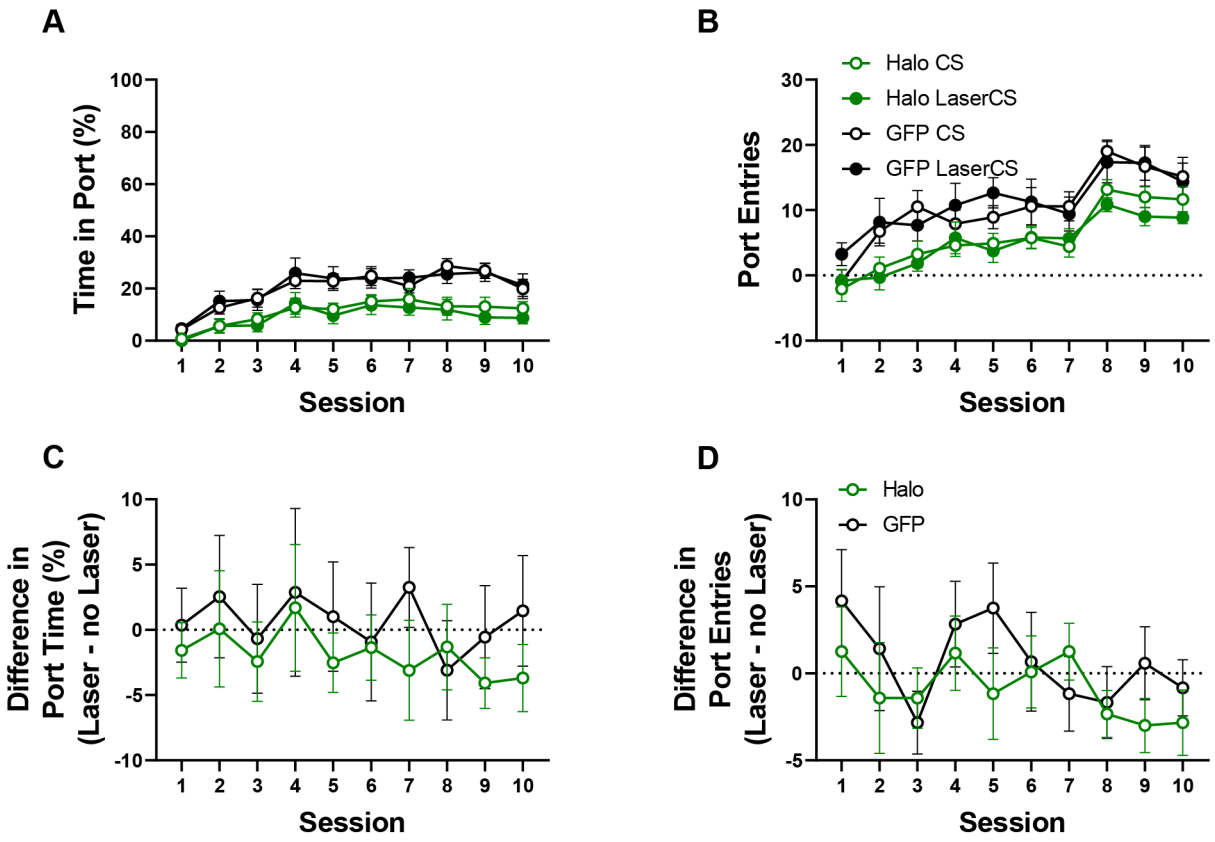

Figure 3.5. VTA dopamine inhibition paired with the consumption of reward associated with a probabilistic CS does not alter learning nor conditioned approach. ***A,*** Time in port during the probabilistic CS with laser-paired reward consumption (solid circles) and non-laser reward-paired (open circles) probabilistic CS. ***B,*** Time in port during the probabilistic CS with laser-paired reward consumption (solid circles) and non-laser reward-paired (open circles) probabilistic CS. ***C,*** Difference in the time in port during the laser paired CS minus the the non-laser paired CS. ***D,*** Difference in port entries made during the laser paired CS minus the the non-laser paired CS. For all figures data points indicate mean ± SEM. Black symbols represent data from the GFP animals and green symbols from rats with cre-dependent halorhodopsin. OS, occasion setter. CS, conditioned stimulus. *p<0.05.

Chapter 4

Hierarchical control of mesolimbic dopamine and striatal encoding of reward-paired cues governs behavioral flexibility

INTRODUCTION

The nucleus accumbens is essential for the generation of motivated behaviors directed towards reward-paired cues. This is thought to arise in part from the integration of glutamatergic signals arising from the amygdala, frontal cortex, thalamus, and hippocampus and a dense dopaminergic input arising from the ventral tegmental area (Mogenson et al., 1980). Moreover, dissociations exist between two primary subdivisions of the nucleus accumbens: the core and shell (Floresco, 2015). The core of the accumbens, in particular dopamine release, is necessary for the attribution of motivational value to reward-paired cues and the generation of approach behavior to Pavlovian conditioned stimuli (Blaiss and Janak, 2009; Chaudhri et al., 2010; Saunders and Robinson, 2012; Fraser and Janak, 2017; Saunders et al., 2018; Valyear et al., 2020). In contrast, the shell has been ascribed a role of inhibiting inappropriate behavioral responding and the retention of such inhibitory behavioral phenomenon like extinction learning (Floresco et al., 2008; Blaiss and Janak, 2009; Chaudhri et al., 2010; Ambroggi et al., 2011; Millan et al., 2015; Valyear et al., 2020).

Despite decades of investigation into the nucleus accumbens as an interface between motivation and action a mechanism linking neural activity and dopamine release in this structure to the control of Pavlovian motivated approach has remained unclear. While dopamine release in the nucleus accumbens is necessary and sufficient for the generation of Pavlovian motivated behavior, lesions and manipulations of neural activity during the learning or performance of Pavlovian conditioned reward-seeking

have minimal impact on behavior (Blaiss and Janak, 2009; Chang et al., 2012; Chang and Holland, 2013; Fraser and Janak, 2017). In contrast, when reward-associated stimuli are linked with the availability of actions to be performed to earn reward there exists widespread neural encoding in nucleus accumbens and alterations in neural activity dramatically affect such cue-motivated actions (Ambroggi et al., 2008, 2011). A potential explanation for this dissociation is that nucleus accumbens neural activity is necessary only for instrumental reward-seeking actions but not Pavlovian conditioned reward-seeking. However, the role of cues to direct instrumental reward-seeking actions centers on the resolution of uncertainty surrounding the relationship between and a given response and reward. In standard Pavlovian conditioning procedures, while conditioned cues resolve uncertainty surrounding when reward will become available, there is no inherent uncertainty to if this cue will deliver rewards as these preparations almost exclusively make use of cues with fixed, all-or-nothing probabilities of reward delivery.

Cue-generated uncertainty is a critical feature of reward-associated stimuli in the real-world and the ability to resolve the current motivational relevance of a given cue is necessary for adaptive behavior. We hypothesized that the nucleus accumbens is essential in generating behaviors as a result of this resolution of uncertainty. To test this, we exploited a Pavlovian paradigm called occasion setting, where the relationship between a given Pavlovian conditioned stimulus and reward is controlled by a separate, non-overlapping, and discrete stimulus called an occasion setter (Fraser and Holland, 2019). Indeed, we find that in this scenario neural activity and dopamine release in the nucleus accumbens is obligatory for occasion setting. Interestingly, we find a

dissociation in the contribution of glutamate release in the core, but not shell, as controlling the accurate processing of occasion setting cues as hierarchical stimuli. By exploiting *in vivo* electrophysiology and calcium imaging we detail correlates of the resolution of uncertainty by both single neurons and by dopamine release in the nucleus accumbens core. Ultimately, these data provide a link between neural activity and dopamine release in the generation of flexible cue-motivated reward-seeking.

MATERIALS AND METHODS

Subjects

Subjects were male and female Long-Evans rats (n=32) aged P60 obtained from ENVIGO. Rats were single-housed in ventilated cages with *ad libitum* access to food and water in a temperature- and humidity-controlled room and maintained on 12:12 light/dark cycle (lights on at 07:00). Despite time allowed for recovery from surgery, feeding was restricted to maintain weights at ~95% of *ad libitum* feeding weights. All procedures were approved by the Animal Care and Use Committee at Johns Hopkins University and the University of Minnesota and followed the recommended guidelines in the Guide for the Care and Use of Laboratory Animals: Eighth Edition, revised in 2011.

Surgery

Rats were anesthetized with isoflurane (5% induction, 1-2.5% maintenance) and standard stereotaxic procedures were used. For microinfusion experiments 22 gauge stainless steel cannula (Plastics One) were implanted 1mm over the nucleus accumbens core (n=12; AP: +1.8 ML: ±1.5 DV: -6) or shell (n=12; AP: +1.6 ML: ±1 DV: -6.5). For electrophysiological recordings, a custom-built microdrive containing 16 50 µm tungsten wires soldered to 8-pin Omnetics connectors was lowered slowly to the

nucleus accumbens core (n=3; AP: +1.8 ML: ±1.5 DV: -6.5) with a silver reference wire wrapped around a screw in contact with the cerebellum. For fiber photometric recordings (n=6), a volume of 800 nL of AAV5-CAG-dLight1.3b-GFP (titer 1.03 x 10^{13} viral particles/mL) was infused per site at a rate of 100 nL/min through a 31-G, gas-tight Hamilton syringe controlled by a Micro4 Ultra Microsyringe Pump 3 (World Precision Instruments) into the nucleus accumbens core (AP: +1.3 ML: ±1.3 DV: -6.9 300nL, -6.7 500nL). The needle was left in place for 10 minutes following the infusion to allow for diffusion of the virus from the injection site. In the same surgery, rats were implanted with optic fiber implants (400 µm glass diameter) targeted just dorsal to the infusion site (AP: +1.3 ML: ±1.3 DV: -6.5). For all surgeries, implants were secured to the skull with 4-8 screws placed in the skull and dental acrylic. After surgery, rats received injections of cefazolin (70 mg/kg, subcutaneous) to prevent infection and carprofen (5 mg/kg, subcutaneous) to relieve pain. Rats were allowed to recover for 7 days prior to the beginning of behavioral procedures or neural recordings.

Apparatus

Behavioral testing occurred in Med Associates conditioning chambers (St. Albans, VT) housed in sound- and light-attenuating cabinets and controlled by a computer running MedPC IV software. In the center of one wall was a fluid receptacle that was located in a recessed port. On the opposite wall near the ceiling of the chamber was a white houselight (28 V) and to the right of the houselight was a white-noise generator (10-25 kHz, 20 dB). Outside of the behavioral chamber but within the sound- and light-attenuating cabinet was a red houselight (28 V) that provided background illumination during each behavioral session. Fluids were delivered to the

port via tubing attached to a 60 mL syringe placed in a motorized pump located outside each cabinet.

Behavioral Procedures

For at least two days prior to any procedures rats were extensively handled and acclimated to the experimenter. Rats were also given free access to 15% sucrose in water for 24 hours in their homecage to prevent any neophobia and accustom them to the future reward solution. At least one day after sucrose pre-exposure, pretraining was conducted in an approximately one-hour session in which rats were able to drink sucrose from the recessed reward port. During this session there were 80 2-second activations of the syringe pump (~0.07 mL per delivery) on a variable time 60 s schedule (30-90 s range). All rats consumed all sucrose delivered in the pretraining session.

Training in the occasion setting task took place in 3 distinct phases with each session lasting ~2 hours on average with 30 total trials and an average inter-trial interval of 3.3 minutes. There was one session a day. In the first phase, all trials were reinforced presentations of the following sequence of events: occasion setter houselight for 5 s, a 5 s empty period, and 5 s of the conditioned stimulus white noise. Reward consisted of 5 s activation of the syringe pump resulting in delivery of ~0.18 mL of 15% sucrose in the reward port occurred immediately upon termination of the conditioned stimulus white noise. Phase one training lasted for 4 sessions. In phase two, 40% of trials were reinforced as before, but the other 60% of trials were conditioned stimulus alone trials consisting of presentation of the white noise for 5 s with no reward. Phase two training lasted for 6 sessions. In the final phase of training, rats were exposed to the full

occasion setting task (Figure 1A) consisting of 10 reinforced trials, 10 conditioned stimulus alone trials, and 10 occasion setter alone trials where the houselight was activated for 5 s with no subsequent stimuli or reward. Note that this task is purely Pavlovian; there was never a response requirement. Training in the final phase lasted for 8 sessions prior to microinfusions or electrophysiological recordings began. Fiber photometric recordings occurred throughout phase 3.

Microinfusion Procedures

Rats were accustomed to the handling and infusion procedure for 1-2 days prior to infusion by being transported to the procedure room after a training session, dummy stylets removed, a flat cut 28 gauge injector inserted into each cannula, and dummy stylets replaced. On the day prior to testing, a regular injector extending 1 mm past the guide cannula was used to confirm cannula patency. At test rats received infusions of either saline, a mixture of the GABA-B and GABA-A agonists, baclofen and muscimol (1.0 mM and 0.1 mM, respectively), the dopamine receptor antagonist flupenthixol (100 mM), or a combination of the AMPA and NMDA receptor antagonists CNQX and MK-801 (20 mM each) infused in a volume of 0.3 µL over 1 minute. After 1 additional minute to allow for diffusion away from the infusion site, injectors were removed, dummy stylets were replaced, and rats returned to their homecage for 5-10 minutes before test. There were four tests for each rat, one in each condition, in a random order and with at least one day of retraining without manipulation between. Test sessions were reinforced.

Fiber Photometric Recordings

Fiber photometry recordings were conducted in a manner similar to previously published (Saunders et al., 2018). A fluorescence mini-cube (Doric Lenses) transmitted

streams of sinusoidally modulated light from a 465 nm LED at 211 Hz through a GFP excitation filter and 405 nm LED at 531 Hz through a 405 nm bandpass filter. Rats were connected to the light source for delivery of light by a ceramic sleeve on their implanted optic fiber via a ceramic sleeve to a low autofluorescence fiber cable (400 nm, 0.48 NA). Fluorescence elicited from dLight1.3b was transmitted via the same cable back to the mini-cube where it was passed through a GFP emission filter, amplified, and focused onto a high-sensitivity photoreceiver (Newport, Model 2151). Demodulation of the brightness produced by 465 nm excitation, which stimulated dLight1.3 fluorescence, versus 405 nm excitation, which elicits fluorescence in a dLight1.3b independent manner, allowed for the correction of motion artifacts and bleaching of the recorded signal. A real-time signal processor (Tucker-Davis Technologies) modulated the output of each LED, recorded photometry signals, as well as logged behavioral events via TTL from the MedAssociates software.

Electrophysiological Recordings

Electrical signals and behavioral events were collected from freely behaving animals with the OmniPlex (Plexon) recording system as in (Ottenheimer et al., 2018, 2020). Waveforms were sorted into units offline using Offline Sorter software (Plexon), and any units that were not recorded throughout a behavioral session were discarded. We only included neurons that were identified as single-units through careful examination of auto and cross correlations, plotting waveform features over time to ensure stability and continuity, and discarded units with more than 0.2% of spikes within a 2-ms refractory period.

Histology

Following the conclusion of experiments, rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with 4% paraformaldehyde. For rats with electrode implants, a brief 10 μA DC current was passed through each electrode to mark its final location in the brain. Brains were post-fixed for 24 hours in 4% paraformaldehyde, cryoprotected in 25% sucrose in 0.1 M NaPB, and then sectioned on a freezing cryostat at -20° C in 50 um sections. Brains with electrode or cannula implants were mounted onto Fisher SuperFrost Plus slides, dried, stained with cresyl violet solution (FD Neurotechnologies) and coverslipped with Permount mounting medium.

Statistics and Data Analysis

Data analysis and visualization were performed with MATLAB (Mathworks) and Prism 9 (Graphpad). Two-way repeated measures ANOVAs were used to analyze the impact of microinfusions at test, with each drug separately being compared to data from the saline control session. For electrophysiology data we first normalized the firing rate for each neuron relative to a 10 s (-12 to -2 s before OS period) period before the OS would be presented on a trial. We then used Wilcoxon signed rank tests to determine significant responses in a period just after each event (0 to 0.5 s for cues) or around -0.5s to 0.1 s for port entries. PSTHs were constructed around each event in 0.01 s bins with a half-normal filter (σ=6.6) that used only activity in previous but not upcoming bins. We sorted neurons by trial based on either significant excitations or inhibitions or additionally by analyzing neurons for whom there were significant differences in activity at the time of the CS for OS+CS versus CS alone trials. We analyzed strength of responses among different cue responses with Wilcoxon rank sum tests. To analyze

activity of neurons across the duration of the CS, z-scored data was analyzed in 10 ms bins with ANOVA from 250 ms before CS onset through the 5 s duration of the CS. Fiber photometry signals were fit to the isosbestic control signal as in (Patriarchi et al., 2018; Saunders et al., 2018) and z-scored relative to a period 5s before the OS period. Photometry data were then analyzed in 100 ms bins with ANOVA, and peak response for each event was analyzed with ANOVA. We only analyzed data from electrophysiology and fiber photometry data when rats exhibited greater responding, evidenced by time in port, during the CS on OS+CS trials relative to both CS alone and OS alone. Depending on the structure of the data, posthocs were performed either with Bonferroni's or Tukey's method. For all statistical tests $\alpha=0.05$.

RESULTS

We trained rats to exploit a hierarchical cue, an occasion setter, to inform whether or not they should expect reward delivery following an auditory conditioned stimulus. If the occasion setting cue or the conditioned stimulus were presented in isolation there was no sucrose delivery. Importantly, in a single session there were equal numbers of presentation of both the occasion setter and the conditioned stimulus, equating the probability of reward delivery amongst the two cues, but only when the occasion setter was presented prior to the conditioned stimulus did rats receive reward (Figure 1A). Following training in this task we performed manipulations and recordings of nucleus accumbens with histology for each experiment in Figure 1B-C.

Contributions of Nucleus Accumbens Core and Shell to Occasion Setting

We tested the contribution of diverse neural signals in the nucleus accumbens core and nucleus accumbens shell to occasion setting. First, we asked whether neural

activity in either region was necessary for performance in this task by reversibly inactivating either structure. Inactivation of the nucleus accumbens core reduced reward-seeking during the conditioned stimulus period compared to behavior under saline (Figure 2A; effect of trial type $F_{(1,18)}$=33.78, p<0.0001; effect of drug $F_{(1,11)}$=92.15, p<0.0001; interaction $F_{(1,13)}$=6.108, p=0.0220). Inactivation significantly reduced time in the port on OS+CS (p=0.0001), CS alone (p=0.0001), and OS alone trials (p=0.003) relative to saline. In contrast to significantly higher port time on OS+CS trials relative to CS alone (p=0.0058) and OS alone trials (p<0.0001) following saline, there was no difference in time on OS+CS trials relative to CS alone (p=0.7028) or OS alone trials (p=0.4240) following inactivation. As a result, inactivation reduced the ability to rats to discriminate amongst reinforce trials and CS alone and OS alone trials (Figure 2B; effect of drug $F_{(1,11)}$=9.974, p=0.0091; within each discrimination p<0.01). Despite cue-triggered reward-seeking, inactivation of the core was without impact on intertrial port entries (Figure 2C; $t_{(11)}$=0.5779, p=0.5750) or intertrial port time (Figure 2D; $t_{(11)}$=1.917, p=0.0816). Surprisingly, we observed a similar impact of inactivation for rats with cannula in the nucleus accumbens shell. Inactivation reduced reward-seeking across all trial types (Figure 2E; effect of trial type $F_{(1,15)}$=38.22, p<0.0001; effect of drug $F_{(1,11)}$=32.09, p=0.0001; interaction $F_{(1,21)}$=5.369, p=0.0139) resulting in significantly reward-seeking relative to saline infusion on OS+CS (p=0.0011) and CS alone trials (p<0.0001) and eliminated differences in responding between OS+CS trials relative to OS alone trials (p=0.1514) but, interestingly, not CS alone trials (p=0.0388). Despite this, inactivation of the shell resulted in significantly reduced overall discrimination in the task (Figure 2F; effect of drug $F_{(1,11)}$=13.10, p=0.0040; within each discrimination

$p<0.05$). In contrast to its effects on cue-triggered responses, inactivation of the nucleus accumbens shell did not have a significant impact on intertrial port entries (Figure 2G; $t_{(11)}=1.697$, $p=0.1178$) or intertrial port time (Figure 2H; $t_{(11)}=0.4630$, $p=0.6524$). Collectively these data indicate neural activity in both nucleus accumbens core and shell is necessary for occasion setting, with a more prominent impact of inactivation within the nucleus accumbens core.

Next, we asked whether dopamine signaling was essential for performance in this task. Flupenthixol delivered into the core significantly reduced cue-elicited reward-seeking (Figure 3A; effect of trial type $F_{(1,21)}=29.58$, $p<0.0001$; effect of drug $F_{(1,11)}=51.65$, $p<0.0001$; interaction $F_{(1,20)}=4.952$, $p=0.0198$). This reduced reward-seeking on all trials relative to saline (OS+CS $p=0.006$; CS alone $p=0.0028$; OS alone $p=0.0008$) and also resulted in rats being unable to elevate reward seeking on OS+CS trials relative to CS alone ($p=0.3447$) trials. In turn, nucleus accumbens core dopamine antagonism reduced overall discrimination in this task between reinforced and non-reinforced trials (Figure 3B; effect of drug $F_{(1,11)}=12.36$, $p=0.0048$; within each discrimination $p<0.05$). As is common for dopamine antagonists, there were small but significant decreases in intertrial port entries (Figure 3C; $t_{(11)}=4.874$, $p=0.0005$) or intertrial port time (Figure 3D; $t_{(11)}=4.705$, $p=0.0006$). Dopamine antagonism within nucleus accumbens shell had an overall similar impact of reducing cue-triggered reward-seeking in the occasion setting task (Figure 3E; effect of trial type $F_{(1,17)}=22.94$, $p<0.0001$; effect of drug $F_{(1,11)}=165.4$, $p<0.0001$; interaction $F_{(1,17)}=15.62$, $p=0.0002$). Following flupenthixol administration reward-seeking was significantly lower on OS+CS ($p<0.0001$), CS alone ($p=0.0007$), and OS alone trials ($p<0.0001$) relative to saline, with

no significant differences following flupenthixol between OS+CS trials and CS alone (p=0.8291) or OS alone (p=0.0667) trials. This was reflected by an overall impact of flupenthixol in reducing discriminations among trial types (Figure 3F; effect of drug $F_{(1,11)}$=51.51, p<0.0001; within each discrimination p<0.01). Dopamine antagonism in the nucleus accumbens shell significantly reduced intertrial port entries (Figure 3G; $t_{(11)}$=7.336, p<0.001) but not intertrial port time (Figure 3H; $t_{(11)}$=1.296, p=0.2215). As a result, functional dopamine signaling in the nucleus accumbens core and shell is equivalently necessary for the performance of occasion setting.

Finally, we assessed whether functional glutamate signaling in either nucleus accumbens subregion was essential for occasion setting. In the nucleus accumbens core, blockade of AMPA and NMDA receptors increased overall responding in the occasion setting task (Figure 4A; effect of trial type $F_{(1,19)}$=32.47, p<0.0001; effect of drug $F_{(1,11)}$=33.16, p=0.0001; interaction $F_{(1,17)}$=2.904, p=0.0904). Analysis of the impact of drug infusion revealed that glutamate antagonism selectively increased reward-seeking on OS alone trials relative to saline (p=0.0069). This resulted in behavior under nucleus accumbens core glutamate antagonism on OS alone trials statistically indistinguishable from behavior on OS+CS trials following saline infusion (p=0.3524). Despite this, rats still discriminated at a similar level under glutamate antagonism (Figure 4B; effect of drug $F_{(1,11)}$=0.0305, p=0.8644) and while there was no impact of treatment on intertrial port time (Figure 4D; $t_{(11)}$=0.6838, p=0.5082) there was a significant elevation in intertrial port entries (Figure 4C; $t_{(11)}$=3.579, p=0.0043). In contrast, glutamate antagonism within the nucleus accumbens shell was without significant effect on reward-seeking (Figure 4E; effect of trial type $F_{(1,14)}$=44.39,

$p<0.0001$; effect of drug $F_{(1,11)}=1.976$, $p=0.1875$; interaction $F_{(1,15)}=0.1706$, $p=0.8315$), discrimination amongst reinforced and non-reinforced trials (Figure 4F; ; effect of drug $F_{(1,11)}=0.3291$, $p=0.5778$), or intertrial port time (Figure 4D; $t_{(11)}=0.2096$, $p=0.8378$). Glutamate antagonism in the shell did however result in a significant increase in intertrial port entries (Figure 4C; $t_{(11)}=3.548$, $p=0.0046$). As a result, it appears that glutamatergic inputs in the nucleus accumbens core, but not shell, constrain the processing of hierarchical cues and are necessary for occasion setters to be recognized as higher-order stimuli and not weakly reinforced conditioned stimuli.

Nucleus Accumbens Core Neurons Encode and Utilize Occasion Setters to Modulate Cue-responses

The surprising impact of inactivation of the nucleus accumbens on eliminating responding to cues in the occasion setting task suggests that single neurons within the nucleus accumbens encode information directly relevant for performance. To assess this we implanted rats with microdrives of 16 tungsten wires in the nucleus accumbens core and recorded neural activity as rats performed the occasion setting task. We recorded 235 putative single nucleus accumbens neurons. We first analyzed the proportions of neurons that encoded task-relevant events: the houselight occasion setter, the white noise conditioned stimulus, port entries, and reward delivery (Figure 5B). We observed that 55% of nucleus accumbens had significant modulation following OS presentation, while only 33% were significantly modulated following CS presentation. We then analyzed the relative strength of excitations and inhibitions to these cues. We found that nucleus accumbens neurons were more strongly strongly excited to the OS than to the CS (Wilcoxon signed rank test $p=0.0281$) but there was no

difference in the strength of the inhibition between the OS and CS (Wilcoxon signed rank test p=0.1461). Overall, these suggest that nucleus accumbens neurons strongly encode occasion setters, and do so more than conditioned stimuli, with primarily excitations.

We then sought to explore potential modulation of CS responses by the prior presence or absence of the OS. We graphed the activity of CS-excited and CS-inhibited neurons by trial type and throughout the duration of the CS. For CS-excited neurons though there was a significant interaction of trial and time during the CS, these were driven by similar changes in CS-excited neurons on OS+CS and CS alone trials relative to OS alone trials (Figure 6A; interaction $F_{(1050,67845)}$=1.543, p<0.0001). Interestingly, we found that for CS-inhibited neurons there was a stronger inhibition to the CS when this cue was preceded by its occasion setter than when this cue was presented alone (Figure 6B; interaction of time x trial type $F_{(1050,67854)}$=4.746, p<0.0001; OS+CS vs CS Alone p<0.05 for 0.64 to 0.84 s post CS). We also identified a subpopulation of neurons whom their activity in the first 500 ms post CS onset was significantly different on OS+CS relative to CS alone trials (n=23 neurons). When we graphed the activity of this subpopulation across trials we observed a striking flip in the response direction of these neurons from excitation to the CS when it was not predictive to inhibitions when the CS was predictive. As a result the average activity of these neurons was significantly different on OS+CS trials relative to CS alone trials (Figure 6C; interaction of time x trial type $F_{(1050,34716)}$=4.910, p<0.0001; OS+CS vs CS Alone p<0.05 for 0.13 to 1.28 s post CS). These collectively indicate that neurons within nucleus accumbens core encode

and utilize hierarchical stimuli to resolve the immediate motivational value of a conditioned stimulus.

Nucleus Accumbens Core Dopamine Release Dynamically Encodes the Motivational Value of Conditioned Stimuli

We previously demonstrated a necessary role for the activity of dopamine neurons during occasion setters for their ability to modulate reward-seeking and here found that long-lasting antagonism of dopamine signaling in the nucleus accumbens occludes the expression of occasion setting. We sought to understand how dopamine release in the nucleus accumbens would be regulated by occasion setters and what aspects of dopamine release, if any, would be related to occasion setting performance. We made use of *in vivo* bulk single-photon imaging of dopamine release with the genetically-engineered fluorescent sensor dLight1.3b which allowed for dopamine release to be recorded as rats freely behaved and performed in the occasion setting task (Patriarchi et al., 2018; Mohebi et al., 2019). We first analyzed the peak dopamine response following OS and CS presentation as well as after reward delivery. Overall, there was significant modulation of peak dopamine release across trials and events (Figure 7B; effect of trial type $F_{(1,7)}=23.64$, $p=0.0012$; effect of event $F_{(1,5)}=89.10$, $p<0.0001$; interaction $F_{(1,9)}=23.18$, $p=0.0003$). Dopamine release was equivalent to the OS when that stimulus was presented as would be expected, yet surprisingly we found that dopamine release was significantly greater to the CS if the OS had previously been presented (OS+CS vs CS alone $p=0.007$; OS+CS vs OS alone $p=0.0118$). We next analyzed the time course of dopamine release throughout a trial and again found similar effects of modulation of dopamine release by trial type to the CS (Figure 7C; effect of

trial type $F_{(1,6)}$=6.478, p=0.0377; effect of time $F_{(3,18)}$=7.275, p=0.0012; interaction $F_{(700,3500)}$=3.691, p<0.0001). Dopamine release to the OS was equivalent for OS alone and OS+CS trials and was greater than CS alone trials beginning 300 ms following OS presentation (p's<0.05). In agreement with the findings of peak fluorescence, dopamine release to the CS was significantly greater than OS alone and CS alone beginning 300 ms after the CS was presented on OS+CS trials and lasting 900 ms following its onset (p's<0.05). These findings indicate that occasion setters drive dopamine release in the nucleus accumbens core and gate dopamine release to conditioned stimuli, with dopamine release only occurring if this cue currently predicts reward.

DISCUSSION

The nucleus accumbens is an interface between glutamatergic forebrain regions like the amygdala, frontal cortex, and hippocampus and midbrain dopaminergic inputs from the ventral tegmental area that integrates these signals to guide cue-elicited behaviors (Mogenson et al., 1980; Nicola, 2010; Floresco, 2015). Despite this, it remains unclear the precise role dopamine release and neural activity in the nucleus accumbens play in Pavlovian cue-triggered reward-seeking. Here we made use of intracranial pharmacology, *in vivo* electrophysiology, and fiber photometry to assess the contributions of nucleus accumbens to the resolution of cue-generated uncertainty. We find that the nucleus accumbens core is an essential neural substrate for the resolution of uncertainty surrounding reward-paired cues, and that both dopamine release and glutamate signaling are essential for the hierarchical control of behavior. We reveal novel neural correlates in the nucleus accumbens core for this process, with widespread encoding of hierarchical cues, and detail dopaminergic substrates that underlie this

occasion setting process. As a result, we provide a number of mechanisms for dopamine release and neural activity in the nucleus accumbens core to allow for the dynamic and rapid updating of the motivational value of reward-paired stimuli and the generation of adaptive reward-seeking.

Our data indicate a similar overall contribution of neural activity in the nucleus accumbens core and shell in occasion setting, although there were nuanced behavioral effects suggesting neural activity in the core was more essential. We were surprised by this, as our lab and others have previously demonstrated a dissociable contribution of nucleus accumbens core neural activity to generating cue-triggered reward-seeking whereas nucleus accumbens shell neural activity was critical for a context to inhibit reward-seeking (Blaiss and Janak, 2009; Chaudhri et al., 2010; Ambroggi et al., 2011; Millan et al., 2015; Fraser and Janak, 2017). However, occasion setting is a process that requires both of these psychological processes – accurately scaling conditioned approach to targets of motivation and inhibiting inappropriate responding when cues will not be rewarded (Holland, 1992; Meyer and Bucci, 2016a; Fraser and Holland, 2019). The manipulations we performed were long-lasting and prevent us from claiming when neural activity nor dopamine release are precisely necessary in either subregion for the hierarchical control of behavior. It is possible then, for future investigations to make use of methods like optogenetics with more precise temporal control to assess whether manipulations of neural activity or dopamine terminals in either region reveal precise contributions of these nucleus accumbens subregions to occasion setting.

By recording single neurons within the nucleus accumbens during occasion setting we discovered a widespread encoding of these hierarchical stimuli that

surpassed the number of neurons modulated by a Pavlovian conditioned stimulus. These data are reminiscent of the encoding of stimuli that indicate the availability of actions to be performed to earn reward (Ambroggi et al., 2008, 2011; Nicola, 2010; Richard et al., 2016; Sicre et al., 2020). In these scenarios, these discriminative stimuli act to instruct when an action will directly lead to reward and resolve uncertainty surrounding the relationship between actions and reward, although the nature of training also results in these stimuli also being directly related to reward. Interestingly, neural activity and dopamine signaling in the nucleus accumbens core is also essential in these scenarios (Ambroggi et al., 2008, 2011; du Hoffmann and Nicola, 2016, 2016; Sicre et al., 2020). This is in contrast to relatively modest effects observed with lesions or inactivations of the nucleus accumbens core, and while dopamine release is necessary in the nucleus accumbens core for Pavlovian conditioned approach, the magnitude of effect is not as extreme as in these scenarios where stimuli disambiguate the relationship between cues and reward or actions and reward (Di Ciano et al., 2001; Blaiss and Janak, 2009; Chang et al., 2012; Saunders and Robinson, 2012; Chang and Holland, 2013; Fraser and Janak, 2017; Sicre et al., 2020). These data in combination with our finding that nucleus accumbens neurons can dynamically encode the motivational value of Pavlovian conditioned stimuli strongly suggest that the resolution of uncertainty is a critical function of neurons within the nucleus accumbens core.

By making use of recent advances in biosensors, we were able to use fiber photometry to record dopamine release as rats performed in the occasion setting task (Patriarchi et al., 2018). Interestingly, we found that dopamine release only occurred during the conditioned stimulus if an occasion setter had indicated that on the current

trial that stimulus would be followed by reward. This is in contrast to theories of dopamine function that ascribe dopamine release as encoding the expected value of the conditioned stimuli, if this were the case we would have observed equivalent dopamine release across trials, and even have expected equivalent magnitudes of release to both the occasion setter and the conditioned stimulus (Schultz and Dickinson, 2000; Fiorillo et al., 2003; Tobler et al., 2005; Watabe-Uchida et al., 2017). The scaling of dopamine release in the nucleus accumbens core suggests the ability of dopamine neurons to dynamically encode the immediate motivational relevance of reward-paired cues (Berridge, 2007, 2012; Smith et al., 2011; Dayan and Berridge, 2014; Aitken et al., 2016). In fact, it has been well-demonstrated that dopamine release in the accumbens can be influenced by alterations in homeostasis, as dopamine release to water-paired cues increases if animals are made thirst or increases in release to food-cues if made hungry (Cone et al., 2016; Fortin and Roitman, 2018; Hsu et al., 2020). Our preparation tested the involvement of a well-controlled and isolated class of hierarchical stimuli, occasion setters, and we were able to parse about neural encoding and dopamine release to the state-defining stimulus, the occasion setter, and the target of its modulation, the conditioned stimuli. This is an obvious advantage compared to the long-lasting and overlapping influence of changes in internal states, like hunger or thirst, have on cue-triggered motivation and in addition allowed for the ability to identify “contextual” encoding within the nucleus accumbens and by dopamine release in this structure. These results suggest that flexibility in encoding reward-associated stimuli is an essential feature of the mesolimbic dopamine system.

Ultimately, our findings provide a mechanism for single neurons and for dopamine release in the nucleus accumbens to allow for the dynamic control of cue-triggered motivation. It remains to be determined the precise cell-types and circuit-defined inputs to the nucleus accumbens that are essential for this process. As inhibitions in dopamine D1-receptor expressing neurons in the nucleus accumbens are essential for approaching and ultimately consuming rewards, it is possible that these neurons are those that are modulated by occasion setters (O'Connor et al., 2015). In contrast, paired recordings and identification have suggested that cue-triggered dopamine release instead rapidly modulates primarily D2-receptor expressing neurons, potentially indicating the modulated neurons we identify here are instead indirect pathway medium spiny neurons. Exploiting cell-type specific viral approaches and transgenic lines will be critical to identifying the precise accumbens neurons that underlie occasion setting. In addition, only when glutamate function was interrupted in the nucleus accumbens core did this manipulation alter behavioral responding to the hierarchical occasion setter. In instrumental scenarios where discriminative stimuli resolve the availability of actions to generate reward, inputs from the basolateral amygdala to the nucleus accumbens core are essential for both neural encoding of these stimuli and behavioral responding (Ambroggi et al., 2008). Investigations into potential contributions of diverse glutamatergic inputs, particularly from the basolateral amygdala, is a critical future direction for understanding the neural circuits of flexible cue-triggered motivation. The inability to accurately regulate motivational responses to ambiguous cues is a hallmark of psychiatric illness and resolving the neural circuitry

underlying this flexibility will undoubtedly lead to novel therapies and approaches for treatment.

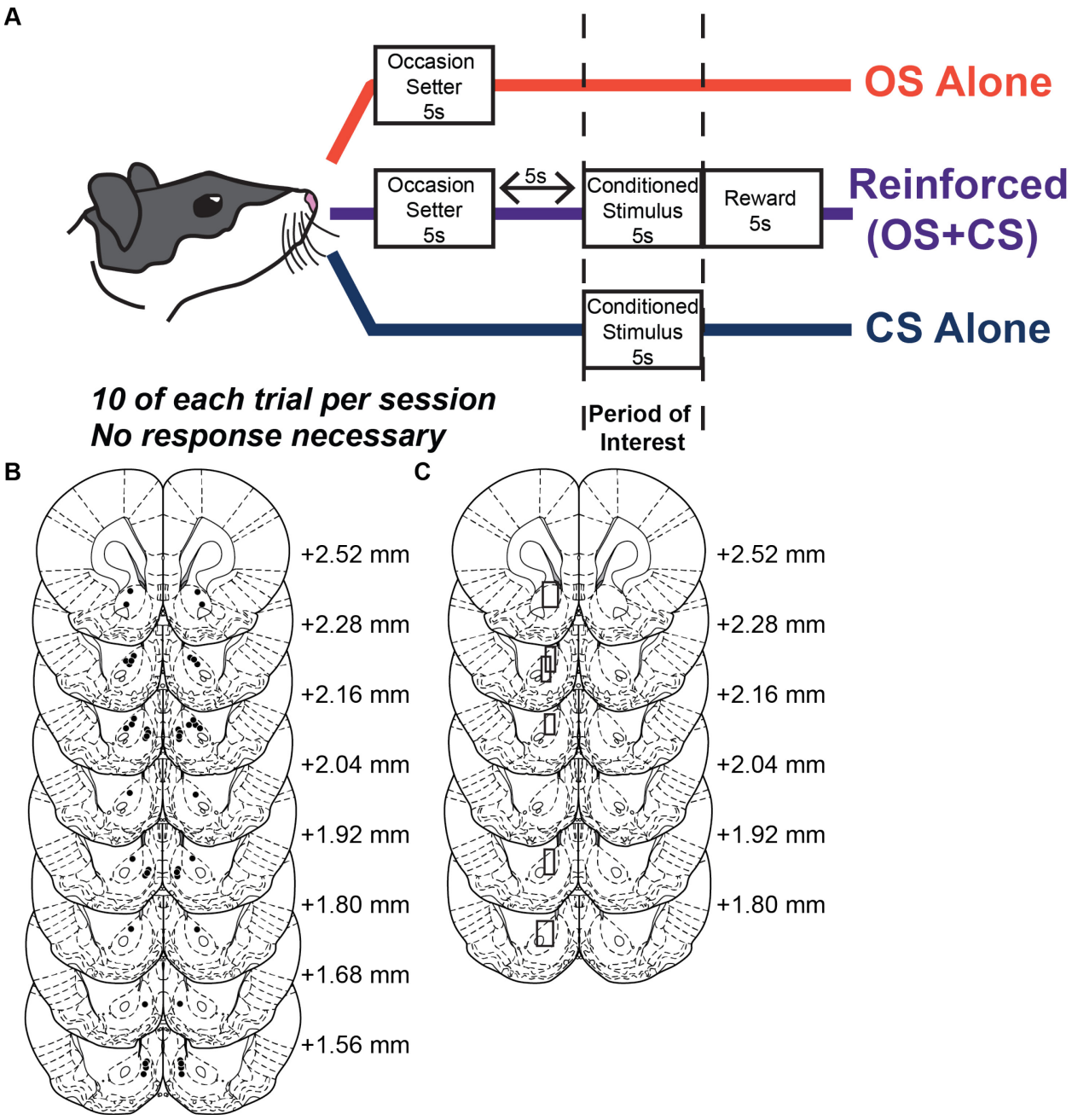

Figure 4.1. Task design and histology. ***A,*** Schematic of the task design used in all experiments. Long-evans rats discriminated whether a conditioned stimulus would be followed by reward delivery by exploiting the prior and non-overlapping presentation of a distinct occasion setting cue. ***B,*** Reconstruction of cannula tips for microinfusion experiments. ***C,*** Reconstruction of locations of electrodes for *in vivo* electrophysiological

recordings. ***D,*** Reconstruction of optic fiber locations and expression of dLight1.3 for fiber photometry recordings. Numbers indicate distance from bregma in millimeters and the coronal sections were obtained from (Paxinos and Watson, 2007).

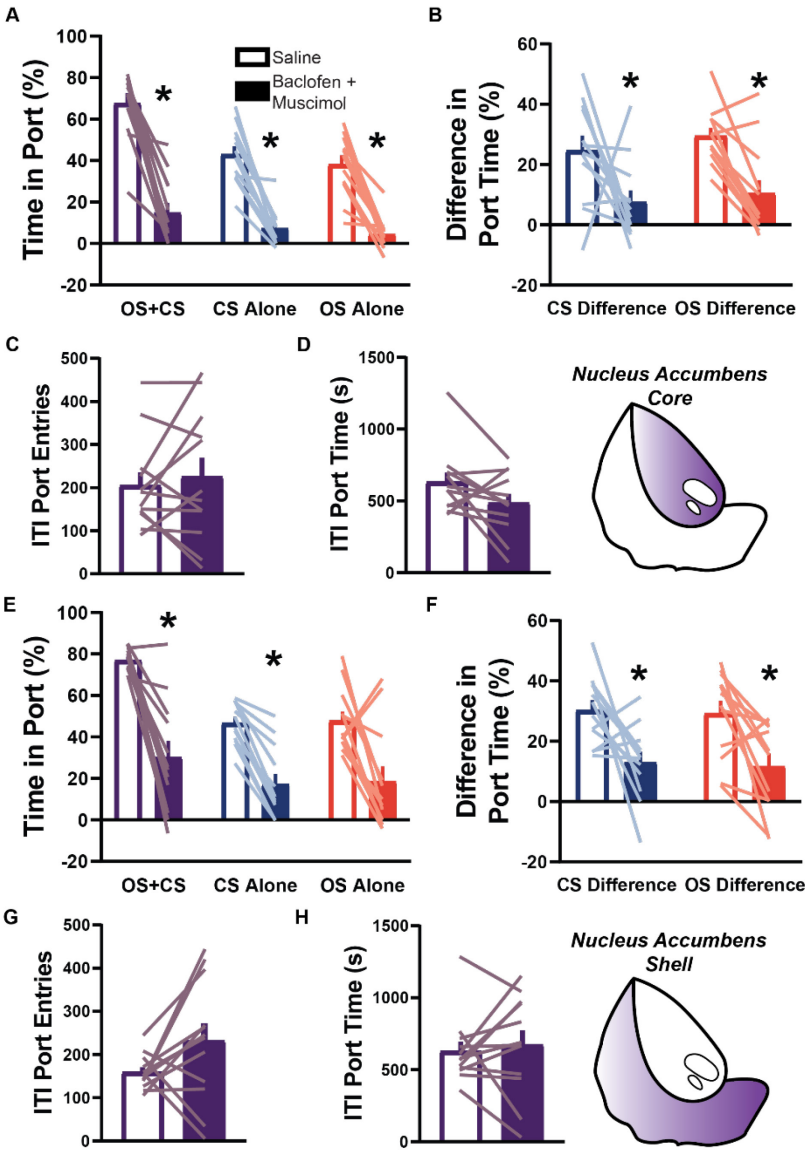

Figure 4.2. Reversible inactivation of either nucleus accumbens core or shell impairs occasion setting. ***A,*** Average time in port during the conditioned stimulus period for each trial type. ***B,*** Individual differences in time in port during the conditioned stimulus period on reinforced trials minus either conditioned stimulus alone or occasion setter alone trials. ***C,*** Intertrial port entries during the behavioral session. ***D,*** Intertrial port time during the behavioral session. ***E-H,*** same as ***A-D*** but for rats with cannula over the nucleus accumbens shell. For all figures bars indicate mean + SEM. Empty bars

represent data from the saline session and filled bars data from the inactivation session. Individual rats are overlaid and represented by the colored lines. OS, occasion setter. CS, conditioned stimulus. *p<0.05.

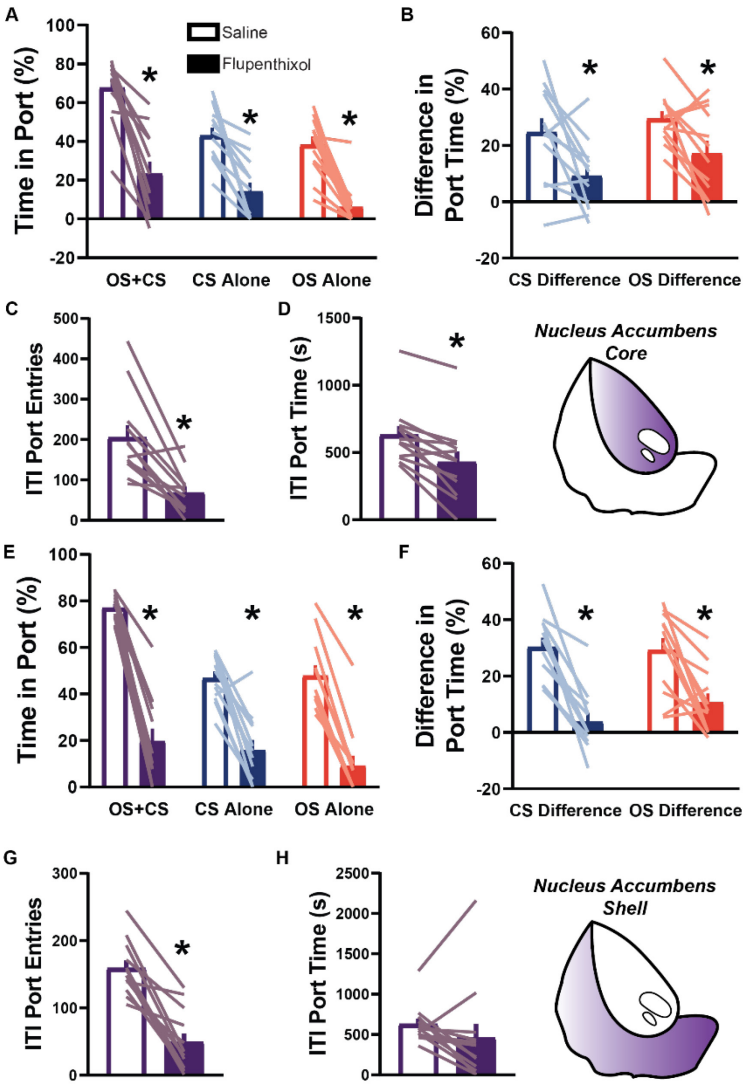

Figure 4.3. Dopamine signaling in the nucleus accumbens core and shell is essential for occasion setting. ***A,*** Average time in port during the conditioned stimulus period for each trial type. ***B,*** Individual differences in time in port during the conditioned stimulus period on reinforced trials minus either conditioned stimulus alone or occasion setter alone trials. ***C,*** Intertrial port entries during the behavioral session. ***D,*** Intertrial port time during the behavioral session. ***E-H,*** same as ***A-D*** but for rats with cannula over the nucleus accumbens shell. For all figures bars indicate mean + SEM. Empty bars represent data from the saline session and filled bars data from the inactivation session.

Individual rats are overlaid and represented by the colored lines. OS, occasion setter. CS, conditioned stimulus. *$p<0.05$.

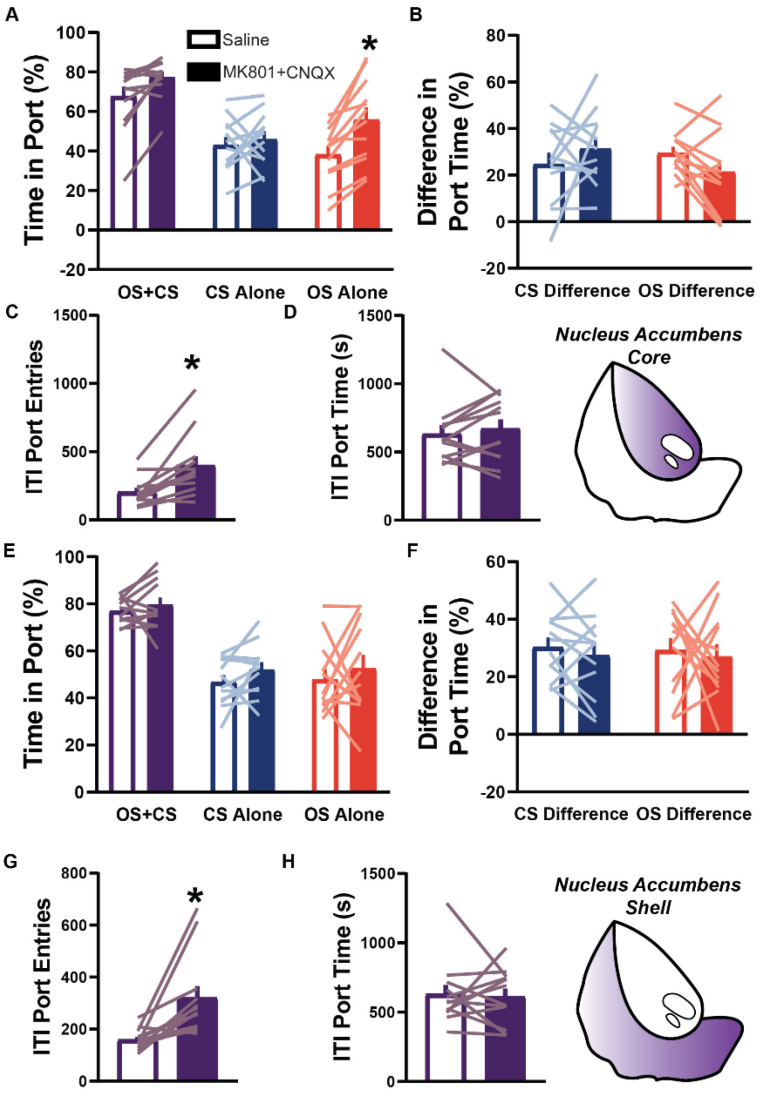

Figure 4.4. Glutamate release in the nucleus accumbens core, but not shell, is necessary for accurate processing of hierarchical stimuli. ***A,*** Average time in port during the conditioned stimulus period for each trial type. ***B,*** Individual differences in time in port during the conditioned stimulus period on reinforced trials minus either conditioned stimulus alone or occasion setter alone trials. ***C,*** Intertrial port entries during the behavioral session. ***D,*** Intertrial port time during the behavioral session. ***E-H,*** same as ***A-D*** but for rats with cannula over the nucleus accumbens shell. For all figures bars indicate mean + SEM. Empty bars represent data from the saline session and filled bars

data from the inactivation session. Individual rats are overlaid and represented by the colored lines. OS, occasion setter. CS, conditioned stimulus. *p<0.05.

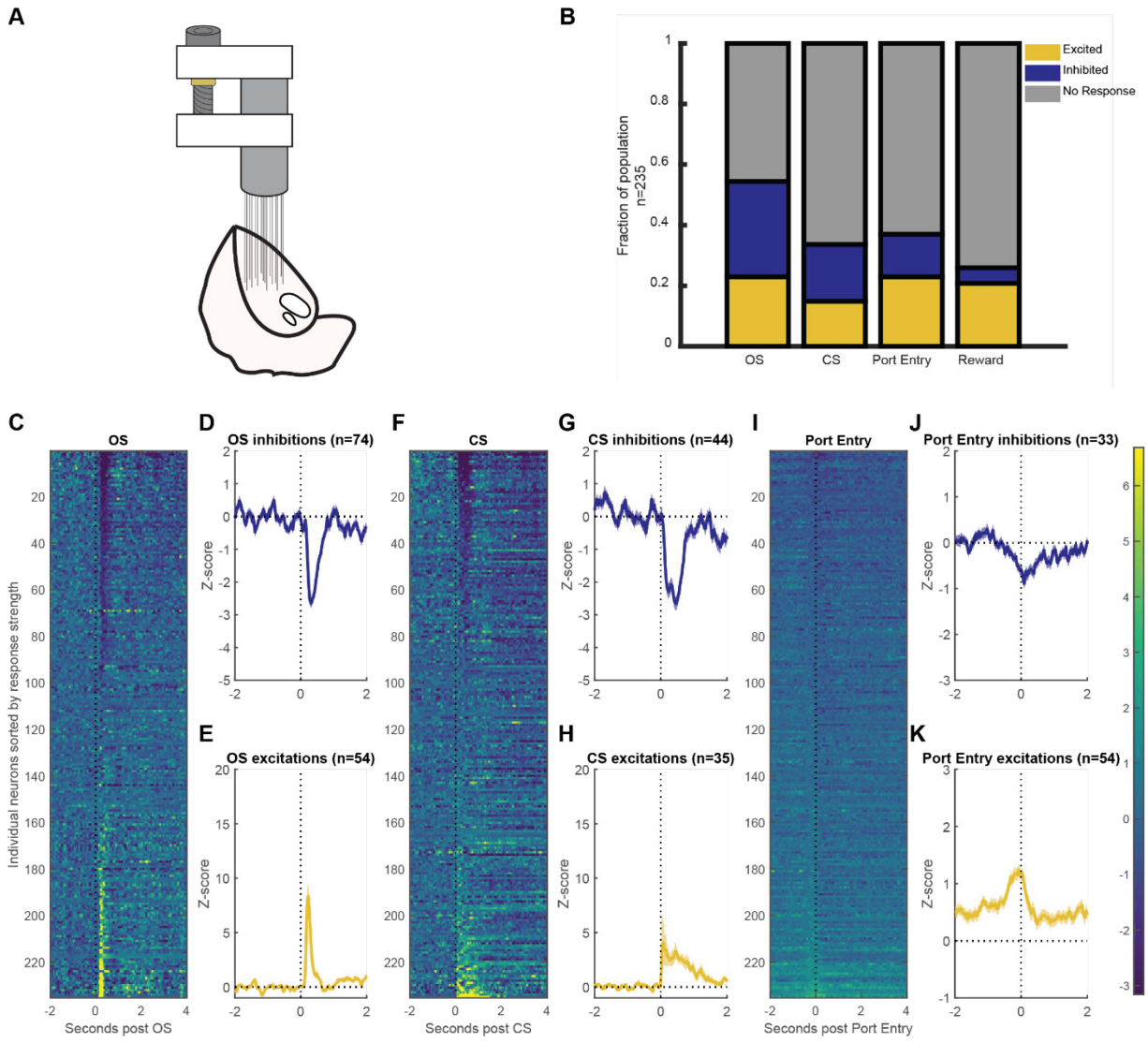

Figure 4.5. Nucleus accumbens core neuronal responses during occasion setting. ***A,*** Schematic of recording approach. ***B,*** Proportion of neurons that are significantly excited (yellow), inhibited (blue), or those with no significant response (gray) for the four relevant task-related events. ***C,*** Heatmap of individual neuron responses to the occasion setting houselight sorted by greatest inhibition at the top to greatest inhibition at the bottom. ***D,*** Mean ± SEM for neurons with significant inhibitions to the OS cue. ***E,*** Mean ± SEM for neurons with significant excitations to the OS cue. ***F-H,*** same as ***C-E*** but for the white noise conditioned stimulus. ***I-K,*** same as ***C-E*** but for entries to the

reward port. Sorting for heatmaps is independent for each event. OS, occasion setter. CS, conditioned stimulus.

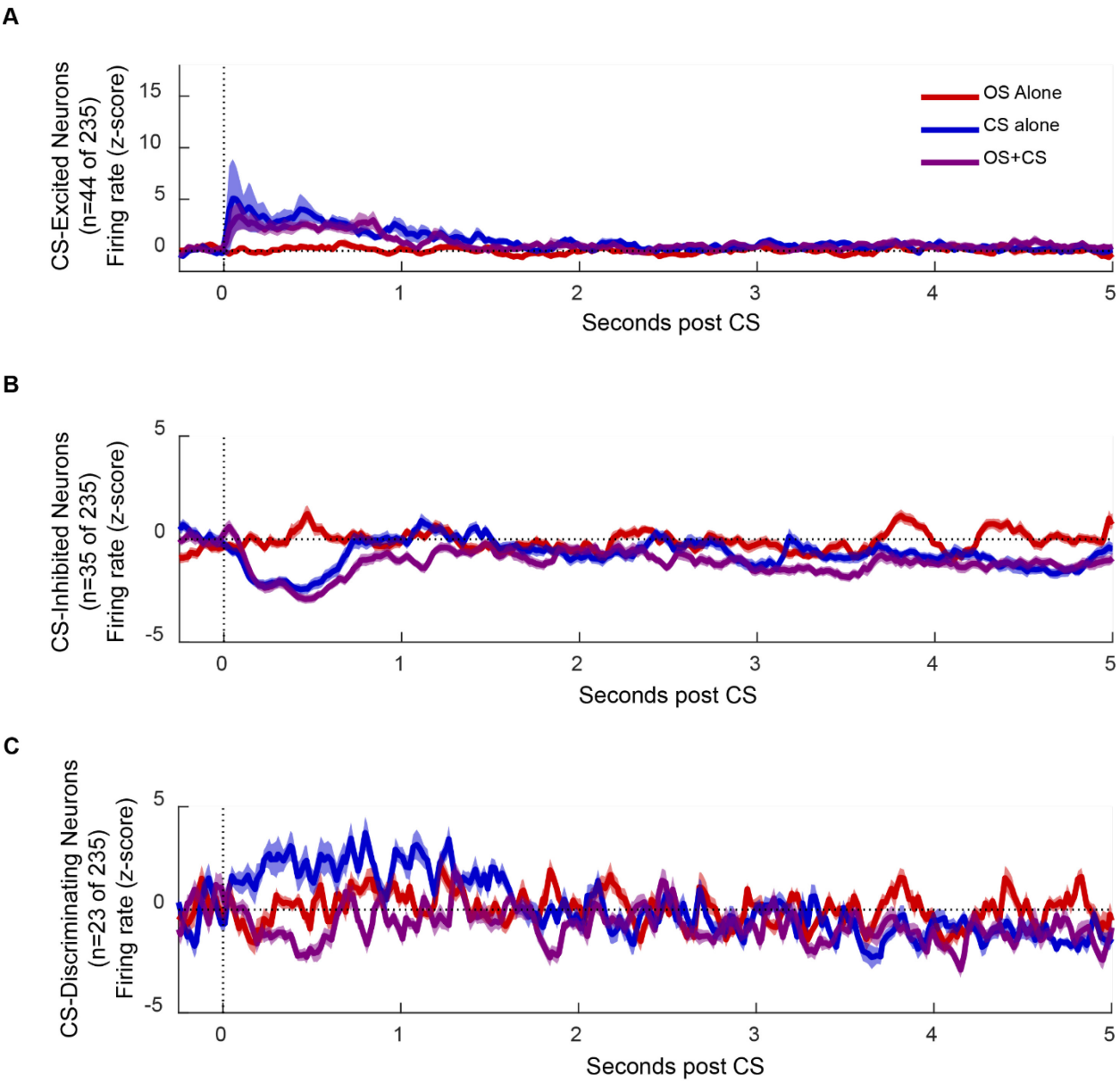

Figure 4.6. Occasion setters determine nucleus accumbens core neuronal responses to an ambiguous conditioned stimulus. ***A,*** Normalized firing rate (z-score) throughout the CS of neurons with significant excitations to the CS that are sorted by trial type. ***B,*** Normalized firing rate (z-score) throughout the CS of neurons with significant inhibitions to the CS that are sorted by trial type. ***C,*** Normalized firing rate (z-score) throughout the CS of neurons whose firing rate was identified as being

significantly different between OS+CS and CS alone trials sorted by trial type. For all figures lines indicate mean ± SEM. OS, occasion setter. CS, conditioned stimulus.

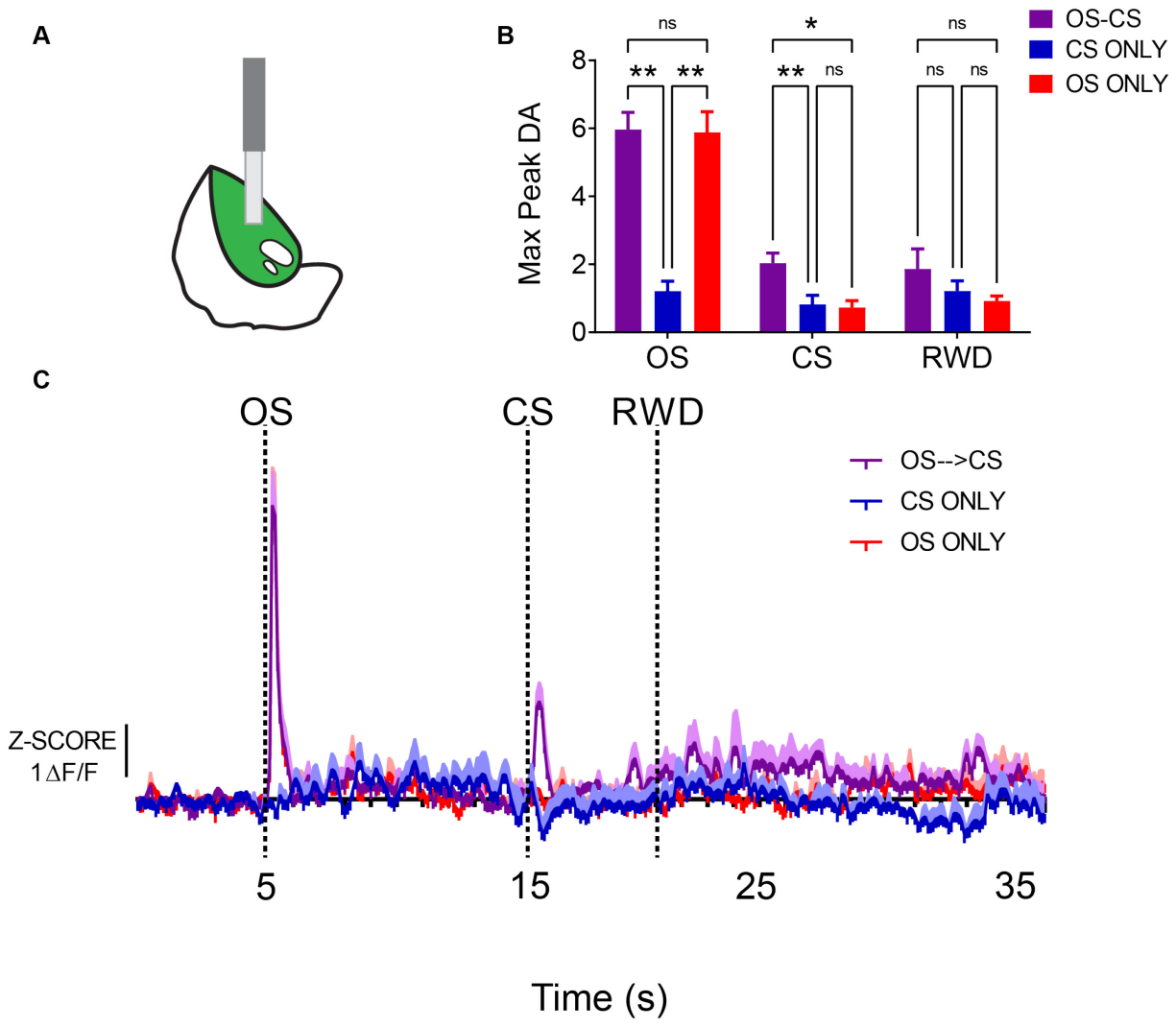

Figure 4.7. Dopamine dynamics in nucleus accumbens core during the hierarchical control of reward-seeking. ***A,*** Schematic of approach to record fluorescence from rats expressing dLight1.3b in nucleus accumbens core. ***B,*** Average peak dopamine response (z-scored ΔF/F; mean + SEM) for each event and across trial types. ***C,*** Average dopamine response (z-scored ΔF/F) throughout a trial expressed as mean +SEM.OS, occasion setter. CS, conditioned stimulus. RWD, reward delivery onset. *p<0.05

Chapter 5

Basolateral amygdala and orbitofrontal cortex, but not dorsal hippocampus, are necessary for the control of reward-seeking by occasion setters

INTRODUCTION

A given reward-paired cue may have myriad relationships with rewards, leading to difficulty ascertaining its motivational and predictive value at any given moment. To overcome this uncertainty, it is critical to make use of other environmental stimuli that resolve ambiguity about reward-predictive cues and promote flexible reward-seeking. These ambiguity-resolving cues are often referred to as occasion setters, historically termed ‘features’, as they act to set the occasion for reward-seeking but do not drive reward-seeking on their own nor acquire a direct relationship with reward (Holland, 1991, 1992; Schmajuk and Holland, 1998; Trask et al., 2017). Occasion setters are thus a unique class of Pavlovian cues that can powerfully modulate the predictive and motivational value of a traditional conditioned stimulus, historically termed the ‘target’, without engendering many of the properties associated with conditioned stimuli. Despite this, little research has explored occasion setting and its relationship to reward-seeking in comparison to standard Pavlovian conditioning preparations where conditioned stimuli have absolute relations with the presence or absence of reward. As a result, very little is known about the neurobiological basis of occasion setting despite its implications across a wide spectrum of neuropsychiatric disorders like schizophrenia and addiction (Lubow and Gewirtz, 1995; Anagnostaras and Robinson, 1996; Anagnostaras et al., 2002; Ramos et al., 2002; Valyear et al., 2017).

The basolateral amygdala (BLA) and orbitofrontal cortex (OFC) are reciprocally connected structures that are critical for exploiting acquired knowledge about reward-

predictive cues to update and guide reward-seeking (Price, 2007; Sharpe and Schoenbaum, 2016). Damage to either structure does not produce a drastic impairment in simple Pavlovian conditioning, but impairment is revealed in a variety of tasks in which acquired cue-based information must be exploited to produce adaptive behavior (Hatfield et al., 1996; Schoenbaum et al., 1998; Burns et al., 1999; Gallagher et al., 1999; Parkinson et al., 2000; Holland et al., 2001, 2002; Setlow et al., 2002; Holland and Gallagher, 2003; Pickens et al., 2003, 2005; Schiller and Weiner, 2004; Corbit and Balleine, 2005; McDannald et al., 2005, 2014; Izquierdo and Murray, 2007; Ostlund and Balleine, 2007; Ambroggi et al., 2008; Ishikawa et al., 2008; Johnson et al., 2009; Jones et al., 2012; Moorman and Aston-Jones, 2014; Lopatina et al., 2015; Lichtenberg et al., 2017; Stolyarova and Izquierdo, 2017). In addition, disruptions in the BLA impair cue-related neural activity in the OFC and damage within the OFC impairs cue-related neural activity within the BLA suggesting communication between these regions is necessary for the proper encoding of cue-reward relationships (Schoenbaum et al., 2003; Saddoris et al., 2005; Lucantonio et al., 2015; Saez et al., 2015, 2017). Within this circuit, the BLA has been proposed to encode the current state value of the environment which is then conveyed to the OFC to integrate into a broader state space that represents multiple features relevant for the current task, that can be used to relay information to downstream targets to guide behavior (Belova et al., 2008; Morrison and Salzman, 2010; Parkes and Balleine, 2013; Wilson et al., 2014; Stalnaker et al., 2015; Sharpe and Schoenbaum, 2016; Wikenheiser and Schoenbaum, 2016; Lichtenberg et al., 2017). We hypothesized that activity within either structure would be critical for occasion setting, as this task includes cue-driven state transitions that require the

constant maintenance and updating of state value to guide reward-seeking. To test this, we trained rats in an occasion setting task and then reversibly inactivated either the BLA or the OFC. These effects were contrasted with inactivation of the dorsal hippocampus (DH), a region implicated in the processing of spatial contexts and in the control of behavioral responding by physical settings (Fuchs et al., 2005; Allen et al., 2016). We reveal that neural activity in both BLA and OFC is necessary for linking cue-triggered expectations across time to resolve ambiguity about a conditioned stimulus and produce adaptive reward-seeking.

MATERIALS AND METHODS

Subjects

Subjects were 73 experimentally naïve male Long-Evans rats (Envigo, Frederick, MD) approximately 60 days of age and weighing 250-300 g on arrival. Upon arrival, rats were single-housed in ventilated cages with *ad libitum* access to food and water in a temperature- and humidity-controlled room and maintained on 12:12 light/dark cycle (lights on at 07:00). After one week of acclimation to the colony room, feeding was restricted to maintain weights at ~95% of *ad libitum* feeding weights. Food restriction was maintained for the duration of the experiment except for the post-surgical recovery period. All behavioral procedures took place between 08:00 and 12:00. All procedures were approved the Animal Care and Use Committee at Johns Hopkins University and followed the recommended guidelines in the Guide for the Care and Use of Laboratory Animals: Eighth Edition, revised in 2011.

Apparatus

Behavioral testing occurred in ten identical Med Associates conditioning chambers (St. Albans, VT) housed in sound- and light-attenuating cabinets and controlled by a computer running MedPC IV software. In the center of one wall was a fluid receptacle that was located in a recessed port. On the opposite wall near the ceiling of the chamber was a white houselight (28 V) and to the right of the houselight was a white-noise generator (10-25 kHz, 20 dB). Outside of the behavioral chamber but within the sound- and light-attenuating cabinet was a red houselight (28 V) that provided background illumination during each behavioral session. Fluids were delivered to the port via tubing attached to a 60 mL syringe placed in a motorized pump located outside each cabinet.

Pretraining

For at least two days prior to any procedures rats were extensively handled and acclimated to the experimenter. Rats were also given free access to 15% sucrose in water for 24 hours in their homecage to prevent any neophobia and accustom them to the future reward solution. At least one day after sucrose pre-exposure, pretraining was conducted in an approximately one-hour session in which rats were able to drink sucrose from the recessed reward port. During this session there were 80 2-second activations of the syringe pump (~0.07 mL per delivery) on a variable time 60 s schedule (30-90 s range). All rats consumed all sucrose delivered in the pretraining session.

Occasion Setting Task

Training in the occasion setting task took place in 3 distinct phases with each session lasting ~2 hours on average with an average inter-trial interval of 3.3 minutes.

There was one session a day. In the first phase, all trials (25 for BLA group, 30 for OFC, DH, and NAc groups) were reinforced presentations of the following sequence of events: occasion setter houselight for 5 s, a 5 s empty period, and 5 s of the conditioned stimulus white noise. Reward consisted of 5 s activation of the syringe pump resulting in delivery of ~0.18 mL of 15% sucrose in the reward port occurred immediately upon termination of the conditioned stimulus white noise. Phase one training lasted for 4 sessions. In phase two, 40% of trials were reinforced as before, but the other 60% of trials were conditioned stimulus alone trials consisting of presentation of the white noise for 5 s with no reward (25 total trials for BLA group, 30 total trials for OFC, DH, and NAc groups). Phase two training lasted for 6 sessions. In the final phase of training, rats were exposed to the full occasion setting task (Figure 1A) consisting of 10 reinforced trials, 10 conditioned stimulus alone trials, and 10 occasion setter alone trials where the houselight was activated for 5 s with no subsequent stimuli or reward. Note that this task is purely Pavlovian; there was never a response requirement. Training in the final phase lasted for 4 sessions prior to surgery.

Simple Conditioning Task

To assess the impact of BLA or OFC inactivation on Pavlovian conditioned responding separate groups of rats were instead trained in a task where a 5 s white noise was followed by 5 s activation of the reward pump containing 15% sucrose. The number of trials (30 per session) and intertrial interval (average 3.3 min) matched that of the occasion setting task and rats were trained for 14 days prior to cannula implantation. Following recovery, rats were retrained for 4 days before microinfusion testing.

Surgery

After 14 sessions of training in the occasion setting task rats were anesthetized with isoflurane (5% induction, 1-2.5% maintenance) and standard stereotaxic procedures were used to implant 22 gauge cannula (Plastics One, Roanoke, VA) 1 mm above the intended infusion site in the BLA (n=30; AP: -3.0 ML: ±5.1 DV: -7.4; with injector DV: -8.4), OFC (n=31; AP: +3.5 ML: ±2.6 DV: -4.5; with injector DV: -5.5), DH (n=12; AP: -3.7 ML: ±2.5 DV: -2.5; with injector DV: -3.5). After surgery, rats received injections of cefazolin (70 mg/kg, subcutaneous) to prevent infection and carprofen (5 mg/kg, subcutaneous) to relieve pain. At all times except for during infusions, dummy stylets were placed in each guide cannula. These coordinates were adapted from previous studies (Chaudhri et al., 2013; Keiflin et al., 2013; Lichtenberg et al., 2017). Rats were allowed to recover for one week after surgery during which they had *ad libitum* access to food and water and then were returned to food restriction. Following this period, rats were retrained in the final phase of the occasion setting or simple conditioning task for at least 4 sessions.

Infusions and Test

Rats were accustomed to the handling and infusion procedure for 1-2 days prior to infusion by being transported to the procedure room after a training session, dummy stylets removed, a flat cut 28 gauge injector inserted into each cannula, and dummy stylets replaced. On the day prior to testing, a regular injector extending 1 mm past the guide cannula was used to confirm cannula patency. At test rats received infusions of either saline or a mixture of the GABA-B and GABA-A agonists, baclofen and muscimol (1.0 mM and 0.1 mM, respectively) infused in a volume of 0.3 µL over 1 minute. After 1 additional minute to allow for diffusion away from the infusion site, injectors were

removed, dummy stylets were replaced, and rats returned to their homecage for 5-10 minutes before test. There were two tests for each rat, one in each condition, with at least one day of retraining without manipulation between. Test sessions were reinforced.

Histology

At the end of behavioral procedures, locations of cannulae were confirmed using standard histological procedures. Brains were extracted and post-fixed in 4% paraformaldehyde in 0.1 M NaPB for at least 24 hours and then cryoprotected in 25% sucrose in 0.1 M NaPB; 50 μm coronal sections were mounted onto Fisher SuperFrost Plus slides, and stained with cresyl violet (FD Neurotechnologies; Ellicott City, MD). Microinjection sites were verified by mapping their locations onto images from a rat brain atlas (Paxinos and Watson, 2007).

Experimental Design and Statistical Analysis

All data were visualized and analyzed in GraphPad Prism 7. For all hypothesis tests, $\alpha=0.05$. The primary behavioral data of interest were the effects of treatment (saline vs baclofen/muscimol) on time in port during the conditioned stimulus period on each trial type (reinforced, conditioned stimulus alone, occasion setter alone) in the occasion setting task or merely time in port between conditions for the simple conditioning task. It is important to note that on occasion setter alone trials, there is no conditioned stimulus presented, but the period analyzed is the corresponding 5 s period when the conditioned stimulus was presented on reinforced trails. These data were analyzed using a repeated measures ANOVA with within-subject factors of treatment

and trial type. Difference scores were calculated to quantify each subject's individual discrimination performance between time in port during the conditioned stimulus period on reinforced and either conditioned stimulus alone or occasion setter alone trials. These data were analyzed with a repeated measures ANOVA with treatment and discrimination (conditioned stimulus alone vs occasion setter alone) as within-subject factors. The observed effects were similar regardless of if time in port was normalized to the 10 s period prior to any stimulus onset. As a result, we present percent time in port data without normalization since there are overall increases in port time throughout the session that could increase the likelihood of observing decreases in responding with normalized measures. Intertial time in port and port entries were analyzed with two-tailed paired t-tests. When appropriate, post hoc comparisons were made with Tukey's or Bonferroni's procedure. Behavioral data for the BLA and OFC experimental groups was collected in separate sequential experiments and therefore were analyzed separately.

RESULTS

To assess the contributions of the BLA and OFC to occasion setting male rats were trained in an occasion setting task and implanted with cannula over either the BLA or OFC. In this task, rats received deliveries of sucrose if the occasion setter (OS) and conditioned stimulus (CS) were linked in time with a 5 s gap between their presentations, whereas presentations of the OS alone or CS alone were non-reinforced (Figure 1A). As a result of this configuration of cues, rats exhibit higher conditioned approach behavior to the reward port on reinforced trials during CS presentation, than on CS alone trials, or during the CS period on OS Alone trials even though the CS was

not presented. Overall, this general pattern of reward-seeking is consistent with previous reports of occasion setting in freely moving rats (Meyer and Bucci, 2016a, 2017). To determine the role of BLA and OFC in this differential responding during the CS period, rats received reversible inactivation of either structure with a mixture of the GABA-A and GABA-B agonists, muscimol and baclofen, or saline vehicle infusions in a counterbalanced manner before a reinforced session identical to that of the final phase of the occasion setting task. Only rats with microinjector tips verified within the bilateral BLA (n=10) or OFC (n=16) were included for analyses and their locations are depicted in Figure 1B and Figure 1C.

Reversible inactivation of the BLA impairs occasion setting

Under control conditions (saline infusion), rats exhibited discriminated reward-seeking, as evidenced by heightened time in the reward port on reinforced trials relative to CS alone and relative to OS alone trials, and inactivation of the BLA abolished this pattern such that rats failed to discriminate among the three trials types, responding equivalently during the CS period regardless of the prior presentation of the OS or not (Figure 2A). These observations are confirmed by a main effect of trial type ($F_{(2,18)}$=9.231, p=0.0017) and treatment ($F_{(1,9)}$=22.6, p=0.001), as well as an interaction of treatment and trial type ($F_{(2,18)}$=15.42, p=0.0001). While responding during the CS was greater following the OS relative to both CS alone (p=0.041) and OS alone trials (p<0.0001), following BLA inactivation rats did not discriminate among trial types, resulting in a similar, low level of reward seeking between reinforced and CS alone trials (p=0.9995) and OS alone trials (p=0.9999). In addition, comparing responding within trial type, inactivation of the BLA significantly reduced reward-seeking during the CS

period on reinforced ($p<0.0001$) and CS alone trials ($p<0.0001$) compared to saline infusions. Conditioned approach to the food cup was already low during OS alone trials, given there was no stimulus present during the CS period, and this was not significantly reduced by BLA inactivation ($p=0.4583$). These effects are also evident when analyzing individual rats' discrimination scores that quantify the difference in responding to the CS on reinforced trials vs either the CS alone or OS alone trials in the occasion setting task, with higher numbers reflecting better discrimination. Inactivation decreased the mean discrimination scores in both cases (Figure 2B; main effect of treatment: $F_{(1,9)}=16.42$, $p=0.0029$; post hoc CS alone discrimination: $p=0.0246$; post hoc OS alone discrimination: $p=0.0040$).These findings collectively indicate that inactivation reduced the ability to use the occasion setter to produce adaptive reward-seeking. We also examined total time in the reward port and total port entries to examine whether inactivation generally impaired activity during the 2 hour session. Inactivation did not significantly alter intertrial port entries (Figure 2C; $t_{(9)}=1.868$, $p=0.0946$) or time in the reward port (Figure 2D; $t_{(9)}=1.783$, $p=0.1083$) indicating that decreased behavior during the CS period cannot be attributable to activity impairment. Rather, the findings suggest that rats failed to organize their reward-seeking appropriately in response to the presented stimuli. In addition, all rats consumed all sucrose delivered during both test sessions, indicating that even the presence of reward was not able to overcome the ability of BLA inactivation to disrupt occasion setting.

Reversible inactivation of the OFC impairs occasion setting

In a separate group of rats, we tested whether inactivation of the OFC would alter occasion setting given its reciprocal connections with the BLA. Notably, we found that

inactivation of the OFC produced a strikingly similar pattern of impairment in the occasion setting task (Figure 3A; main effect of trial type: $F_{(2,30)}$=12.01, p=0.0001; main effect of treatment: $F_{(1,15)}$=25.95, p=0.0001; interaction of treatment and trial type: $F_{(2,30)}$=7.486, p=0.001). As above, rats exhibited discriminative reward-seeking in the task by using the occasion setter to increase reward-seeking on reinforced trials relative to CS alone (post hoc comparisons of reinforced versus CS alone: p<0.0001) and OS alone trials (p<0.0001) following saline infusions into the OFC. Similar to the findings above, inactivation of the OFC eliminated the ability of rats to use the occasion setter to guide reward-seeking and produced an overall low level of conditioned behavior during the test session (Figure 3A) as evidenced by no significant difference in time in port during the CS period between reinforced trials and either CS alone (p=0.9999) and OS alone trials (p=0.4234). For OFC, inactivation significantly reduced responding on reinforced (p<0.0001) and CS alone trials (p=0.0045), but did not reduce the already low level of responding on OS alone trials (p=.459). As a result, rats' individual discrimination between reinforced trials and CS alone or OS alone trials was abolished following reversible inactivation of the OFC (Figure 3B; main effect of treatment: $F_{(1,15)}$=0.0024; post hoc CS alone discrimination: p=0.0024; post hoc OS alone discrimination: p=0.0001). We analyzed total time in port and total port entries to assess whether the findings could be attributed to an overall lack of engagement during the test session. As with BLA inactivation, OFC inactivation did not impair overall activity during the session, but instead significantly increased intertrial port entries (Figure 3C; $t_{(15)}$=3.266, p=0.0052) and time in the reward port (Figure 3D; $t_{(15)}$=5.793, p<0.0001). In addition, all rats consumed all sucrose delivered during the test sessions. Together

these results indicate that inactivation of the OFC impaired the ability of rats to use an occasion setter to resolve ambiguity about conditioned stimuli to guide reward-seeking.

Reversible inactivation of the DH has no impact on occasion setting

We next asked whether the dorsal hippocampus, a region critical for the encoding of discrete locations in space, sequences of stimuli, and implicated in the control of responding by physical contexts may be critical in occasion setting. We trained 12 naïve rats in the occasion setting task as before and 2 rats were excluded for incorrect cannula placement (final n=10 for DH; placements in Figure 4E). In contrast to the impact of reversible inactivation with baclofen and muscimol in the BLA or OFC, there was no significant effect of inactivation of the DH on occasion setting assessed with time in port during the conditioned stimulus across trials (Figure 4A; main effect of treatment $F_{(1,9)}$=0.068, p=0.7997; main effect of trial type $F_{(2,18)}$=16.03, p=0.0001; interaction of treatment and trial type $F_{(2,18)}$=5.111, p=0.0175). Under both saline and inactivation rats exhibited more food cup responding on OS+CS trials relative to either CS Alone (saline p=0.0010; inactivation p<0.0001) and OS Alone trials (saline p<0.0001; inactivation p=0.0002), but there was no impact of inactivation on responding within any given trial (OS+CS p>0.9999; CS Alone p=0.6239; OS Alone p=0.2675). In agreement, analysis of the ability of each rat to discriminate between reinforced conditioned stimuli and non-reinforced trials revealed no significant effect of treatment (Figure 4B; $F_{(1,9)}$=0.006, p=0.9386). Despite a lack of impact on occasion setting performance, inactivation of the DH significantly increased intertrial port entries (Figure 4C; $t_{(9)}$=3.57, p=0.0060) but had no effect on the time rats spent in the port in the intertrial interval (Figure 4D; $t_{(9)}$=1.536 p=0.1589). Together these indicate that the

contributions of the DH to occasion setting are minimal, but that inactivation of the dorsal hippocampus can increase locomotor activity and disinhibit behavior in the absence of conditioned stimuli.

Inactivation of either BLA or OFC fails to impair Pavlovian conditioned responding

Finally, we sought to confirm whether the impact of inactivation of the BLA or OFC on occasion setting could be explained by a potential requirement for activity in either structure for generating conditioned responding to reward-predictive cues. Despite evidence from multiple laboratories demonstrating that lesions of either structure or reversible inactivation are generally without effect on responding to Pavlovian cues, we wondered if the procedures employed here with a brief 5s cue followed by the non-overlapping delivery of reward might alter the involvement of either structure in Pavlovian conditioned approach. Rats were implanted with cannula over either the BLA (n=8 after exclusions) or the OFC (n=10 after exclusions) and trained for an identical number of sessions in a simple Pavlovian conditioning task where a 5s white noise was always followed by sucrose reward, with matched trial timing and trial number as the occasion setting task (Figure 5A). Reversible inactivation of the BLA after training for 18 sessions in this task was without effect on conditioned responding to the white noise (Figure 5B; $t_{(8)}$=1.037, p=0.3344) but did significantly increase intertrial port time (Figure 5C; $t_{(8)}$=2.476, p=0.0425). For OFC, there was similar a lack of impairment of reversible inactivation on conditioned responding to the white noise (Figure 5E; $t_{(11)}$=2.012, p=0.0719), but inactivation did also significantly increase intertrial port time (Figure 5F; $t_{(11)}$=2.868, p=0.0167). Ultimately, there is minimal contribution of either BLA or OFC to Pavlovian conditioned approach which suggests

the extreme impairment in the occasion setting task is not due to their involvement in the generation of conditioned food cup approach.

DISCUSSION

Resolving ambiguity about reward-predictive cues is an essential tool for survival, including the proper organization of reward-seeking behavior. However, in many experiments Pavlovian reward-predictive cues are deterministic with absolute relations with the presence or absence of reward and as a result are unambiguous. To better understand situations in which conditioned stimuli are ambiguous, we took advantage of an occasion setting task that required rats to use a discrete occasion-setting cue which signaled that if a conditioned stimulus was presented shortly after the occasion setter it would be followed by reward (Holland, 1992; Fraser and Holland, 2019; Fraser and Janak, 2019), while conditioned stimulus presentations not preceded by the occasion setting cue would not be followed by reward. We assessed the contributions of the BLA and OFC in regulating behavior under these conditions as these structures have been shown to be important for the generation and exploitation of cue-elicited expectations. We demonstrate that reversible inactivation of either structure produced a profound inability of rats to use an occasion setting cue to guide adaptive reward-seeking. Inactivation also increased overall time in the reward port, particularly following OFC inactivation, suggesting an inability in using cues to guide reward-seeking as opposed to a motor impairment. These data suggest activity within these reciprocally-connected structures, and likely communication within this circuit, is critical for resolving ambiguity about reward-predictive cues.

Occasion setting mechanisms in conditioned reward-seeking

Despite extensive behavioral work, little is known with respect to the neural systems and circuits that are critical to occasion setting. Occasion setting is a rich and complex behavioral scenario that requires animals to maintain multiple cue-generated representations and link them in time to resolve the predictive and motivational meaning of a conditioned stimulus. In recent years there has been a renewed focus on negative occasion setting, a task in which the occasion-setting cue signals a conditioned stimulus will not be reinforced, thereby inhibiting reward-seeking (Holland, 1992; Meyer and Bucci, 2016a). These experiments have suggested that the OFC and nucleus accumbens, as well as the dorsal hippocampus, are involved in the acquisition of this behavior (Holland et al., 1999; Meyer and Bucci, 2016b; Shobe et al., 2017). Negative occasion setting differs in a significant number of ways from the positive occasion setting task that we used here, in which the occasion setting cue instructs the animal that it may soon encounter a conditioned stimulus that will spur reward-seeking (Holland, 1992; Meyer and Bucci, 2016a; Trask et al., 2017). Negative occasion setting requires behavioral inhibition, and negative occasion setting cues may act as conditioned inhibitors (Meyer and Bucci, 2017; Trask et al., 2017). Interestingly, evidence suggests distinct neural systems may underlie negative and positive occasion setting (Holland et al., 1999). In support of this distinction, we observed no impact of reversible inactivation of the dorsal hippocampus on positive occasion setting. To our knowledge ours is one of the first reports of a neurobiological manipulation to selectively affect positive occasion setting, laying a groundwork for future investigations into the neural circuits regulating the acquisition and expression of positive versus negative occasion setting.

Basolateral amygdala and orbitofrontal cortex are critical for occasion setting

In many situations the BLA is not necessarily critical for the acquisition and expression of simple Pavlovian conditioned responses, but is necessary for exploiting and updating previously acquired cue-based information (Hatfield et al., 1996; Setlow et al., 2002; Holland and Gallagher, 2003; Pickens et al., 2003; Johnson et al., 2009; Chang et al., 2012). In the present study, in the absence of a functioning BLA, rats were not able to retrieve the appropriate motivational information associated with either cue in the occasion setting task (Averbeck and Costa, 2017), and as a result exhibited a flat, low-level of reward-seeking regardless of the cue presented. In contrast to studies of the BLA in context-based renewal of Pavlovian conditioned responding (Chaudhri et al., 2013; Millan et al., 2015; Sciascia et al., 2015), a situation in which a context must be used to inform reward-seeking, we observed significant impairments despite testing under reinforced conditions. This suggests perhaps that the nature of the occasion setting task, characterized by the need to rapidly update cue values every few minutes, more strongly engages the BLA. That inactivation of the BLA impairs occasion setting is in agreement with suggestions the BLA encodes state value, as occasion setting requires subjects to appropriately recognize and transition between cue-driven states (Morrison and Salzman, 2010). Neurons within the BLA respond to Pavlovian cues and to discriminative stimuli that signal the availability of an action to produce reward (Paton et al., 2006; Tye and Janak, 2007; Ambroggi et al., 2008; Morrison and Salzman, 2009; Shabel and Janak, 2009; Sangha et al., 2013), but whether or how they encode occasion setters themselves remains to be observed.

The OFC, like the BLA, is largely not necessary for simple Pavlovian conditioning, but is necessary for exploiting and updating acquired cue-based representations in new situations (Gallagher et al., 1999; Pickens et al., 2003, 2005; McDannald et al., 2005; Ostlund and Balleine, 2007; Chang, 2014). We found that inactivation of the OFC impaired occasion setting in well-trained subjects, suggesting an essential role of the OFC in the ongoing expression of this behavior. While neurons in the OFC acquire responses to reward-predictive stimuli, this information is encoded and used in a manner distinct from the BLA (Schoenbaum et al., 1998; Morrison and Salzman, 2011; Morrison et al., 2011; Takahashi et al., 2013; Moorman and Aston-Jones, 2014; Lopatina et al., 2017; Shobe et al., 2017). Neurons in the OFC have been argued to encode task space and ultimately construct a composite cognitive map of all possible states and their transitions (Wilson et al., 2014; Stalnaker et al., 2015; Wikenheiser and Schoenbaum, 2016). Accordingly, we hypothesize that inactivation of the OFC eliminated the ability of rats to use state value information, perhaps arising from the BLA (Schoenbaum et al., 2003; Sharpe and Schoenbaum, 2016; Lichtenberg et al., 2017), to maintain and/or transition in this state space appropriately, seemingly rendering all cues to have similar low significance to the subject as evidenced by the similar low level of responding across trials following OFC inactivation.

BLA and OFC interactions in occasion setting

With the current approach we are unable to ascertain how communication between the OFC and BLA contributes to occasion setting. However, it is striking that the impairments in occasion setting observed after inactivation of either structure were almost identical. This suggests communication between OFC and BLA is critical for

linking cue-triggered expectations across time to produce adaptive and flexible reward-seeking. Indeed, in an odor-guided decision-making task, neural encoding of cue-based information in the OFC is dependent on neural activity within the BLA (Schoenbaum et al., 2003), and encoding in the BLA is dependent on the OFC (Saddoris et al., 2005). A recent report suggests that the BLA encodes and retrieves cue-triggered expectations and the OFC exploits this information to guide decision-making (Lichtenberg et al., 2017). It is possible, however, that the identical effects we observed on occasion setting may be mediated by a shared downstream target of the BLA and OFC, such as the nucleus accumbens, but this remains to be explored (Heilbronner et al., 2016). Paired recordings in the OFC and BLA during this occasion setting task would give insight into the unique contributions of each structure and clarify the computations occurring in each, while future investigations using chemogenetic or optogenetic tools to restrict manipulations to BLA terminals in the OFC or OFC terminals in the BLA to resolve contributions of directionality in this circuit for occasion setting.

Conclusions

The ability to resolve ambiguity surrounding reward-paired cues is essential for survival but often neglected in studies of Pavlovian conditioning. Occasion setting allows for a complex understanding of the dynamic regulation of cue-triggered reward-seeking by discrete events. The present data demonstrate that the BLA and OFC are essential neural substrates for exploiting occasion setting cues to produce flexible conditioned reward-seeking. Excessive and inappropriate pursuit of rewards is a hallmark of neuropsychiatric disorders, like addiction, that may arise from deficits in

occasion setting processes, and a better understanding of the neural and behavioral mechanisms of occasion setting could provide insights into new clinical interventions.

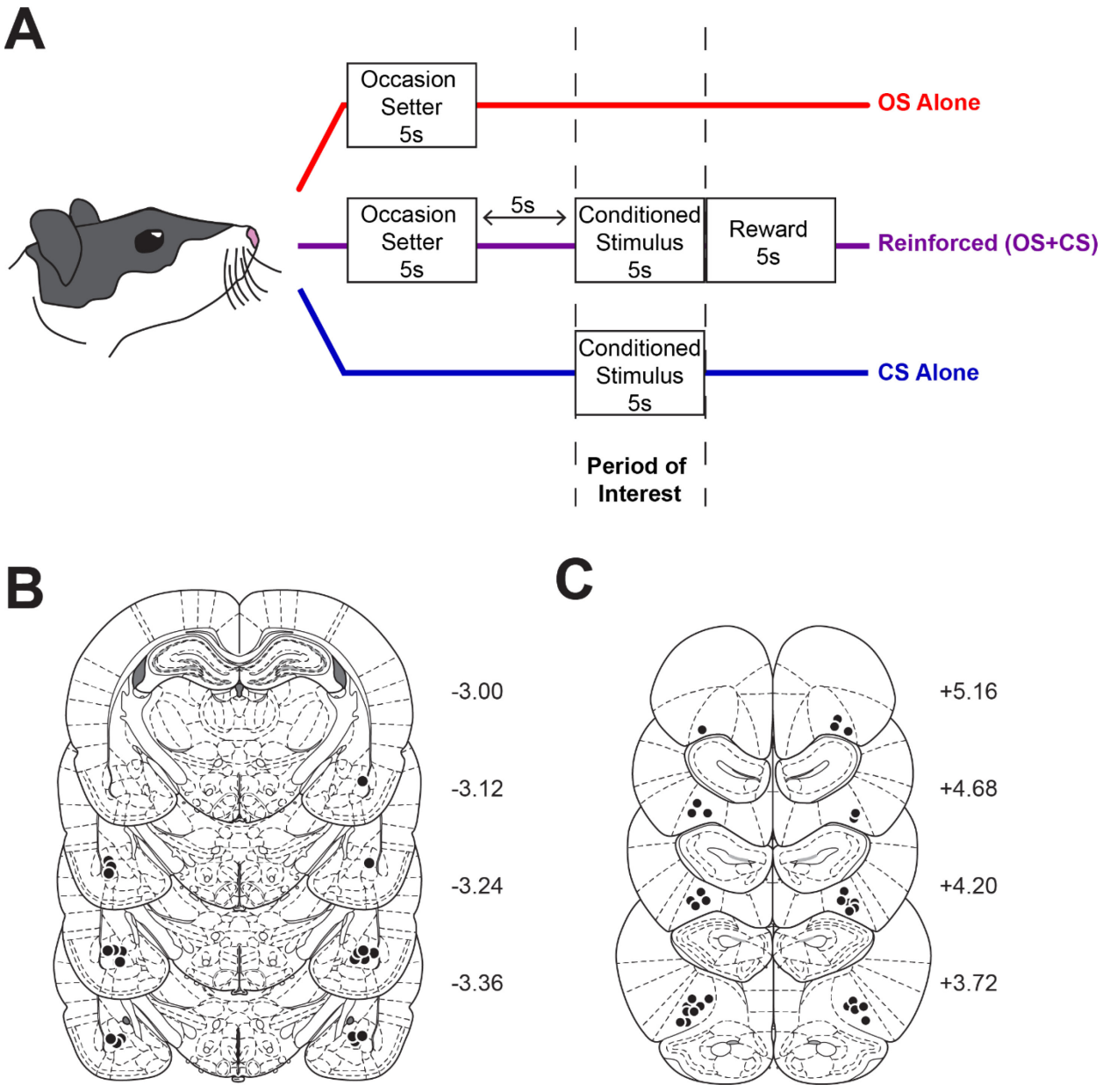

Figure 5.1. Experimental design and histological verification of microinjector tips. ***A,*** Schematic of the occasion setting task. ***B,*** Cannulae placements for rats in the BLA group (n=10). ***C,*** Cannulae placements for rats in the OFC group (n=16). Numbers indicate distance from bregma in millimeters and the coronal sections were obtained from (Paxinos and Watson, 2007).

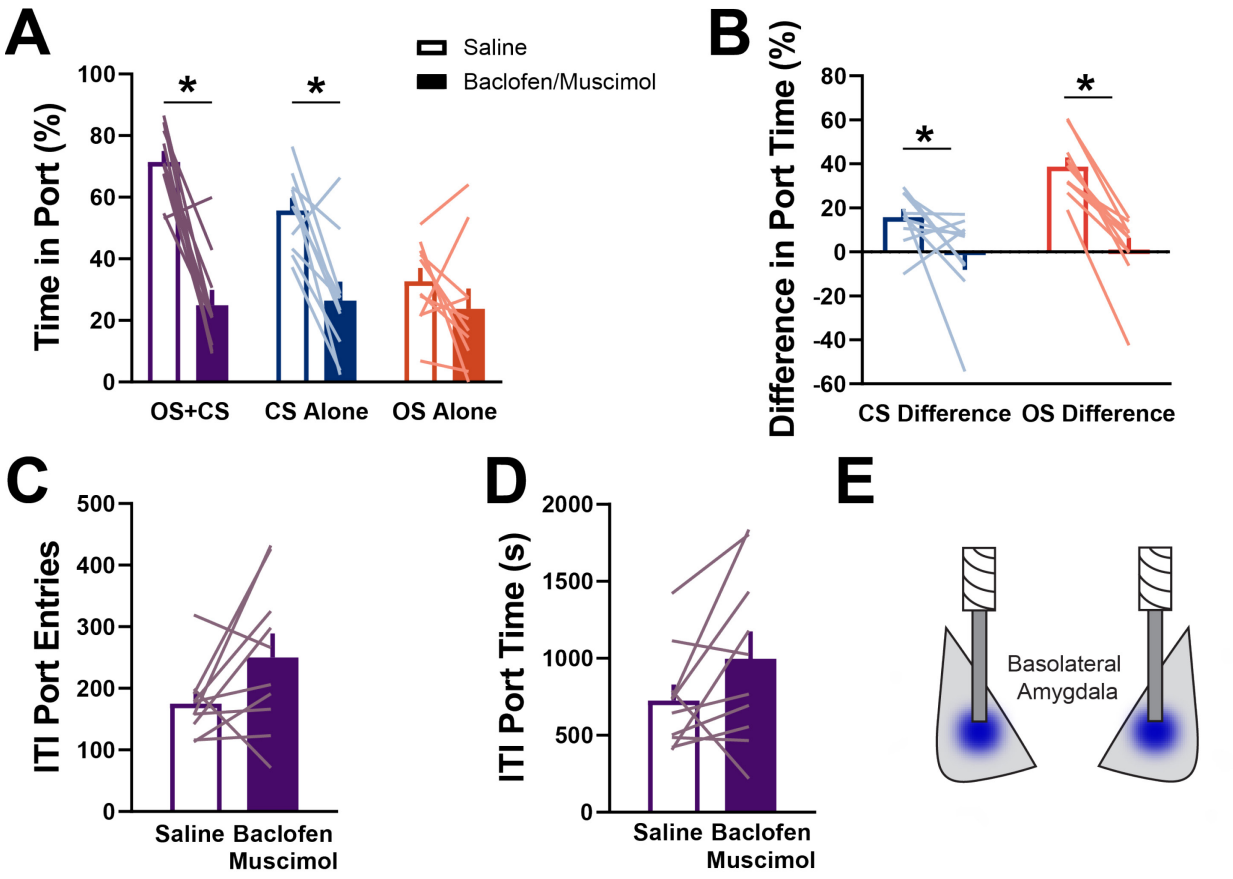

Figure 5.2. Reversible inactivation of the basolateral amygdala impairs occasion setting. ***A,*** Average time in port during the conditioned stimulus period for both test sessions. ***B,*** Individual differences in time in port during the conditioned stimulus period on reinforced trials minus either conditioned stimulus alone or occasion setter alone trials. ***C,*** Intertrial port entries during the behavioral session. ***D,*** Intertrial port time during the behavioral session. For all figures bars indicate mean ± SEM. Empty bars represent data from the saline session and filled bars from the inactivation session. Individual rats are overlaid and represented by the colored lines. OS, occasion setter. CS, conditioned stimulus. *p<0.05.

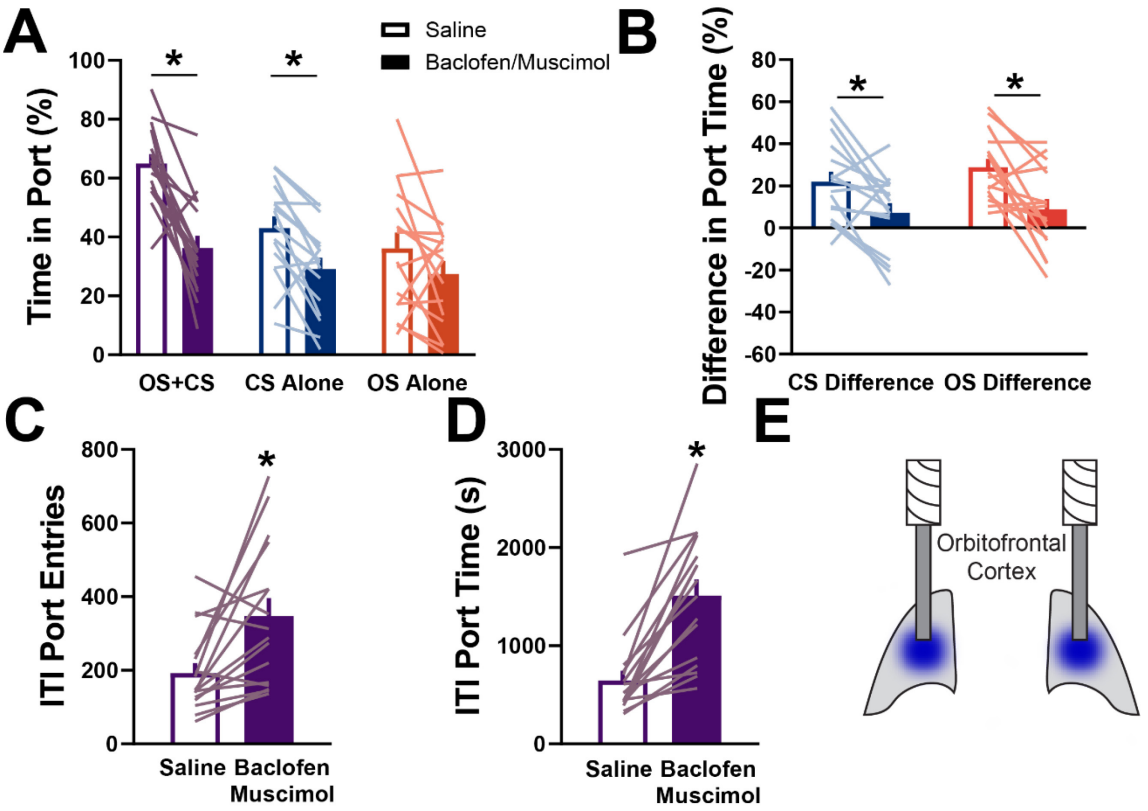

Figure 5.3. Reversible inactivation of the orbitofrontal cortex impairs occasion setting. ***A,*** Average time in port during the conditioned stimulus period for each trial type. ***B,*** Individual differences in time in port during the conditioned stimulus period on reinforced trials minus either conditioned stimulus alone or occasion setter alone trials. ***C,*** Intertrial port entries during the behavioral session. ***D,*** Intertrial port time during the behavioral session. For all figures bars indicate mean + SEM. Empty bars represent data from the saline session and filled bars data from the inactivation session. Individual rats are overlaid and represented by the colored lines. OS, occasion setter. CS, conditioned stimulus. *p<0.05.

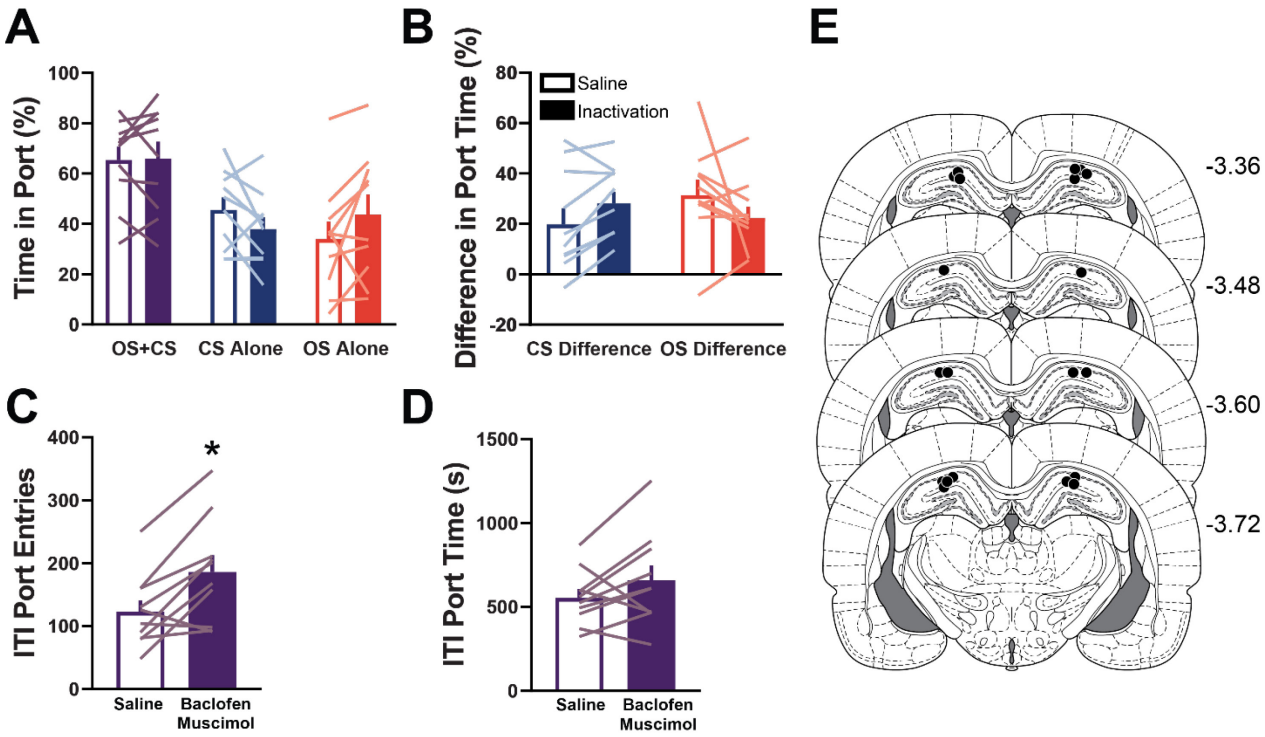

Figure 5.4. Dorsal hippocampus inactivation is without effect on occasion setting. ***A,*** Average time in port during the conditioned stimulus period for each trial type. ***B,*** Individual differences in time in port during the conditioned stimulus period on reinforced trials minus either conditioned stimulus alone or occasion setter alone trials. ***C,*** Intertrial port entries during the behavioral session. ***D,*** Intertrial port time during the behavioral session. ***E,*** Cannulae placements for DH rats (n=10). Numbers indicate distance from bregma in millimeters and the coronal sections were obtained from (Paxinos and Watson, 2007). For all figures bars indicate mean + SEM. Empty bars represent data from the saline session and filled bars data from the inactivation session. Individual rats are overlaid and represented by the colored lines. OS, occasion setter. CS, conditioned stimulus. *p<0.05.

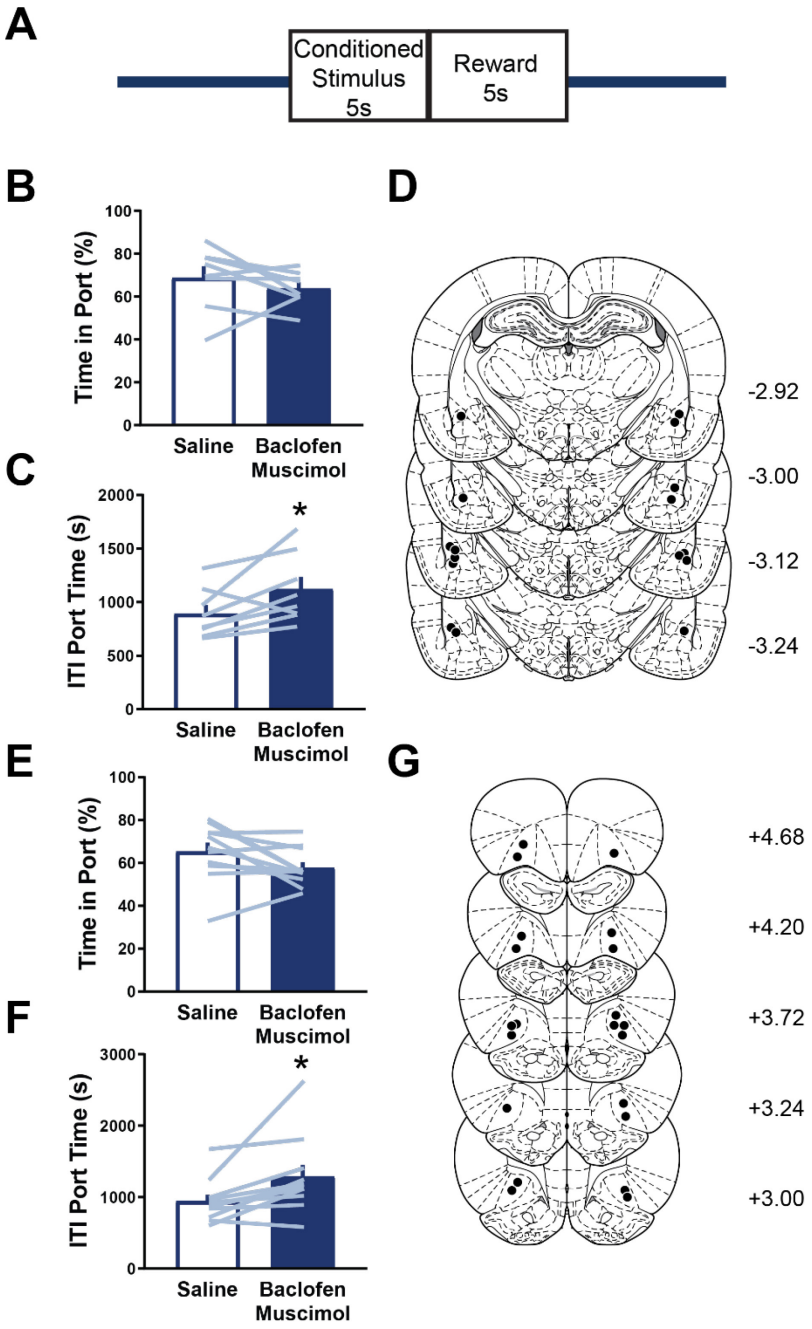

Figure 5.5. Inactivation of neither basolateral amygdala nor orbitofrontal cortex alters Pavlovian conditioned approach. ***A,*** Schematic of task design where 30 trials per session were presented with each trial being the presentation of white noise for 5 s followed by 5 s activation of a pump containing 15% sucrose. ***B,*** Time in port expressed as a percentage during the white noise. ***C,*** Intertrial time in the port during the

behavioral session. ***D,*** Cannulae placements for BLA rats (n=8). ***E,*** Time in port expressed as a percentage during the white noise. ***F,*** Intertrial time in the port during the behavioral session. ***G,*** Cannulae placements for OFC rats (n=11). Numbers indicate distance from bregma in millimeters and the coronal sections were obtained from (Paxinos and Watson, 2007). For all figures bars indicate mean + SEM. Empty bars represent data from the saline session and filled bars data from the inactivation session. Individual rats are overlaid and represented by the colored lines. *p<0.05.

Chapter 6

The basolateral amygdala encodes higher-order contextual cues and is essential for the hierarchical control of conditioned reward-seeking

INTRODUCTION

Accurately assessing the likelihood of reinforcement associated with a given conditioned stimulus is essential for adaptive behavior and survival. One way in which the significance of a given conditioned stimulus can be resolved is by exploiting the scenario in which it has been encountered. By making use of other cues, internal states, and reinforcement history it is possible to discern the immediate motivational significance of conditioned cues and set the occasion for the appropriate behavioral response (Holland, 1992; Fraser and Holland, 2019). This occasion setting process is crucial to the proper organization of reward-seeking yet its underlying neural basis is poorly investigated as it has been primarily investigated by making use of distinctly arranged operant chambers that make impossible the parsing of neural systems essential for the encoding and utilization of hierarchical stimuli that modulate reward-seeking (Holland and Bouton, 1999; Bouton et al., 2006; Fraser and Janak, 2019).

The basolateral amygdala (BLA) is one critical site proposed to underlie the proper organization of cue-controlled reward-seeking (Baxter and Murray, 2002; Morrison and Salzman, 2010; Janak and Tye, 2015). Despite a lack of role in generating conditioned approach to reward-paired cues, the BLA is essential for flexibly responding to cues whose relationship with reward has been altered either by devaluing the reward or in scenarios in which inferences must be made regarding its current relationship with an outcome (Gallagher and Holland, 1994; Hatfield et al., 1996; Holland and Gallagher, 2003; Lindgren et al., 2003; Saddoris et al., 2005; Chang et al., 2012; Sharpe and

Schoenbaum, 2016). Given this, it seems likely that the BLA is critical for the hierarchical control of reward-seeking and the resolution of uncertainty as a result of this structure encoding the current motivational value of reward-associated stimuli. Indeed, we previously reported that inactivations of the BLA were sufficient to impair occasion setting. However, the mechanisms within the BLA and its precise contributions to occasion setting have remained unclear as these prior studies have made use of manipulations that lack temporal-specificity. Here we sought to test the precise role the BLA plays in the hierarchical control of reward-seeking and identify potential mechanisms within the BLA that could explain its contribution to this higher-order process. These findings were contrasted with investigations into the contributions of the orbitiofrontal cortex (OFC) as these regions are interconnected and have been ascribed similar roles in the control of Pavlovian conditioned reward-seeking (Schoenbaum et al., 2003; Saddoris et al., 2005; McDannald et al., 2012; Lucantonio et al., 2015; Sharpe and Schoenbaum, 2016). Interestingly, we find that the BLA and not OFC is necessary for occasion setters to update conditioned responding to an ambiguous conditioned stimulus and that neural encoding of occasion setters is more profound in BLA neurons than OFC neurons. These findings point to an essential role for BLA, but not OFC, and its related circuitry in the hierarchical control of reward-seeking.

MATERIALS AND METHODS

Subjects

Subjects were 12 experimentally naïve male Long-Evans rats obtained from ENVIGO. Rats were single-housed in ventilated cages with *ad libitum* access to food and water in a temperature- and humidity-controlled room and maintained on 12:12

light/dark cycle (lights on at 07:00). After recovery from surgery, feeding was restricted to maintain weights at ~95% of *ad libitum* feeding weights. All behavioral procedures took place between 08:00 and 13:00. All procedures were approved by the Animal Care and Use Committee at Johns Hopkins University and followed the recommended guidelines in the Guide for the Care and Use of Laboratory Animals: Eighth Edition, revised in 2011.

Surgery

Rats were anesthetized with isoflurane (5% induction, 1-2.5% maintenance) and standard stereotaxic procedures were used. Rats for the optogenetics experiments were infused with a viral vector containing archaerhodopsin (AAV5-CamKIIa-ArchT-eYFP; titer 1.9 x 10^{13} viral particles/mL; Addgene) or a control virus (AAV5-CamKIIa-eYFP; titer 4.3 x 10^{12} viral particles/mL; Addgene) bilaterally into the BLA (AP: -2.8 ML: ±5.1 DV: -8.4) or OFC (AP: +3.5 ML: ±2.6 DV: -5.5). A volume of 500 nL of virus was infused per site at a rate of 100 nL/min through a 31-G, gas-tight Hamilton syringe controlled by a Micro4 Ultra Microsyringe Pump 3 (World Precision Instruments). The needle was left in place for 10 minutes following the infusion to allow for diffusion of the virus from the injection site. In the same surgery, rats were bilaterally implanted with custom made optic fiber implants (300 µm glass diameter) targeted 200 µM dorsal to the infusion site. For electrophysiological recordings, a custom-built microdrive containing 16 50 µm tungsten wires soldered to 8-pin Omnetics connectors was lowered slowly to the OFC (n=7; AP: +3.5 ML: ±2.6 DV: -4.5) or BLA (n=5; AP: -2.8 ML: ±5.1 DV: -7.4) with a silver reference wire wrapped around a screw in contact with the cerebellum. Implants were secured to the skull with 4-5 screws placed in the skull and

dental acrylic. After surgery, rats received injections of cefazolin (70 mg/kg, subcutaneous) to prevent infection and carprofen (5 mg/kg, subcutaneous) to relieve pain. Rats were allowed to recover for 10 days prior to the beginning of behavioral procedures, with at least 4 weeks passing before any optogenetic manipulations were performed.

Apparatus

Behavioral testing occurred in Med Associates conditioning chambers (St. Albans, VT) housed in sound- and light-attenuating cabinets and controlled by a computer running MedPC IV software. In the center of one wall was a fluid receptacle that was located in a recessed port. On the opposite wall near the ceiling of the chamber was a white houselight (28 V) and to the right of the houselight was a white-noise generator (10-25 kHz, 20 dB). Outside of the behavioral chamber but within the sound- and light-attenuating cabinet was a green houselight (28 V) that provided background illumination during each behavioral session. Fluids were delivered to the port via tubing attached to a 60 mL syringe placed in a motorized pump located outside each cabinet.

Behavioral Procedures

Behavioral procedures were generally identical to those we have previously published (Fraser and Janak, 2019). Briefly, Training in the occasion setting task took place in 3 distinct phases with each session lasting ~2 hours on average with 30 total trials and an average inter-trial interval of 3.3 minutes. There was one session a day. In the first phase, all trials were reinforced presentations of the following sequence of events: occasion setter houselight for 5 s, a 5 s empty period, and 5 s of the conditioned

stimulus white noise. Reward consisted of 5 s activation of the syringe pump resulting in delivery of ~0.18 mL of 15% sucrose in the reward port occurred immediately upon termination of the conditioned stimulus white noise. Phase one training lasted for 4 sessions. In phase two, 40% of trials were reinforced as before, but the other 60% of trials were conditioned stimulus alone trials consisting of presentation of the white noise for 5 s with no reward. Phase two training lasted for 6 sessions. In the final phase of training, rats were exposed to the full occasion setting task (Figure 1A) consisting of 10 reinforced trials, 10 conditioned stimulus alone trials, and 10 occasion setter alone trials where the houselight was activated for 5 s with no subsequent stimuli or reward. Note that this task is purely Pavlovian; there was never a response requirement. Training in the final phase lasted for 8 sessions prior to optogenetic manipulations or electrophysiological recordings began. Fiber photometric recordings occurred throughout phase 3.

Optogenetic Manipulations

Following training rats were habituated to the procedures necessary for light delivery through their surgically implanted optic fibers. Rats were held gently by the experiment and connected via a ceramic mating sleeve bilaterally to 200 μm core patch cords (Doric), which were connected to a fiber optic rotary join (Doric), connected to a separate 200 μm patch cord that interfaced with a 532 nm DPSS laser (Opto-Engine LLC). Laser delivery was controlled by transistor-transistor logic pulses from MedPC SmartCTRL cards that interfaced with a Master9 Stimulus Controller (AMPI), which dictated the duration of stimulation. During tests, constant laser light (15-20 mW) was delivered bilaterally for a constant duration of either 15.5s, being presented 0.5s prior to

the OS period and lasting until the end of the CS period, or for 5.5s beginning 0.5 s before either the houselight or white noise (or the time in which they would be presented in CS Alone and OS alone trials). During optogenetic tests the number of trials per session was doubled, with 60 total trials (20 of each), during which half of the trials were associated with light delivery. Optogenetic testing was done under extinction conditions without reward delivery. The order of testing was counterbalanced.

Electrophysiological Recordings

Electrical signals and behavioral events were collected from freely behaving animals with the OmniPlex (Plexon) recording system as we have previously described (Ottenheimer et al., 2018, 2020). Waveforms were sorted into units offline using Offline Sorter software (Plexon), and any units that were not recorded throughout a behavioral session were discarded. We only included neurons that were identified as single-units through careful examination of auto and cross correlations, plotting waveform features over time to ensure stability and continuity, and discarded units with more than 0.2% of spikes within a 2-ms refractory period.

Histology

Following the conclusion of experiments, rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with 4% paraformaldehyde. Brains were post-fixed for 24 hours in 4% paraformaldehyde, cryoprotected in 25% sucrose in 0.1 M NaPB, and then sectioned on a freezing cryostat at -20° C in 50 um sections. Sections from rats in the optogenetic experiments were processed for detection of GFP using fluorescent immunohistochemistry. Sections were first washed in 0.1 M PBS containing 0.2% Triton-X (PBST) for 20 minutes and blocked for 30 minutes in 0.1M

PBST containing 10% normal donkey serum. Primary antibody incubation (mouse anti-GFP, 1:1500, Invitrogen A11120, Lot#2180270) occurred overnight at 4C. The following day sections were washed in PBST for 15 minutes, blocked in PBS containing 2% normal donkey serum for 10 minutes, then secondary antibody incubation (Alexafluor 488 donkey anti-mouse, 1:200, Invitrogen A21202, Lot# 2229195) occurred at room temperature for 2 hours. Following 15 minutes of washing in PBS sections were mounted onto Fisher SuperFrost Plus slides and coverslipped with Vectashield mounting medium containing DAPI. Brain sections were imaged with a Zeiss Axio 2 microscope for the reconstruction of final placements of optic fibers and virus expression. Final positions of electrodes in the brain were visualized by staining with cresyl violet (FD Neurotechnologies).

Statistics and Analysis

The primary behavioral metrics of interest for all tests was the time spent in port (indicated here as a percentage of the 5s stimulus) during the white noise in the occasion setting task or the tones and lights in the probabilistic conditioning tasks. We normalized behavioral responding by subtracting average time in port measured in a period 10 s prior to the presentation of any stimuli. We also computed differences between responding on trials in which the white noise would be reinforced (OS→CS trials) and trials in which the white noise (CS Alone) was presented alone or the houselight presented alone (OS Alone) to allow for an assessment of each individual rat's representative ability to discriminate amongst the trial types. Repeated measure ANOVAs were used to compare time in port across trial types or difference scores within virus groups by light delivery for each test separately. When applicable, post hoc

comparisons were made with Sidak's correction. Electrophysiological analysis was identical to as described in Chapter 4. Statistical comparisons and graphics were made with Prism 9 (GraphPad) and MATLAB (Mathworks). For all statistical tests α=0.05.

RESULTS

Optogenetic inhibition of the BLA impairs occasion setting

We tested the impact of brief inhibitions of the basolateral amygdala (BLA) during occasion setting performance. Following exclusions for misplaced virus and cannula the final group sizes were BLA ArchT n=8 and BLA GFP n=12 (Figure 1A). We first tested the ability of light delivery throughout both the OS and CS on occasion setting performance. For rats with ArchT there was no apparent effect of light delivery on reward-seeking overall (Figure 1B; effect of laser $F_{(1,7)}$=1.407, p=0.2742; effect of trial type $F_{(1,8)}$=6.576, p=0.0292; interaction $F_{(1,9)}$=3.131, p=0.0988) yet when examining the average discrimination for each rat there was an overall impact of light delivery reducing discrimination amongst to be rewarded OS→CS trials and CS alone and OS alone trials (Figure 1C; effect of laser $F_{(1,7)}$=13.93, p=0.0073). There was no impact of this same manipulation for rats with GFP in the BLA on either overall reward-seeking (Figure 1D; effect of laser $F_{(1,11)}$=0.4562, p=0.5134; effect of trial type $F_{(1,19)}$=15.38, p=0.0002; interaction $F_{(1,18)}$=3.131, p=0.4027) or discrimination in the occasion setting task (Figure 1E; effect of laser $F_{(1,11)}$=1.312, p=0.2763). We next tested whether light delivery just the OS could recapitulate these effects. Surprisingly, there was no significant effect of laser delivery during the OS on performance (Figure 1F; effect of laser $F_{(1,7)}$=2.321, p=0.1714; effect of trial type $F_{(1,8)}$=15.22, p=0.0031; interaction $F_{(1,12)}$=0.8667, p=0.4293) or discrimination for ArchT rats (Figure 1G; effect of laser $F_{(1,7)}$=0.7851, p=0.4050).

Interestingly, GFP rats exhibited higher levels of responding when light delivery was presented during the OS (Figure 1H; effect of laser $F_{(1,11)}$=8.568, p=0.0138; effect of trial type $F_{(1,17)}$=19.08, p<0.0001; interaction $F_{(1,19)}$=1.7, p=0.2095) but this did not impact discrimination for these rats (Figure 1I; effect of laser $F_{(1,11)}$=2.351, p=0.1535). Finally, we tested the impact of light delivery during the CS. For ArchT rats, inhibition of the BLA did not have an overall impact on conditioned reward-seeking (Figure 1J; effect of laser $F_{(1,7)}$=1.061, p=0.3372; effect of trial type $F_{(111)}$=9.982, p=0.0045; interaction $F_{(1,9)}$=2.188, p=0.1684) but planned comparisons revealed an impact of light delivery on significantly reducing discrimination achieved (Figure 1K; p<0.05 for both CS and OS difference). There was no effect of light delivery during the CS for reward-seeking (Figure 1L; effect of laser $F_{(1,11)}$=0.7690, p=0.3993; effect of trial type $F_{(1,20)}$=18.31, p<0.0001; interaction $F_{(1,19)}$=1.7, p=0.3938) or discriminations among trials (Figure 1M; effect of laser $F_{(1,14)}$=0.8776, p=0.5218) for GFP rats. Taken together these data suggest a role for the BLA in utilizing occasion setters to update conditioned responding at the time of an ambiguous conditioned stimulus.

Neural correlates of occasion setting within BLA

To assess potential correlates of occasion within BLA itself, we recorded 115 neurons from 5 rats during occasion setting performance (Figure 2A). Interestingly comparable numbers of neurons in the BLA were significantly modulated by the OS and CS (Figure 2B; 34% vs 41%) and cues evoked the most modulation among task events within neurons that we recorded. There was no significant difference in the degree of excitation (p=0.7039) or inhibition (p=0.1292) between BLA neurons with significant responses to the OS or CS. We then sought to determine whether BLA neurons might

exhibit variations in responses to the CS depending on the prior presentation of the occasion setter.

We found that although neurons that were excited (Figure 3A) and inhibited (Figure 3B) in their response to the CS by trial type (excited $F_{(2,47340)}$=1894, p<0.0001; inhibited $F_{(2,26826)}$=459.7, p<0.0001) these were reflective of significant differences in activity common to OS+CS and CS alone trials (p<0.0001 against OS alone trials for each comparison). We wondered if potentially a subset of neurons may exhibit modulation as the BLA and turned to neurons that would exhibit significant modulation to both the OS and CS as plasticity of sensory specific modalities into the BLA is a hallmark of learning and this nucleus frequently exhibits multisensory responses (Quirk et al., 1995; Tye et al., 2008; Morrow et al., 2019). We identified 17 neurons that were CS- and OS-excited (54% of CS-excited neurons) and no neurons that were CS- and OS-inhibited. When analyzing these neurons in their response to the CS across trials we found that these neurons exhibited a greater CS-evoked excitation on OS+CS trials relative to CS alone trials (Figure 3C; interaction of time and trial type $F_{(1050,25248)}$=2.771, p<0.0001; p<0.05 from 0.12s to 0.27s post CS onset). These data, coupled with the finding that optogenetic inhibition of BLA activity during the CS reduces discrimination in this task, suggest that the BLA encodes and resolves the uncertainty of an ambiguous CS by exploiting hierarchical stimuli to modulate cue-generated excitations.

Optogenetic inhibition of the OFC is without effect on occasion setting

We previously demonstrated that reversible inactivation of OFC is able to impair occasion setting and asked when neural activity in OFC may be critical for occasion setting performance. Following exclusions for misplaced cannula or virus infusions final

group sizes were OFC ArchT n=8 and OFC GFP n=12 (Figure 4A). First we tested if light delivery throughout both stimuli would replicate the effect of reversible inactivation. There was no impact of light delivery on reward-seeking for either rats with ArchT (Figure 4B; effect of laser $F_{(1,7)}$=1.032, p=0.3436; effect of trial type $F_{(1,13)}$=18.32, p=0.0002; interaction $F_{(1,13)}$=1.532, p=0.2505) or GFP (Figure 4D; effect of laser $F_{(1,11)}$=0.003, p=0.9560; effect of trial type $F_{(1,21)}$=15.38, p<0.0001; interaction $F_{(1,16)}$=0.2475, p=0.7159). As a result discriminations among trial types were unaffected for both ArchT (Figure 4C; effect of laser $F_{(1,7)}$=1.538, p=0.2548) and GFP (Figure 4E; effect of laser $F_{(1,11)}$=0.3212, p=0.5823) groups. We then tested if light delivery during the OS would impair occasion setting but again found a lack of effect on reward-seeking for both ArchT (Figure 4F; effect of laser $F_{(1,7)}$=0.0389, p=0.8492; effect of trial type $F_{(2,14)}$=17.66, p=0.0001; interaction $F_{(2,14)}$=1.482, p=0.2608) and GFP rats (Figure 4H; effect of laser $F_{(1,11)}$=0.009, p=0.9237; effect of trial type $F_{(2,22)}$=25.90, p<0.0001; interaction $F_{(2,22)}$=0.7208, p=0.4975). There was no effect of light delivery on discrimination for ArchT (Figure 4G; effect of laser $F_{(1,7)}$=1.356, p=0.2824) or GFP rats (Figure 4I; effect of laser $F_{(1,11)}$=0.8745, p=0.3698). Finally we tested the impact of light delivery during the CS on occasion setting, yet observed no impact on reward-seeking for ArchT rats (Figure 4J; effect of laser $F_{(1,7)}$=0.4693, p=0.5153; effect of trial type $F_{(2,14)}$=13.76, p=0.0005; interaction $F_{(2,14)}$=1.782, p=0.2044) nor GFP rats (Figure 4L; effect of laser $F_{(1,11)}$=0.1322, p=0.7231; effect of trial type $F_{(1,21)}$=23.05, p<0.0001; interaction $F_{(1,19)}$=2.078, p=0.1555) nor on discriminations for either ArchT (Figure 4K; effect of laser $F_{(1,7)}$=2.462, p=0.1606) or GFP rats (Figure 4M; effect of laser $F_{(1,11)}$=3.285, p=0.0972). These data suggest that if the OFC is critical for occasion

setting then its contributions are resistant to transient manipulations of neural activity in this region.

Recordings of OFC neural activity during occasion setting

We turned to *in vivo* electrophysiological recordings of individual OFC neurons to potentially identify neural correlates that would reveal what the contribution, if any, of OFC is to occasion setting (Figure 5A). Surprisingly, the event that evoked the highest degree of modulation within OFC neurons was entries to the reward port (Figure 5B; 52%), with relative degrees of modulation to the OS (15%) and CS (16%) being quite low in comparison to what we observed in BLA and also in what we have reported for nucleus accumbens. We next analyzed whether the overall strength of response in the 500 ms post cue-onset differed among the CS and OS (Figure 5D-E; G-H). There were no significant differences in the degree of excitation ($p=0.7730$) or inhibition ($p=0.9626$) between OFC neurons with significant responses to the OS and CS. As an additional attempt to observe potential correlates of occasion setting we examined neurons with either CS-excitations or CS-inhibitions across trial types. Despite overall modulation of CS-excited (Figure 6A) and CS-inhibited (Figure 6B) OFC neurons by trial type (excited neurons $F_{(2,20514)}=595.3$, $p<0.0001$; inhibited neurons $F_{(2,11046)}=94.55$, $p<0.0001$) these were reflected by similar changes in activity for OS+CS and CS alone trials relative only to OS alone trials ($p's<0.05$), which is to expected as there is no CS presented on OS alone trials, with no difference between OS+CS and CS alone trials. Given the minimal number of CS-modulated neurons and the lack of effect of optogenetic inhibition of OFC on occasion setting, the contribution of OFC to the hierarchical control of Pavlovian cue-triggered reward-seeking remains unclear.

DISCUSSION

The neural circuits underlying the flexible control of cue-triggered motivation remain unclear. Here we made use of optogenetics and *in vivo* electrophysiology to assess the contributions of basolateral amygdala and orbitofrontal cortex to the regulation of conditioned reward-seeking by occasion setters. Despite a similar impact of reversible inactivation of either structure on performance in this task, we find a selective effect of optogenetic inhibition of the BLA on the utilization of occasion setters to disambiguate the relationship between a conditioned stimulus and reward. Further, we identify correlates of occasion setting in single neurons within the BLA and not OFC. Collectively these data suggest that the BLA and its related circuitry, but not OFC, provide an essential contribution to the ongoing resolution of cue-generated uncertainty.

The essential contribution of the BLA to occasion setting builds on a number of studies demonstrating the necessity in a number of behaviors that are considered “model-based”. BLA neural activity is necessary for reinforcer devaluation, outcome-specific Pavlovian-to-instrumental transfer, and second-order conditioning as has been demonstrated with primarily lesions of the BLA (Gallagher and Holland, 1994; Hatfield et al., 1996; Baxter and Murray, 2002; Holland and Gallagher, 2003; Lindgren et al., 2003; Sharpe and Schoenbaum, 2016). Despite this, the precise contributions of BLA to these processes has remained unclear as lesions interrupt BLA activity permanently. Exploiting the temporal precision of optogenetics we reveal a time-limited contribution of BLA activity to occasion setting as only inhibitions overlapping with the conditioned stimulus impaired performance. This suggests BLA utilizes a previously presented occasion setter to resolve uncertainty during a conditioned stimulus. This was

accompanied by findings of single neurons in the BLA that strongly encoded these occasion setting cues and evidence that BLA neurons show trial-to-trial modulation of responses to the conditioned stimulus. These findings suggest a complex contribution of BLA to the resolution of uncertainty in potentially disambiguating the current motivational significance of Pavlovian cue for downstream circuits.

Despite a role for the BLA, we found little evidence of neural encoding nor a time-limited contribution of activity in the OFC for occasion setting. This was surprising to us given a number of demonstrations that interactions between OFC and BLA shape cue and outcome related neural activity in each structure and that activity in the OFC is necessary for a number of higher-order phenomena in Pavlovian conditioning (Schoenbaum et al., 2003; Saddoris et al., 2005; McDannald et al., 2012; Takahashi et al., 2013; Lucantonio et al., 2015). However, the contributions of the OFC to occasion setting may be resistant to transient manipulations of OFC neural activity, and that limited removal of this structure is without effect given that its functions can be overtaken by other regions like the BLA (Sharpe and Schoenbaum, 2016). This may potentially explain the discrepancy between our prior finding that reversible inactivation of the OFC impaired occasion setting, as this manipulation likely was profound enough to interrupt not only OFC activity but also that of its downstream structures like the BLA. It is possible that optogenetic manipulations of OFC performed during learning, when rats are expected to construct a map of possible relations among the stimuli in this task, could affect behavior. Interestingly, this hypothesis would support a recent demonstration that optogenetic inactivation of the OFC is without effect on value-guided choice behavior once it has been established, but can have effects when rats are

learning about their relative preferences among options (Gardner et al., 2017, 2019, 2020; Zhou et al., 2020). Combining manipulations of OFC during learning and potentially in combination with circuit-defined optogenetic manipulations will be critical to assessing its role in hierarchical reward-seeking.

That the BLA encoded occasion setters was suggested by numerous findings that this region is essential in utilizing contexts to influence conditioned reward-seeking (Chaudhri et al., 2013; Millan et al., 2015; Sciascia et al., 2015). By exploiting the ability of occasion setters to have precise and discrete presentations that are non-overlapping with their conditioned stimuli we report for the first time the widespread encoding of these hierarchical stimuli in the BLA. Interestingly, the BLA also encodes discriminative stimuli, which disambiguate when a given action will lead to reward (Ambroggi et al., 2008; Ishikawa et al., 2008). The manner in which occasion setters and discriminative stimuli influence reward-seeking is similar in that they resolve the uncertainty surrounding a separate discrete event, although discriminative stimuli themselves are essentially always reward-predictive while we made use of occasion setters that on their own are not predictive of reward. The BLA is critical for the encoding of discriminative stimuli within the nucleus accumbens, where inactivation of the BLA reduces excitations in the nucleus accumbens to discriminative stimuli (Ambroggi et al., 2008; Ishikawa et al., 2008). We previously reported that a majority of nucleus accumbens neurons are significantly modulated by occasion setting stimuli which suggests that input from the BLA is additionally necessary for both these neural responses in the nucleus accumbens and that projection-specific manipulations of this circuit will affect occasion setting.

In conclusion, we exploited the ability of occasion setting to reduce a context to a brief and phasic event to assess the precise contributions of the BLA and OFC to the hierarchical control of reward-seeking. These findings leave open numerous possibilities for how the BLA and its related circuitry control the resolution of cue-generated uncertainty, potentially by interacting with downstream striatal circuits. Paired recordings of neural activity within BLA and OFC during training will be essential for clarifying discrepancies among the impacts of reversible inactivation and optogenetic inhibition on the contribution of OFC to this dynamic process. Collectively an increased investigation into the circuits responsible for the modulation of the motivational value of reward-paired cues has the potential to identify novel treatments for substance use disorders and post-traumatic stress disorders which are characterized by the improper regulation of motivation.

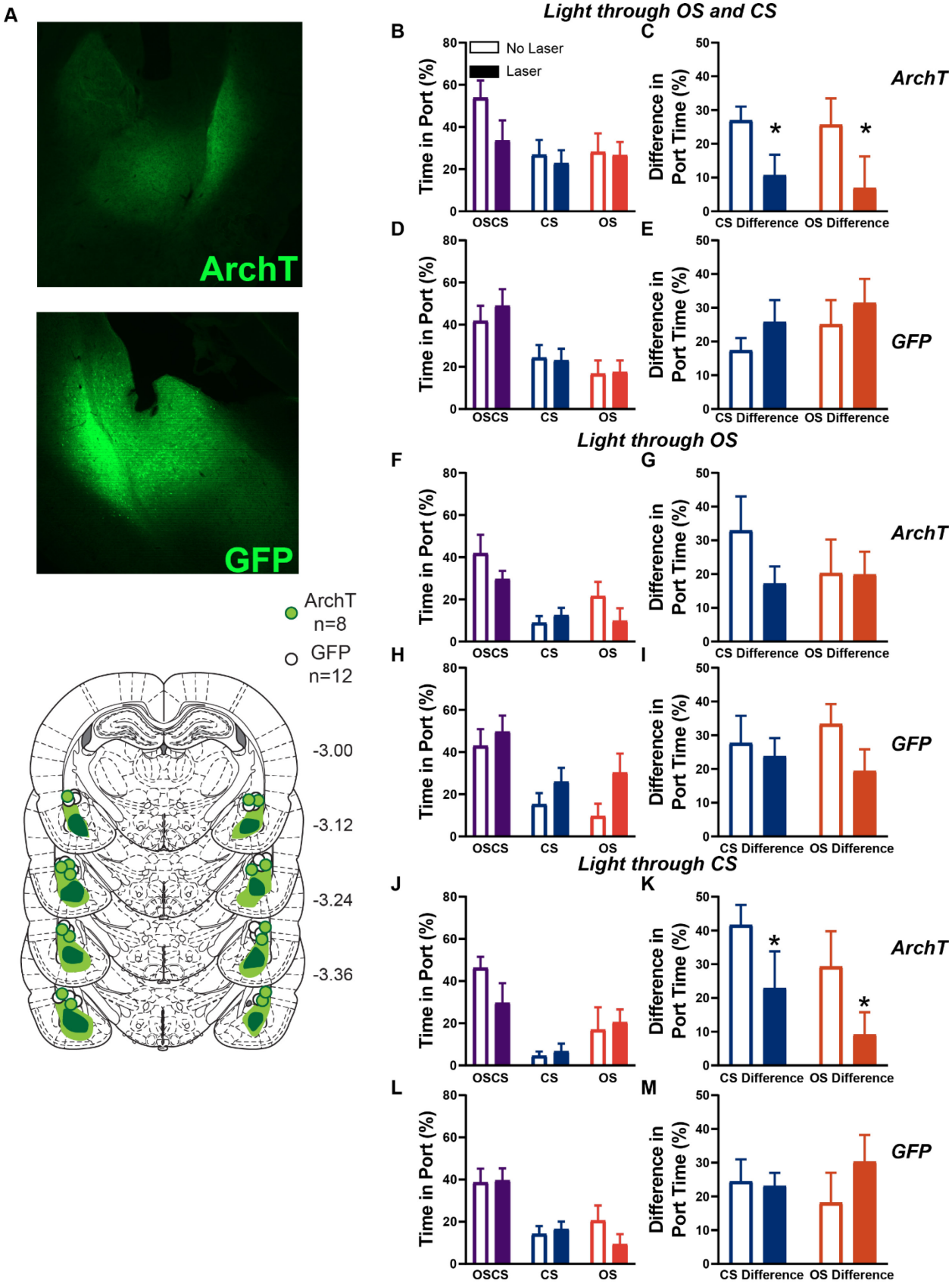
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Figure 6.1. Basolateral amygdala neural activity is essential for occasion setters to influence conditioned reward-seeking. ***A,*** Representative expression of ArchT and GFP in the BLA and reconstruction of optic fiber placements and the largest, in light green, and smallest, in dark green, degree of expression of ArchT in the BLA. ***B,*** Time in the reward port during the CS period across trials normalized to a period 10s before a trial started for ArchT rats when 532 nm light was delivered throughout the OS and CS periods during half of all trials. ***C,*** Individual differences in time in port during the conditioned stimulus period on reinforced trials minus either conditioned stimulus alone or occasion setter alone trials for ArchT rats when light was delivered throughout the OS and CS on half of the trials. ***D-E,*** same as ***B-C*** but for rats with GFP in the BLA. ***F-I,*** same as ***B-E*** but when light was delivered overlapping with half of all OS presentations. ***J-M,*** same as ***B-E*** but when light was delivered overlapping with half of all CS presentations. Numbers indicate distance from bregma in millimeters and the coronal sections were obtained from (Paxinos and Watson, 2007). Open bars indicate trials without laser delivery, closed bars indicate trials with laser delivery. OS, occasion setter. CS, conditioned stimulus. *p<0.05.

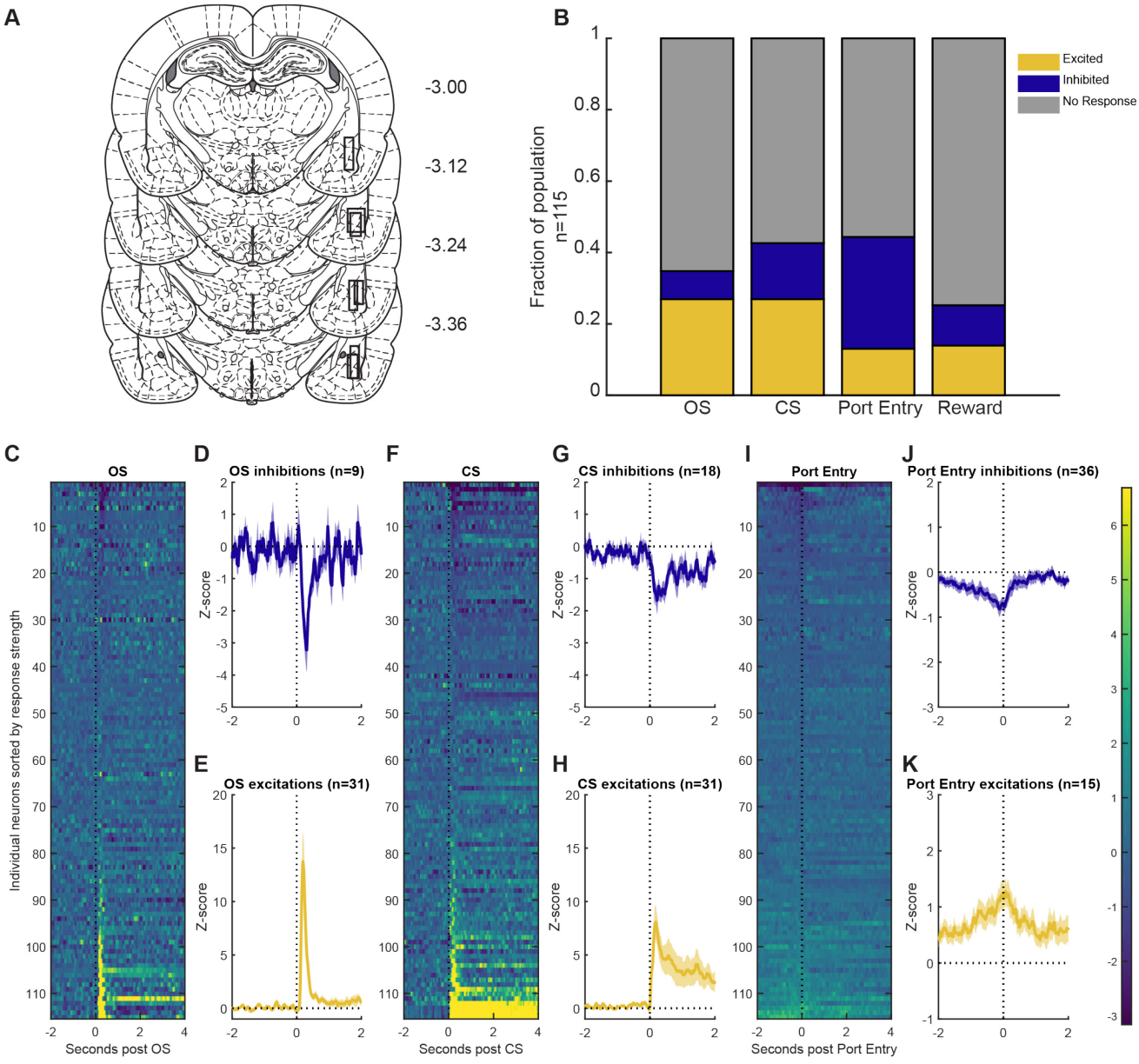


Figure 6.2. Basolateral amygdala neurons encode occasion setters. ***A,*** Reconstruction of electrode tracks throughout the BLA. ***B,*** Proportion of neurons that are significantly excited (yellow), inhibited (blue), or those with no significant response (gray) for the four relevant task-related events. ***C,*** Heatmap of individual neuron responses to the occasion setting houselight sorted by greatest inhibition at the top to greatest inhibition at the bottom. ***D,*** Mean ± SEM for neurons with significant inhibitions

to the OS cue. ***E,*** Mean ± SEM for neurons with significant excitations to the OS cue. ***F-H,*** same as ***C-E*** but for the white noise conditioned stimulus. ***I-K,*** same as ***C-E*** but for entries to the reward port. Sorting for heatmaps is independent for each event. Numbers indicate distance from bregma in millimeters and the coronal sections were obtained from (Paxinos and Watson, 2007). OS, occasion setter. CS, conditioned stimulus.

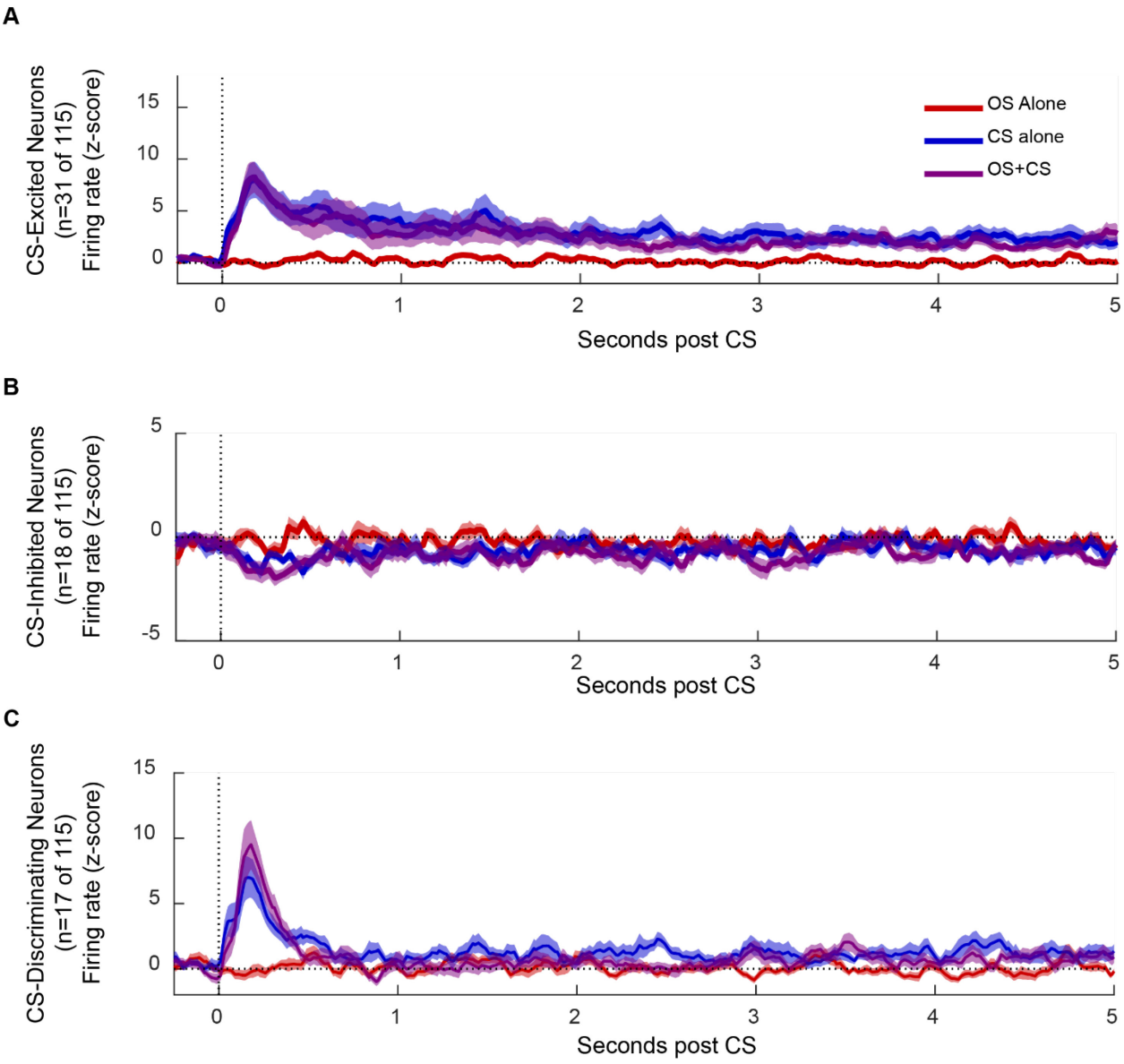
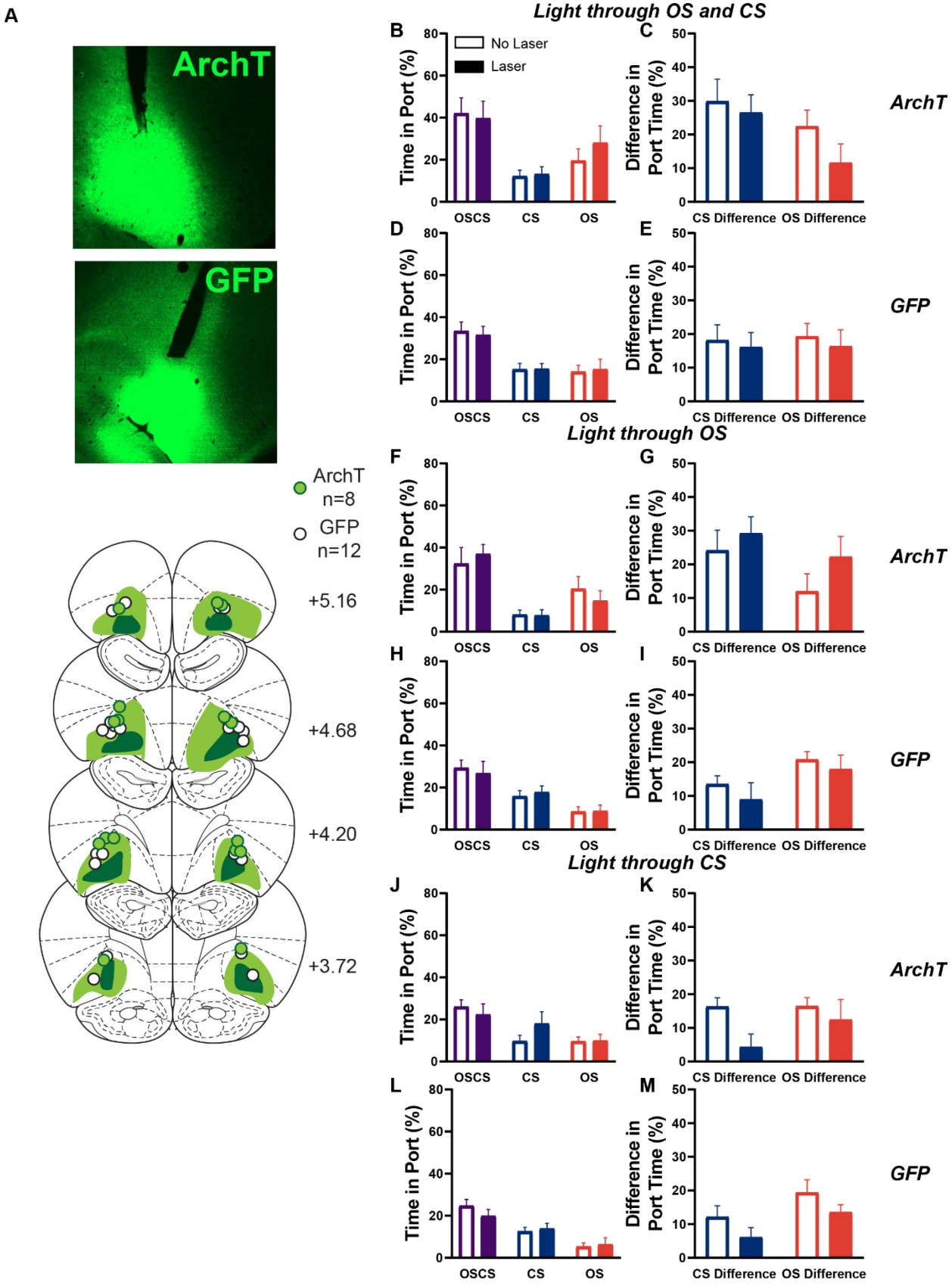


Figure 6.3. Responses to conditioned stimuli in the basolateral amygdala are influenced by occasion setters. ***A,*** Normalized firing rate (z-score) throughout the CS of neurons with significant excitations to the CS that are sorted by trial type. ***B,*** Normalized firing rate (z-score) throughout the CS of neurons with significant inhibitions to the CS that are sorted by trial type. ***C,*** Normalized firing rate (z-score) throughout the CS of neurons that were significantly excited to both the CS and OS sorted by trial type.

For all figures lines indicate mean ± SEM. OS, occasion setter. CS, conditioned stimulus.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+5.16
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Light through OS and CS
Light through OS
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Time in Port (%)
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OSCS
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ArchT
GFP

Figure 6.4. Lack of impact of optogenetic inhibition of orbitofrontal cortex on occasion setting. ***A,*** Representative expression of ArchT and GFP in the OFC and reconstruction of optic fiber placements and the largest, in light green, and smallest, in dark green, degree of expression of ArchT in the OFC. ***B,*** Time in the reward port during the CS period across trials normalized to a period 10s before a trial started for ArchT rats when 532 nm light was delivered throughout the OS and CS periods during half of all trials. ***C,*** Individual differences in time in port during the conditioned stimulus period on reinforced trials minus either conditioned stimulus alone or occasion setter alone trials for ArchT rats when light was delivered throughout the OS and CS on half of the trials. ***D-E,*** same as ***B-C*** but for rats with GFP in the OFC. ***F-I,*** same as ***B-E*** but when light was delivered overlapping with half of all OS presentations. ***J-M,*** same as ***B-E*** but when light was delivered overlapping with half of all CS presentations. Numbers indicate distance from bregma in millimeters and the coronal sections were obtained from (Paxinos and Watson, 2007). Open bars indicate trials without laser delivery, closed bars indicate trials with laser delivery. OS, occasion setter. CS, conditioned stimulus. *p<0.05.

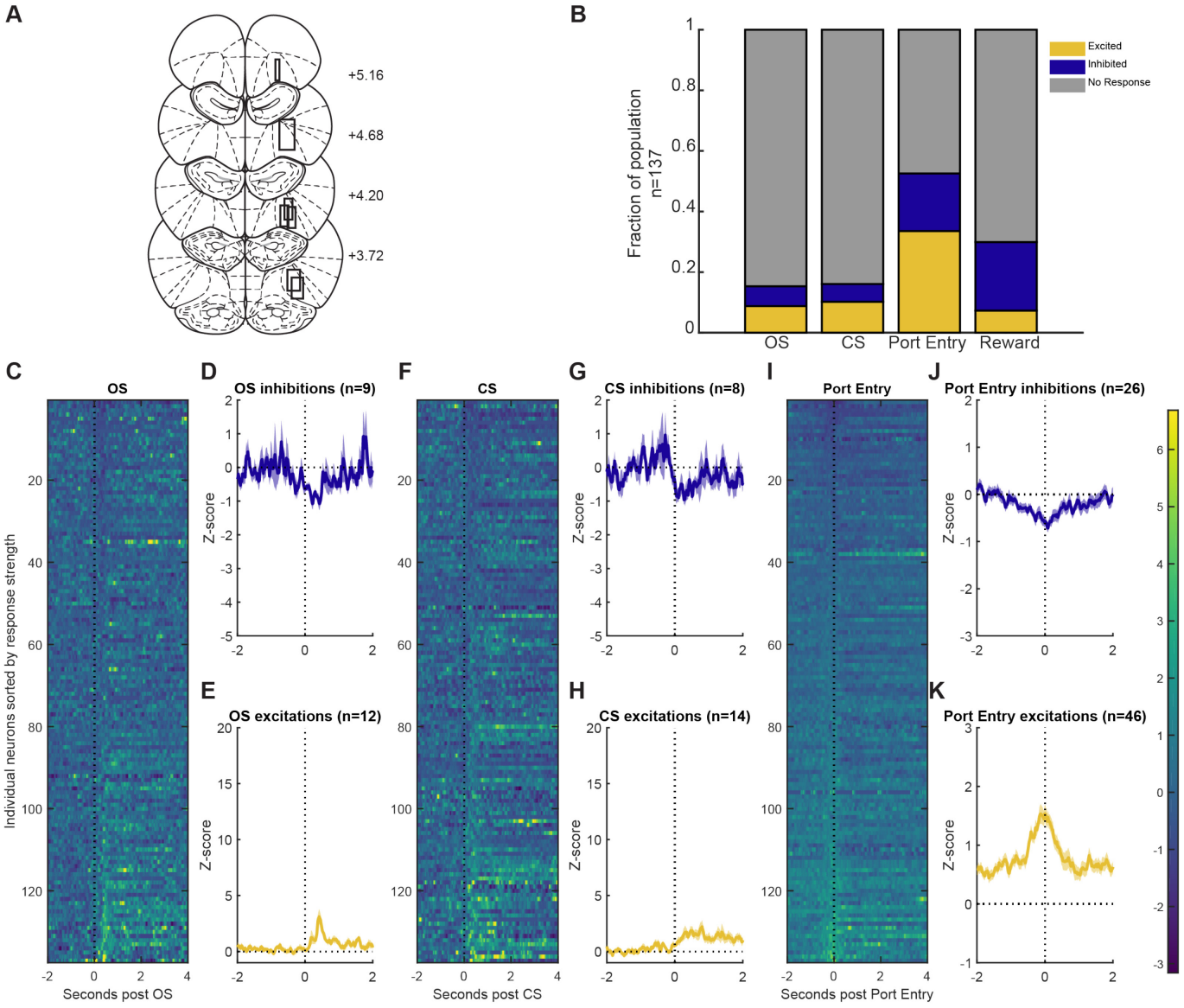

Figure 6.5. Minimal encoding of task-relevant cues in orbitofrontal cortex during occasion setting. ***A,*** Reconstruction of electrode tracks through the OFC. ***B,*** Proportion of neurons that are significantly excited (yellow), inhibited (blue), or those with no significant response (gray) for the four relevant task-related events. ***C,*** Heatmap of individual neuron responses to the occasion setting houselight sorted by greatest inhibition at the top to greatest inhibition at the bottom. ***D,*** Mean ± SEM for neurons with

significant inhibitions to the OS cue. ***E,*** Mean ± SEM for neurons with significant excitations to the OS cue. ***F-H,*** same as ***C-E*** but for the white noise conditioned stimulus. ***I-K,*** same as ***C-E*** but for entries to the reward port. Sorting for heatmaps is independent for each event. Numbers indicate distance from bregma in millimeters and the coronal sections were obtained from (Paxinos and Watson, 2007). OS, occasion setter. CS, conditioned stimulus.

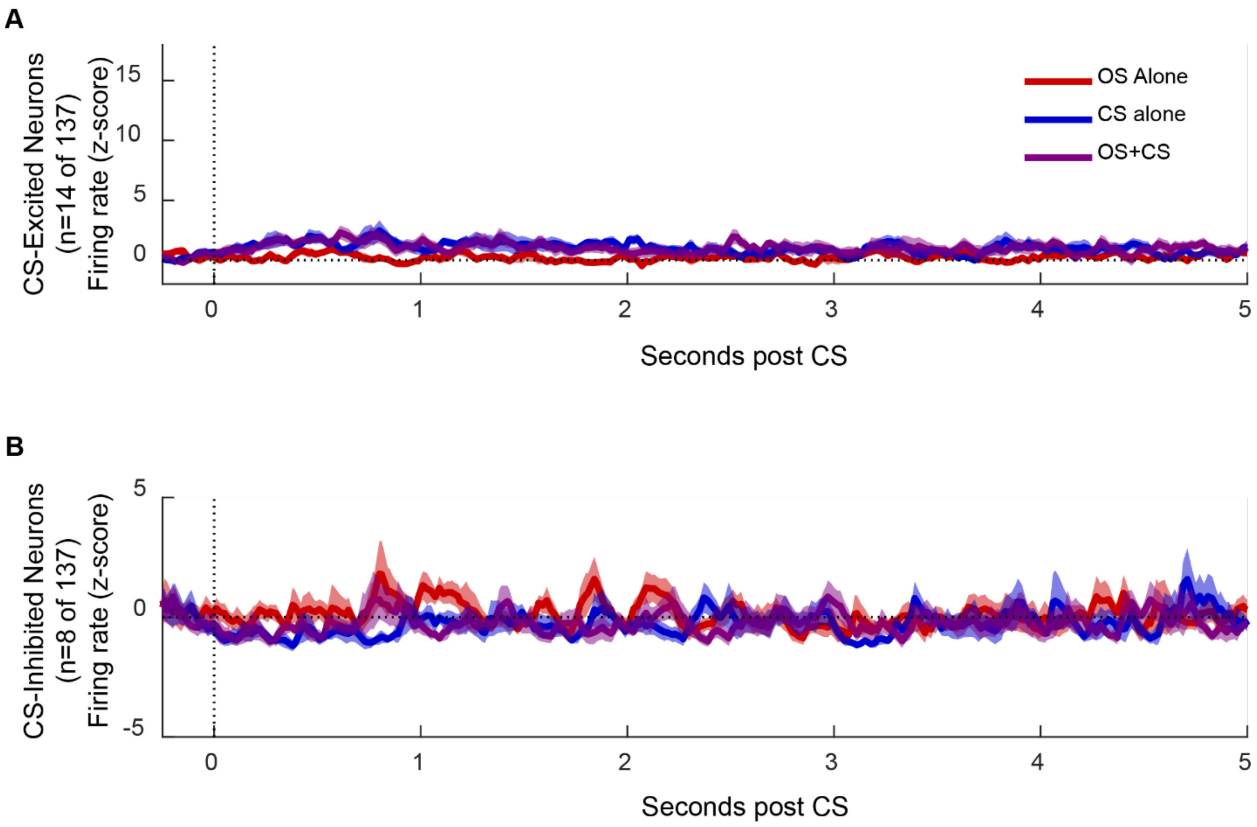

Figure 6.6. Lack of influence of occasion setters on orbitofrontal neural activity during an ambiguous conditioned stimulus. . *A,* Normalized firing rate (z-score) throughout the CS of neurons with significant excitations to the CS that are sorted by trial type. ***B,*** Normalized firing rate (z-score) throughout the CS of neurons with significant inhibitions to the CS that are sorted by trial type. For all figures lines indicate mean ± SEM. OS, occasion setter. CS, conditioned stimulus.

Chapter 7

General Discussion

In the research described here, I exploited a unique behavioral approach to resolve neural and behavioral mechanisms underlying the resolution of uncertainty. This work has a number of implications for our understandings of complex interactions among stimuli in the world in generating motivation, craving, and approach to reward-associated stimuli. In particular, I have identified a number of brain regions that are essential for this process and described mechanisms within these essential nuclei that contribute to this occasion setting process. I will provide first a brief overview of each chapter and then turn to a more general implication of the collective findings with attention to critical next steps.

Occasion setters have motivational value

In Chapter 2 I developed and characterized a novel behavioral approach to capture occasion setting in freely-behaving rodents. These efforts were motivated to overcome difficulties in the time required for training, minimal discrimination achieved between occasion set and non-occasion set trials, and the reduced trial count in previous demonstrations of occasion setting. It is hopefully apparent that the occasion setting paradigm I developed captures the notion that in essence the occasion setter is akin to acontext, but allows the experimenter to have direct control over the presentation and actions of this context. Exploiting this approach, I validated that the behavioral performance in this paradigm meets criteria for observing occasion setting as extinction of the occasion setting cue was without effect on behavioral discrimination. I then asked what are the actions of occasion setters: do they modulate predictive

relationships between cues and rewards or do they attain incentive motivational value and in turn regulate the motivational value of cues? I demonstrate that the answer to both of these questions is yes. Together these resolve a long-standing assumption yet difficult to ascertain property of traditional operant-box contexts and that by capturing the underlying process suggest that these contexts themselves are desirable, obtain motivational value, and in turn act in some way to gate the motivational and predictive properties of conditioned stimuli.

Dopamine neuron activity is essential for occasion setting

Dopamine neurons are one of the most intensely investigated neuromodulatory systems in the brain yet we continue to lack a detailed understanding of their contributions to cue-triggered behavior. In Chapter 3 I made use of optogenetics in combination with TH-Cre transgenic rats to allow for the precise inhibition of dopamine neuron activity on a timescale relevant to processing reward-associated cues. These experiments revealed a surprising and selective effect of dopamine neuron inhibition during the presentation of occasion setters as interrupting the ability of rats to use these hierarchical stimuli to resolve the ambiguity of a conditioned stimulus. In control experiments I attempted to rule out potential explanations concerning the well-appreciated encoding of expected value, a combination of the probability and magnitude of the reward predicted by a conditioned stimulus, by these neurons. In scenarios in which expected value was stable and unchanging, there was no apparent impact of optogenetic inhibition of dopamine neurons during these probabilistic conditioned stimuli. This selective impact on occasion setting is in agreement with a growing body of work arguing that dopamine neurons contribute to conditioned behavior by integrating

factors beyond simply the value of a conditioned stimulus (Sharpe et al., 2017b; Babayan et al., 2018; Langdon et al., 2018; Gershman and Uchida, 2019).

Nucleus accumbens mechanisms mediating occasion setting

That manipulations of dopamine activity impacted occasion setting made apparent that investigations into downstream targets of dopamine neurons could reveal how such modulation is utilized. In Chapter 4 I found that manipulations of neural activity or dopamine release in the nucleus accumbens disrupted occasion setting performance. To better understand these pharmacological effects, I first recorded single neurons within the nucleus accumbens. I found widespread encoding of occasion setters within the nucleus accumbens core which suggests a critical contribution of neurons within this specialized subregion is to recognize such hierarchical stimuli. By analyzing the activity of neurons on a trial by trial basis I discovered that there existed two potential mechanisms for occasion setters to modulate neural activity at the time of a conditioned stimulus. One was that the prior presentation of an occasion setter generated more intense inhibitions at the time of the conditioned stimulus. The other was that there existed a population of about 10% of recorded nucleus accumbens core neurons for whom their response flipped from excitation to inhibition to the conditioned stimulus if the occasion setter had been previously presented. I collaborated with colleagues at the University of Minnesota and we recorded dopamine release in near real-time with the biosensor dLight1.3b. We observed a striking gating function of occasion setters on dopamine release at the time of the conditioned stimulus such that we only observed significant dopamine release to this ambiguous cue if the occasion setter had been previously presented. Overall these suggest that both neurons and

dopamine release in the nucleus accumbens core are highly dynamic, encode hierarchical stimuli, and are necessary for adaptive behavior.

Basolateral amygdala and orbitofrontal cortex are critical for occasion setting

In Chapter 4 I observed an interesting effect of glutamate antagonism within the core, but not shell, of the nucleus accumbens in increasing responding following presentation of an occasion setter – an effect that I interpret as improper processing of this cue as a higher-order stimulus. This finding spurred investigations into potential glutamatergic inputs to the nucleus accumbens core that may also be critical for occasion setting. On the basis of a large literature investigating the selective contribution of activity within the basolateral amygdala and orbitofrontal cortex in adjusting responding to cues when their value has changed in Pavlovian conditioning these regions were obvious candidates to probe. In Chapter 5 I found that reversible inactivation of either basolateral amygdala or orbitofrontal cortex impaired occasion setting performance in a manner reminiscent of the same manipulation in the nucleus accumbens core. In a series of control experiments, I found that these regions are not necessary in the performance of conditioned responding to a conditioned stimulus with a fixed and unchanging relationship to reward. I also demonstrate that dorsal hippocampus inactivation is without effect on occasion setting performance supporting that there is not general state-dependency effects of inactivation in any brain region that will affect occasion setting.

Basolateral amygdala neurons encode and are necessary for occasion setting

In Chapter 6 I sought to explore more precise contributions and mechanisms within the basolateral amygdala and orbitofrontal cortex that would underlie their essential contribution to occasion setting. In electrophysiological recordings I found that basolateral amygdala neurons, and not necessarily orbitofrontal neurons, encoded occasion setters. This was commonly observed as a significantly strong and brief excitation in basolateral amygdala neurons and I found evidence that for some neurons in basolateral amygdala the strength of excitation to the ambiguous conditioned stimulus was gated by occasion setters. To test what aspects and, more specifically, *when* neural activity in either structure was necessary I made use of optogenetics to precisely inhibit either basolateral amygdala or orbitofrontal cortex. Interestingly, optogenetic inhibition of the basolateral amygdala throughout a trial or just during the conditioned stimulus decreased discrimination in the occasion setting task. In contrast, there was no effect of optogenetic inhibition of the orbitofrontal cortex, despite previously demonstrating in Chapter 5 that reversible inactivation of this region impaired responding. This discrepancy suggests a complex contribution of orbitofrontal cortex to occasion setting that is potentially resistant to brief alterations in orbitofrontal neural activity. Overall these data provide strong support for the basolateral amygdala itself as critical locus for occasion setting and open up a number of potential future investigations into circuit mechanisms underlying its contribution to the hierarchical control of cue-motivated behavior.

Going forward versus holding back with occasion setting

In this dissertation I've exploited a specific form of occasion setting, positive occasion setting, that disambiguates when a conditioned stimulus will predict reward as

opposed to negative occasion setting, where the occasion setter acts to inhibit responding to a conditioned stimulus that will not lead to reward on the current trial. There is an emerging body of work that is concerned with the neural circuits underlying this inhibitory process that has focused on the orbitofrontal cortex primarily (Meyer and Bucci, 2016b; Shobe et al., 2017). Perhaps the lack of effect of optogenetic inhibition I observed is due to a selective contribution of the orbitofrontal cortex to negative occasion setting. I opted to pursue positive occasion setting as there are concerns that negative occasion setters themselves typically become conditioned inhibitors and act to broadly inhibit all conditioned responding in a general manner (see Chapter 1). Despite this, it is interesting to note that in most traditional studies of the contextual control of behavior that the process at play is thought to be negative occasion setting by the extinction context (Grahame et al., 1990; Holland and Bouton, 1999; Bouton, 2004; Bouton et al., 2006; Trask et al., 2017). Despite this, in our preparation and investigations into positive occasion setting we found overlap in brain regions that have previously been implicated in classical contextual control of conditioned responding. This suggests there is potential overlap in the neural systems supporting these processes – but caution is noted in that physical settings as contexts may not act exclusively as occasion setters. In the future it will be critical for better behavioral paradigms to be developed for the reliable observation of negative occasion setting and work to distinguish the overlap in neural circuits between this form of hierarchical control and that of positive occasion setting.

Dopamine, uncertainty, and nucleus accumbens dynamics

One of the most fascinating aspects of these findings is the precise contribution of dopamine neuron activity to occasion setting and in turn the modulation of dopamine signaling by occasion setters. This was surprising given the finding that dopamine neurons encoding the probability of reward delivery associated with conditioned stimuli (Fiorillo et al., 2003; Tobler et al., 2005). In addition, it has been demonstrated that dopamine release in the accumbens core is sensitive to probability of reinforcement (Hart et al., 2015). Despite this, how such expected value interacted to guide behavior was not apparent and required technical and behavioral innovation. The findings in Chapters 3 and 4 are in agreement with a developing notion that dopamine functions in a manner consistent with “model-based” reinforcement learning algorithms (Dayan and Berridge, 2014). That is to say, neither dopamine neurons, nor dopamine release, nor activity in their downstream striatal targets are exclusively involved in signaling Rescorla-Wagner or temporal difference learning derived prediction errors. What then could be the contribution of dopamine neurons to occasion setting?

Perhaps occasion setters function to scale the amount of dopamine release in response to conditioned stimuli. In a modification of temporal difference reinforcement learning put forth by Zhang and colleagues, they propose a scaling factor κ that allows for changes in internal state to alter the value of a given conditioned stimulus (Zhang et al., 2009; Berridge, 2012; Dayan and Berridge, 2014). An issue arises however in imaging how κ could translate to situations of either uncertainty or where a separate stimulus like an occasion setter is acting to modify value. The proposal of κ to describe occasion setters follows a common theme in this research in the difficult distinction in whether alterations in internal state are occasion setters. It is certainly apparent that

alterations in homeostasis result in motivated behavior that deviate from traditional reinforcement learning algorithms and that κ can indeed capture these given its primary assumption that this scaling occurs for all presentations of the stimulus. It is important to emphasize, however, that these approaches to manipulate internal states are akin to the application of distinct physical contexts to modulate behavior – they make it difficult to understand the neural correlates that react to these modulatory states and stimuli. The occasion setting approach we have developed has revealed dopaminergic correlates that certainly indicate exquisite tuning of this system to hierarchical information and then its utilization to gate dopamine release, striatal signaling, and behavior at the time of ambiguous conditioned stimuli. As I highlighted in the introduction, this presents a conundrum in that no current computational model of Pavlovian conditioning appears suited to capture the nuances of occasion setting behavior. As we now provide evidence for neural correlates of occasion setting, a renewed interest in developing computational models of hierarchical cue control will undoubtedly be transformative for our understanding of the function of the mesolimbic dopamine system.

Hierarchical regulation of appetitive versus aversive cues

I have focused exclusively on occasion setting for cues that predict appetitive stimuli with little to no evidence for occasion setting for aversion-predictive cues. It is certainly possible for occasion setters to act on stimuli that predict aversive outcomes. Whether the underlying neural substrates are similar or distinct for appetitive versus aversive occasion setting is one of the most pressing future directions that is ripe for exploration. It is interesting to note that recent evidence has revealed unique

dopaminergic contributions to reward and aversion and a segregation of dopamine release to rewarding and aversive simple Pavlovian cues in distinct striatal subregions (de Jong et al., 2019; Verharen et al., 2020). These findings suggest that there may be distinctions in how such release patterns across these striatal subregions are sensitive to hierarchical control and if there are any differences in such control for rewarding and aversive stimuli. Indeed, this is one direction that I am most excited about and hope to explore in the future.

Apart from dopamine, basolateral amygdala neurons are able to segregate the encoding of appetitive and aversive cues that is to some extent captured by their projection-target (Paton et al., 2006; Morrison and Salzman, 2009, 2010; Shabel and Janak, 2009; Beyeler et al., 2016). These neurons are also able to encode “safety” cues, a unique form of conditioned inhibitor for aversion-predictive stimuli, suggesting a mechanism within basolateral amygdala for the hierarchical control of aversion (Sangha et al., 2013). Translating the model of occasion setting developed here to capture such aversive occasion setting and even demonstrating feasibility of such a behavior in mice will facilitate the use of novel neuroscientific tools for investigation into flexible cue-driven behavior.

Translational implications of occasion setting process in psychiatric illness

All of the studies described here made use of traditional sucrose reinforcers to first define the neural substrates supporting hierarchical control of reward-seeking. These studies were all motivated by a pressing need to better capture in animal models the control of cue-elicited motivation that occurs for humans in daily life. It should be apparent that our behaviors are highly dynamic and we are not driven to respond to

cues solely as a result of learning the precise probability and contingency of their relationship to outcomes. It is certainly pressing to develop models in which drug associated stimuli are under hierarchical control. An analogue of this process is the use of drug availability cues in self-administration paradigms like the intermittent access approach (Zimmer et al., 2012; Kawa et al., 2016). Whether these availability cues act as occasion setters or are discriminative stimuli is not totally clear, yet evidence suggests there are individual differences in the way in which these hierarchical cues influence reinstatement either directly or by modulating the motivational value of drug-paired cues – reminiscent of the findings in Chapter 3 (Collins and Saunders, 2019). In line with this, the use of distinct operant boxes to signal extinction or non-extinction is a classic model for context-based control (Crombag et al., 2008; Janak and Chaudhri, 2010; Valyear et al., 2017). As noted previously, this has limited neuroscientific investigations into how these higher-order stimuli influence reward-seeking in real-time. Adapting operant and Pavlovian occasion setting approaches for drug self-administration will be essential for identifying novel neural circuits for drug use, extinction, and relapse.

Apart from modifying the value of drug- and reward-paired cues, occasion setting is also applicable to a number of physiological processes resulting from drug use. In critical experiments that have largely been overlooked occasion setting processes were identified as being essential for the expression of both sensitization and tolerance – two of the criteria necessary for a diagnosis of a substance use disorder (Anagnostaras and Robinson, 1996; Anagnostaras et al., 2002; Ramos et al., 2002). Despite this, little investigation has spurred from these initial findings leaving many open questions

pertaining to the neurobiology of sensitization and tolerance that may be essential for lessening the harm and concerns of overdosing in human substance users. Moreover, interoceptive states evoked by drugs of abuse are able to act as occasion setters and investigations into this form of occasion setting will be essential for factors regulating continued intake once drug use has been initiated (Randall et al., 2019). Beyond addiction, disordered occasion setting has been implicated in schizoaffective disorders and in post-traumatic stress disorders (Fraser and Holland, 2019). It is clear that embracing a neuroscientific study of occasion setting has pressing implications for our approach to the treatment of numerous disorders.

Conclusion

Collectively the work in this dissertation captures a number of initial investigations into the hierarchical control of cue-motivated behavior. I provide a framework for modeling occasion setting and an optimization of this approach for neuroscientific investigation. As a result, I have identified a number of brain regions and mechanism within that are essential for flexibility in responding to reward-paired cues. Particularly poignant outstanding questions concern the interactions between the neural systems explored here, how dopamine release and neural activity interact in the resolution of uncertainty, the generality of such findings to outcomes of opposing valence, and how experiences such as drug use and stress impede on the neural and behavioral mechanisms of hierarchical control. Given the critical influence cues have over our behavior and that cues in the real-world are dynamic and have ever varying motivational value it is apparent that hierarchical processes regulating cue-driven behavior are the norm rather than the exception. Expanding our understanding of how

the brain generates and rapidly fine-tunes motivation at an ever more complex and mechanistic level will undoubtedly transform our insight into behavior and its disorder in psychiatric illness.

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Tye KM, Stuber GD, de Ridder B, Bonci A, Janak PH (2008) Rapid strengthening of thalamo-amygdala synapses mediates cue-reward learning. Nature 453:1253–1257.

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Valyear MD, Villaruel FR, Chaudhri N (2017) Alcohol-seeking and relapse: A focus on incentive salience and contextual conditioning. Behavioural Processes 141:26–32.

Verharen JPH, Zhu Y, Lammel S (2020) Aversion hot spots in the dopamine system. Curr Opin Neurobiol 64:46–52.

Wassum KM, Izquierdo A (2015) The basolateral amygdala in reward learning and addiction. Neuroscience and Biobehavioral Reviews 57:271–283.

Watabe-Uchida M, Eshel N, Uchida N (2017) Neural circuitry of reward prediction error. Annual Review of Neuroscience 40:373–394.

Wikenheiser AM, Schoenbaum G (2016) Over the river, through the woods: cognitive maps in the hippocampus and orbitofrontal cortex. Nature Reviews Neuroscience 17:513–523.

Wilson RC, Takahashi YK, Schoenbaum G, Niv Y (2014) Orbitofrontal cortex as a cognitive map of task space. Neuron 81:267–279.

Witten IB, Steinberg EE, Lee SY, Davidson TJ, Zalocusky KA, Brodsky M, Yizhar O, Cho SL, Gong S, Ramakrishnan C, Stuber GD, Tye KM, Janak PH, Deisseroth K

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Zhang J, Berridge KC, Tindell AJ, Smith KS, Aldridge JW (2009) A neural computational model of incentive salience. PLoS Computational Biology 5:e1000437.

Zhou J, Jia C, Montesinos-Cartagena M, Gardner MPH, Zong W, Schoenbaum G (2020) Evolving schema representations in orbitofrontal ensembles during learning. Nature.

Zimmer BA, Oleson EB, Roberts DC (2012) The Motivation to Self-Administer is Increased After a History of Spiking Brain Levels of Cocaine. Neuropsychopharmacology 37:1901–1910.

Zinn R, Leake J, Krasne FB, Corbit LH, Fanselow MS, Vissel B (2020) Maladaptive Properties of Context-Impoverished Memories. Current Biology:S0960982220305546.

Zironi I, Burattini C, Aicardi G, Janak PH (2006) Context is a trigger for relapse to alcohol. Behavioural Brain Research 167:150–155.

Kurt Michael Fraser, MA

CURRENT POSITION

Johns Hopkins University, Baltimore, MD — Expected February 2021
PhD Candidate in Psychological & Brain Sciences (Biopsychology)
Supervisor: Patricia H. Janak, PhD
Dissertation Topic: Setting the Occasion for Reward-seeking in Brain and Behavior

EDUCATION

Johns Hopkins University, Baltimore, MD — 2017
MA in Psychological & Brain Sciences (Biopsychology)
Supervisor: Patricia H. Janak, PhD
Master's Thesis: Dorsal and Ventral Striatal Systems in the Attribution of Incentive Salience to Reward-Paired Cues

University of Michigan, Ann Arbor, MI — 2015
BS in Neuroscience with High Honors & Distinction
Supervisor: Shelly B. Flagel, PhD
Thesis: Contributions of Dopamine D_2 and D_3 Receptors to Pavlovian Conditioned Approach

RESEARCH INTERESTS

Neurobiological mechanisms of cue-triggered motivated behavior:

- How are reward learning and conditioned motivation separable in both behavior and neurobiology?
- What neural circuits underlie the attribution of conditioned motivation to reward-paired cues?
- How is ambiguity about the motivational value of conditioned cues in the environment resolved to produce reward-seeking?
- What is the content of learning?
- How do neural circuits supporting adaptive reward-seeking go awry in mental health disorders such as alcohol abuse and addiction?

RESEARCH SUPPORT AND FELLOWSHIPS

NIH National Research Service Award Predoctoral Fellowship (F31 DA046136) 2019-present
The role of the basolateral amygdala in occasion setting

PUBLICATIONS

Ottenheimer DJ, Wang K, Tong X, **Fraser KM**, Richard JM, & Janak PH. (2020). Reward activity in ventral pallidum tracks satiety-sensitive preference and drives choice behavior. *Science Advances*. 6(45). doi: 10.1126/sciadv.abc9321

Ottenheimer DJ, Bari BA, Sutlief E, **Fraser KM**, Kim TH, Richard JM, Cohen JY & Janak PH. (2020). A quantitative reward prediction error signal in the ventral pallidum. *Nature Neuroscience*. 23(10): 1267-1276. doi: 10.1038/s41593-020-0688-5

**Originally a preprint on bioRxiv*

Fraser KM & Janak PH. (2019). Occasion setters attain incentive motivational value: implications for contextual influences on reward-seeking. *Learning & Memory*. 26:291-298. doi: 10.1101/lm.049320.119

**Originally a preprint on bioRxiv*

Fraser KM & Janak PH. (2019). How does drug use shift the balance between model-based and model-free control of decision-making? *Biological Psychiatry*. 85(11): 886-888. doi: 10.1016/j.biopsych.2019.04.106

**Commentary on Groman et al. 2019*

Fraser KM & Holland PC. (2019). Occasion Setting. *Behavioral Neuroscience*. 133(2):145-175. doi: 10.1037/bne0000306

Fraser KM & Janak PH. (2018). Stressing the other paraventricular nucleus. *Nature Neuroscience*. 21(7):901-902. doi: 10.1038/s41593-018-0178-1

**News & Views on Beas, Wright et al. 2018*

Fraser KM & Janak PH. (2017). Long-lasting contribution of dopamine in the nucleus accumbens core, but not dorsal lateral striatum, to sign-tracking. *European Journal of Neuroscience*. 46(4):2047-2055. doi: 10.1111/ejn.13642

**Originally a preprint on bioRxiv*

Haight JL, Fuller ZL, **Fraser KM** & Flagel SB. (2017). A food-predictive cue attributed with incentive salience engages subcortical afferents and efferents of the paraventricular nucleus of the thalamus. *Neuroscience*. 340:135-152. doi: 10.1016/j.neuroscience.2016.10.043

Fraser KM & Haight JL. (2016). Diminished Dopamine: Timing, Neuroanatomy, or Drug History?*The Journal of Neuroscience*. 36(18):4907-4909. doi: 10.1523/JNEUROSCI.0731-16.2016

** Journal Club review of Saddoris et al. 2016*

Fraser KM, Haight JL, Gardner EL & Flagel SB. (2016). Examining the role of dopamine D_2 and D_3 receptors in Pavlovian conditioned approach behaviors. *Behavioural Brain Research*. 305:87-99. doi: 10.1016/j.bbr.2016.02.022

Haight JL, **Fraser KM,** Akil H & Flagel SB. (2015). Lesions of the paraventricular nucleus of the thalamus differentially affect sign- and goal-tracking conditioned responses. *European Journal of Neuroscience.* 42(7):2478-2488. doi: 10.1111/ejn.13031

HONORS AND AWARDS

Enoch Gordis Award Finalist, Research Society on Alcoholism	2020
Outstanding Poster Award, Winter Conference on Brain Research	2019

Travel Award, Winter Conference on Brain Research 2019
Trainee Professional Development Award, Society for Neuroscience 2018
Robert S. Waldrop Junior Investigator Award, Johns Hopkins University 2018
Special Opportunities for Undergraduate Learning Course Award, Johns Hopkins University2017
Collaborative Research Award, Psychological & Brain Sciences, Johns Hopkins University2017
Walter L. Clark Service Award, Psychological & Brain Sciences, Johns Hopkins University 2017
Honorable Mention, National Science Foundation Graduate Research Fellowship Program2017
Owen Scholars Award, Johns Hopkins University 2015-2018
Graduated with Distinction, University of Michigan 2015
University of Michigan Wilson P. "Spike" Tanner Memorial Award 2014
Faculty for Undergraduate Neuroscience Travel Award 2014
University of Michigan Literature, Science, and Arts Internship Scholarship 2014
Michigan Schools and Government Credit Union Scholarship 2011
Michigan Education Association Scholarship 2011
National Multiple Sclerosis Society Scholarship 2011

TEACHING EXPERIENCE

Johns Hopkins University:

Guest Lecture, AS.200.335 *How Does the Brain Predict the Future?* 2019
- "Reward Prediction Error-based Learning"

Guest Lecture, AS.200.369 *Neuroscience of Motivation and Reward* 2019
- "Social Reward"

Teaching Assistant, AS.200.369 *Neuroscience of Motivation and Reward* 2018
- Responsible for grading, developing assessments and exams, private tutoring

Instructor, AS.360.111, *Setting up for Success in Systems Neuroscience* 2017
- Independently developed and taught 6-week course designed for underclassmen

Teaching Assistant, AS.200.368 *Sleep, Dreams, and Altered States of Consciousness* 2017
- Responsible for grading, private tutoring, developing course materials, overseeing IRB approval for independent research projects
- Lectures Given: "Statistics for Psychological Sciences" & "Sleep and Memory: A Neural Perspective"

Teaching Assistant, AS.200.314 *Advanced Statistical Methods* 2016
- Responsible for developing exams and homework, grading, private tutoring

Guest Lecture, AS.200.303 *Psychobiology of Addiction* 2016
- "Individual differences in the attribution of incentive salience: implications for addiction"

Teaching Assistant, AS.200.369 *Neuroscience of Motivation and Reward* 2016
- Responsible for grading, developing assessments and exams, private tutoring
- Lectures Given: "Introduction to Addiction Theory: Opponent Process and Habits" & "Introduction to Addiction Theory: Incentive Sensitization"

Laboratory mentor to 6 undergraduate research assistants 2016-2019

Department of Neuroscience, Icahn School of Medicine at Mount Sinai 2020,2021

Guest Lecture, BSR 1707 *Behavioral and Cognitive Neuroscience*
- "Incentive Salience, Wanting, and Liking"

University of Michigan Science Learning Center

Study Group Leader, Biology 225 *Introduction to Animal Physiology and Neurobiology* 2014

Study Group Leader, Chemistry 210 *Structure and Reactivity I* 2013-2014

SERVICE

Johns Hopkins University
Co-Chair, School Outreach Committee, Department of Psychological & Brain Sciences
2018
Chair, Colloquium Committee, Department of Psychological & Brain Sciences
2016-2017
Student Chair, PhD Admissions Committee, Department of Psychological & Brain Sciences
2016-2017

University of Michigan
Vice President, University of Michigan Neuroscience Student Association
2017

VOLUNTEERING AND OUTREACH

Coordinator, Baltimore Brain Series 2016-2019
Editor, Interstellate Magazine 2016-2017
Contributor for PLoS Neurocommunity Society for Neuroscience 2016 Coverage 2016
Brain Awareness Day, Baltimore Polytechnic Institute, Baltimore, MD 2016-2017
Brains Rule! Neuroscience Youth Outreach, University of Michigan, Ann Arbor, MI 2014-2015
Student Volunteer, University of Michigan Cardiovascular Center, Ann Arbor, MI 2013-2014
Peer Mentor, University of Michigan Office of New Student Programs 2012-2015

PROFESSIONAL MEMBERSHIPS

Research Society on Alcoholism (2019-present), Associate Faculty Member F1000 (2018-present), Pavlovian Society (2017-present), Society for Neuroscience (2014-present)

PEER REVIEW SERVICE (* WITH SUPERVISORS)

Addiction Biology*, Behavioral Neuroscience, Behavioural Brain Research*, Biological Psychiatry*, eNeuro, Journal of Neuroscience, Nature Nanotechnology*, Nature Neuroscience*, Neurobiology of Learning & Memory, Pharmacology Biochemistry and Behavior*, Psychopharmacology

INVITED TALKS

"Encoding and enhancement of the motivation to consume alcohol by the central nucleus of the amygdala" (2020, June) Research Society on Alcoholism Annual Meeting, New Orleans, LA
*Virtual Enoch Gordis Award Talk due to Covid-19

"Dynamic coding of the motivational value of reward-paired cues in the limbic system" (2020, June) Research Society on Alcoholism Annual meeting, New Orleans, LA
*Cancelled due to COVID-19

"Encoding and enhancement of the motivation to consume alcohol by the central nucleus of the amygdala" (2020, March) Gordon Research Seminar on Alcohol, Galveston, TX

"Setting the Occasion for Reward Seeking" (2019, June) Minnesota Discovery Team on Addiction, University of Minnesota, Minneapolis, MN

"Cortico-amygdala circuits for flexible cue-triggered motivation" (2019, February) Winter Conference on Brain Research, Snowmass, CO

"Setting the Occasion for Reward Seeking" (2018, June) Central Atlantic Meeting on Associative Learning, Princeton University, Princeton, NJ

"Setting the Occasion for Incentive Motivation" (2018, April) Flagel Lab, University of Michigan, Ann Arbor, MI

"Setting the Occasion for Reward Seeking" (2018, April) Department of Neuroscience, University of Maryland Medical School, Baltimore, MD

SYMPOSIUM CHAIRED

Ch-ch-changes: Redefining the Role of the Basolateral Amygdala and Orbitofrontal Cortex in Tracking Value. (2019, February). Winter Conference on Brain Research, Snowmass, CO

IN THE MEDIA

"Training the Next Generation of Reviewers" Society for Neuroscience
https://www.sfn.org/Publications/Neuroscience-Quarterly/Winter-2019/SfN-Journals
"Quality in Peer Review" Society for Neuroscience eNeuro Blog
https://blog.eneuro.org/2019/09/peer-review-week

ABSTRACTS AND POSTER PRESENTATIONS

Fraser KM, Collins V, Pat F, Janak PH, Saunders BT. (2020, December). Hierarchical control of mesolimbic dopamine and striatal encoding of reward-paired cues governs behavioral flexibility. 59th Annual meeting of the American College of Neuropsychopharmacology.
*Virtual due to COVID-19.

Fraser KM, Collins V, Pat F, Janak PH, Saunders BT. (2020, September). A role for mesolimbic dopamine and striatal encoding of reward-paired cues in the hierarchical control of behavior. Pavlovian Society Annual Meeting.
*Virtual due to COVID-19.

Fraser KM, Kim TH**,** Ottenheimer DJ, & Janak PH. (2020, June). Encoding and enhancement of The motivation to consume alcohol by the central nucleus of the amygdala. Research Society on Alcoholism & ISBRA Joint Meeting.
*Cancelled due to COVID-19

Fraser KM & Janak PH. (2019, October). Amygdalocortical circuits for the dynamic regulation of conditioned reward-seeking. 49th Annual Meeting of the Society for Neuroscience. Chicago, IL.

Kim TH, **Fraser KM** & Janak PH. (2019, October). Ventral hippocampus, basolateral amygdala,

and nucleus accumbens inactivation impairs cue responding in Pavlovian conditioned contextual discrimination. 49th Annual Meeting of the Society for Neuroscience. Chicago, IL.

Janak PH, Kim TH**,** Ottenheimer DJ, **& Fraser KM**. (2019, October). Encoding and enhancement of the motivation to consume alcohol by the central nucleus of the amygdala. 49th Annual Meeting of the Society for Neuroscience. Chicago, IL.

Fraser KM, Pat F, Janak PH. (2019, August). Role of nucleus accumbens core and shell in flexible cue-triggered reward-seeking. Gordon Research Seminar and Conference on Catecholamines. Newry, ME.

Fraser KM & Janak PH. (2019, August). Amygdalocortical circuits for the dynamic regulation of conditioned reward-seeking. Gordon Research Seminar and Conference on the Amygdala. Easton, MA.

Fraser KM & Janak PH. (2019, February). Dynamic regulation of cue-triggered reward seeking by the basolateral amygdala and orbitofrontal cortex. Winter Conference on Brain Research, Snowmass, CO

**Selected for the Prize Poster Session, Received Outstanding Poster Award*

Fraser KM, Kong E, Pat F & Janak PH. (2018, November). Dynamic regulation of cue-triggered reward seeking by the basolateral amygdala and orbitofrontal cortex. 48th Annual Meeting of the Society for Neuroscience, San Diego, CA

DiBartolo MM, **Fraser KM**, Nicholas V, Janak PH & Courtney SM. (2018, November). Incentive salience attribution predicts task-irrelevant attention biases in human sign- and goal-trackers. 48th Annual Meeting of the Society for Neuroscience, San Diego, CA

Fraser KM & Janak PH. (2018, March). Setting the occasion for incentive motivation in the basolateral amygdala: implications for alcohol addiction. Gordon Research Seminar and Conference on Alcohol & the Nervous System, Galveston, TX

Fraser KM & Janak PH. (2017, November). Extended experience does not alter the role of dopamine in the nucleus accumbens core, nor recruit dorsal lateral striatum, to facilitate Pavlovian cue approach. 47th Annual Meeting of the Society for Neuroscience, Washington, D.C.

Fraser KM & Janak PH. (2017, October). Feature-positive occasion setting is dependent upon neuronal activity in the basolateral amygdala. Pavlovian Society, Philadelphia, PA

Fraser KM & Janak PH. (2017, August). Dorsal and ventral striatal systems and the attribution of incentive salience to reward-paired cues. Gordon Research Seminar and Conference on Catecholamines, Newry, ME

Haight JL, Fuller ZL, **Fraser KM** & Flagel SB. (2016, November). Differential activity in the circuitry of the paraventricular nucleus of the thalamus following presentation of an incentive vs. a reward-predictive stimulus. 46th Annual Meeting of the Society for Neuroscience, San Diego,

CA

Richard JM, Armstrong A, Rosenthal HB, **Fraser KM** & Janak PH. (2016, February). Role of ventral pallidum in conditioned alcohol seeking and the effect of stress. Gordon Research Conference on Alcohol & the Nervous System, Galveston, TX

Fraser KM, Haight JL, Gardner EL & Flagel SB. (2015, October). Investigating contributions of dopamine D_2 and D_3 receptors to Pavlovian conditioned approach behaviors. 45th Annual Meeting of the Society for Neuroscience, Chicago, IL

Haight JL, **Fraser KM** & Flagel SB. (2015, October). Differential activity in afferents to the paraventricular nucleus of the thalamus in response to incentive and predictive stimuli. 45th Annual Meeting of the Society for Neuroscience, Chicago, IL

Covelo IR, Haight JL, **Fraser KM**, Kuhn BN, Ferguson SM & Flagel SB. (2015, October). Chemogenetic manipulation of prelimbic afferents to the paraventricular nucleus of the thalamus alter cue-oriented responses in a Pavlovian conditioned approach task. 45th Annual Meeting of the Society for Neuroscience, Chicago, IL

Haight JL**, Fraser KM**, Akil H, Ferguson SM & Flagel SB. (2015, June). Establishing a role for cortico-thalamic circuitry in cue-driven behaviors. 24th Annual International Behavioral Neuroscience Society Meeting, Victoria, British Columbia, Canada

Haight JL, **Fraser KM**, Akil H, Ferguson SM & Flagel SB. (2015, January). Establishing a role for the paraventricular nucleus of the thalamus in Pavlovian conditioned approach behavior. 48th Annual Winter Conference on Brain Research, Big Sky, MT
**Selected for the Prize Poster Session*

Haight JL, **Fraser KM**, Akil H, Ferguson SM & Flagel SB. (2014, December). Parsing the role of the paraventricular nucleus of the thalamus in mediating individual variation in incentive salience attribution. 53rd Annual Meeting of the American College of Neuropsychopharmacology, Phoenix, AZ

Fraser KM, Haight JL & Flagel SB. (2014, November). Stimulation of dopamine D_3 receptors attenuates the expression of Pavlovian conditioned approach responses and motivation for incentive cue presentation. 44th Annual Meeting of the Society for Neuroscience, Washington, D.C.

Haight JL, **Fraser KM**, Kuhn BN, Akil H & Flagel SB. (2014, November). Lesions of the paraventricular nucleus of the thalamus differentially affect the acquisition and expression of Pavlovian-conditioned responses. 44th Annual Meeting of the Society for Neuroscience, Washington, D.C.

Fraser KM, Haight JL & Flagel SB. (2014, April). Stimulation of dopamine D_3 receptors attenuates the expression of Pavlovian conditioned approach responses and motivation for incentive cue presentation. Department of Psychology Spring Research Symposium, University of Michigan, Ann Arbor, MI

REFERENCES

Patricia H. Janak, PhD: Bloomberg Distinguished Professor of Psychology & Neuroscience
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Peter C. Holland, PhD: Krieger-Eisenhower Professor of Psychology & Neuroscience
Johns Hopkins University, Baltimore, MD 21218
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Shelly B. Flagel, PhD: Associate Professor of Psychiatry
Molecular and Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI 48109
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