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The Role Of The Prelimbic, Infralimbic, And Cerebellar Cortices In Operant Behavior

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THE ROLE OF THE PRELIMBIC, INFRALIMBIC, AND CEREBELLAR CORTICES IN OPERANT BEHAVIOR

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Abstract

Operant (instrumental) conditioning is a laboratory method for investigating voluntary behavior and involves training a particular response, such as pressing a lever, to earn a reinforcer. Operant behavior is generally divided into two categories: actions and habits. Actions are goal-directed and controlled by response-outcome (R-O) associations. Habits are stimulus-driven and controlled by stimulus-response associations (S-R). Behavior is determined to be goal-directed or habitual by whether or not it is sensitive (action) or insensitive (habit) to reinforcer/outcome devaluation. Many brain regions have been linked to the learning and/or expression of actions and/or habits. This dissertation investigates a few different brain regions in goal-directed and habitual behavior, and determines more specific roles for the prelimbic cortex, infralimbic cortex, prelimbic cortex to dorsomedial striatum pathway, and Crus I/II of the cerebellum.

Chapter two investigates the prelimbic and infralimbic cortices in goal-directed behavior. We trained rats on a two-response paradigm, where one response was extensively-trained, and a second response was minimally-trained in a separate context. This maintained both responses as goal-directed. In experiment 1, inactivation of the prelimbic cortex at time of test resulted in an attenuation of responding, but only for the minimally-trained response. This implicates the prelimbic cortex in the expression of goal-directed behavior, but only when that goal-directed behavior is minimally-trained. In experiment 2, we repeated the procedure with infralimbic cortex inactivation and found an attenuation of the extensively-trained response. This implicates the infralimbic cortex in the expression of extensively-trained behavior that is goal-directed.

The third chapter examines the role of the prelimbic cortex-to-dorsomedial striatal pathway in minimally-trained operant behavior. Both regions have been implicated in operant behaviors and have strong anatomical connections, but few studies have directly linked them together in the mediation of operant behaviors. After minimal instrumental conditioning, we silenced projections from the prelimbic cortex to the dorsomedial striatum and found that instrumental behavior was reduced, implicating this PL-DMS pathway in the expression of minimally-trained operant responding.

The final chapter examines the role of Crus I/II of the cerebellar cortex in the expression of goal-directed and habitual behavior. The cerebellum is well-characterized as a mediator of motor coordination via its connections with the motor cortex. There is also evidence of connections between Crus I/II and non-motor regions of the prefrontal cortex. Additionally, recent studies have pointed towards a role for Crus I/II in non-motor function. In experiment 1, rats learned one minimally-trained and one extensively-trained response, and both responses were goal-directed. Inactivation of Crus I/II attenuated responding only in rats that had undergone reinforcer devaluation. Residual responding in rats that have undergone reinforcer devaluation is attributed to habit, suggesting that Crus I/II may be involved in habit expression. In a follow-up experiment, we extensively-trained a single response and verified that it was expressed as a habit. This time, Crus I/II inactivation at time of test had no effect. Overall, this complex pattern of results suggests the possibility that Crus I/II of the cerebellar cortex is only engaged in habit expression when two responses are trained, but further experiments will be necessary to verify this.

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Chapter 1: Introduction

Chapter 1: Section 1, Overview

The work in this dissertation is broadly directed at an understanding of the neurobiology of learning and memory. This dissertation focuses on some of the brain regions involved in operant (instrumental) responding. A more specific categorization of behavior within operant responding is whether behavior can be classified as an action or a habit, which can be determined by whether or not responding is sensitive to reinforcer devaluation. A substantial literature has determined brain regions involved in actions or habits. These regions seem to be specific to involvement in either actions or habits, and it is thought that both action and habit circuitries exist. Amongst known regions important for actions are the prelimbic cortex and dorsomedial striatum. However, our work here demonstrates that the prelimbic cortex is not involved in all types of actions. We also show that the prelimbic cortex to dorsomedial striatum projections work to modulate operant responding during the early phase of conditioning. Opposingly, the infralimbic cortex has been implicated in habitual responding. However, we demonstrate here that it is also involved in goal-directed behavior that is extensively-trained. Finally, we investigate a region that has not been previously examined for involvement in actions and habits, Crus I/II of the cerebellar cortex, and show that it seems to be involved in a habitual element of responding.

Chapter 1: Section 2, Development and interaction of actions and habits

Operant conditioning is a way in which voluntary behavior is modeled in a lab. In a basic paradigm, rats must perform a behavior in order to earn an outcome. Thus, we can

examine how behavior that is performed to earn a reinforcer can be affected by different experimental parameters. Operant behavior is generally categorized as either goal-directed or habitual, though there is considerable evidence that both processes are involved in tandem (Balleine & O'Doherty, 2010). The manner in which goal-directed versus habitual responding is tested is reinforcer devaluation. In a typical paradigm, following acquisition, rats are either satiated on a particular reinforcer or the reinforcer is paired with lithium chloride (LiCl) induced illness. Rats are then tested on responding in the absence of the ability to earn the reinforcer (in extinction). Responses that are goal directed, meaning that they are supported by a response-outcome (R-O) association, are sensitive to reward devaluation; rats reduce responding because the outcome is undesirable, or if it is a choice test, respond for an alternative reward. However, responses that are habitual, meaning that they are supported by a stimulus-response (S-R) association, are insensitive to reward devaluation; rats continue to lever press. Another way of testing this is contingency degradation. In one of these paradigms, making a response no longer has any effect on receiving a reinforcer. Therefore, if animals are still responding based on the outcome (i.e. goal directed), then they will reduce responding since receiving the reinforcer is no longer contingent upon their making that response. If animals are responding habitually, they will continue to make the response, because responding is driven by a reinforcer-strengthened association between S and R (S-R rather than R-O; Balleine & O'Doherty, 2010). This habitual responding appears to be sensitive to context switches while goal-directed responding is not (Thrailkill and Bouton, 2015; See Figure 1). These patterns of responding

are based on the ability of the rat to maintain the understanding that a particular response leads to a particular outcome, and about the representation of that particular outcome.

Cognitive habits are thought to develop as an adaptive means of transitioning from processing that is goal-directed and effortful to habitual and automatic (Ramnani, 2006; Lingawi et al., 2016). This allows for rapid and fluid processing to proceed without increasing the cognitive load in working memory, by engaging behavior that predicts the next step to make without conscious thought. One way that this transition from actions to habits occurs is with continued training. In rats, overtraining a response can result in the formation of a habit (insensitivity to reward devaluation), while undertraining maintains responding as goal-directed (Dickinson, 1985). This is dependent partially on reward schedule, as ratio schedules, in which the rat receives reinforcers paired closely with the responses they make, results in continued goal-directed responding. Conversely, interval schedules, where response performance is less important than time passing, promote the formation of habitual behavior (Dickinson et al., 1983). Though it is not generally agreed upon as to why these schedules of reinforcement promote these particular behaviors, it has been suggested that reinforcer

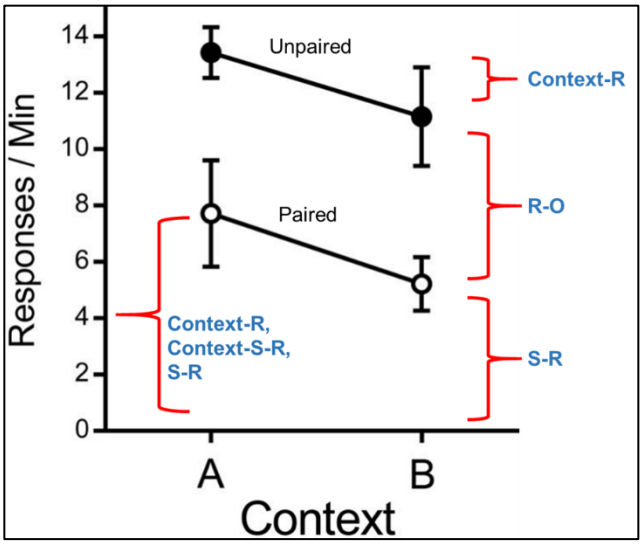

Figure 1. Modified from Thrailkill and Bouton, 2015. Associations formed during training in Context A, subsequent devaluation, and test in Context A and neutral context B. Reduced paired responding could indicate a reduction of Context-S-R associations.

predictability is a particularly important aspect of habit formation (Thrailkill et al., 2018). Across training, responding is likely a mixture of both S-R and R-O associations, as it is generally accepted that both actions and habits develop in tandem, with habits growing in influence across training, while actions dominate responding initially before tapering off (Dickinson et al., 1995).

Chapter 1: Section 2, Anatomy and function of the rodent medial prefrontal cortex (mPFC)

2.1 Anatomy of the mPFC

The rodent prefrontal cortex is divided into five different subregions based on connectivity with other structures. Our research concerns regions within the ventral medial prefrontal cortex, specifically the prelimbic and infralimbic cortices. The ventral medial region of the rat prefrontal cortex is made up of the prelimbic, infralimbic, and medial orbital cortices (see Figure 2). The prelimbic and infralimbic cortices receive projections from the thalamus (medial dorsal nucleus, paratenial nucleus, and

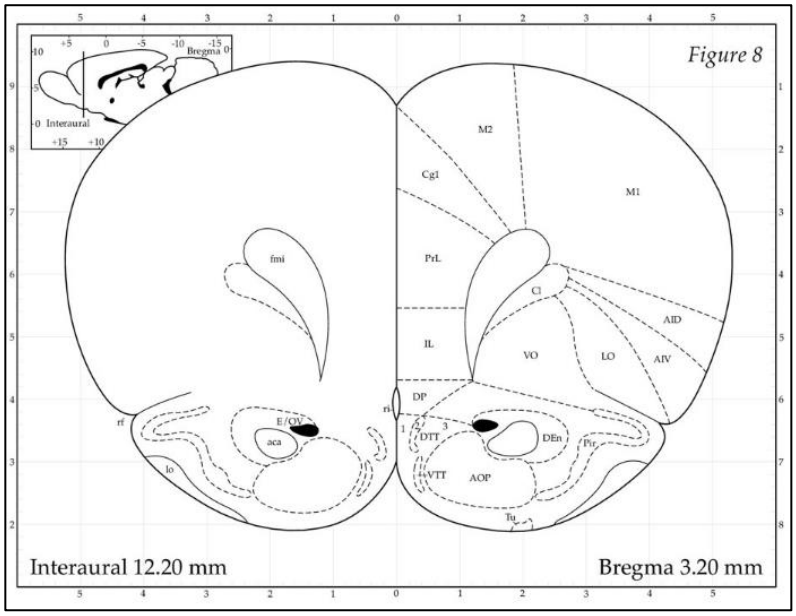

Figure 2. Anatomy of the rat prefrontal cortex. Cg1=cingulate cortex area 1, PrL=prelimbic cortex, IL=infralimbic cortex, VO = ventral orbital cortex. (Paxinos & Watson, 2006).

midline thalamic nuclei) and limbic-related regions (hippocampus, perirhinal cortex, entorhinal cortex, amygdala, and basal forebrain). These regions share connectivity primarily with the limbic system, as the prelimbic cortex projects to the dorsomedial striatum (Sesack et al., 1989). Structurally, the prelimbic cortex is beneath the anterior cingulate while the infralimbic cortex is ventral to the prelimbic cortex. The prelimbic and infralimbic regions are strongly interconnected (Vertes, 2004), and increasing evidence suggests that the infralimbic cortex may inhibit the prelimbic cortex while simultaneously activating its subcortical targets (Riga et al., 2014).

The majority of the medial prefrontal cortex is comprised of excitatory pyramidal neurons (about 90%) while the remaining neurons are GABAergic interneurons. Like much of the neocortex, the medial prefrontal cortex is made up of five different cellular layers. Afferents arrive in the more superficial layers I, II, and III. Pyramidal neurons in layer II of the prelimbic cortex receive functional inputs from the basolateral amygdala, the ventral hippocampus, the contralateral medial prefrontal cortex, and the midline thalamic nucleus. The prelimbic cortex also receives thalamic input from layer I. Projection sites from the prelimbic cortex primarily indicate that it plays a role in limbic and cognitive functions, much like the primate dorsolateral prefrontal cortex. Conversely, efferents from the infralimbic cortex synapse in regions that imply a primary role for it as a controller of visceral and autonomic activity like that of the primate orbitalmedial prefrontal cortex (Vertes, 2004).

Cortical input to the dorsal striatum innervates two neuronal types about equally (Doig et al., 2010; Kress et al., 2013; Wall et al., 2013; Huerta-Ocampo et al., 2014): direct

pathway spiny projection neurons (dSPNs), which express excitatory D1 receptors, and indirect pathway spiny projection neurons (iSPNs), which express D2 inhibitory receptors (Matamales et al., 2009). These GABAergic spiny projection neurons make up approximately 95% of cells in the dorsal striatum. The rodent PL sends excitatory dense connections to the DMS (Groenewegen & Uylings, 2010; Hunnicutt, Jongbloets, Birdsong, Gertz, Zhong, & Mao, 2016; Mailly, Aliana, Groenewegen, Haber, & Deniau, 2013; Sesack, Deutch, Roth, & Bunney, 1989), and these are particularly dense in the anterior portion of the DMS (Hunnicutt et al., 2016; Mailly et al., 2013). This region of the striatum also receives direct input from other regions of the prefrontal cortex, the amygdala, and thalamus, making it a crucial hub for behavior.

2.2 General functions of prelimbic and infralimbic cortices

The rat medial prefrontal cortex has been implicated in many areas of executive functioning and is generally considered to be functionally homologous to, though not as complex as, the primate dorsolateral prefrontal cortex and anterior cingulate (Seamans et al., 2008; Uylings, Groenewegen, & Kolb, 2003). The prelimbic cortex as well as the infralimbic cortex have been implicated in drug seeking behaviors including renewal of extinguished instrumental responding (Eddy et al., 2016; Willcocks & McNally, 2013; Bossert et al., 2011) and extinction of instrumental responding (Peters et al., 2008; LaLumiere et al., 2010). Instrumental renewal is a type of relapse that occurs following extinction training (where reinforcers are absent) that is dependent on context. The most common type of renewal is ABA, in which acquisition of a behavior for a reinforcer occurs in context A, is extinguished in context B, and followed by testing in context A. The

prelimbic cortex appears to be consistently involved in contextual renewal for all reinforcers (cocaine, sucrose and alcohol) (Eddy et al., 2016; Willcocks & McNally, 2013) except for heroin (Bossert et al., 2011). Though, this may be attributable to its role in excitatory contextual associations in operant conditioning (Trask et al., 2017). The infralimbic cortex may be involved in extinction renewal for cocaine and sucrose reinforcers (Eddy et al., 2016; Peters et al., 2008), consolidation of extinction memory for cocaine reinforcement (LaLumiere et al., 2010), context induced renewal for heroin (Bossert et al., 2011) but not for alcohol (Willcocks & McNally, 2013). There is also an extensive literature that implicates the mPFC (implying the prelimbic and infralimbic cortices nonspecifically) in set-shifting (Stefani et al., 2003; Floresco et al., 2008). In set-shifting paradigms, rules for receiving a reward on a particular task change and a new strategy must be adapted. A failure of this cognitive flexibility, or the regions that promote it, results in perseverative errors, or continued usage of the strategy that was initially successful.

The prelimbic and infralimbic cortices are frequently implicated as functional opposites, as in fear conditioning and renewal, in which the prelimbic cortex is involved in responding while the infralimbic cortex is involved in extinction (for a review of prelimbic and infralimbic cortices in both fear and addiction circuits, see Peters et al., 2009). The prelimbic cortex drives behavior, whether it is drug or reward seeking, or expression of conditioned fear, while the infralimbic cortex is necessary for extinction memory (Gourley & Taylor, 2016). Though both areas appear to be important for different aspects of the same phenomena, their roles may actually be more complex than reciprocal. For one, recent

electrophysiological research found that neurons in both the prelimbic and infralimbic cortices fire in response to contextually appropriate behavior such as initiating reward seeking during acquisition and inhibiting responding during extinction (Moorman & Aston-Jones, 2015). Further, the stop-go dichotomy doesn't seem to hold up when comparing different literatures; the infralimbic cortex is involved in both maintaining extinction behavior (not responding) in Pavlovian and operant conditioning, as well as habitual responding (responding despite a devalued reinforcer). Thus, there is considerable interest in further developing our understanding of the contributions of these brain regions to these behaviors (Barker, Taylor, & Chandler, 2014; Sharpe & Killcross, 2018).

2.3 Prelimbic and infralimbic involvement in actions and habits

The prelimbic and infralimbic cortices have been well-established for their roles in goal-directed and habitual responding. Generally, the prelimbic cortex has been implicated in goal-directed learning, as prelimbic lesions prior to training result in insensitivity to reward devaluation (Killcross & Coutureau, 2003; Corbit & Balleine, 2003). Lesion following training or temporary inactivation of the prelimbic cortex by muscimol at the time of test doesn't result in any difference between groups while inactivation during training does (Ostlund & Balleine, 2005; Tran-Tu-Yen et al., 2009). The infralimbic cortex has conversely been implicated in habitual responding. Killcross and Coutureau (2003) found that infralimbic lesions had no effect early on in training when responding was still goal directed. However, following overtraining, lesions of the infralimbic cortex maintained behavior as goal directed, even though controls now responded habitually. Further, pharmacological inactivation by muscimol at the time of test also resulted in a

sensitivity to devaluation that wasn't present in controls (Coutureau & Killcross, 2003). This implies that the infralimbic cortex may be important in the acquisition (Killcross & Coutureau, 2003) and expression (Coutureau & Killcross, 2003) of habitual (S-R) responding.

Chapter 1: Section 3, Neural correlates of actions and habits

3.1 Overview of action and habit circuitry

Generally, the prelimbic cortex (Killcross & Coutureau, 2003; Corbit & Balleine, 2003; Tran-Tu-Yen, 2009), dorsomedial striatum (Yin et al., 2005; Corbit & Janak, 2010; Yin et al., 2005), mediodorsal thalamic nucleus (Corbit et al., 2003; Ostlund & Balleine, 2008) and basolateral amygdala (Ostlund & Balleine, 2008; Balleine et al., 2003; Corbit & Balleine, 2005; Johnson et al., 2009) have been implicated in goal-directed responding. These structures may act in a circuit to encode and express R-O associations and are anatomically connected (Corbit, 2018; Peak, Hart, & Balleine, 2018). Conversely, the infralimbic cortex (Coutureau & Killcross, 2003; Killcross & Coutureau, 2003), dorsolateral striatum (Corbit et al., 2013; Yin et al., 2004; Yin et al., 2006), and the

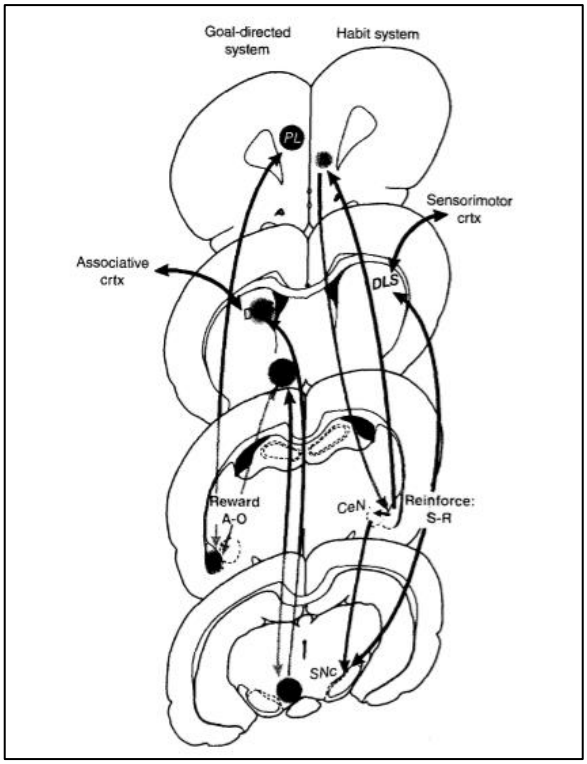

Figure 3. Known action and habit circuitry (Lingawi, Dezfouli, & Balleine, 2016).

central nucleus of the amygdala (Lingawi & Balleine, 2012) have been implicated in habitual responding (See Figure 3 for a summary of these circuits). Additionally, one study has implicated the interpositus nuclei of the cerebellum as involved in habitual responding (Callu et al., 2007).

Brain circuits supporting actions and habits likely interact in a way in which the habitual circuitry inhibits the goal-directed circuitry over training, but where goal-directed circuitry is also able to inhibit habits if they are no longer adaptive (Lingawi et al., 2016). Similarly, goal-directed regions may tonically inhibit habit regions until enough strengthening of these regions across training overcomes this inhibition, and habit execution occurs (Keramati et al., 2011; Peak, Hart, & Balleine, 2018). The balance between the systems is "all-or-none" (Corbit, 2018; Lingawi et al., 2016), meaning interruption of one system results in performance by the other. This is evident in lesion studies, for instance, lesion of the prelimbic cortex early on in training results in behavior that looks habitual (i.e. insensitive to devaluation; Corbit & Balleine, 2003; Killcross & Coutureau, 2003) and infralimbic lesion results in behavior later on in training that is maintained as goal directed even when controls respond habitually (Killcross & Coutureau, 2003; but see the discussion in Shipman et al., 2018). However, these circuits are thought to strengthen together, and inactivation of the DLS has been shown to enhance learning early on in training and affect PL-DMS neural activity (Bergstrom et al., 2018). These circuits may also be hierarchical in that failure of one system (action or habit) to achieve reinforcement may result in switching to the other (Dezfouli et al., 2014).

3.2 Discrepancies in the action/habit literature

Though much is known about the brain regions involved in action and habit, there is debate about when particular areas are involved in learning as well as if specific regions of known areas are involved in behavior. One unknown is the precise time that the prelimbic cortex is involved during behavior: acquisition, expression, or recall. Another unknown is if the anterior portion of the dorsomedial striatum is involved in goal-directed responding in the same way as the posterior region. We detail these discrepancies, which our work addresses, below. In understanding how the action and habit circuits function, it is necessary to determine precise roles for all regions involved.

One discrepancy in the action/habit literature is whether the prelimbic cortex is involved in acquisition, expression, or both. The majority of the literature has utilized permanent lesions prior to acquisition sessions, meaning that the prelimbic cortex is inactivated throughout the duration of training and test. This could mean that the prelimbic cortex is involved in acquisition, recall, or expression of this action-outcome association. Corbit and Balleine (2003) argue that the prelimbic cortex lesion effect is specific to recall rather than acquisition since a test conducted in which rats can earn reinforcers (not in extinction) results in no habitual responding of prelimbic lesioned rats following devaluation. In this case, presence of the outcome maintains responding as sensitive to devaluation (i.e., as an action) despite prelimbic lesion, indicating that lesion of the prelimbic cortex doesn't alter how the response was learned or expressed, but rather, the recall of the action-outcome association. This aligns with our previous results in which pharmacological inactivation at time of test resulted in a reduction of responding on a

minimally-trained (4 days) action (Shipman et al., 2018). However, others have found that lesion following training or temporary inactivation of the prelimbic cortex by muscimol at the time of test doesn't result in any difference between groups while inactivation during training does (Ostlund & Balleine, 2005; Tran-Tu-Yen et al., 2009). Ostlund and Balleine (2005) examined goal-directed responding to compare pre vs post training lesions after 11 acquisition sessions. They found an impairment in responding in the groups with medial prefrontal cortex lesions before training, but no difference between post-training lesions and controls. Similarly, Tran-Tu-Yen et al. (2009) found that pharmacological inactivation of the prelimbic cortex prior to each of six days of acquisition sessions resulted in attenuated responding following devaluation that was not apparent when inactivation occurred only during an extinction test.

Another discrepancy in the literature is that although the posterior DMS has been studied extensively in goal-directed responding, study of the anterior DMS has led to mixed results. The posterior DMS mediates goal-directed responding (Shiflett, Brown, & Balleine, 2010; Yin, Ostlund, Knowlton, & Balleine, 2005; Yin & Knowlton, 2006). The anterior DMS has been shown to both be involved in action expression and acquisition (Corbit & Janak, 2010; Corbit, Nie, & Janak, 2012) or to play no role in actions (Yin et al., 2005). Yet despite this, the aDMS receives denser connections from another region implicated in goal-directed responding, the PL (Hunnicutt et al., 2016; Mailly et al., 2013). Thus, more work needs to be done to determine if the aDMS does indeed participate in actions in the same way that the pDMS does.

Chapter 1: Section 4, Cerebellar anatomy and function

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4.1 Basic cerebellar anatomy

The cerebellum is grossly divided into anatomical zones. Larsell delineated ten distinct lobules, labelled I-X that correspond to vermal sections. This organization varies slightly but is consistent across mammals. The superior posterior lobe is also divided into two parts. Anterior to the horizontal fissure is Crus I while Crus II lies posteriorly (see Figure 4; Voogd & Glickstein, 1998). The cerebellar cortex is made up of five distinct cell types: Purkinje cells, granule cells, Golgi cells, stellate cells and basket cells. Golgi, stellate, and basket cells are inhibitory interneurons while granule cells are glutamatergic. Purkinje cells are the largest cells and sit in between the molecular and granule cell layers. They are GABAergic and directly inhibit the deep cerebellar nuclei or project to brainstem nuclei. The cerebellum can receive information via both the inferior olive and mossy fibers. The inferior olive relays information via climbing fibers to Purkinje cells. Mossy fibers arriving from pontine nuclei synapse on granule cells whose axons become

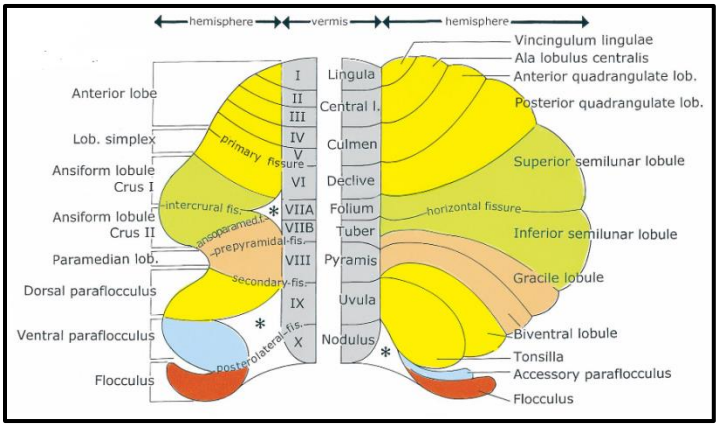

Figure 4. Gross anatomy of the cerebellum (Voogd and Glickstein, 1998). The cerebellar cortex is separated into a central vermis with lateral hemispheres on either side. Lobules V and VI are separated by the primary fissure, which delineates the anterior from the posterior cortex. More posterior are Crus I and Crus II, which are separated by the intercrural fissure.

parallel fibers in the molecular layer. These inputs all eventually arrive at Purkinje cells (Voogd & Glickstein, 1998). The Purkinje cells project to the deep nuclei, which also receive collaterals from mossy fibers and climbing fibers, and integrate information that they receive from the pontine nuclei and the inferior olive. This organization is homogenous across the entire cerebellar cortex and is only distinct based on the inputs received within a particular lobule and where the corresponding deep nuclei project to. Information coming into the cerebellum from the cerebral cortex arrives via cortico-ponto-cerebellar projections (Ramnani, 2006).

There are three pairs of cerebellar nuclei: the fastigial nuclei, the interpositus nuclei and the dentate nuclei. These are located, respectively, more medial to more lateral, with the fastigial nucleus located within the vermis, and the dentate nuclei in the lateral hemispheres. The deep cerebellar nuclei are the only means of output from the cerebellum to the cerebral cortex, and projections from them leave to synapse in the ventrolateral or medial dorsal nuclei of the thalamus. From there, cerebellar information can be sent to other areas of the cerebral cortex including the primary motor cortex, the striatum, and at least in monkeys, the prefrontal cortex (Ramnani, 2006).

4.2 Anatomical evidence for cerebellar role in "cognitive" function

In non-human primates, anatomical research has identified "cognitive" pathways, distinct from motor pathways, that link cerebral cortical structures to the cerebellum (for reviews, see Bostan, Dum, & Strick, 2013; Strick et al., 2009). The lateral hemispheres of the cerebellum can be divided into ten lobules and considered extensions of the ten vermal lobules (Larsell, 1952), although there are a number of organizational schemes and

nomenclatures (Schmahmann et al., 1999; Voogd & Glickstein, 1998). Using *Cebus apella* monkeys, Kelly and Strick (2003) showed that injection of a transneuronal retrograde tracer (rabies virus) into the arm area of primary motor cortex labeled Purkinje cells in mediolateral regions of lobules IV-VI, and also some Purkinje cells in the hemispheric portions of lobules VIIb and HVIII. Injections of a transneuronal anterograde tracer (H129 strain of herpes simplex virus type 1) into the arm area of primary motor cortex labeled granule cells in these same regions of cerebellar cortex, suggesting a closed "motor" loop between at least the arm area of primary motor cortex and select regions of cerebellar cortex. More importantly, they showed that injection of a transneuronal retrograde tracer into area 46 of cerebral cortex labeled Purkinje cells in lateral regions of Crus II (along with Crus I, the hemispheric extension of lobule VIIa), and also a few Purkinje cells in vermal lobule X and vermal parts of lobule VII; injection of a transneuronal anterograde tracer into area 46 labeled granule cells in these same regions of cerebellar cortex, except that granule cells were labeled in lobule IX rather than X. Area 46 of cerebral cortex has been shown to be involved in working memory, decision making, temporal processing and other "cognitive" functions (e.g., Barbey, Koenigs, & Grafman, 2013). Overall, the results of Strick and colleagues suggested a "cognitive" loop between area 46 and select regions of cerebellar cortex that is distinct from a "motor" loop between primary motor cortex and separate regions of cerebellar cortex.

More recently, Bernard and colleagues used resting-state functional connectivity magnetic resonance imaging (fcMRI) to show similarly separate "motor" and "cognitive" corticocerebellar loops in humans based on separate connections of the dorsal and ventral

dentate nucleus; the dentate nucleus is the lateral-most deep cerebellar nucleus (Bernard et al., 2014). More specifically, fcMRI revealed functional connectivity between the dorsal dentate nucleus and lobules I-VI of cerebellum on the one hand, and the dorsal dentate nucleus and primary motor cortex and premotor cortex, as well as the putamen and the inferior parietal lobule, on the other. This corresponds to a "motor" loop. A separate "cognitive" loop (with the exception of overlap with the motor loop in lobule VI) was revealed between the ventral dentate nucleus and lobule VI, Crus II, lobule VIIb, and vermal VIIIb of cerebellar cortex on the one hand, and the anterior cingulate cortex, as well as the caudate nucleus and the thalamus, on the other (Bernard et al., 2014). Sub-millimeter diffusion MRI combined with probabilistic tractography demonstrated in humans that hemispheric portions of lobules IV, V, and VI connect to the dorsal dentate nucleus and Crus I and Crus II connect to the ventrolateral dentate nucleus (Steele et al., 2017). Meta-analytic connectivity modeling showed that, across studies, the hemispheric portions of lobules V, VI, VIIb, and VIII were activated together with motor and somatosensory regions of cortex; behaviorally, these regions were activated by motor tasks such as finger tapping and overt reading (Balsters, Laird, Fox, & Eickhoff, 2014). In contrast, Crus I and Crus II were activated together with prefrontal cortex and parietal cortex; these regions were activated by "cognitive" tasks, such as passive listening, the Stroop task, and the Simon task (Balsters, Laird, Fox, & Eickhoff, 2014).

The primate cerebellum has been implicated in the acquisition and storage of internal forward and inverse models, which predict the outcomes of movements and transform goals into movements, respectively (Ito, 2008; Koziol, Budding, & Chidekel,

2012). The movement-related functions of the primate cerebellum have been extended to thought or cognition (Leiner et al., 1986; Schmahmann, 1991), based on both its connections with non-motor regions of prefrontal cortex (as detailed above) and on its uniform internal circuitry (Ito, 2008; Katz & Steinmetz, 2002; Koziol et al., 2014; Leiner et al., 1989; Popa, Hewitt, & Ebner, 2014; Ramnani, 2006; Schmahmann, 1991). The general idea is that the uniform internal circuitry of the cerebellar cortex suggests that what a given region of cerebellar cortex computes is determined by its input and output connections. Lateral cerebellar cortical areas, particularly Crus I and Crus II, receive projections from prefrontal cortex (via pontine nuclei) and project back to these same regions (via ventral dentate nucleus and thalamic nuclei). Since these regions of prefrontal cortex are believed to have non-motor, "cognitive" functions, then the regions of cerebellar cortex that they are reciprocally connected to must also be engaged in processing cognitive data. For example, Ito (2008) proposed that an implicit, internal model is formed in the cerebellum that mimics an explicit, mental model formed in the cerebral cortex, analogous to an internal model formed in the cerebellum that models a movement; both are subject to error correction. A related idea is that the cerebellum contributes to the automatization of both thought and action (Ramnani, 2014). Some researchers have proposed that there is no real distinction between motor and non-motor functions of the cerebellum (Bloedel & Bracha, 1997; Katz & Steinmetz, 2002; Koziol et al., 2012).

In rodents, as in non-human primates, multiple regions of the cerebral cortex have been shown to project to the pontine nuclei. Retrograde tracing studies using horseradish peroxidase injections into the pontine nuclei have shown that the heaviest projections to

the rat pontine nuclei come from motor, somatosensory, and visual cortical areas, but there are also significant projections from the cingulate cortex and the retrosplenial cortex (Legg, Mercier, & Glickstein, 1989; Wiesendanger & Wiesendanger, 1982). There are also projections from auditory cortex and insular cortex to the pontine nuclei (Legg et al., 1989; Wiesendanger & Wiesendanger, 1982). Retrograde tracing using horseradish peroxidase injections into the cerebellar hemispheres have mapped pontine nuclei projections to hemispheric portions of lobule VI (lobulus simplex), Crus I, Crus II, and the paramedian lobule (Mihailoff, Burne, Azizi, Norell, & Woodward, 1981). More recently, transsynaptic rabies virus retrograde tracer has been used to map outputs from the cerebral cortex to four regions of the cerebellar cortex: Crus IIb, the vermal portion of lobule VII, the paramedian lobule, and lobule VIII (Suzuki, Coulon, Sabel-Goedknegt, & Ruigrok, 2012). This study showed that injections of retrograde tracer into Crus IIb labeled neurons in the face region of somatosensory cortex; injections into the paramedian lobule labeled neurons in primary and secondary motor cortex and in the forelimb region of somatosensory cortex; injections into lobule VIII labeled neurons in the primary and secondary motor cortex and in the hindlimb region of somatosensory cortex (Suzuki et al., 2012). Especially interesting were the results of injections into vermal lobule VII, which revealed intense labeling of neurons in ventrolateral orbital cortex, as well as retrosplenial cortex (Suzuki et al., 2012). It is worth nothing that it has recently been suggested that Crus I in rodents, an area not investigated by Suzuki et al. (2012), is homologous to Crus I/II in primates (Sugihara, 2018). Functionally, studies using rodent eyeblink conditioning have shown that medial

prefrontal cortex inputs to pontine nuclei can modulate this cerebellar-dependent form of learning (Siegel et al., 2015).

In terms of projections from the cerebellum that are in a position to influence cerebral cortex, it is well established that the rodent cerebellum, like the primate cerebellum, projects to various thalamic nuclei via the deep cerebellar nuclei (Houck & Person, 2015; Locke et al., 2018; for a review, see Voogd, 2004). What is less well understood in rodents, compared to primates, is the extent to which the cerebellum is connected, via thalamic nuclei, to non-motor regions of cerebral cortex and is therefore in a position to influence non-motor functions traditionally associated with cerebral cortex. We are aware of only a few published studies that have attempted to address this question. In one study, co-infusion of a retrograde tracer into posterior parietal cortex and an anterograde tracer into lateral (dentate) nucleus of rats yielded co-localization in centrolateral and ventrolateral thalamic nuclei (Giannetti & Molinari, 2002). An experiment reported by Parker and colleagues showed that co-infusion of a retrograde tracer into the anterior cingulate cortex and anterograde tracer into the lateral (dentate) nucleus of rats yielded co-localization in the ventrolateral thalamic nuclei, as well as ventral tegmental area nuclei (Parker, Narayanan, & Andreasen, 2014). Microstimulation of the prelimbic cortex evoked field potentials in cerebellar cortical lobule VII along the vermis and caused complex spikes in Purkinje cells in the same area, suggesting prelimbic cortex activation of climbing fibers in the inferior olive (Watson, Jones, & Apps, 2009). Microstimulation of the medial (fastigial) nucleus elicited local field potentials in the prelimbic cortex (Watson, Becker, Apps, & Jones, 2014). Similarly, lateral (dentate)

nucleus stimulation in mice resulted in dopamine efflux in the prelimbic region of medial prefrontal cortex (Mittleman, Goldowitz, Heck, & Blaha, 2008; Rogers, Dickson, Heck, Goldowitz, Mittleman, & Blaha, 2011; Rogers et al., 2013). More work is needed to directly determine rodent cerebellar afferents to the cerebrum.

4.3 Overview of cerebellar role in "cognitive" function

In humans compared to other species, the expansion in size of both association neocortex and the lateral cerebellum (particularly the ventrolateral portion of the lateral-most deep cerebellar nucleus, the dentate nucleus), as well as evidence from patient case studies, suggested that at least the human cerebellum might be involved in more than movement (Leiner, Leiner, & Dow, 1986, 1989; Schmahmann, 1991). Research in the past several decades has supported this view for the non-human primate cerebellum in general.

Early on, the non-human animal literature suggested the possibility of cerebellar involvement in non-motor functions such as sensory processing, discrimination learning, spatial learning, motivation, and emotion (Berntson & Torello, 1982; Lalonde, 1994; Lalonde & Botez, 1990; Watson, 1978). Further, clinical observations by Schmahmann and colleagues of non-motor, "cognitive" dysfunctions in cerebellar patients with damage to lateral cerebellum, including deficits in executive function, visuo-spatial processing, and linguistic processing, and "emotional" dysfunctions in cerebellar patients with damage to the cerebellar vermis, led to the proposal of Cerebellar Cognitive Affective Syndrome (Schmahman, 2004; Schmahmann & Sherman, 1998; Schmahmann, Weilburg, & Sherman, 2007; Stoodley & Schmahmann, 2010). The development of functional neuroimaging allowed well-controlled experiments to be conducted on motor vs. non-

motor functions of the human cerebellum (Buckner, 2013). Functional neuroimaging studies have also found that the cerebellum appears to be involved in cognitive performance in a way that cannot be explained solely by motor function (Balsters, Whelan, Robertson, & Ramnani, 2013; Desmond, Gabrieli, Wagner, Ginier, & Glover, 1997; Kim, Uğurbil, & Strick, 1994; Küper et al., 2011; Riedel et al., 2015; Thurling et al., 2012). In addition, the advent of multi-synaptic tract tracing techniques provided additional evidence that the primate cerebellum is disynaptically connected with both motor and non-motor areas of the frontal cortex (Buckner, 2013; Strick, Dum, & Fiez, 2009). Even so, not all researchers have been convinced that the primate cerebellum has non-motor functions; for example, an alternative proposal is that most of the association cortex input to the primate lateral cerebellum is visual in nature and most of the output of the primate lateral cerebellum to "non-motor" cortical regions is actually to regions controlling eye movements (Glickstein, 1993, 2006, 2007). Thus, the larger size of the lateral cerebellum in primates might be due to increased demand on coordination between the visual and motor systems. However, it has also been argued that even eye movements can be shown to involve "cognitive" components; for example, saccadic eye movements can be influenced by decisions about where to look (i.e., can be viewed as a goal-directed behavior; cf. Hutton, 2008).

Recent consensus papers suggest that the view that the cerebellum contributes to non-motor functions, at least in humans and non-human primates, is now widespread (Bodranghien et al., 2016; Caligiore et al., 2017; Koziol et al., 2014). Koziol et al. (2014) concluded that there was unanimous agreement among the 14 co-authors that the

cerebellum contributes to cognition, in addition to movement. They suggested that researchers must now come to agreement on how the cerebellum contributes to cognition, as current conclusions are inferential based on our knowledge of the cerebellum's contributions to motor function, the cerebellum's uniform internal circuitry, and the cerebellum's connections with non-motor cortical areas. Bodranghien et al. (2016) also concluded that the cerebellum contributes to non-motor functions, both cognitive and emotional, and that cognitive deficits in patients result from damage to lateral cerebellum. Caligiore at al. (2017) stressed anatomical findings that identify separate motor and cognitive loops and urged researchers to consider the broader systems-level role of the cerebellum in relation to the cerebral cortex, as well as the basal ganglia.

4.4 Role of cerebellum in actions and habits

Almost no work has examined a possible involvement of the rodent cerebellum in goal-directed and/or habitual behavior, yet there is solid evidence that the rodent prefrontal cortex is important for both (Corbit & Balleine, 2003; Coutureau & Killcross, 2003; Hart, Bradfield, & Balleine, 2018; Hart, Bradfield, Fok, Chieng, & Balleine, 2018; Killcross & Coutureau, 2003; Ostlund & Balleine, 2005; Shipman, Trask, Bouton, & Green, 2018; Tran-Tu-Yen, Marchand, Pape, DiScala, & Coutureau, 2009). This is a potentially interesting line of future work.

Goal-directed behavior involves knowledge of the outcome that will result from a particular action (Dickinson, 1985). Behaviorally, reinforcer devaluation is a way to determine if a behavior is goal-directed; a reduction in behavior after reinforcer devaluation is indicative of behavior guided by a response-outcome (R-O) association (Dickinson,

1985). Though the cerebellum has not been directly linked with goal-directed behavior, a recent study has shown that it may be involved in reinforcer expectation. Wagner and colleagues used two-photon calcium imaging *in vivo* to examine the same cerebellar granule cells over a span of days in mice who genetically expressed a fluorescent calcium indicator specific to cerebellar granule cells (GCaMP6f) (Wagner, Kim, Savall, Schnitzer, & Luo, 2017). These mice were trained to push a manipulandum forward with their forelimb for a sucrose liquid reinforcer. Different granule cells responded to expected reward, unexpected reward, and omitted reward. Over trials, cells that initially responded to reward started to respond in anticipation of reward and cells that responded to omitted rewards continued to do so. This pattern was also evident in a simple Pavlovian task, when a tone was associated with sucrose delivery. These results were not explained by motor responses, as different operant responses (push vs pull) resulted in different "motor" granule cell activity but failed to affect "reward anticipation" or "reward omission" cells. This activity also could not be explained by sensory input, as "reward" cells but not "anticipatory reward" cells showed a calcium response when an unexpected reward occurred and "reward omission" activity occurred in the absence of reward, implicating a role for the cerebellum in tracking conditioned reinforcers.

Habitual behavior is generally determined by a behavior's insensitivity to reinforcer devaluation. Stimulus-response (S-R) associations are thought to underlie this behavior (Dickinson, 1985), as stimuli associated with an operant response are enough to elicit that response despite the outcome no longer being valued. Thus, habitual behavior is insensitive to reinforcer devaluation. Much like cognitive flexibility, habitual behavior involves an

element of perseveration, and tends to be more prevalent following overtraining. One human fMRI study found that when behavior was manipulated to be more habitual (as opposed to goal-directed), stronger cerebellar activation over the first two acquisition blocks predicted an increased likelihood of responding on devalued trials later on in the experiment (Liljeholm, Dunne, & O'Doherty, 2015). A similar fMRI study examined action-habit conflicts by training responses and then devaluing some responses. Subsequent tests put participants under time pressure to choose a response, favoring the less effortful habit response. It was found that goal-directed performance during the test was negatively correlated with cerebellar activation during acquisition, implicating the cerebellum in habit formation (Watson, van Wingen, & de Wit, 2018).

To our knowledge, only one rodent study has examined a role for the cerebellum in habitual behavior; the results of the study suggested a role for the interpositus nucleus in the transition of instrumental behavior from goal-directed to habitual (Callu, Puget, Faure, Guegan, & Massioui, 2007). Callu et al. (2007) made bilateral electrolytic interpositus nuclei lesions in some rats, and a thin midline lesion of the vermis in other rats to mimic a common surgical injury to the cerebellum when posterior fossa tumors are removed. Rats were trained on a discriminated operant conditioning procedure across many acquisition sessions in which they learned to press a lever for a food pellet during a 10-sec tone. There was no difference in acquisition of lever pressing between groups, similar to a previous study that observed no effect of dentate-interpositus nucleus lesions on discriminated (1-sec tone) operant lever-press conditioning for a food pellet (Steinmetz, Logue, & Miller, 1993). Subsequently, the rats in Callu et al. were given a conditioned taste aversion

procedure to devalue the food pellets. In a test phase, control rats and rats with vermis lesions continued to lever-press for the now-devalued pellets, indicating habitual responding. In contrast, rats with interpositus nuclei lesions reduced lever-pressing in the test phase, suggesting that their lever-pressing remained goal-directed, and had never transitioned to habitual.

In summary, though there is evidence hinting at a cerebellar role in goal-directed and/or habitual behavior, there is not enough research that has directly measured its involvement in these behaviors.

Chapter 1: Section 4, DREADDs as a technique

Behavioral neuroscience research has benefited from techniques that allow more precise ways of targeting and manipulating different brain regions and neural pathways such as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). This technology works by surgically infusing a viral construct carrying the DREADD transgene, a promoter element, and a reporter element into the brain region of interest. The most widely-used DREADDs are mutations of muscarinic receptors that no longer bind acetylcholine but instead are activated solely by clozapine-N-oxide (CNO). Using a Gi-coupled DREADD, injection of CNO causes a sustained but temporary inactivation of the infected neurons (Rogan & Roth, 2011). Mechanistically, inhibitory DREADD receptor (hM4Di) activation suppresses this pathway-specific firing through inhibition of presynaptic terminal neurotransmitter release (Mahler et al., 2014; Stachniak et al., 2014). This may be due to inhibition of c-AMP signaling, thus affecting voltage-gated calcium channels or by inhibiting SNARE fusion proteins via G-protein Bγ-subunits (Zhu &

Roth, 2014). In behavioral experiments, CNO can be injected intraperitoneally, allowing inactivation (or activation) of DREADD-expressing cells for the duration of a training session. In comparison to a cannula infusion, intraperitoneal injections are minimally invasive and may also circumvent issues that arise with repeated drug infusions such as sensitization and tissue damage. Additionally, this sustained inactivation may be preferable to optogenetics, which can be too temporally precise if the exact timing of a specific brain region's involvement in a complex behavior is unknown or a broader aspect of behavior is being investigated.

DREADDs also allow for inactivation of specific neural pathways. There are two ways in which this can be done: utilizing Cre-technology or intracranial infusions of CNO. With the first method, infusion of a Cre-dependent DREADD virus into a projecting region, and subsequent infusion of a Cre-retrograde virus into a region that the first region projects to allows for DREADD expression only in cell bodies in the region that receives retrograde transport from the Cre- vectors and that has received the Cre-dependent DREADD. Intraperitoneal injections of CNO then can silence this pathway. One potential issue with this is that the Cre-dependent DREADD virus may also inactivate collaterals, though the extent to which this occurs is unknown. However, it has been argued that this may actually be a more biologically relevant means of pathway inactivation (Campbell & Marchant, 2018).

The other way of inactivating a neural pathway with DREADDs is by infusing a viral vector into a projecting region and implanting cannulae into a region that receives projections from the first region. Some types of vectors transport DREADD receptors

down axons and allow for expression in axon terminals. Thus, subsequent intracranial CNO infusion via cannulae into a region that receives projections from the region of virus infusion will result in inactivation of DREADDs being expressed on the axon terminals of projecting neurons. This means of pathway-specific intervention is more invasive than the former method and reliant on higher doses of CNO, though direct intracranial infusion circumvents concerns that CNO may not be crossing the blood-brain-barrier (BBB) (Campbell & Marchant, 2018).

One caveat to DREADD use is that CNO, or its metabolite clozapine, may have unintentional behavioral effects. However, it is well-agreed upon by behavioral neuroscientists that including a non-DREADD group that receives CNO as a control can mitigate concerns (Campbell & Marchant, 2018; Smith et al., 2016). Additionally, CNO may not readily cross the BBB, and thus, IP injections of CNO can mean that clozapine, its metabolite, is activating any brain receptors (Gomez et al., 2017).

Chapter 1: Section 5, The Current Report

This collective research seeks to expand upon our current understanding of the neural correlates of operant responding, particularly in the expression of goal-directed behavior. For one, we attempt to address some of the discrepancies in the action/habit literature, including determining the involvement of the prelimbic cortex in the expression of operant behavior. We also attempt to further this understanding by expanding the role of the PL in expression to the role of the PL-to-anterior DMS, which addresses a disagreement in anterior DMS involvement in operant responding. Finally,

we explore a novel role in actions and habits of Crus I/II of the cerebellar cortex, a region that may be linked to higher cognitive functions (see Shipman & Green, in press).

Specifically, Chapter 2 seeks to clarify the roles of the prelimbic and infralimbic cortices in goal-directed behavior. Though the prelimbic cortex has previously been implicated in the acquisition of goal-directed responding and the infralimbic cortex has been implicated in habit, we examine the effect of inactivation of each of these regions following different amounts of training on two responses in separate contexts. These experiments aim to parse out prelimbic vs. infralimbic functions in the expression of minimally vs. extensively trained behavior that is still goal-directed. Thus, this research expands our understanding of the roles of these two brain regions in specific aspects of actions and habits.

Chapter 3 aims to expand our understanding of prelimbic function beyond the PL to its projection to the anterior region of the dorsomedial striatum. We have previously shown that the PL is important for operant responding in the acquisition context (Trask et al., 2017). Utilizing the same paradigm and a relatively new technique, DREADDs, to selectively inactivate the PL-to-DMS pathway, we explore its role in the expression of operant responding.

Finally, Chapter 4 examines a potential role for Crus I/II in the expression of actions and habits. An extensive human literature and a few rodent studies have implicated the cerebellum in "cognition" and hinted at its involvement in actions and/or habits. We explore this by minimally and extensively training separate goal-directed responses and also extensively training behavior to the point of habit, then inactivating

Crus I/II at time of test. These results are highly novel and expand our understanding of the circuitry that may be driving actions and habits.

Together, these experiments increase our understanding of PL, IL, PL-to-anterior dorsomedial striatum, and Crus I/II involvement in different aspects of operant responding.

References

Balleine, B. W., Killcross, A., & Dickinson, A. (2003). The effect of lesions of the basolateral amygdala on instrumental conditioning. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *23*(2), 666–75.

Balleine, B. W., & O'Doherty, J. P. (2010). Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *35*(1), 48–69.

Balsters, J. H., Laird, A. R., Fox, P. T., & Eickhoff, S. B. (2014). Bridging the gap between functional and anatomical features of cortico-cerebellar circuits using meta-analytic connectivity modeling. *Human Brain Mapping, 35*, 3152-3169.

Balsters, J. H., Whelan, C. D., Robertson, I. H., & Ramnani, N. (2013). Cerebellum and cognition: Evidence for the encoding of higher order rules. Cerebral Cortex, 23, 1433-1443.

Barbey, A. K., Koenigs, M., & Grafman, J. (2013). Dorsolateral prefrontal contributions to human working memory. *Cortex, 49*, 1195-1205.

Barker, J. M., Taylor, J. R., & Chandler, L. (2014). A unifying model of the role of the infralimbic cortex in extinction and habits. *Learning & memory (Cold Spring Harbor, N.Y.)*, *21*(9), 441–8.

Bergstrom, H. C., Lipkin, A. M., Lieberman, A. G., Pinard, C. R., Gunduz-Cinar, O., Brockway, E. T., ... & Rubio, F. J. (2018). Dorsolateral Striatum Engagement Interferes with Early Discrimination Learning. *Cell reports*, *23*(8), 2264-2272.

Bernard, J. A., Peltier, S. J., Benson, B. L., Wiggins, J. L., Jaeggi, S. M., Buschkuehl, M., . . . Seidler, R. D. (2014). Dissociable functional networks of the human dentate nucleus. *Cerebral Cortex, 24*, 2151-2159.

Berntson, G. G., & Torello, M. W. (1982). The paleocerebellum and the integration of behavioral function. *Physiological Psychology, 10*, 2-12.

Bloedel, J. R., & Bracha, V. (1997). Duality of cerebellar motor and cognitive functions. *International Review of Neurobiology, 41*, 613-634.

Bodranghien, F., Bastian, A., Casali, C., Hallett, M., Louis, E. D., Manto, M., . . . Dun, K. v. (2016). Consensus paper: Revisiting the symptoms and signs of cerebellar syndrome. *Cerebellum, 15*, 369-391.

Bossert, J. M., Stern, A. L., Theberge, F. R., Cifani, C., Koya, E., Hope, B. T., & Shaham, Y. (2011). Ventral medial prefrontal cortex neuronal ensembles mediate context-induced relapse to heroin. *Nature neuroscience*, *14*(4), 420.

Bostan, A. C., Dum, R. P., & Strick, P. L. (2013). Cerebellar networks with the cerebral cortex and basal ganglia. *Trends in Cognitive Sciences, 17*, 241-254.

Buckner, R. L. (2013). The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging. *Neuron, 80*, 807-815.

Caligiore, D., Pezzulo, G., Baldassarre, G., Bostan, A. C., Strick, P. L., Doya, K., . . . Herreros, I. (2017). Consensus paper: Towards a systems-level view of cerebellum function: the interplay between cerebellum, basal ganglia, and cortex. *Cerebellum, 16,* 203-229.

Callu, D., Puget, S., Faure, A., Guegan, M., & Massioui, N. (2007). Habit learning dissociation in rats with lesions to the vermis and the interpositus of the cerebellum. *Neurobiology of disease*, *27*(2), 228–37.

Campbell, E. J., & Marchant, N. J. (2018). The use of chemogenetics in behavioural neuroscience: receptor variants, targeting approaches and caveats. *British journal of pharmacology*, *175*(7), 994-1003.

Corbit, L. H. (2018). Understanding the balance between goal-directed and habitual behavioral control. *Current opinion in behavioral sciences*, *20*, 161-168.

Corbit, L. H., & Balleine, B. W. (2003). The role of prelimbic cortex in instrumental conditioning. *Behavioural brain research*, *146*(1–2), 145–57.

Corbit, L. H., & Balleine, B. W. (2005). Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *25*(4), 962–70.

Corbit, L. H., & Janak, P. H. (2010). Posterior dorsomedial striatum is critical for both selective instrumental and Pavlovian reward learning. *The European journal of neuroscience*, *31*(7), 1312–21.

Corbit, L. H., Leung, B. K., & Balleine, B. W. (2013). The role of the amygdala-striatal pathway in the acquisition and performance of goal-directed instrumental actions. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *33*(45), 17682–90.

Corbit, L. H., Muir, J. L., & Balleine, B. W. (2003). Lesions of mediodorsal thalamus and anterior thalamic nuclei produce dissociable effects on instrumental conditioning in rats. *The European journal of neuroscience*, *18*(5), 1286–94.

Corbit, L. H., Nie, H., & Janak, P. H. (2012). Habitual Alcohol Seeking: Time Course and the Contribution of Subregions of the Dorsal Striatum. *Biological Psychiatry*, *72*(5), 389–395.

Coutureau, E., & Killcross, S. (2003). Inactivation of the infralimbic prefrontal cortex reinstates goal-directed responding in overtrained rats. *Behavioural brain research*, *146*(1–2), 167–74.

Desmond, J. E., Gabrieli, J. D. E., Wagner, A. D., Ginier, B. L., & Glover, G. H. (1997). Lobular patterns of cerebellar activation in verbal working-memory and finger-tapping tasks as revealed by functional MRI. *Journal of Neuroscience, 17*, 9675-9685.

Dezfouli, A., Lingawi, N. W., & Balleine, B. W. (2014). Habits as action sequences: hierarchical action control and changes in outcome value. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *369*(1655).

Dickinson, A. (1985). Actions and habits: the development of behavioural autonomy. *Philosophical Transactions of the Royal Society of London, 308,* 67-78.

Dickinson, A., Balleine, B., Watt, A., Gonzalez, F., & Boakes, R. A. (1995). Motivational control after extended instrumental training. *Animal Learning & Behavior*, *23*(2), 197-206.

Dickinson, A., Nicholas, D. J., & Adams, C. D. (1983). The effect of the instrumental contingency on susceptibility to reinforcer devaluation. *Quarterly Journal of Experimental Psychology*, 35B, 35–51.

Eddy, M. C., Todd, T. P., Bouton, M. E., & Green, J. T. (2016). Medial prefrontal cortex involvement in the expression of extinction and ABA renewal of instrumental behavior for a food reinforcer. *Neurobiology of learning and memory*, *128*, 33–9.

Floresco, S. B., Block, A. E., & Maric, T. L. (2008). Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. *Behavioural brain research, 190*(1), 85-96.

Giannetti, S., & Molinari, M. (2002). Cerebellar input to the posterior parietal cortex in the rat. *Brain Research Bulletin, 58*, 481-489.

Glickstein, M. (1993). Motor skills but not cognitive tasks. *Trends in Neurosciences, 16*, 450-451.

Glickstein, M. (2006). Thinking about the cerebellum. *Brain, 129,* 288-292.

Glickstein, M. (2007). What does the cerebellum really do? *Current Biology, 17*, R824-R827.

Gomez, J. L., Bonaventura, J., Lesniak, W., Mathews, W. B., Sysa-Shah, P., Rodriguez, L. A., ... & Pomper, M. G. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science*, *357*(6350), 503-507.

Gourley, S. L., & Taylor, J. R. (2016). Going and stopping: dichotomies in behavioral control by the prefrontal cortex. *Nature neuroscience*, *19*(6), 656–64.

Groenewegen, H. J., & Uylings, H. B. (2010). Organization of prefrontal-striatal connections. In *Handbook of Behavioral Neuroscience*, 20, 353-365. Elsevier.

Hart, G., Bradfield, L. A., & Balleine, B. W. (2018). Prefrontal cortico-striatal disconnection blocks the acquisition of goal-directed action. *Journal of Neuroscience*, *38*, 1311-1322.

Hart, G., Bradfield, L. A., Fok, S. Y., Chieng, B., & Balleine, B. W. (2018). The bilateral prefronto-striatal pathway is necessary for learning new goal-directed actions. *Current Biology, 28*, 1-12.

Houck, B. D., & Person, A. L. (2015). Cerebellar premotor output neurons collateralize to innervate the cerebellar cortex. *Journal of Comparative Neurology*, *523*, 2254-2271.

Hunnicutt, B. J., Jongbloets, B. C., Birdsong, W. T., Gertz, K. J., Zhong, H., & Mao, T. (2016). A comprehensive excitatory input map of the striatum reveals novel functional organization. *eLife, 5*.

Hutton, S. B. (2008). Cognitive control of saccadic eye movements. *Brain and Cognition, 68*, 327-340.

Ito, M. (2008). Control of mental activities by internal models in the cerebellum. *Nature Reviews Neuroscience, 9*, 304-313.

Johnson, A. W., Gallagher, M., & Holland, P. C. (2009). The basolateral amygdala is critical to the expression of pavlovian and instrumental outcome-specific reinforcer devaluation effects. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 29*(3), 696–704.

Katz, D. B., & Steinmetz, J. E. (2002). Psychological functions of the cerebellum. *Behavioral and Cognitive Neuroscience Reviews, 1*, 229-241.

Keramati, M., Dezfouli, A., & Piray, P. (2011). Speed/accuracy trade-off between the habitual and the goal-directed processes. *PLoS computational biology, 7*(5), e1002055.

Killcross, S., & Coutureau, E. (2003). Coordination of actions and habits in the medial prefrontal cortex of rats. *Cerebral cortex (New York, N.Y. : 1991), 13*(4), 400–8.

Kim, S. G., Uğurbil, K., & Strick, P. L. (1994). Activation of a cerebellar output nucleus during cognitive processing. *Science, 265*, 949-951.

Koziol, L. F., Budding, D., Andreasen, N., D'Arrigo, S., Bulgheroni, S., Imamizu, H., . . . Yamazaki, T. (2014). Consensus paper: The cerebellum's role in movement and cognition. *Cerebellum, 13,* 151-177.

Koziol, L. F., Budding, D. E., & Chidekel, D. (2012). From movement to thought: Executive function, embodied cognition, and the cerebellum. *Cerebellum, 11*, 505-525.

Kuper, M., Dimitrova, A., Thurling, M., Maderwald, S., Roths, J., Elles, H. G., . . . Timmann, D. (2011). Evidence of a motor and a non-motor domain in the human dentate nucleus: An fMRI study. *Neuroimage, 54,* 2612-2622.

Lalonde, R. (1994). Cerebellar contributions to instrumental learning. *Neuroscience and Biobehavioral Reviews, 18,* 161-170.

Lalonde, R., & Botez, M. I. (1990). The cerebellum and learning processes in animals. *Brain Research Reviews, 15,* 325-332.

LaLumiere, R. T., Niehoff, K. E., & Kalivas, P. W. (2010). The infralimbic cortex regulates the consolidation of extinction after cocaine self-administration. *Learning & memory (Cold Spring Harbor, N.Y.), 17*(4), 168–75.

Larsell, O. (1952). The morphogenesis and adult pattern of the lobules and fissures of the cerebellum of the white rat. *Journal of Comparative Neurology, 97*, 281-356.

Legg, C. R., Mercier, B., & Glickstein, M. (1989). Corticopontine projection in the rat: The distribution of labelled cortical cells after large injections of horseradish

peroxidase in the pontine nuclei. *Journal of Comparative Neurology, 286,* 427-441.

Leiner, H. C., Leiner, A. L., & Dow, R. S. (1986). Does the cerebellum contribute to mental skills? *Behavioral Neuroscience, 100,* 443-454.

Leiner, H. C., Leiner, A. L., & Dow, R. S. (1989). Reappraising cerebellum: What does the hindbrain contribute to the forebrain? *Behavioral Neuroscience, 103,* 998-1008.

Liljeholm, M., Dunne, S., & O'Doherty, J. P. (2015). Differentiating neural systems mediating the acquisition vs. expression of goal-directed and habitual behavioral control. *European Journal of Neuroscience, 41,* 1358-1371.

Lingawi, N. W., Dezfouli, A., & Balleine, B. W. (2016). The psychological and physiological mechanisms of habit formation. In R. A. Murphy & R. C. Honey (Ed) *The Wiley Handbook on the Cognitive Neuroscience of Learning,* 411-440.

Lingawi, N. W., & Balleine, B. W. (2012). Amygdala central nucleus interacts with dorsolateral striatum to regulate the acquisition of habits. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 32*(3), 1073–81.

Locke, T. M., Soden, M. E., Miller, S. M., Hunker, A., Knakal, C., Licholai, J. A., . . . Carlson, E. S. (2018). Dopamine D1 receptor-positive neurons in the lateral nucleus of the cerebellum contribute to cognitive behavior. *Biological Psychiatry*.

Mahler, S. V., Vazey, E. M., Beckley, J. T., Keistler, C. R., McGlinchey, E. M., Kaufling, J., ... & Aston-Jones, G. (2014). Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. *Nature neuroscience, 17*(4), 577.

Mihailoff, G. A., Burne, R. A., Azizi, S. A., Norell, G., & Woodward, D. J. (1981). The pontocerebellar system in the rat: An HRP Study. II. Hemispheral components. *Journal of Comparative Neurology, 197*, 559-577.

Mittleman, G., Goldowitz, D., Heck, D. H., & Blaha, C. D. (2008). Cerebellar modulation of frontal cortex dopamine efflux in mince: Relevance to autism and schizophrenia. *Synapse, 62*, 544-550.

Moorman, D. E., & Aston-Jones, G. (2015). Prefrontal neurons encode context-based response execution and inhibition in reward seeking and extinction. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(30), 9472–7.

Ostlund, S. B., & Balleine, B. W. (2008). Differential involvement of the basolateral amygdala and mediodorsal thalamus in instrumental action selection. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *28*(17), 4398–405.

Parker, K. L., Narayanan, N. S., & Andreasen, N. C. (2014). The therapeutic potential of the cerebellum in schizophrenia. *Frontiers in Systems Neuroscience, 8*, Article 163.

Paxinos, G., & Watson, C. (2006). The rat brain in stereotaxic coordinates: hard cover edition. *Elsevier*.

Peak, J., Hart, G., & Balleine, B. W. (2018). From learning to action: the integration of dorsal striatal input and output pathways in instrumental conditioning. *The European journal of neuroscience*.

Peters, J., Kalivas, P. W., & Quirk, G. J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learning & memory (Cold Spring Harbor, N.Y.)*, *16*(5), 279–88.

Peters, J., LaLumiere, R. T., & Kalivas, P. W. (2008). Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. *The Journal of Neuroscience*, *28*(23), 6046-6053.

Popa, L. S., Hewitt, A. L., & Ebner, T. J. (2014). The cerebellum for jocks and nerds alike. *Frontiers in Systems Neuroscience, 8,* Article 113.

Ramnani, N. (2006). The primate cortico-cerebellar system: anatomy and function. *Nature reviews. Neuroscience*, *7*(7), 511–22.

Riedel, M. C., Ray, K. L., Dick, A. S., Sutherland, M. T., Hernandez, Z., Fox, P. M., . . . Laird, A. R. (2015). Meta-analytic connectivity and behavioral parcellation of the human cerebellum. *Neuroimage, 117,* 327-342.

Riga, D., Matos, M. R., Glas, A., Smit, A. B., Spijker, S., & den Oever, M. C. (2014). Optogenetic dissection of medial prefrontal cortex circuitry. *Frontiers in systems neuroscience*, *8*, 230.

Rogan, S. C., & Roth, B. L. (2011). Remote control of neuronal signaling. *Pharmacological reviews*, *63*(2), 291-315.

Rogers, T. D., Dickson, P. E., Heck, D. H., Goldowitz, D., Mittleman, G., & Blaha, C. D. (2011). Connecting the dots of the cerebro-cerebellar role in cognitive function: Neuronal pathways for cerebellar modulation of dopamine release in the prefrontal cortex. *Synapse, 65*, 1204-1212.

Rogers, T. D., Dickson, P. E., McKimm, E., Heck, D. H., Goldowitz, D., Blaha, C. D., & Mittleman, G. (2013). Reorganization of circuits underlying cerebellar modulation of prefrontal cortical dopamine in mouse models of Autism Spectrum Disorder. *Cerebellum, 12*, 547-556.

Schmahmann, J. D. (1991). An emerging concept: The cerebellar contribution to higher function. *Archives of Neurology, 48,* 1178-1187.

Schmahmann, J. D. (2004). Disorders of the cerebellum: Ataxia, Dysmetria of Thought, and the Cerebellar Cognitive Affective Syndrome. *Journal of Neuropsychiatry and Clinical Neurosciences, 16*, 367-378.

Schmahmann, J. D., Doyon, J., McDonald, D., Holmes, C., Lavoie, K., Hurwitz, A. S., . . . Petrides, M. (1999). Three-dimensional MRI atlas of the human cerebellum in proportional stereotaxic space. *Neuroimage, 10*, 233-260.

Schmahmann, J. D., & Sherman, J. C. (1998). The cerebellar cognitive affective syndrome. *Brain, 121*, 561-579.

Schmahmann, J. D., Weilburg, J. B., & Sherman, J. C. (2007). The neuropsychiatry of the cerebellum -- insights from the clinic. *Cerebellum, 6*, 254-267.

Seamans, J. K., Lapish, C. C., & Durstewitz, D. (2008). Comparing the prefrontal cortex of rats and primates: insights from electrophysiology. *Neurotoxicity research*, *14*(2–3), 249–62.

Sesack, S., Deutch, A., Roth, R., & Bunney, B. (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *The Journal of comparative neurology*, *290*(2), 213–42.

Sharpe, M. J., & Killcross, S. (2018). Modulation of attention and action in the medial prefrontal cortex of rats. *Psychological review*, *125*(5), 822.

Shiflett, M. W., Brown, R. A., & Balleine, B. W. (2010). Acquisition and Performance of Goal-Directed Instrumental Actions Depends on ERK Signaling in Distinct Regions of Dorsal Striatum in Rats. *The Journal of Neuroscience*, *30*(8), 2951–2959.

Shipman, M. L., Trask, S., Bouton, M. E., & Green, J. T. (2018). Inactivation of prelimbic and infralimbic cortex respectively affects minimally-trained and extensively-trained goal-directed actions. *Neurobiology of Learning and Memory*, *155*, 164-172.

Siegel, J. J., Taylor, W., Gray, R., Kalmbach, B., Zemelman, B. V., Desai, N. S., . . . Chitwood, R. A. (2015). Trace eyeblink conditioning in mice is dependent upon the dorsal medial prefrontal cortex, cerebellum, and amgydala: Behavioral characterization and functional circuitry. *eNeuro, 2*, e0051-0014.2015.

Smith, K. S., & Graybiel, A. M. (2013). A dual operator view of habitual behavior reflecting cortical and striatal dynamics. *Neuron, 79*(2), 361–74.

Smith, K. S., Virkud, A., Deisseroth, K., & Graybiel, A. M. (2012). Reversible online control of habitual behavior by optogenetic perturbation of medial prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(46), 18932–7.

Stachniak, T. J., Ghosh, A., & Sternson, S. M. (2014). Chemogenetic synaptic silencing of neural circuits localizes a hypothalamus→ midbrain pathway for feeding behavior. *Neuron*, *82*(4), 797-808.

Steele, C. J., Anwander, A., Bazin, P.-L., Trampel, R., Schaefer, A., Turner, R., . . . Villringer, A. (2017). Human cerebellar sub-millimeter diffusion imaging reveals the motor and non-motor topography of the dentate nucleus. *Cerebral Cortex, 27*, 4537-4548.

Stefani, M. R., Groth, K., & Moghaddam, B. (2003). Glutamate receptors in the rat medial prefrontal cortex regulate set-shifting ability. *Behavioral neuroscience, 117*(4), 728.

Steinmetz, J. E., Logue, S. F., & Miller, D. P. (1993). Using signaled barpressing tasks to study the neural substrates of appetitive and aversive learning in rats: Behavioral manipulations and cerebellar lesions. *Behavioral Neuroscience, 107,* 941-954.

Stoodley, C. J., & Schmahmann, J. D. (2010). Evidence for topographic organization in the cerebellum of motor control versus cognitive and affective processing. *Cortex, 46,* 831-844.

Strick, P. L., Dum, R. P., & Fiez, J. A. (2009). Cerebellar and nonmotor function. *Annual Review of Neuroscience, 32,* 413-434.

Sugihara, I. (2018). Crus I in the rodent cerebellum: Its homology to Crus I and II in the primate cerebellum and its anatomical uniqueness among neighboring lobules. *Cerebellum, 17,* 49-55.

Suzuki, L., Coulon, P., Sabel-Goedknegt, E. H., & Ruigrok, T. J. H. (2012). Organization of cerebral projections to identified cerebellar zones in the posterior cerebellum in the rat. *Journal of Neuroscience, 32,* 10854-10869.

Thrailkill, E. A., & Bouton, M. E. (2015). Contextual control of instrumental actions and habits. Journal of Experimental Psychology: *Animal Learning and Cognition, 41,* 69–80.

Thurling, M., Hautzel, H., Kuper, M., Stefanescu, M. R., Maderwald, S., Ladd, M. E., & Timmann, D. (2012). Involvement of the cerebellar cortex and nuclei in verbal and visuospatial working memory: A 7T fMRI study. *Neuroimage, 62,* 1537-1550.

Tran-Tu-Yen, D. A., Marchand, A. R., Pape, J.-R. R., Scala, G., & Coutureau, E. (2009). Transient role of the rat prelimbic cortex in goal-directed behaviour. *The European journal of neuroscience*, *30*(3), 464–71.

Trask, S., Shipman, M. L., Green, J. T., & Bouton, M. E. (2017). Inactivation of the prelimbic cortex attenuates context-dependent operant responding. *Journal of Neuroscience*, 3361-16.

Uylings, H. B., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal cortex?. *Behavioural brain research*, *146*(1-2), 3-17.

Vertes, R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse (New York, N.Y.)*, *51*(1), 32–58.

Voogd, J. (2004). Cerebellum. In G. Paxinos (Ed.), *The Rat Nervous System* (3rd ed., pp. 205-242). Amsterdam: Elsevier Academic Press.

Voogd, J., & Glickstein, M. (1998). The anatomy of the cerebellum. *Trends in neurosciences*, *21*(9), 370–5.

Wagner, M. J., Kim, T. H., Savall, J., Schnitzer, M. J., & Luo, L. (2017). Cerebellar granule cells encode the expectation of reward. *Nature, 544*, 96-100.

Watson, P. J. (1978). Nonmotor functions of the cerebellum. *Psychological Bulletin, 85*, 944-967.

Watson, P., van Wingen, G., & de Wit, S. (2018). Conflicted between goal-directed and habitual control, an fMRI investigation. *eNeuro;* ENEURO.0240-18.2018.

Watson, T. C., Becker, N., Apps, R., & Jones, M. W. (2014). Back to front: Cerebellar connections and interactions with the prefrontal cortex. *Frontiers in Systems Neuroscience, 8,* Article 4.

Watson, T. C., Jones, M. W., & Apps, R. (2009). Electrophysiological mapping of novel prefrontal-cerebellar pathways. *Frontiers in Integrative Neuroscience, 3,* Article 18.

Wiesendanger, R., & Wiesendanger, M. (1982). The corticopontine system in the rat. II. The projection pattern. *Journal of Comparative Neurology, 208,* 227-238.

Willcocks, A. L., & McNally, G. P. (2013). The role of medial prefrontal cortex in extinction and reinstatement of alcohol-seeking in rats. *The European journal of neuroscience*, *37*(2), 259–68.

Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2005). Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. *The European journal of neuroscience*, *22*(2), 505–12.

Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2006). Inactivation of dorsolateral striatum enhances sensitivity to changes in the action-outcome contingency in instrumental conditioning. *Behavioural brain research*, *166*(2), 189–96.

Yin, H. H., Ostlund, S. B., Knowlton, B. J., & Balleine, B. W. (2005). The role of the dorsomedial striatum in instrumental conditioning. *The European journal of neuroscience*, *22*(2), 513–23.

Zhu, H., & Roth, B. L. (2014). Silencing synapses with DREADDs. *Neuron*, *82*(4), 723-725.

Chapter 2: Inactivation of prelimbic and infralimbic cortex respectively affects minimally-trained and extensively-trained goal-directed actions

Shipman, M. L., Trask, S., Bouton, M. E., & Green, J. T. (2018). *Neurobiology of Learning and Memory, 155,* 164-172.

Abstract

Several studies have examined a role for the prelimbic cortex (PL) and infralimbic cortex (IL) in free operant behavior. The general conclusion has been that PL controls goal-directed actions (instrumental behaviors that are sensitive to reinforcer devaluation) whereas IL controls habits (instrumental behaviors that are *not* sensitive to reinforcer devaluation). To further examine the involvement of these regions in the expression of instrumental behavior, we first implanted male rats with bilateral guide cannulae into their PL, then trained two responses to produce a sucrose pellet reinforcer, R1 and R2, each in a distinct context. R1 received extensive training and R2 received minimal training. Rats then received lithium chloride injections either paired or unpaired with sucrose pellets in both contexts until paired rats rejected all pellets. Following acquisition, in Experiment 1, rats received either an infusion of saline or baclofen/muscimol into the PL and were tested (in extinction) on both R1 and R2. In vehicle controls, both responses were goal-directed actions, as indicated by their sensitivity to reinforcer devaluation. PL inactivation decreased expression of the minimally-trained action without affecting expression of the extensively-trained action. Experiment 2 utilized the same experimental design but with IL inactivation at test. The extensively-trained response was again a goal-directed action. However, now expression of the extensively-trained goal-directed action was suppressed by IL inactivation. The overall pattern of results suggests that the PL is involved in expression of minimally trained goal-directed behavior while the IL is involved in expression of extensively trained goal-directed behavior. This implies that the PL does not control all types of

actions and the IL can control some types of actions. These results expand upon the traditional view that the PL controls action while the IL controls habit.

Introduction

Rodent operant conditioning provides a laboratory analogue to human voluntary behavior. In a typical paradigm, performing a response (e.g., lever pressing or chain pulling) produces a reinforcing outcome. Operant responding can be classified as either goal-directed (performed to produce a specific outcome) or habitual (automatic, not outcome-driven). One common method used to separate the two types of responding is a reinforcer devaluation procedure that involves pairing the outcome with a lithium-chloride (LiCl) induced illness so that the animal develops a taste aversion to the outcome (e.g., Colwill & Rescorla, 1990). When the instrumental response is then tested in extinction, goal-directed actions are sensitive to reinforcer devaluation (i.e., responding is suppressed following reinforcer devaluation), whereas habits are not. Thus, actions are said to depend on the organism's knowledge of the response-outcome (R-O) association and reflect the current outcome value; habits depend on stimulus-response (S-R) associations and are not dependent on the outcome value. It is thought that early in training, responding is controlled primarily by R-O associations (although some S-R behavior likely develops early in training; see Thrailkill & Bouton, 2015). After many response-reinforcer pairings, behavior becomes habitual (Dickinson, 1985). Additionally, habits are more likely to develop with interval reinforcement schedules, while behavior can remain goal-directed with ratio reinforcement schedules (Dickinson, Nicholas, & Adams, 1983).

Brain structures involved in instrumental behavior are often described as belonging to either goal-directed or habitual circuitry (Lingawi, Dezfouli, & Balleine,

2016; Smith & Graybiel, 2016). Within the medial prefrontal cortex (mPFC), the prelimbic cortex (PL) has been implicated in goal-directed responding. Pre-training lesions or inactivation of the PL result in insensitivity of a behavior to the effects of reinforcer devaluation, whereas control animals suppress responding, suggesting that it supports goal-directed behavior (Corbit & Balleine, 2003; Killcross & Coutureau, 2003; Ostlund & Balleine, 2005; Tran-Tu-Yen et al., 2009). A typical finding is that the amount of responding in the PL-lesioned animals appears to be similar to the sham-lesioned animals' level of responding for the devalued reinforcer. While pre-training lesions or inactivation of the PL decrease sensitivity to reinforcer devaluation, pre-*test* lesions or inactivation often have no effect (Ostlund & Balleine, 2005; Tran-Tu-Yen et al., 2009).

The infralimbic cortex (IL), a second region of the mPFC, is generally thought to have an opposing role from the PL in controlling instrumental behavior. IL lesions or temporary inactivation at time of test following overtraining results in goal-directed operant responding, implicating the IL in habit expression (Coutureau & Killcross, 2003; Killcross & Coutureau, 2003). In a T-maze task, IL inactivation results in a change from habitual to goal-directed performance (Smith, Virkud, Deisseroth, & Graybiel, 2012) and optogenetic IL inactivation during training can prevent habit formation (Smith & Graybiel, 2013). These results suggest a role for the IL in controlling habit.

Two previous studies have demonstrated that goal-directed and habitual circuitries can be dissociated in individual subjects using free operant designs. In these studies, an action was produced in one context while a different response was trained as habit in another context. Lesions selectively affected one type of response but not the

other. In the first of these studies, Killcross and Coutureau (2003) made sham lesions or excitotoxic lesions of the PL or IL. They then trained rats to make one type of response (left or right lever-press) for one type of reward (food pellet or sucrose solution) in one context and the other response for the second reward in another context. One response was extensively trained (20 sessions) while the other response was minimally trained (5 sessions). Prior to the test, one reinforcer was devalued by allowing free access to it for an hour. In control rats, the sensory-specific satiety that resulted revealed that the extensively trained response was habitual and the minimally trained response was goal-directed. Pre-training lesions of the PL selectively impaired goal-directed responding whereas pre-training lesions of the IL selectively impaired habitual responding. In the second study in which an action was produced in one context and a habit was produced in another context, Gremel and Costa (2013) reinforced lever pressing in mice in two contexts; the response was reinforced on a random ratio schedule in one context (which produced an action) and a random interval schedule in the other context (which produced a habit). Goal-directed and habitual behavior were again dependent on dissociable brain regions (in this case, orbitofrontal cortex for goal-directed behavior and dorsolateral striatum (DLS) for habitual behavior).

Comparing two responses with different histories in the same animal is a powerful way to examine the neural substrates of instrumental behavior. We therefore made use of this type of design to further examine how the amount of instrumental training affects the underlying brain circuitry. In the current study, rats learned to press a lever and pull a chain for food reward in two different contexts. One response was extensively trained

(approximately 1,440 response-reinforcer pairings), and the other was only minimally trained (approximately 240 response-reinforcer pairings). Somewhat surprisingly, however, both were shown to be goal-directions actions in that they were both sensitive to a reinforcer devaluation treatment. In Experiment 1, PL inactivation at the time of testing suppressed the minimally trained action, but not the extensively trained action. In Experiment 2, inactivation of the IL suppressed only the extensively trained action. Together, these results suggest that the PL does not control all types of actions and the IL can control some types of actions.

Experiment 1

Method

Subjects. The subjects were 48 male Wistar rats purchased from Charles River Laboratories (St. Constance, Quebec). They were between 59 and 63 days old at arrival and were individually housed in a room maintained on a 12:12-h light: dark cycle. Experimentation took place during the light period of the cycle. Following post-surgery recovery, a baseline weight was obtained, and the rats were food-deprived to 90% of their baseline body weight throughout the experiment.

Surgery. Following acclimation to the colony, rats were anesthetized with isoflurane and stereotaxic surgery was performed to bilaterally implant guide cannulae (26 gauge, Plastics One) in the PL. Rats were given 0.1ml/mg of carprofen for analgesia both during surgery and one day post-operatively. During surgery, bupivacaine was also administered as a local anesthetic (0.15 ml) and 1 ml of lactated Ringers was administered for hydration. Coordinates used were +3.0 mm from bregma, ± 0.75 mm

from midline, and -3.0 mm ventral from bregma. Following surgery, rats were given 5-6 days of recovery. After recovery, a new baseline weight was taken and rats began food deprivation.

Apparatus. Two sets of four conditioning chambers housed in separate rooms of the laboratory served as the two contexts (counterbalanced). Each chamber was housed in its own sound attenuation chamber. All boxes were of the same design (Med Associates model ENV-008-VP, St. Albans, VT) and measured 30.5 cm × 24.1 × 21.0 cm (*l* × *w* × *h*). A recessed 5.1 cm × 5.1 cm food cup was centered in the front wall approximately 2.5 above the level of the floor. A retractable lever (Med Associates model ENV-112CM) positioned to the left of the food cup protruded 1.9 cm into the chamber. The chain pull manipulandum (Med Associates model ENV-111C) was a chain suspended from a micro switch mounted on top (outside) of the ceiling panel of each operant chamber. The chain hung 1.9 cm from the front wall, 3 cm to the right of the food cup, and 6.2 cm above the grid floor. The chambers were illuminated by one 7.5-W incandescent bulb mounted to the ceiling of the sound attenuation chamber, approximately 34.9 cm from the grid floor at the front wall of the chamber. Ventilation fans provided background noise of 65 dBA.

Each set of boxes had unique features to create discernably different contexts. In one set, the side walls and ceiling were made of clear acrylic plastic, while the front and rear walls were made of brushed aluminum. The floor was made of stainless steel grids (0.48 cm diameter) staggered such that odd- and even-numbered grids were mounted in two separate planes, one 0.5 cm above the other. This set of boxes had no distinctive visual cues on the walls or ceilings of the chambers. A dish containing 5 ml of Rite Aid

lemon cleaner (Rite Aid Corporation, Harrisburg, PA) was placed outside of each chamber near the front wall.

The second set of boxes was similar to the lemon-scented boxes except for the following features. In each box, one side wall had black diagonal stripes, 3.8 cm wide and 3.8 cm apart. The ceiling had similarly spaced stripes oriented in the same direction. The grids of the floor were mounted on the same plane and were spaced 1.6 cm apart (center-to-center). A distinct odor was continuously presented by placing 5 ml of Pine-Sol (Clorox Co., Oakland, CA) in a dish outside the chamber.

The reinforcer was a 45-mg sucrose-based food pellet (5-TUT: 1811251, TestDiet, Richmond, IN, USA) delivered to the magazine. The apparatus was controlled by computer equipment located in an adjacent room.

Procedure. The design used in both experiments is summarized in Table 1.

Magazine Training. On the first day of the experiment, all rats were assigned to a box within each set of chambers. They then received one 30-min session of magazine training in Context A. On the same day, the animals also received a second 30-min session of magazine training in Context B. Half the animals were trained first in Context A, and half were trained first in Context B. The sessions were separated by approximately 1 hr. Once all animals were placed in their respective chambers, a two-minute delay was imposed before the start of the session. In each session, approximately 60 reinforcers were delivered freely on a random time 30-s (RT 30-s) schedule. The levers were not present during this training.

R1 Acquisition. On each of the next 12 days, all rats received two 30-min sessions of instrumental training with R1 in Context A. R1 was counterbalanced so that for half the animals it was the lever and for half it was the chain. Throughout the sessions, R1 responding delivered reinforcers on a variable interval 30-s (VI 30-s) schedule of reinforcement. No hand shaping was necessary.

R2 Acquisition. On the final four days of R1 acquisition, all rats received an additional 30-min session of instrumental training with R2 in Context B. R2 was the chain for animals whose R1 was the lever and vice versa. As before, R2 responding delivered reinforcers on a VI 30-s schedule of reinforcement and no hand shaping was necessary. These daily sessions occurred after the final R1 acquisition session on days 9 – 12 of training.

Reinforcer Devaluation. Over the next 12 days, animals were given 6 two-day reinforcer devaluation cycles (3 in each context, alternating; see Trask & Bouton, 2014). Half the rats received the contexts in the order of AABBAABBAABB, and half received them in the order of BBAABBAABBAA. On the first day of each cycle, rats were all given an injection of 20mg/kg .15M lithium chloride (LiCl) following time in the acquisition context. For half the animals, Group Paired B/M and Group Paired Vehicle, LiCl injections were given following exposure to the sucrose reinforcer presented on a random time 30-s (RT 30-s) schedule into the magazine. For the other half, Group Unpaired B/M and Group Unpaired Vehicle, no reinforcer presentations occurred prior to LiCl injections. On the second day of each cycle, rats were given no injection following time in the appropriate context. Now, Group Paired received no reinforcers and Group

Unpaired received an equivalent number of reinforcers as had been consumed by a yoked animal in Group Paired the day before. On the first cycle, rats in Group Paired were given 30 reinforcers. On subsequent cycles, they were given the amount that they had consumed on the last cycle.

Baclofen/Muscimol Infusions. On the final day of the experiment, rats were given a bilateral infusion into the PL via Hamilton syringes of 0.9% saline vehicle (control) or baclofen/muscimol (B/M) (1.0mM/0.1mM; Sigma Aldrich, St Louis, MO) dissolved in 0.9% saline to temporarily inactivate the PL region. Internal cannulae (33 gauge, Plastics One) were inserted bilaterally into guide cannulae. Internal cannula tips protruded 1 mm below the guide cannula tip. An infusion of 0.5 µL per side was delivered at a rate of 0.25 µL per minute using a microinfusion pump. Following completion of the infusion, the internal cannulae were left in place for 1 min to allow diffusion of the drug or saline away from the cannula tips. Internal cannulae were then removed and dummy cannulae replaced. Each rat was then placed in the transportation container. Time between the end of infusion and the start of testing was 15-30 minutes.

Test. Following infusions, all rats were given two 10-min extinction tests, one in Context A (where R1 was tested) and one in Context B (where R2 was tested). Responding did not produce any pellets. Testing order was counterbalanced such that half the animals in each group were tested first in Context A and half were tested first in Context B. There was a delay of 30 min between tests for each animal.

Consumption Test. On the next day, animals all received 10 reinforcers delivered freely to the magazine on an RT 30-s schedule in each context (order counterbalanced) and pellet consumption was recorded.

Reacquisition Test. Following the consumption test, all animals were then given one 15-min reacquisition session in each context (with its respective response) in which reinforcers were delivered contingent on responding on a VI 30-s schedule. Half the animals were tested first with R1 and the other half were tested first with R2.

Statistical Data Analysis. All data were subjected to analysis of variance. The rejection criterion was set at $p < .05$. Training data for each response were analyzed with three-way ANOVAs that included dummy factors of drug and devaluation, in addition to session. Devaluation (consumption) data were analyzed with two-way ANOVAs that included the dummy factor of drug, in addition to session. Test data were analyzed with three-way ANOVAs that included the factors of drug, devaluation, and response. Reacquisition data were analyzed with three-way ANOVAs that included the dummy factor of drug, as well as devaluation and minute.

Eight animals were euthanized during the experiment due to lost head caps. Five animals were removed because we could not localize one or both cannulae to the PL (see Figure 1 for cannulae verification). All groups were left with an *n* of 9, except Group Unpaired Vehicle, which had an *n* of 8.

Results

R1 Acquisition. Acquisition results are summarized in Figure 2. The animals increased their R1 responding over the 24 sessions of acquisition. This was confirmed by

a 2 (Drug: B/M vs. Vehicle) x 2 (Devaluation: LiCl vs. Vehicle) x 24 (Session) ANOVA which revealed a main effect of session, F (23, 713) = 50.98, MSE = 61.10, $p < .01$, η_p^2 = .62, but no other main effects or interactions, largest F = 1.64.

R2 Acquisition. Animals also increased their R2 responding over the 4 sessions of R2 acquisition. This was confirmed by a 2 (Drug: B/M vs. Vehicle) x 2 (Devaluation: LiCl vs. Vehicle) x 4 (Session) ANOVA which again revealed a main effect of session, F (3, 93) = 101.26, MSE = 15.39, $p < .01$, η_p^2 = .77, but no other main effects or interactions, largest F = 1.14.

Devaluation. As shown in Figure 3, animals in both Groups Paired B/M and Paired Vehicle decreased their consumption of the pellets during the reinforcer devaluation phase. This was confirmed by a 2 (Drug: B/M vs. Vehicle) x 6 (Session) ANOVA, which revealed a main effect of session, F (5, 80) = 66.93, MSE = 45.90, $p <$ .01, η_p^2 = .81, but no other main effects or interactions, Fs < 1.

Test. The results of testing are summarized in Figure 4. R1 and R2 response rates were expressed as a proportion of the response rates each rat achieved on the final day of acquisition (see also Killcross & Coutureau, 2003). Somewhat unexpectedly, both responses were actions. But PL inactivation reduced expression of the minimally-trained response and not the extensively-trained response. A 2 (Drug: B/M vs. Vehicle) x 2 (Devaluation: LiCl vs. Vehicle) x 2 (Response: R1 vs. R2) ANOVA was conducted to compare R1 and R2 responding during the R1 and R2 tests. This found a main effect of devaluation, F (1, 31) = 6.17, MSE = 0.04, $p < .02$, η_p^2 = .17. The drug by response interaction approached, but did not attain, statistical significance, F (1, 31) = 2.92, p =

.097. Our *a priori* hypothesis was that R2, but not R1, would be affected by PL inactivation. This was confirmed by planned comparisons that revealed that animals in Group Unpaired B/M differed from Group Unpaired Vehicle in the R2 test, F (1, 31) = 4.63, MSE = .02, $p < .04$, $\eta_p^2 = .13$, but not during the R1 test, $F < 1$. Groups in the paired conditions did not differ based on PL inactivation during either test, Fs < 1.

The same planned comparisons were conducted using responses per minute as the dependent measure, rather than proportion baseline. Group Unpaired B/M again showed lower responding than Group Unpaired Vehicle in the R2 test, F (1, 31) = 6.09, MSE = 13.15, $p < .02$, $\eta_p^2 = .16$ but not in the R1 test, $F < 1.4$. Groups in the paired conditions did not differ based on PL inactivation during either test, Fs < 1. For the minimally-trained R2 response, responses per minute (mean ± SEM) were: Group Unpaired B/M = 5.60 ± 1.22; Group Unpaired Vehicle = 9.95 ± 1.41; Group Paired B/M = 3.34 ± 1.13; Group Paired Vehicle = 4.71 ± 1.17. For the extensively-trained R1 response, responses per minute were: Group Unpaired B/M = 10.02 ± 2.04; Group Unpaired Vehicle = 13.91 ± 3.35; Group Paired B/M = 6.20 ± 2.21; Group Paired Vehicle = 6.32 ± 1.44.

Consumption Test. No animals in the paired condition ate pellets, whereas all animals in the unpaired condition ate all of the pellets in both contexts, confirming that the reinforcer devaluation treatment was successful.

R1 Reacquisition. Results of the reacquisition tests are summarized in Figure 5. A 2 (Drug: B/M vs. Vehicle) x 2 (Devaluation: LiCl vs. Vehicle) x 15 (Minute) ANOVA was conducted to assess R1 responding during the reacquisition phase. This revealed a main effect of minute, F (14, 434) = 8.71, MSE = 54.43, $p < .01$, $\eta_p^2 = .22$, and a main

effect of LiCl, F (1, 31) = 52.14, MSE = 1942.80, $p < .01$, $\eta_p^2 = .63$. These effects were qualified by a minute by LiCl interaction, F (14, 434) = 10.09, MSE = 54.43, $p < .01$, $\eta_p^2 = .25$. No other main effects or interactions were significant, F's < 1. Follow-up one-way ANOVAs compared LiCl paired vs. unpaired conditions at each minute of reacquisition. This analysis revealed significantly lower responding in rats that underwent reinforcer devaluation in all minutes of reacquisition (largest $p = .007$). Lower responding in the LiCl paired condition in minute 1 of reacquisition strengthens the conclusion that R1 was a goal-directed action.

R2 Reacquisition. A similar 2 (Drug: B/M vs. Vehicle) x 2 (Devaluation: LiCl vs. Vehicle) x 15 (Minute) was conducted to assess R2 responding during the reacquisition phase. This revealed a main effect of minute, F (14, 434) = 5.25, MSE = 25.74, $p < .01$, $\eta_p^2 = .14$, and a main effect of LiCl, F (1, 31) = 75.54, MSE = 579.01, $p < .01$, $\eta_p^2 = .71$. These effects were qualified by a minute by LiCl interaction, F (14, 434) = 7.50, MSE = 25.74, $p < .01$, $\eta_p^2 = .19$. No other main effects or interactions were significant, although the minute by drug interaction was borderline significant, F (14, 434) = 1.70, MSE = 25.74, $p = .053$, $\eta_p^2 = .05$ because of a difference in responding between the two unpaired groups during minutes 2-4 of reacquisition. Follow-up one-way ANOVAs compared LiCl paired vs. unpaired conditions at each minute of reacquisition. This analysis revealed significantly lower responding in rats that underwent reinforcer devaluation in all minutes of reacquisition (p's < .001). Lower responding in the LiCl paired condition in minute 1 of reacquisition strengthens the conclusion that R2 was a goal-directed action.

Experiment 2

Because Experiment 1 found that inactivation of the PL selectively attenuated a minimally-trained (but not an extensively-trained) goal-directed action, the results begged the question of what brain structure might control the more extensively-trained action. One clear candidate was the IL, because studies that have implicated IL in the control of habit have done so via extensive training. Could the extensive training, rather than the behavior's actual status as habit, be the important variable? Experiment 2 therefore used the same experimental design to ask whether inactivation of the IL would have an effect opposite to that of the PL and attenuate an extensively trained, but not a minimally trained, goal-directed action.

Method

Subjects. The subjects were 48 male Wistar rats purchased, housed, and maintained exactly as in Experiment 1.

Surgery. Following acclimation to the colony, rats were anesthetized with isoflurane and stereotaxic surgery was performed to bilaterally implant guide cannulae (22 gauge, Plastics One) in the IL. As before, rats were given 0.1ml/mg of carprofen for analgesia both during surgery and one day post-operatively. Surgery proceeded as in Experiment 1, except that coordinates used were +2.8 mm from bregma, ± 2.66 mm from midline, and -4.71 mm ventral from bregma. Cannula were implanted at a 24-degree angle to avoid the PL.

Procedure. R1 acquisition, R2 acquisition, reinforcer devaluation, infusions,

testing, consumption testing, and reacquisition proceeded exactly as in Experiment 1.

Statistical Data Analysis. All data were subjected to analysis of variance as in Experiment 1. The rejection criterion was set at $p < .05$. Fifteen animals were removed based on inability to localize one or both cannulae to the IL (see Figure 6 for cannulae verification). Two additional animals were removed, one for ceasing to respond partway through the experiment and one for being an outlier in the R2 test (Z = 2.03, see Field, 2005). This left Groups Paired B/M and Unpaired B/M with *n*s = 8, Group Paired vehicle with $n = 6$, and Group Unpaired Vehicle with $n = 9$.

Results

R1 Acquisition. Acquisition of both responses is shown in Figure 7. As in Experiment 1, animals increased responding on R1 across the 24 acquisition sessions. This was confirmed by a 2 (Drug: B/M vs. Vehicle) x 2 (LiCl: Paired vs. Unpaired) x 24 (Session) ANOVA that revealed a main effect of session, F (23, 621) = 47.88, MSE = 52.94, $p < .01$, $\eta_p^2 = .64$. We found no other main effects or interactions, largest $F = 3.10$, $p = .09$.

R2 Acquisition. As in Experiment 1, animals increased responding on R2 across the 4 acquisition sessions. This was confirmed by a 2 (Drug: B/M vs. Vehicle) x 2 (LiCl: Paired vs Unpaired) x 4 (Session) ANOVA that revealed a main effect of session, F (3, 81) = 96.45, MSE = 14.26, $p < .001$, $\eta_p^2 = .78$, but no other main effects or interactions, largest $F = 2.63$, $p = .12$.

Devaluation. Devaluation across sessions is shown in Figure 8. As in Experiment 1, both Paired B/M and Paired Vehicle groups decreased pellet consumption across devaluation sessions. This was confirmed by a 2 (Drug: B/M vs. Vehicle) x 6 (Session) ANOVA which revealed a main effect of session, F (5, 60) = 85.91, MSE = 30.32, p < .01, η_p^2 = .88. There were no other main effects or interactions, Fs < 1.

Test. The crucial test results are shown in Figure 9. As in Experiment 1, response rates were expressed as a proportion (see also Killcross & Coutureau, 2003) of the final rates achieved in the last session of acquisition. The extensively-trained response was again an action and IL inactivation reduced expression of the extensively-trained response but not the minimally-trained response. A 2 (Drug: B/M vs. Vehicle) x 2 (LiCl: Paired vs. Unpaired) x 2 (Response: R1 vs. R2) ANOVA was conducted to assess R1 and R2 responding during the test. This found a drug by LiCl by response interaction, F (1, 27) = 9.49, MSE = .007, p < .01, η_p^2 = .26, but no other main effects or interactions, largest F = 2.26, p = .14. Surprisingly, no main effect of the lithium chloride treatment was observed, F = 0.77, p = .39, likely due to floor responding in Group Unpaired Vehicle during the R2 test. However, the Paired Vehicle group responded less than the Unpaired Vehicle group during the R1 test, F (1, 27) = 4.63, MSE = .02, p = .04, η_p^2 = .15, again indicating that R1 responding was still goal-directed even after extensive training. Recall that R1 received substantially more training than R2, leaving it reasonable to conclude that R2 was also still goal-directed (see also "R2 Reacquisition" below, which supports this conclusion), even if not detected during this test. Our *a priori* hypothesis was that R1, but not R2, responding would be suppressed by IL inactivation.

This was true: Group Unpaired B/M responded less than Group Unpaired Vehicle during the R1 test, F (1, 27) = 6.41, MSE = .02, $p < .02$, $\eta_p^2 = .19$, but not during the R2 test, $F < 1$. Animals in the paired conditions did not differ from each other, largest $F = 2.68$, $p = .11$.

The same planned comparisons were conducted using responses per minute as the dependent measure, rather than proportion baseline. Group Unpaired B/M again showed lower responding than Group Unpaired Vehicle in the R1 test, F (1, 27) = 10.06, MSE = 11.05, $p < .01$, $\eta_p^2 = .27$ but not in the R2 test, $F < 1$. There was a trend towards lower responding in Group Paired B/M compared to Group Paired Vehicle in the R2 test, F(1, 27) = 3.48, MSE = 3.14, $p = .07$, $\eta_p^2 = .11$. For the minimally-trained response, responses per minute (mean ± SEM) were: Group Unpaired B/M = 2.49 ± 0.65; Group Unpaired Vehicle = 2.61 ± 0.58; Group Paired B/M = 1.40 ± 0.58; Group Paired Vehicle = 3.18 ± 0.78. For the extensively-trained response, responses per minute were: Group Unpaired B/M = 2.99 ± 1.12; Group Unpaired Vehicle = 8.11 ± 1.16; Group Paired B/M = 2.21 ± 1.44; Group Paired Vehicle = 2.62 ± 0.73.

Consumption Test. The rats in the paired group who received B/M ate an average of zero pellets in Context A and .25 pellets in Context B. The rats in the paired vehicle group ate an average of .17 pellets in Context A and .17 pellets in Context B. All rats in the unpaired groups ate all pellets in both contexts.

R1 Reacquisition. Results of the reacquisition tests are summarized in Figure 10. A 2 (Drug: B/M vs. Vehicle) x 2 (LiCl: Paired vs. Unpaired) x 15 (Minute) ANOVA was conducted to examine R1 responding. This revealed a main effect of minute, F (14, 378)

= 7.24, MSE = 54.39, $p < .01$, $\eta_p^2 = .21$, and a main effect of LiCl, F (1, 27) = 18.248, MSE = 3424.68, $p < .01$, $\eta_p^2 = .63$. These effects were qualified by a minute by LiCl interaction, F (14, 378) = 6.14, MSE = 54.39, $p < .01$, $\eta_p^2 = .18$. No other main effects or interactions were significant, largest $F = 2.96$, $p = .10$. Follow-up one-way ANOVAs compared LiCl paired vs. unpaired conditions at each minute of reacquisition. These revealed significantly lower responding in rats that underwent reinforcer devaluation in all minutes of reacquisition (largest $p = .005$). Lower responding in the LiCl paired condition in minute 1 of reacquisition strengthens the conclusion that R1 was a goal-directed action.

R2 Reacquisition. To assess R2 responding during reacquisition, a 2 (Drug: B/M vs. Vehicle) x 2 (Devaluation: LiCl vs. Vehicle) x 15 (Minute) ANOVA was conducted to examine R2 responding. This revealed a main effect of minute, F (14, 378) = 5.07, MSE = 20.26, $p < .01$, $\eta_p^2 = .16$, and a main effect of LiCl, F (1, 27) = 34.21, MSE = 849.55, $p < .01$, $\eta_p^2 = .56$. These effects were qualified by a minute by LiCl interaction, F (14, 378) = 8.82, MSE = 20.263, $p < .001$, $\eta_p^2 = .25$. No other main effects or interactions were significant, largest $F = 1.10$. Follow-up one-way ANOVAs compared LiCl paired vs. unpaired conditions at each minute of reacquisition. This analysis revealed significantly lower responding in rats that underwent reinforcer devaluation in all minutes of reacquisition (largest $p = .003$). Lower responding in the LiCl paired condition in minute 1 of reacquisition strongly suggests that R2 was a goal-directed action, even though the results of the previous extinction test were somewhat less clear.

General Discussion

In Experiment 1, PL inactivation during testing suppressed the expression of a minimally trained goal-directed action, but not an extensively trained goal-directed action in the same animal. In Experiment 2, IL inactivation during testing in turn suppressed expression of an extensively trained goal-directed action. PL and IL inactivation suppressed responding only in the unpaired (non-devalued) groups in the current study. Since any responding remaining in the paired (devalued) groups might theoretically represent some habit that was learned along with action (Thrailkill & Bouton, 2015), this may provide additional evidence that both PL and IL play a role in expression of goal-directed actions; the part of the response that might have been controlled by habit was not affected by inactivation of either PL or IL. Finally, since inactivation occurred at the time of testing, the results imply a role for the PL and IL in the expression, rather than just acquisition, of these goal-directed responses (cf. Ostlund & Balleine, 2005; Tran-Tu-Yen et al., 2009). Our results therefore suggest that the PL is involved in expression of minimally trained goal-directed responding, and the IL is involved in expression of extensively trained goal-directed responding. It is worth noting that the only other study to examine the role of both PL and IL in demonstrably goal-directed responding suggested a split in the function of the PL and IL based on action vs. habit, respectively (Killcross & Courtureau, 2003). Our results importantly expand on this observation by suggesting that the involvement of IL might depend on a behavior's extensive training, rather than its actual status as a habit. Furthermore, our results suggest that the PL is not important in expression of all actions, but rather only in expression of minimally-trained

ones. It is also notable that while aversive Pavlovian conditioning of the context resulting from a reinforcer devaluation procedure can suppress extensively-trained instrumental responding (Jonkman, Kosaki, Everitt, & Dickinson, 2010), the possible involvement of such a mechanism here would not change our observation that amount of training is an important factor in whether the PL or IL mediates responding.

The implication of our results that PL and IL control minimally-trained and extensively-trained actions is not incongruent with current thinking if we note that the transition from action to habit may be progressive rather than sudden. Consistent with this possibility, Smith and Graybiel (2013) found that on a T-maze task, neurons in the DLS, a brain region associated with habitual behavior, developed a "task bracketing pattern" (i.e. firing at the beginning and end of a maze run, rather than at a decision point) early in training, at a point where the behavior was still sensitive to reinforcer devaluation (i.e., was a goal-directed action) and before the behavior had become a habit. This pattern was also observed in the IL (but not the PL) after further training around the time when behavior transitioned from sensitive to reinforcer devaluation to insensitive to reinforcer devaluation (i.e., a habit). Thus, one possibility is that the current experiments assessed the role of the IL at a point in training where the instrumental behavior was beginning to become automatic but did not yet fulfill the habitual behavior criterion of being completely insensitive to reinforcer devaluation.

The many differences between our procedure and that of Killcross and Coutureau (2003) make it challenging to determine why our extensively-trained behavior was an action and theirs was a habit. Our unpublished observations suggest that our extensively-

trained response may actually have been a habit prior to the introduction of training of the second response (Trask, Shipman, Green, & Bouton, unpublished observations). Recall that in the current study, rats were trained on R2 in context B on the final four days of R1 training in context A. Both R1 (24 sessions of training) and R2 (4 sessions of training) were expressed as actions at test. However, we have observed that an extensively-trained R1 is expressed as a habit at test if rats are merely exposed to context B on the final four days of R1 training in context A. Regardless of exactly why our extensively-trained response was expressed as an action in the current study, it does not change the conclusion that the amount of training is an important factor in whether or not the PL or the IL controls responding.

Determining the reason why our extensively trained response was not expressed as a habit will be an important next step in refining our view of PL and IL function in instrumental behavior. It is important to note that previous behavioral work suggests that even extensively-trained instrumental responses can be actions under some conditions. Interestingly, as suggested by our observations described above, a common thread may be that intermixed training of two responses may often discourage the acquisition of habit. For example, Colwill and Rescorla (1985) used a within-subjects training and testing procedure to show that both a minimally-trained (1 session) and an extensively-trained (13 sessions) instrumental response were sensitive to reinforcer devaluation (and were both thus actions). In their experiments, more than one response was associated with the same to-be-devalued reinforcer, and sessions in which the extensively-trained response and minimally-trained response were reinforced were intermixed (see also

Colwill & Rescorla, 1988). Concurrent training procedures, in which two different response-reinforcer contingencies are available simultaneously, are also known to discourage extensively-trained responses from becoming habits (Kosaki & Dickinson, 2010).

Previous results have suggested that the PL is involved only in the learning, and not in the expression, of a minimally-trained action. Ostlund and Balleine (2005) found that only pre-training mPFC lesions (centered on the PL), and not post-training lesions, resulted in an impairment of goal-directed responding. Tran-Tu Yen et al. (2009) also found that pharmacological inactivation prior to acquisition sessions but not prior to test resulted in impaired goal-directed responding. In contrast, we found here that inactivation of the PL can suppress responding when inactivation occurs prior to testing. One possibility is that the previous experiments that failed to find a role for PL in action expression were effectively testing manipulation of the PL on the expression of a more extensively-trained response; we show here that while the PL is necessary for expression of a minimally-trained response, it is not necessary for expression of an extensively-trained response.

One anomaly in the current findings was our failure to see a reinforcer devaluation effect on the minimally-trained response in Experiment 2. However, given that the extensively-trained behavior was sensitive to reinforcer devaluation in that experiment, there is little reason to think that a response that had received less training could have been habitual. The same method used in Experiment 1 revealed that the minimally-trained response was sensitive to devaluation; unpublished results using the

same paradigm have replicated that observation. Moreover, an analysis of the first minute of reacquisition suggested that the minimally-trained response was indeed an action at that time; rats that had undergone reinforcer devaluation showed less responding than rats that had not undergone reinforcer devaluation. There is little reason to question that the minimal-training procedure used here in both the current experiments produces a goal-directed action.

Nevertheless, the failure to find that the minimally-trained response in Experiment 2 was a goal-directed action is a limitation of that experiment's results, as they do not allow us to determine whether or not the IL is involved in the expression of minimally-trained instrumental responses. A second limitation of our results is that, in Experiment 1, the interaction between drug (B/M vs. vehicle) and response (minimally-trained action vs. extensively-trained action) approached, but did not attain, statistical significance. While planned comparisons between drug conditions for each response did reveal that B/M suppressed a minimally-trained action but not an extensively-trained action, the lack of a drug by response interaction does mean that we have to temper our conclusions a bit that PL is involved *only* in expression of minimally-trained actions and not extensively-trained actions. As with all novel results, it will be important to replicate our observations.

In summary, the present results show that inactivation of PL or IL results in the suppression of instrumental responses that differed in their amount of training, but not in their status as goal-directed actions and habits. Our results suggest a role for the PL and

IL in expression of minimally trained and extensively trained operant responses, respectively.

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References

Bouton, M. E., Todd, T. P., Vurbic, D., Winterbauer, N. E. (2011). Renewal after the extinction of free operant behavior. *Learning and Behavior, 39,* 57–67.

Colwill, R. M., & Rescorla, R. A. (1985). Instrumental responding remains sensitive to reinforcer devaluation after extensive training. *Journal of Experimental Psychology: Animal Behavior Processes, 11,* 520-536.

Colwill, R. M., & Rescorla, R. A. (1990). Effect of reinforcer devaluation on discriminative control of instrumental behavior. *Journal of Experimental Psychology: Animal Behavior Processes, 16,* 40-47.

Corbit, L. H., & Balleine, B. W. (2003). The role of prelimbic cortex in instrumental conditioning. *Behavioural Brain Research, 146,* 145-157.

Coutureau, E., & Killcross, S. (2003). Inactivation of the infralimbic prefrontal cortex reinstates goal-directed responding in overtrained rats. *Behavioural Brain Research, 146,* 167-174.

Dickinson, A. (1985). Actions and habits: the development of behavioural autonomy. *Philosophical Transactions of the Royal Society of London, 308,* 67-78.

Dickinson, A., Nicholas, D. J., & Adams, C. D. (1983). The effect of the instrumental contingency on susceptibility to reinforcer devaluation. *Quarterly Journal of Experimental Psychology, 35B,* 35-51.

Eddy, M. C., Todd, T. P., Bouton, M. E., & Green, J. T. (2016). Medial prefrontal cortex involvement in the expression of extinction and ABA renewal of instrumental

behavior for a food reinforcer. *Neurobiology of Learning and Memory*, *128*, 33-39.

Field, A. (2005). Discovering statistics using SPSS. Thousand Oaks, CA: Sage.

Fuchs, R. A., Evans, K. A., Ledford, C. C., Parker, M. P., Case, J. M., Mehta, R. H., & See, R. E. (2005). The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. *Neuropsychopharmacology 30*, 296-309.

Gremel, C. M., & Costa, R. M. (2013). Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions. *Nature Communications*, *4*, 2264.

Jonkman, S., Kosaki, Y., Everitt, B. J., & Dickinson, A. (2010). The role of contextual conditioning in the effect of reinforcer devaluation on instrumental performance by rats. *Behavioural Processes, 83,* 276-281.

Killcross, S., & Coutureau, E. (2003). Coordination of actions and habits in the medial prefrontal cortex of rats. *Cerebral Cortex*, *13*, 400-408.

Kosaki, Y., & Dickinson, A. (2010). Choice and contingency in the development of behavioral autonomy during instrumental conditioning. *Journal of Experimental Psychology: Animal Behavior Processes, 36*, 334-342.

Lingawi, N. W., Dezfouli, A., & Balleine, B. W. (2016). The psychological and physiological mechanisms of habit formation. In R. A. Murphy & R. C. Honey (Eds.), *Wiley Handbook on the Cognitive Neuroscience of Learning* (pp. 411-440). Chichester, UK: John Wiley and Sons.

Ostlund, S. B., & Balleine, B. W. (2005). Lesions of medial prefrontal cortex disrupt the acquisition but not the expression of goal-directed learning. *Journal of Neuroscience, 25,* 7763–7770.

Smith K. S. & Graybiel, A. M. (2016). Habit formation. *Dialogues in Clinical Neuroscience, 18,* 33–43.

Smith, K. & Graybiel, A. (2013) A dual operator view of habitual behavior reflecting cortical and striatal dynamics. *Neuron, 79,* 361–374.

Smith, K. S., Virkud, A., Deisseroth, K., & Graybiel, A. M. (2012). Reversible online control of habitual behavior by optogenetic perturbation of medial prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America, 109,* 18932–7.

Thrailkill, E. A., & Bouton, M. E. (2015). Contextual control of instrumental actions and habits. *Journal of Experimental Psychology: Animal Learning and Cognition, 41*, 69-80.

Tran-Tu-Yen, D. A., Marchand, A. R., Pape, J.-R. R., Di Scala, G., & Coutureau, E. (2009). Transient role of the rat prelimbic cortex in goal-directed behaviour. *European Journal of Neuroscience, 30,* 464–71.

Trask, S., Shipman, M. L., Green, J. T., & Bouton, M. E. (2017). Inactivation of the prelimbic cortex attenuates context-dependent operant responding. *Journal of Neuroscience, 37,* 2317-2324.

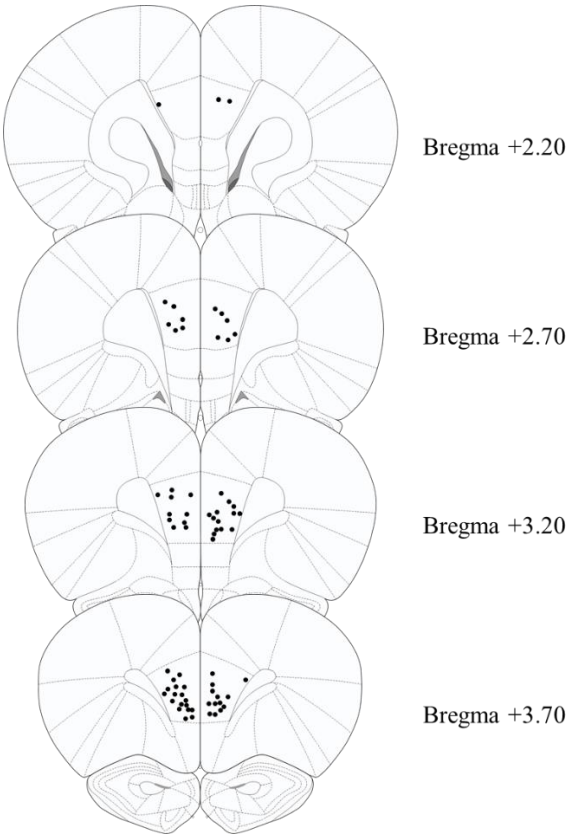
Trask, S., & Bouton, M. E. (2014). Contextual control of operant behavior: evidence for hierarchical associations in instrumental learning. *Learning & Behavior 42*, 281–288.

Vandaele Y., Pribut, H. J., & Janak, P. H. (2017). Lever insertion as a salient stimulus promoting insensitivity to outcome devaluation. *Frontiers in Integrative Neuroscience*, *11*, 1-23.

Willcocks AL, McNally GP (2013) The role of medial prefrontal cortex in extinction and reinstatement of alcohol-seeking in rats. *European Journal of Neuroscience, 37*, 259-268.

Figures

Figure 1. Cannulae tip placement in the prelimbic cortex in Experiment 1 and a representative image (scale bar = 1 mm). In the image, infusion sites are indicated by arrows. Infusions were made 1 mm below the guide cannula tip.



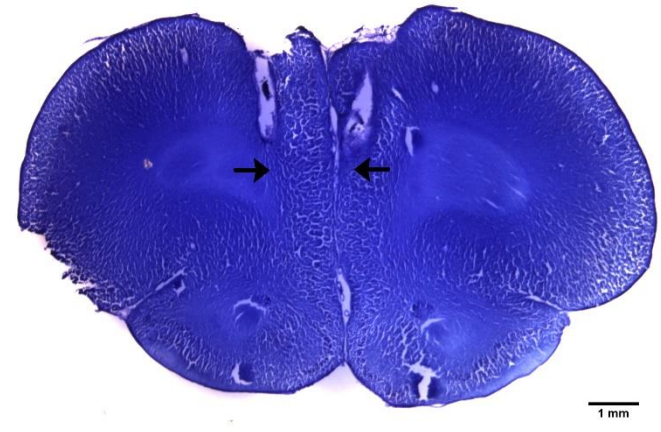


Figure 2. R1 and R2 responding throughout acquisition in Experiment 1.

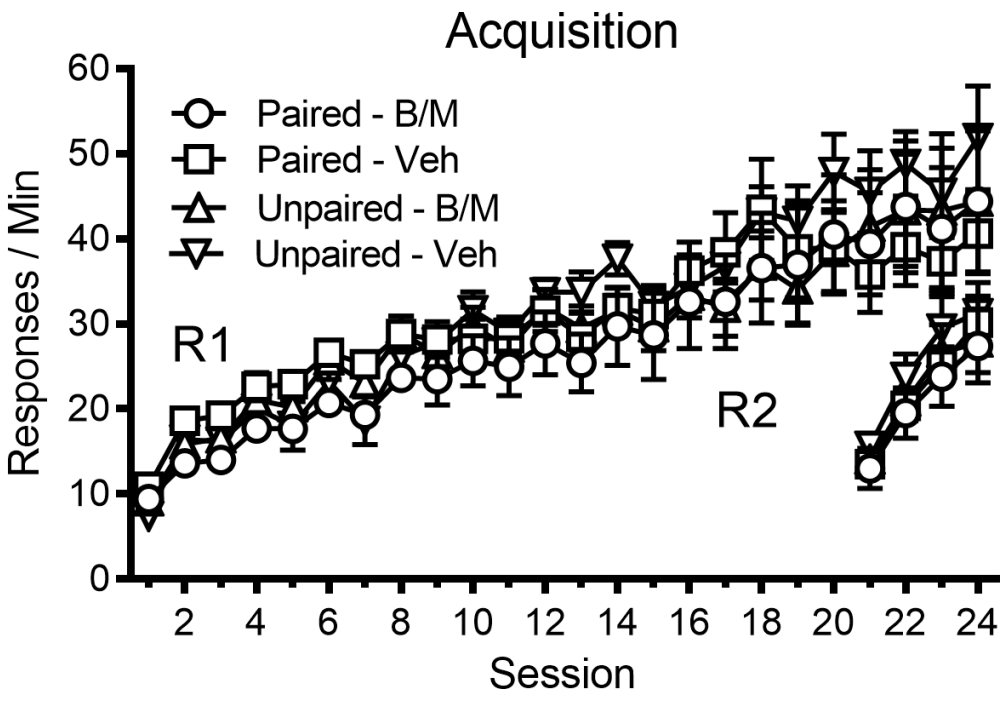


Figure 3. Reinforcers consumed throughout the devaluation phase of Experiment 1.

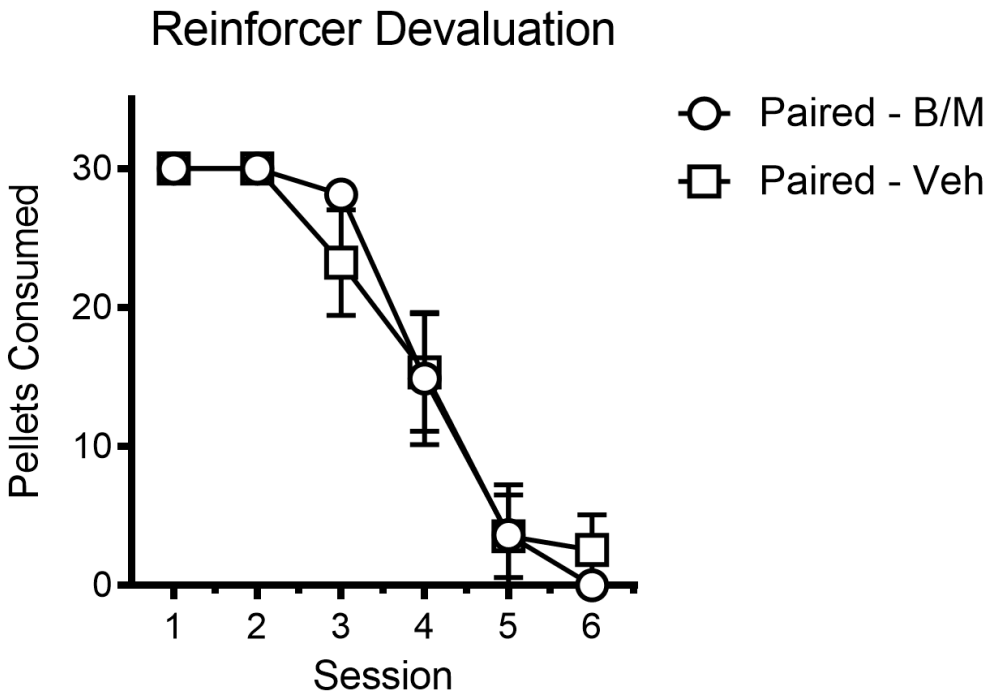


Figure 4. Responding during the testing phase of Experiment 1. R1 is depicted in the left panel and R2 is depicted in the right panel. Proportion baseline is calculated as the average of responding (per minute) during the test divided by responding (per minute) on the last day of acquisition on the respective response. * = $p < .05$.

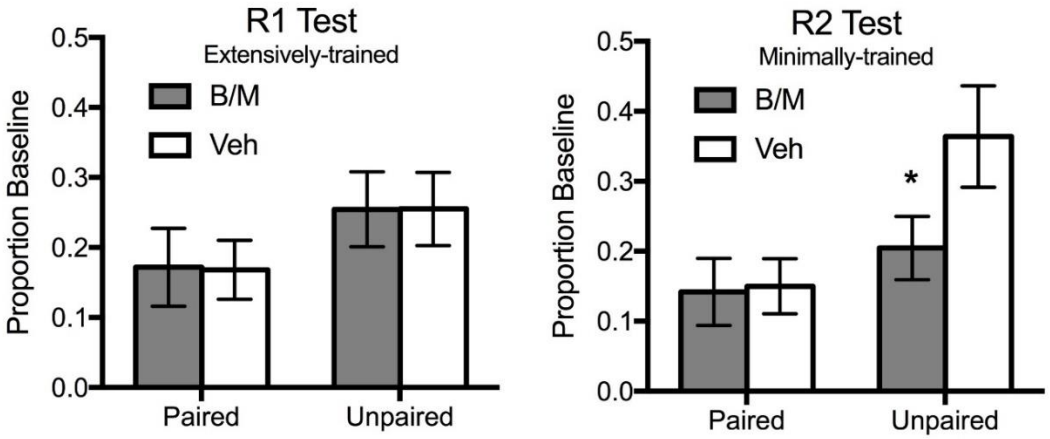


Figure 5. Responding during the reacquisition test of Experiment 1.

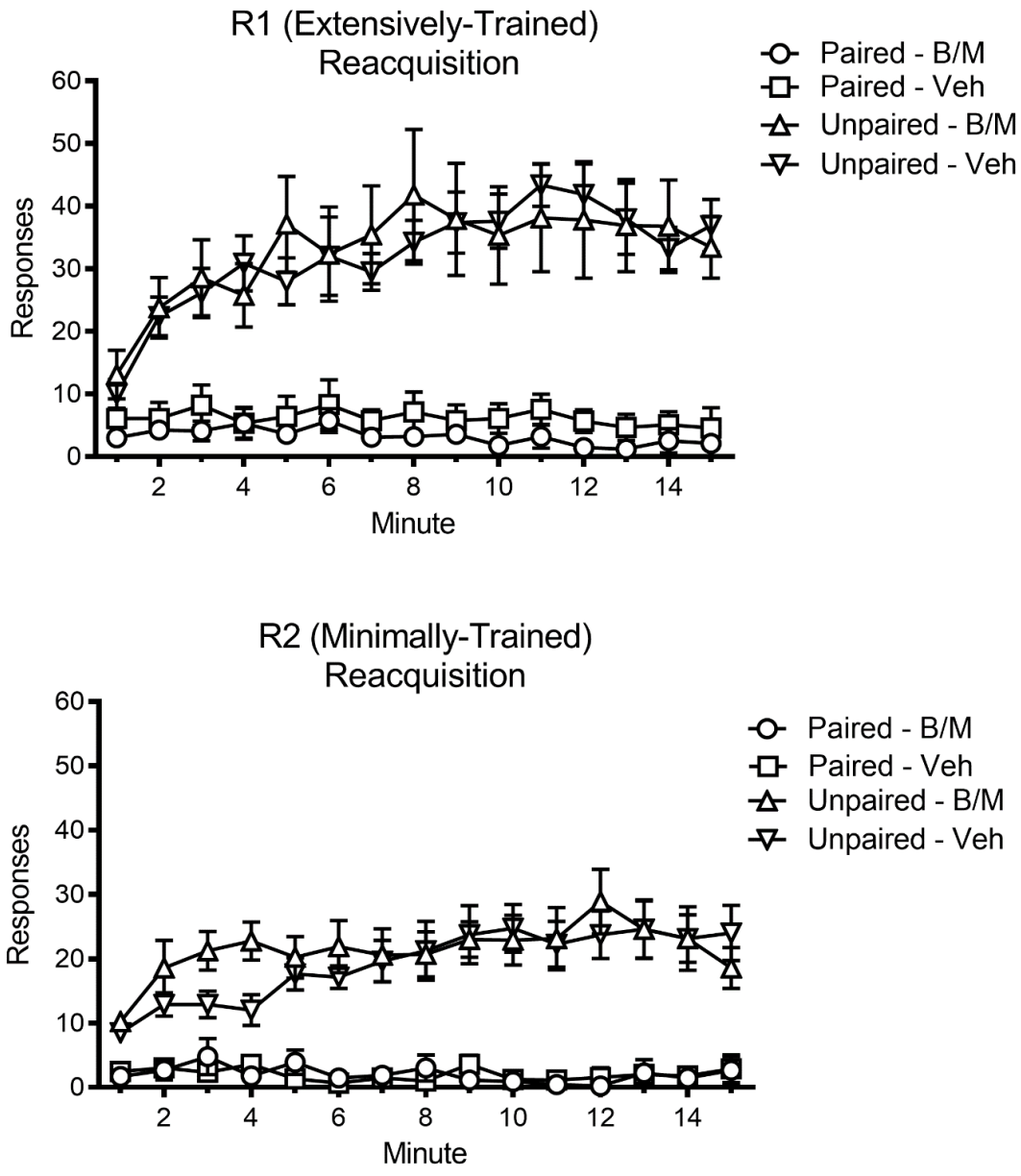
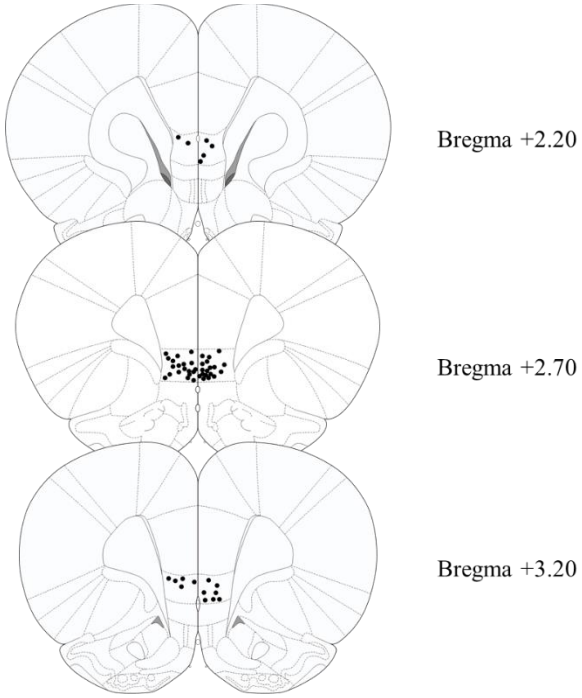


Figure 6. Cannulae tip placement in the infralimbic cortex in Experiment 2 and a representative image (scale bar = 1 mm). In the image, infusion sites are indicated by arrows. Infusions were made 1 mm below the guide cannula tip.



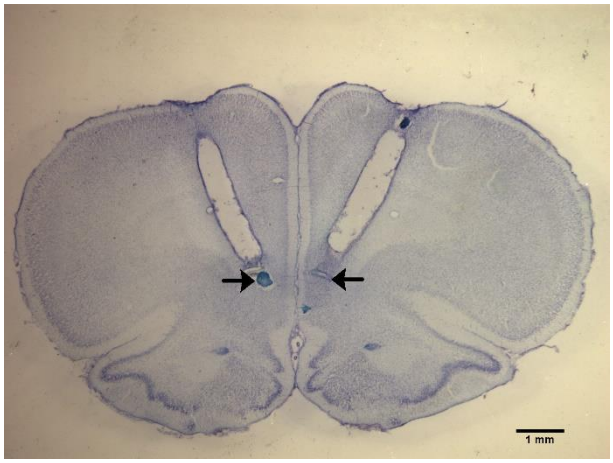


Figure 7. R1 and R2 responding during acquisition in Experiment 2.

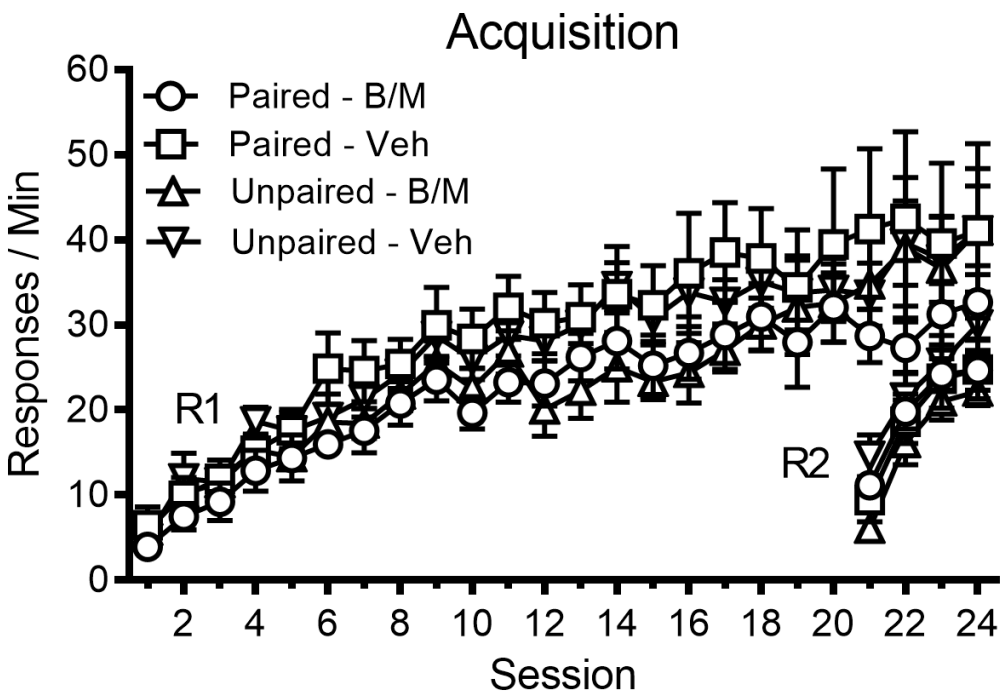


Figure 8. Reinforcers consumed during the devaluation phase of Experiment 2.

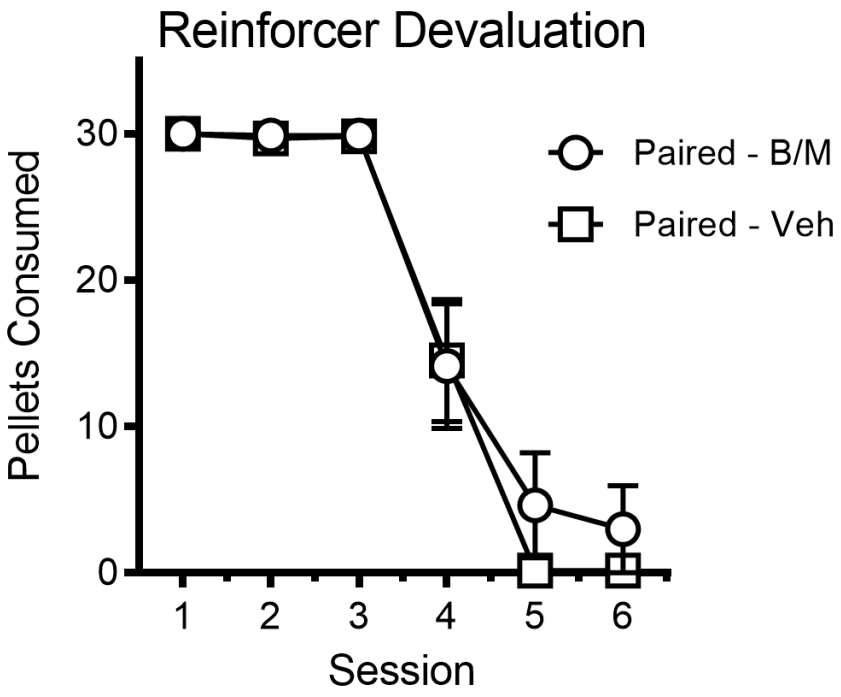


Figure 9. Responding during the testing phase of Experiment 2. R1 is depicted in the left panel and R2 is depicted in the right panel. Proportion baseline is calculated as the average of responding (per minute) during the test divided by responding (per minute) on the last day of acquisition on the respective response. * = $p < .05$.

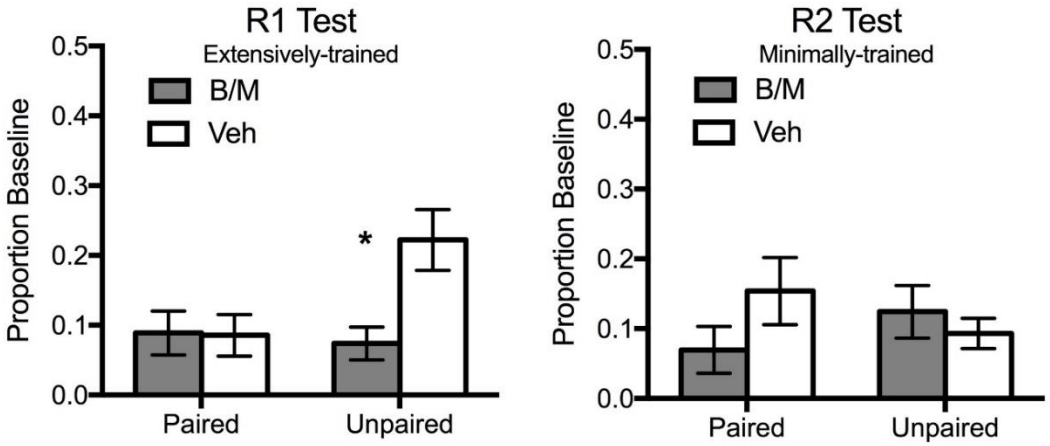
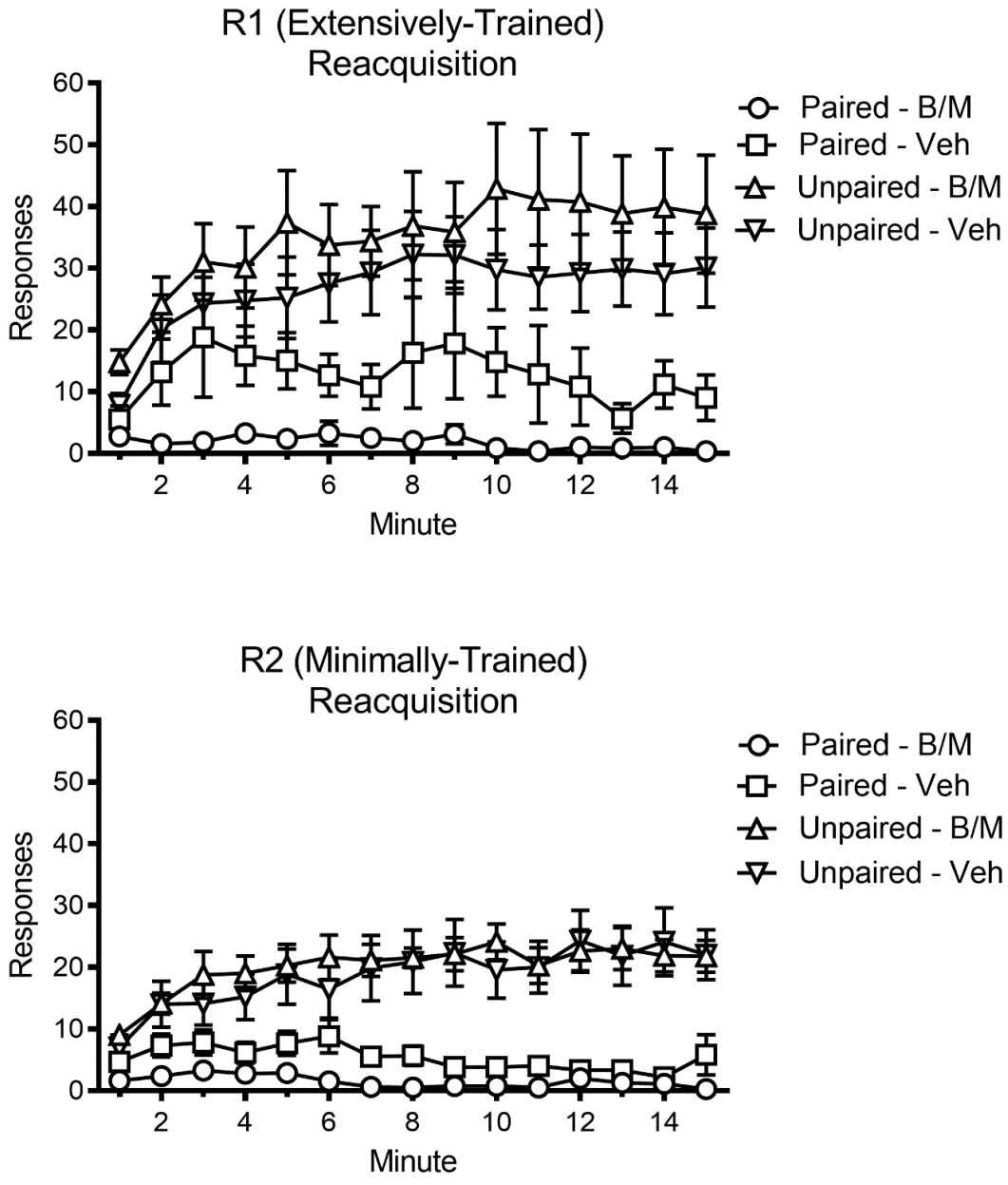


Figure 10. Responding during the reacquisition test of Experiment 2.



Tables

Table 1. Experimental Design. The experiments utilized a within-subjects design to test rats on expression of both extensively trained and minimally trained responses. Half of all rats received baclofen/muscimol and half received a saline vehicle infusion prior to test. Half also received LiCl paired with sucrose pellets during devaluation while the other half received LiCl on days where they did not receive sucrose pellets prior to injection. Devaluation occurred in both contexts.

Group	Instrumental Acquisition	Reinforcer Devaluation	Infusion	Test
B/M-Paired	Context A: R1 (24 sessions) Context B: R2 (4 sessions)	Paired (devalued)	B/M	Context A: R1? Context B: R2?
B/M-Unpaired		Unpaired (non-devalued)	B/M	
Vehicle-Paired		Paired (devalued)	Saline	
Vehicle-Unpaired		Unpaired (non-devalued)	Saline	

Chapter 3: Chemogenetic inhibition of prelimbic cortex projections to anterior dorsomedial striatum attenuates operant responding

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Abstract

Operant (instrumental) conditioning is a laboratory analog for voluntary behavior and involves learning to make a response for a reinforcing outcome. The prelimbic cortex (PL), a region of the rodent medial prefrontal cortex, and the dorsomedial striatum (DMS), have been separately established as important in the acquisition of minimally-trained operant behavior. Despite dense anatomical connections between the two regions, experimenters have only recently linked actual projections from the PL to the posterior DMS in the acquisition of an operant response. Yet, it is still unknown if these projections mediate behavioral expression, and if more anterior regions of the DMS (aDMS), which receive denser projections from the PL, are also involved. Therefore, we utilized designer receptors exclusively activated by designer drugs (DREADDs) to test whether or not projections from the PL to the anterior DMS influence the expression of operant behavior. Rats underwent bilateral PL-targeted infusions of either a DREADD virus (AAV8-hSyn-hM4D(Gi)-mCherry) or a control virus (AAV8-hSyn-GFP) and guide cannulae implanted bilaterally in the aDMS. Rats were tested with both CNO (DREADD ligand) and vehicle infusions into the aDMS. Animals that had received the DREADD virus, but not the control virus, showed attenuated responding when they received CNO microinfusions into the aDMS, compared to vehicle infusions. Patch clamp electrophysiology verified the inhibitory effect of CNO on virally infected PL neurons in acute brain slices. The results add to the recent literature suggesting that connections between the PL and aDMS are important for the expression of minimally-trained operant responding.

Significance statement

A recent study has only directly connected the prelimbic to posterior dorsomedial striatum pathway in the acquisition of operant responding. Here, we show that the prelimbic to *anterior* dorsomedial striatum pathway is also important in the *expression* of operant responding.

Introduction

The prelimbic cortex (PL) has been well established as a mediator of operant (instrumental) responses early in training (Corbit & Balleine, 2003; Killcross & Coutureau, 2003; Ostlund & Balleine, 2005; Shipman, Trask, Bouton, & Green, 2018; Tran-Tu-Yen, Marchand, Pape, Di Scala, & Coutureau, 2009; Trask, Shipman, Green, & Bouton, 2017). The dorsomedial striatum (DMS) has similarly been implicated in the acquisition and expression of operant responding, with a particular emphasis on the posterior DMS (pDMS) (Shiflett, Brown, & Balleine, 2010; Yin, Ostlund, Knowlton, & Balleine, 2005; Yin,Knowlton, and Balleine, 2006). Because the PL and pDMS have both been implicated in the early acquisition of operant responding, it has been suggested that they may function together as part of a greater circuit supporting goal-directed operant responding (see Corbit, 2018). Indeed, pharmacological disconnection of these two regions prior to acquisition sessions disrupts the expression of operant responding at test (Hart, Bradfield, & Balleine, 2018).

Traditional disconnection studies do not address the question of whether or not function is mediated by a direct vs. an indirect connection between two brain regions. Recent research using Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) has shown that PL to pDMS projections are important for the acquisition of operant responding (Hart, Bradfield, Fok, Chieng, & Balleine, 2018). Hart, Balleine, and colleagues utilized a dual-virus approach to inactivate the PL-pDMS pathway by infusing AAV-Cre recombinase into the pDMS, and a Cre-dependent DREADDs viral construct into the PL. They found that silencing the PL-pDMS pathway during acquisition, via

systemic injection of the DREADDs ligand clozapine-N-oxide (CNO), reduced operant responding during test (Hart, Bradfield, Fok, Chieng, & Balleine, 2018).

The PL has been implicated in the expression of minimally-trained operant responding when testing occurs in the acquisition context (Shipman et al., 2018; Trask et al., 2017). Temporary inactivation of the PL with baclofen/muscimol at the time of test following six daily sessions of acquisition (lever press training) resulted in an attenuation of operant responding in the context where training had been conducted, but not in another context (Trask et al., 2017). Hart et al. (2018) showed that PL projections to posterior DMS are important in the acquisition of operant behavior, but they did not examine whether PL projections to the DMS are important for the expression of operant behavior. In addition, Hart et al. did not examine the function of PL projections to the anterior DMS (aDMS); some studies suggest that PL projections to the anterior DMS (aDMS) are at least as dense as PL projections to pDMS (Hunnicutt et al., 2016; Mailly et al., 2013).

In the current experiment, we hypothesized that PL projections to the anterior DMS are involved in the expression of operant responding in the acquisition context. Six weeks prior to training, we infused an AAV8-DREADDs or control viral construct bilaterally into the PL and implanted bilateral guide cannulae into the aDMS. Rats underwent six days of instrumental conditioning followed by infusion of CNO or vehicle into the anterior DMS prior to test. We found that silencing projections from the PL to a relatively anterior region of the DMS attenuated lever-press responding, implicating this pathway for the first time in the expression of operant responding. Patch-clamp

electrophysiology in a separate group of rats confirmed that CNO suppressed spiking in DREADDs-expressing, layer 5 PL pyramidal neurons.

Methods

All animal procedures were performed in accordance with the University of Vermont's animal care committee's regulations.

Subjects

The subjects were 24 male Wistar rats from Charles River Laboratories (St. Constance, Quebec). Rats were 59-63 days old and initially housed in pairs upon arrival. They were given at least 3 days to acclimate to the colony before undergoing surgery. Following surgery, rats were housed individually in a room maintained on a 12:12-h light: dark cycle. Experimentation occurred during the light portion of the cycle.

Surgery

Rats were anaesthetized with isoflurane. AAV8-hSyn-hM4D(Gi)-mCherry viral construct (AddGene; Watertown, MA) or the control AAV8-hSyn-GFP viral construct (AddGene; Watertown, MA) was infused bilaterally into the PL with a Hamilton syringe (stereotaxic coordinates AP: +3.0, ML: +/-0.75, DV: -4.0) at a rate of 0.1µl/min. Each side received an infusion of 0.8µl. The needle was in place for two minutes prior to the start of the infusion to allow the brain to settle, and 10 minutes following completion of the infusion to allow for diffusion away from the needle tip. Guide cannulae (22 gauge, Plastics One) were targeted bilaterally to the anterior DMS at stereotaxic coordinates AP: +1.0, ML:+/-2.0, DV: -3.6. Rats were given carprofen (5.0 mg/kg) for analgesia, as well

as bupivacaine around the scalp incision, and Ringer's solution (1.0ml) following surgery. A second dose of carprofen was administered the following day. Rats were weighed and reduced to 90% free feeding weight four days prior to magazine training, and were maintained at 90% free feeding weight throughout the experiment.

Apparatus

Two sets of four operant chambers were utilized for this experiment (Med Associates model ENV-008-VP, St. Albans, VT). The sets were separated by room and differed slightly in their features. Differentiation of contexts was not required for this experiment, but rats were counterbalanced on vector type and the contexts where they received training/testing. Operant chambers measured 30.5 x 24.1 x 21.0 cm (l x w x h) and the food cup (measuring 5.1 x 5.1 cm) was located within the center of the front wall at a height of 2.5 cm above the floor. All chambers also featured a lever to the left of the magazine (Med Associates model ENV-112CM) that was inserted following a time-out period of two minutes at the beginning of each session. Within each room, each of the four chambers was housed in a sound attenuation chamber. These chambers were lit by a single incandescent bulb (7.5 W) located on the sound attenuation chamber ceiling. Ventilation fans provided white noise (65 dBA).

Half the operant chambers featured clear, acrylic plastic on the walls and a ceiling with brushed aluminum on the front and rear walls. Floor panels were stainless steel grids (0.48 cm diameter) that were staggered so that every other bar was in the opposite of two planes from the previous bar (one plane was 0.5 cm above the other). The other half of the chambers had all floor grids mounted in the same plane with each bar spaced 1.6 cm

from the previous bar. The walls in these boxes were also acrylic plastic but featured black, diagonal stripes that were 3.8 cm wide and 3.8 cm apart.

The reinforcer utilized for this experiment was a 45-mg sucrose pellet (5-TUT:1811251, TestDiet, Richmond, IN). The pellet was delivered to the magazine by instruction through a computer located in an adjacent room.

Procedure

All behavioral procedures were conducted so that both tests occured 6-7 weeks following vector infusion. Rats were run in cohorts of 4 or 8 and counterbalanced across conditions.

Magazine training

All rats received one half-hour session of magazine training. Once all animals were placed in their respective operant chambers, a two-minute time-out period began. During this period, no reinforcers were available. Following that, sucrose reinforcers were freely delivered to the food magazine on a RT 30 schedule. No levers were present during this training.

Acquisition training

Rats then received six daily acquisition sessions. At the start of each session, once all rats were in their respective operant chambers, left levers were inserted into boxes after two min and rats were reinforced on a VI-30 schedule for lever presses. Levers retracted following completion of the half hour session. If rats initially failed to eat

sucrose pellets, levers were baited with mashed pellets. One rat had to be removed from further analysis because it failed to eat any pellets and thus failed to acquire the operant lever-pressing response.

Test

After acquisition, all rats underwent two test sessions, separated by a day of retraining. Prior to the first test session, half the rats received a 0.5 µl bilateral intracranial infusion of CNO (1.0 mM) and the other half received a vehicle infusion (artificial CSF (ACSF)) into the DMS (see slice preparation section for more specifics about ACSF composition). The CNO concentration of 1 mM was based on Mahler et al. (2014) and Lichtenberg et al. (2017). For infusions, dummy cannulae were removed and internal cannulae were inserted into guide cannulae. Internal cannulae tips protruded 1 mm below the tip of guide cannulae. Infusions were delivered over 2 minutes (0.25 µl/minute) by internal cannulae attached to tubing (Intramedic) that connected to Hamilton syringes driven by a microinfusion pump (Kd Scientific). Internal cannulae were allowed to remain in place for one minute following infusions before removal and reinsertion of dummy cannulae. Rats were then placed in transport containers and put into operant chambers 5-15 min after the infusion. After a 2-min period, levers were inserted into the operant chambers (as usual). The test ran for 10 min; lever press responses had no scheduled consequences (i.e., the test was conducted in extinction). The following day, rats received a session of retraining with the VI-30 reinforcement schedule. A second test was given the day after, in which rats received the opposite infusion of the

first test. Other than receiving the opposite infusate, testing proceeded exactly as on the first test day.

Histology

Following the second test, rats were injected with a lethal dose of sodium pentobarbital (150 mg/kg, i.p.) and transcardially perfused with phosphate-buffered saline (PBS) followed by 4% paraformaldehyde. Brains were removed and postfixed for one hour before being transferred to a 30% sucrose/PBS solution. After sinking, brains were embedded in OCT and flash-frozen in 2-methyl butane that had been cooled with dry ice. The PL and DMS of each brain were sectioned at 60µm and floated in phosphate buffer onto slides. Sections were dried in the dark before being mounted with Vectashield mounting medium with DAPI and coverslipped. Viral transfection was examined using a confocal microscope (Nikon C-2) (see representative images in Figure 1D-E). Viral expression was examined for accuracy by comparing the location of PL cell expression to the PL location in the Paxinos and Watson (1998) rat brain atlas. Axon terminals were examined for expression directly underneath the deepest part of the cannulae, which were confirmed to be in the DMS.

Slice Preparation for Electrophysiology

Adult Wistar rats, of the same age and from the same supplier as above, were used for patch clamp electrophysiology. Rats underwent PL infusion of viral construct AAV8-hSyn-hM4D(Gi)-mCherry as described above. Following at least six weeks of recovery, electrophysiology experiments were performed. On the experimental day, rats

were deeply anesthetized with sodium pentobarbitol and transcardially perfused with cold, sucrose-replaced artificial cerebrospinal fluid. The brain was then quickly removed and sliced in the coronal plane on a Leica VT1000S (Leica Instruments) vibratome. Brain slices were then allowed to recover in warmed sucrose-replaced artificial cerebrospinal fluid at 32° C for 30 minutes, and then equilibrated in room temperature artificial cerebrospinal fluid (ACSF) for at least 30 minutes prior to recording. ACSF was composed of the following in mM: NaCl (124), KCl (2.8), CaCl (2), NaH_2PO_4 (1.25), Glucose (10), Sodium Ascorbate (0.4), Sodium Pyruvate (2), $MgSO_4$ (2), and $NaHCO_3$ (26). Sucrose-replaced ACSF was similar to recording-ACSF with the following exceptions in mM: NaCl (0), Sucrose (206), CaCl (1), MgCl (1). Each was pH adjusted to 7.3-7.4 with HCl and osmolarity was 310 ±5 mOsM.

Recording Procedures

Slices were transferred to a recording chamber (Warner Instruments) and continuously perfused with oxygenated, 32° C ACSF at a rate of 3-4 ml/minute. Virally-infected cells were identified under fluorescent illumination in layer 5 of the PL (Figure 2B) using a Leica DM-LFSA microscope and Rolera Bolt 3000 CCD camera. Cells were then patched under brightfield/infrared illumination in current clamp mode. Electrodes were made from thin-walled borosilicate glass capillaries (World Precision Instruments) and pulled on a Sutter P-97 micropipette puller and filled with a K-glu intracellular solution composed of the following in mM: potassium gluconate (140), KCl (2), MgCl (3), HEPES (10), Phosphocreatine (5), K-ATP (2), Na-GTP (0.2) and pH adjusted to 7.3-7.4. Cells were clamped with a Multiclamp 700B controller and Multiclamp software

(Molecular Devices). Data from patched cells was acquired using a Digidata 1440 interface (Molecular Devices) and pClamp software (Molecular Devices). Patched neurons equilibrated for approximately 5 minutes following successful whole cell configuration. Access resistance was monitored throughout experiments and if it reached above 25 MΩ, or changed by >20%, recordings were discarded. Patched neurons were considered acceptably healthy with a resting membrane potential below -50 mV and an action potential overshoot greater than +10 mV. Excitability curves were generated by injecting progressively larger positive current at 50 pA increments from 0-450 pA at the highest level of stimulation and counting the number of spikes at each level. This was done prior to CNO exposure, and after 4-6 minutes of 10 μM CNO exposure. Spike curves were analyzed using Clampfit software (Molecular Devices).

Statistical analysis

IBM SPSS 25.0 was used for data analysis. A repeated measures ANOVA was used to examine responses per minute across acquisition sessions and test sessions. The rejection criterion was set at $p<.05$. Following a significant interaction, within-subjects comparisons (two-tailed paired-samples t-tests) were performed to determine the source of the interaction. Effect size was calculated as Cohen's d for all significant effects (see statistical table) (Cohen, 1988; Rosenthal, 1994).

Table 2: Statistical tests and effect sizes for tests run.

	Data Structure	Type of test	Power (Cohen's d)

a	Normal distribution	Repeated Measures ANOVA	Main effect Session: 1.784
b	Normal distribution	Repeated Measures ANOVA	Interaction (Drug x Vector): 0.247
c	Normal distribution	Paired Samples *T* test	Main effect Drug: 0.745
d	Normal distribution	Paired Samples *T* test	Not significant
e	Normal distribution	Repeated Measures ANOVA	Main effect CNO: 0.388 Interaction (Drug x Current): 0.262
f	Normal distribution	Paired samples *T* tests	Main effects Current: 200 pA: 1.204 250 pA: 3.095 300 pA: 2.807
g	Normal distribution	Repeated Measures ANOVA	Main effect CNO: 0.048

			Interaction (Drug x Current): 0.082
h	Normal distribution	Paired samples *T* tests	Not significant

Results

Four rats (2 DREADD, 2 GFP) were removed prior to analysis: one rat did not acquire the lever-press response, two rats had a viral vector infusion site dorsal to the PL, and one rat had extensive cannula-related damage to the DMS (see further explanation in histology section). This left 10 rats in each group.

Acquisition

All rats increased responding (lever presses/minute) across training sessions, indicating successful learning of the operant response (see Figure 1A). A 2 (Vector: DREADD vs GFP) x 6 (session) repeated-measures ANOVA yielded a main effect of session, F (5,90)= 56.18, MSE= 9.78, p<.001[a], but no main effect of vector or a vector x session interaction (F's<1).

Test

Inactivation of the PL-anterior DMS pathway attenuated the expression of operant responding during the test (see Figure 1B). A 2 (Vector: DREADD vs GFP) x 2 (Drug: CNO vs vehicle) repeated-measures ANOVA yielded a significant vector x drug

interaction,F (1,18)= 5.08, MSE= 1.95, p= 0.04[b]. Follow-up paired-samples t-tests compared lever-press responding after CNO vs vehicle for each vector group separately. The DREADD group showed an attenuation of responding when tested with a CNO infusion, t (9)= 2.36, p= 0.04[c]. In contrast, the rats that had received the GFP vector showed no difference in responding following CNO vs vehicle infusions into the DMS, t (9)= 1.31, p= 0.22[d]. The pattern indicates that intra-DMS CNO effects were selective to the rats that had received PL DREADD transfection.

Histology

DREADD-mCherry expression and control GFP expression were verified in the cell bodies of the PL and axon terminals of the DMS in all rats. Examples are shown in Figures 1D and 1E. Two rats were removed because the viral-vector infusion site in the PL was too shallow. Cannula placements in the DMS were also verified (Figure 1C). No rats had to be excluded from analysis for incorrect cannula placement, though one brain showed extensive damage from a cannula (possibly from infection) that affected tissue well beyond the cannula tract and DMS. This rat was excluded from analysis. Thus, three rats were removed during verification of viral expression, leaving the DREADD group with a final n of 10 and the GFP group with a final n of 10.

Electrophysiology

To confirm the effect of CNO on DREADDs-expressing PL pyramidal neurons, we used whole-cell patch-clamp electrophysiology to compare spike activity (number of spikes to 10 current steps, 0-450 pA) before and after CNO exposure (see Figure 2).

DREADDs-expressing PL neurons showed fewer spikes than non-expressing neurons after CNO exposure. In contrast, non-DREADDs expressing PL neurons spiked slightly more after CNO exposure, possibly because of CNO suppression of nearby DREADDs-expressing inhibitory interneurons.

A 2 (Drug: CNO vs vehicle) x 10 (Current: 0-450 pA) repeated-measures ANOVA on DREADDs-expressing PL neurons revealed a significant main effect of CNO on neuron spiking, F (1, 4)= 7.83, MSE= 31.49, p= 0.049[e], and a significant drug x current interaction, F (9, 36)= 4.52, MSE= 2.82, p= 0.001[e]. Follow-up paired-samples t-tests comparing CNO vs. vehicle at each current step revealed significantly fewer spikes with CNO at current steps of 200-, 250-, and 300-pA (p's < 0.046[f]) (see Figures 2A and 2C). The same analyses on non-DREADDs expressing PL neurons revealed a significant main effect of CNO on neuron spiking, F (1, 3)= 4.05, MSE= 0.32, p= 0.037[g], and a significant drug x current interaction, F (9, 27)= 1.33, MSE= 0.30, p= 0.001[g]. Follow-up paired-samples t-tests comparing CNO vs. vehicle at each current step revealed a trend towards significantly *more* spikes with CNO at current steps of 200- and 400-pA (p's= 0.058[h]).

Discussion

The present results suggest that PL projections to a relatively anterior region of the DMS are involved in the expression of operant responding. This finding expands upon the work by Trask et al. (2017) that had found involvement of the PL in expression of operant responding in the same paradigm, as well as that of Hart et al. (2018), who demonstrated a role for PL-to-*posterior* DMS projections in the *acquisition* of goal-

directed operant responding. The current results contrastingly show that a PL-to-a more *anterior* DMS pathway is important in the *expression* of operant responding early in training. This is unlikely to be a motor-related effect, given that studies have demonstrated that pharmacological inactivation of the PL (and therefore all of its projections) reduces only minimally-trained responding, and only in the acquisition context, while leaving other responses unaffected (Killcross & Coutureau, 2003; Shipman et al., 2018; Trask et al., 2017). Additionally, we confirmed with *ex vivo* patch-clamp electrophysiology that cells in layer 5 of the PL expressing the DREADD construct reporter showed attenuated spiking in the presence of CNO.

Though statistically significant, the size of the reduction in responding was numerically small in our DREADDs-expressing rats. However, there are several important points to keep in mind. First, we inactivated only a subset of projections from the PL to the aDMS, and the inactivation was probably less than total, as suggested by our electrophysiology results. Second, it is likely that other PL projections, besides just those to the aDMS, are important in expression of minimally-trained operating responding in the acquisition context; indeed, others (e.g., Trask et al., 2017) have shown a fairly large attenuation of responding with pharmacological inactivation of PL, which would inactivate all PL projections. Finally, it is worth comparing the magnitude of our effects to those of Hart et al., (2018), who used a dual-vector approach and intraperitoneal injections of CNO during acquisition to silence PL-pDMS projections. Hart et al. (2018) reported that in a 5-min choice (still-valued R2 vs. devalued R1) test session, DREADDs-expressing rats that had received vehicle injection prior to

acquisition sessions emitted an average of approximately 18 R2 lever-presses; a separate group of DREADDs-expressing rats that had received CNO injection prior to acquisition sessions emitted an average of approximately 12 R2 lever-presses. This translates to a reduction of approximately 1 lever-press per min. We found that during a 10-min test session, vehicle-infused DREADDs-expressing rats emitted an average of approximately 86 responses while CNO-infused DREADDs-expressing rats emitted an average of approximately 76 responses. This also translates to a reduction of 1 lever-press per min. Thus, despite a difference in methods, the magnitude of operant response reduction as a result of DREADDs-mediated inactivation of PL-DMS terminals was similar in Hart et al. (2018) and our experiment.

A common concern with the use of DREADDs is that CNO does not appear to cross the blood-brain barrier; instead, the effects of systemic injections of CNO may be via the CNO metabolite clozapine, which binds with high affinity to DREADDs and binds with endogenous receptors (Gomez et al., 2017). We avoided this issue here by using intracranial CNO infusions. However, there may still be off-target effects caused by the use of a relatively high concentration of CNO in this method (Gomez et al., 2017). Therefore, we included two control procedures: (1) a group of rats that did not express DREADDs and (2) all rats received CNO and vehicle, in separate tests. Thus, we controlled for CNO effects as well as for potential vector effects. We also note here that an additional caveat to circuit-specific manipulation using DREADDs is that it may be difficult to completely isolate a specific pathway. For example, collateral projections of

projection neurons expressing DREADDs may also be activated/inactivated by CNO. However, it is unclear how likely this is given that CNO is infused directly into the DMS.

Like Hart et al. (2018), we examined a role for PL-to-DMS projections in minimally trained operant responding, though our methods differ on a few critical points. First, we only trained one response with one outcome. Hart et al. trained two lever-press responses, each with its own unique outcome, and both levers were available during (choice) testing. Second, we did not devalue our reinforcer; thus we did not distinguish between goal-directed vs. habitual behavior. Third, we examined the PL-DMS pathway in a more anterior portion of the DMS, rather than the PL projections to posterior DMS regions that have more frequently been associated with acquisition of goal-directed behavior. Fourth, we examined expression of responding, rather than the acquisition of responding, by inactivating the PL-DMS pathway prior to test rather than prior to each acquisition session. Finally, we utilized a different means of pathway-specific chemogenetic inactivation, implanting cannulae into the DMS to inactivate PL axon terminals after AAV8-DREADD infusion into the PL. In contrast, Hart et al. utilized a dual-virus approach, infusing a Cre-dependent DREADD viral construct into the PL and a Cre recombinase viral construct into the pDMS, and then inactivating the PL-pDMS pathway with intraperitoneal injection of CNO. Overall, our findings complement those of Hart et al. (2018) who showed that the PL-pDMS pathway is important for the acquisition of goal-directed behavior. We show here that the PL-aDMS pathway is also important for expression of minimally-trained operant behavior.

Many of the studies investigating the role of the PL in operant behavior have additionally confirmed whether responding was goal-directed or habitual (Corbit & Balleine, 2003; Killcross & Coutureau, 2003; Ostlund & Balleine, 2005; Shipman et al., 2018; Tran-Tu-Yen et al., Coutureau, 2009). Behavior is considered goal-directed if it is sensitive to reinforcer devaluation, whereas habitual behavior is insensitive to reinforcer devaluation. Though we did not utilize reinforcer devaluation to examine if our behavior was goal-directed, it is reasonable to assume that our minimally-trained operant response was goal-directed, as habit typically develops across many training sessions (Dickinson, 1985). This is further supported by the findings of Shipman et al. (2018), who showed that the PL plays a transitory role in the development of operant responding: inactivation of PL reduced minimally-trained goal-directed instrumental behavior, but not more extensively-trained instrumental behavior that is goal-directed. The PL has never been linked to habit.

Despite dense anatomical connections from the PL to the aDMS, research has tended to focus on the pDMS in goal-directed behavior. This focus is largely based on an early study by Yin and colleagues (Yin et al., 2005). Yin et al. (2005) found that pre-training or post-training lesions of the posterior region of the DMS impaired the acquisition and expression of goal-directed behavior (target posterior coordinates at -0.4 mm AP relative to bregma, compared to +1.0 mm AP in the current study). However, the effects of aDMS lesions were actually somewhat inconclusive, as pre-training aDMS lesions did not affect expression of goal-directed behavior at test but post-training aDMS lesions did. Other research has provided support for the idea that the pDMS, but not the

aDMS, is important for goal-directed responding. For example, functional disconnection of the parafascicular thalamus and pDMS disrupts goal-directed responding, whereas disconnection of the parafascicular thalamus and aDMS has no effect (Bradfield, Bertran-Gonzalez, Chieng, & Balleine, 2013).

Nonetheless, other studies have found that the aDMS, in addition to the pDMS, is important for goal-directed behavior. Corbit and Janak (2010) trained two different lever-press responses and then used satiation to devalue the outcome associated with one response. They found that temporary inactivation with baclofen/muscimol of either the anterior DMS or posterior DMS during acquisition resulted in insensitivity to outcome devaluation at time of test in an operant task (coordinates at +1.2 mm and -0.3 mm AP relative to bregma, respectively). This result suggests that aDMS and pDMS both seem to be involved in goal-directed responding. Further studies by this lab also showed a role for the anterior DMS in goal-directed behavior with an alcohol reinforcer (Corbit, Nie, & Janak, 2012). Thus, there is some evidence for aDMS involvement in goal-directed behavior despite a literature that focuses largely on the pDMS.

In conclusion, we found that the PL-aDMS pathway is important in the expression of operant responding. Thus, we expand upon previous research to show, using circuit-specific chemogenetic silencing, a role for a PL-to-anterior DMS pathway in the expression of operant behavior to complement the demonstrated role of a PL-to-posterior DMS pathway in the acquisition of operant behavior.

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References

Bradfield, L. A., Bertran-Gonzalez, J., Chieng, B., & Balleine, B. W. (2013). The Thalamostriatal Pathway and Cholinergic Control of Goal-Directed Action: Interlacing New with Existing Learning in the Striatum. *Neuron*, *79*(1), 153–166.

Corbit, L. H. (2018). Understanding the balance between goal-directed and habitual behavioral control. *Current opinion in behavioral sciences*, *20*, 161-168.

Corbit, L. H., & Balleine, B. W. (2003). The role of prelimbic cortex in instrumental conditioning. *Behavioural brain research*, *146*(1–2), 145–57.

Corbit, L. H., & Janak, P. H. (2010). Posterior dorsomedial striatum is critical for both selective instrumental and Pavlovian reward learning. *The European journal of neuroscience*, *31*(7), 1312–21.

Corbit, L. H., Nie, H., & Janak, P. H. (2012). Habitual Alcohol Seeking: Time Course and the Contribution of Subregions of the Dorsal Striatum. *Biological Psychiatry*, *72*(5), 389–395.

Dickinson, A. (1985). Actions and habits: the development of behavioural autonomy. *Philosophical Transactions of the Royal Society of London, 308*, 67-78.

Gomez, J. L., Bonaventura, J., Lesniak, W., Mathews, W. B., Sysa-Shah, P., Rodriguez, L. A., ... & Pomper, M. G. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. Science, 357(6350), 503-507.

Hart, G., Bradfield, L. A., & Balleine, B. W. (2018). Prefrontal corticostriatal disconnection blocks the acquisition of goal-directed action. *Journal of Neuroscience*, *38*(5), 1311-1322.

Hart, G., Bradfield, L. A., Fok, S. Y., Chieng, B., & Balleine, B. W. (2018). The Bilateral Prefronto-striatal Pathway Is Necessary for Learning New Goal-Directed Actions. *Current Biology*, *28*.

Hunnicutt, B. J., Jongbloets, B. C., Birdsong, W. T., Gertz, K. J., Zhong, H., & Mao, T. (2016). A comprehensive excitatory input map of the striatum reveals novel functional organization. *eLife*, *5*.

Killcross, S., & Coutureau, E. (2003). Coordination of actions and habits in the medial prefrontal cortex of rats. *Cerebral cortex (New York, N.Y. : 1991)*, *13*(4), 400–8.

Lichtenberg, N. T., Pennington, Z. T., Holley, S. M., Greenfield, V. Y., Cepeda, C., Levine, M. S., & Wassum, K. M. (2017). Basolateral amygdala to orbitofrontal cortex projections enable cue-triggered reward expectations. *Journal of Neuroscience*, *37*(35), 8374-8384.

Mahler, S. V., Vazey, E. M., Beckley, J. T., Keistler, C. R., McGlinchey, E. M., Kaufling, J., ... & Aston-Jones, G. (2014). Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. *Nature neuroscience*, *17*(4), 577.

Mailly, P., Aliane, V., Groenewegen, H. J., Haber, S. N., & Deniau, J. M. (2013). The rat prefrontostriatal system analyzed in 3D: evidence for multiple interacting functional units. *Journal of Neuroscience*, *33*(13), 5718-5727.

Ostlund, S. B., & Balleine, B. W. (2005). Lesions of medial prefrontal cortex disrupt the acquisition but not the expression of goal-directed learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *25*(34), 7763–70.

Paxinos, G., & Watson, C. (1998). The Rat Brain in Stereotaxic Coordinates, 4th edn Academic Press: New York.

Shiflett, M. W., Brown, R. A., & Balleine, B. W. (2010). Acquisition and Performance of Goal-Directed Instrumental Actions Depends on ERK Signaling in Distinct Regions of Dorsal Striatum in Rats. *The Journal of Neuroscience*, *30*(8), 2951–2959.

Shipman, M. L., Trask, S., Bouton, M. E., & Green, J. T. (2018). Inactivation of prelimbic and infralimbic cortex respectively affects minimally-trained and extensively-trained goal-directed actions. *Neurobiology of Learning and Memory*.

Tran-Tu-Yen, D. A., Marchand, A. R., Pape, J.-R. R., Scala, G., & Coutureau, E. (2009). Transient role of the rat prelimbic cortex in goal-directed behaviour. *The European journal of neuroscience*, *30*(3), 464–71.

Trask, S., Shipman, M. L., Green, J. T., & Bouton, M. E. (2017). Inactivation of the Prelimbic Cortex Attenuates Context-Dependent Operant Responding. *The Journal of Neuroscience*, *37*(9), 2317–2324.

Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2005). Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. *The European journal of neuroscience*, *22*(2), 505–12.

Yin, H. H., Ostlund, S. B., Knowlton, B. J., & Balleine, B. W. (2005). The role of the dorsomedial striatum in instrumental conditioning. *The European journal of neuroscience*, *22*(2), 513–23.

Figures

Figure 1. A. Acquisition of lever-press response over six training sessions with one retraining session. B. Test of rats with DREADD vector and CNO vector when receiving both CNO and vehicle infusions into the aDMS. C. Cannula placements in aDMS. D. Representative image of cell-bodies expressing mCherry DREADDs-mCherry viral construct (left) or GFP control construct (right) in the PL at 40X. Blue is DAPI stain of cell bodies. E. Representative images of axon terminals in aDMS expressing DREADDs-mCherry (left) or GFP (right) at 60X. Scale bars are 50 µm.

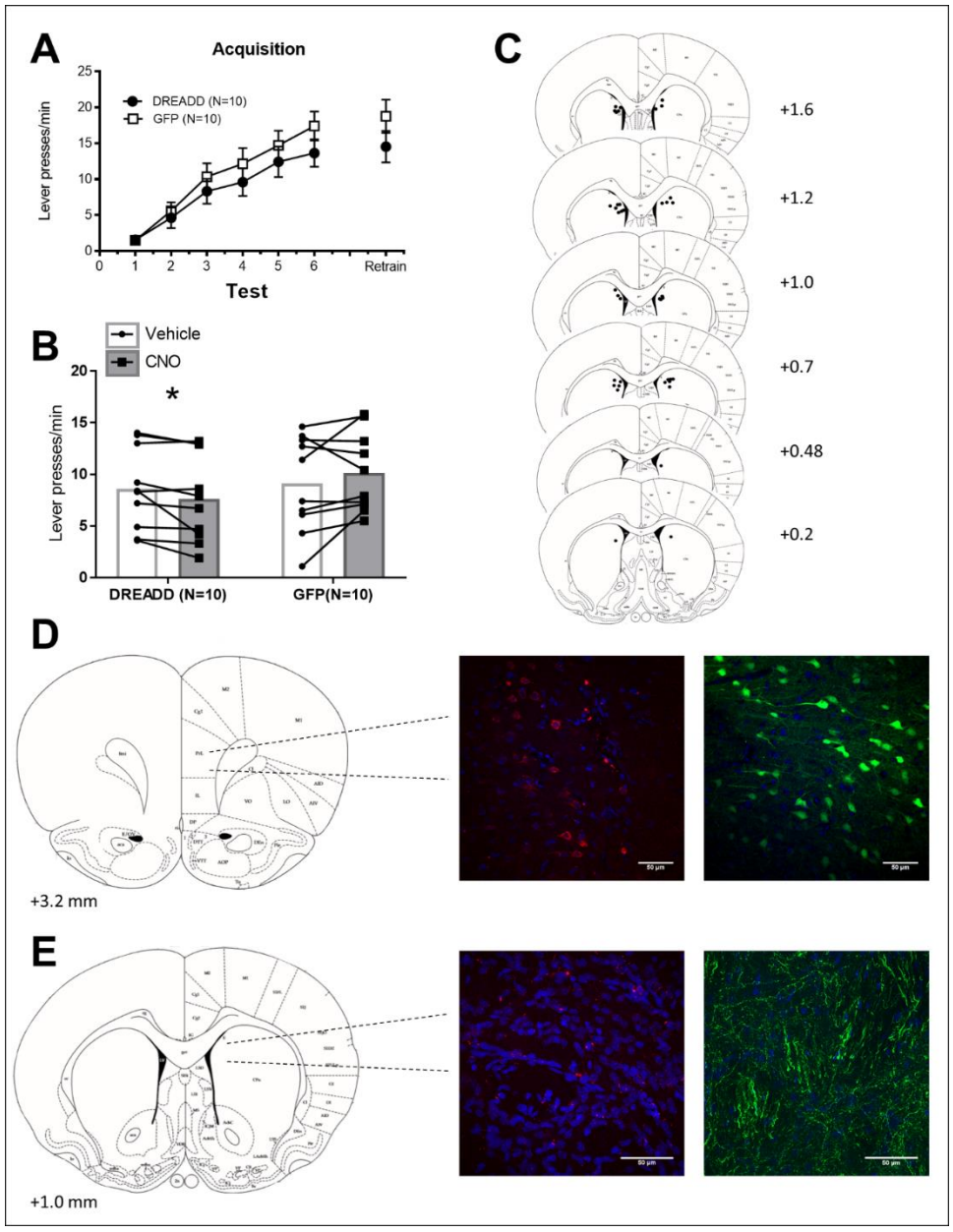
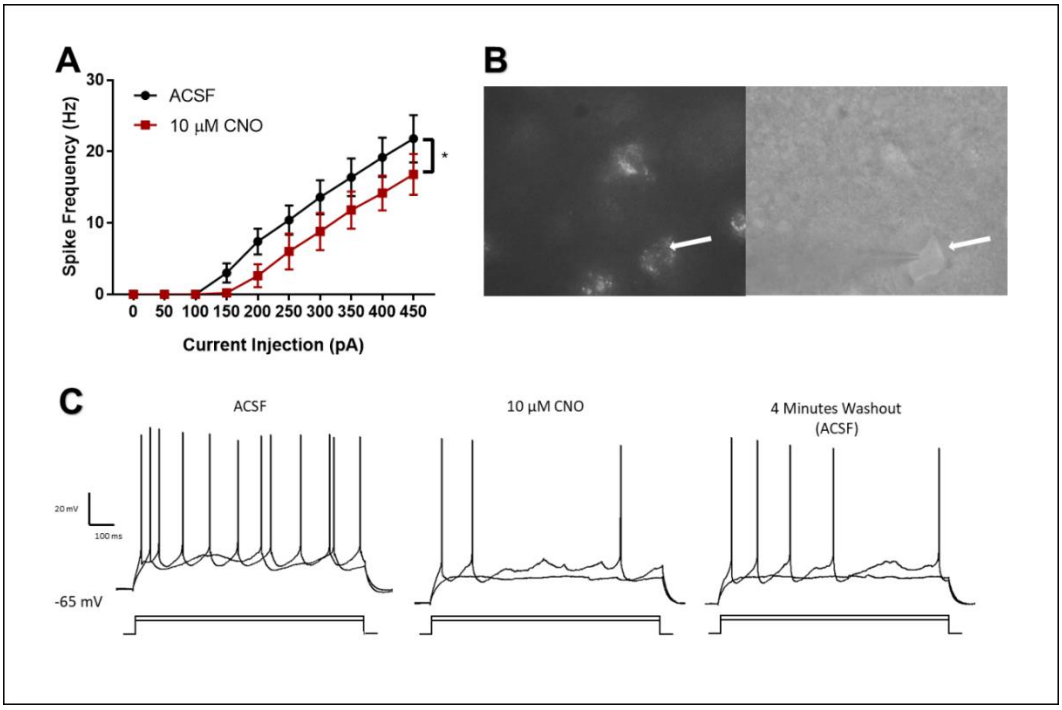


Figure 2. A, Excitability curve shows spikes elicited to progressively larger current injection of PL pyramidal cells before and after CNO (10 µM) exposure. B, Example image of virally infected PL pyramidal cell in fluorescent (left), and infrared (right). C, Example trace of neuron, 4 minutes of CNO exposure caused a reduction in spike frequency to current injection compared to baseline, while removal of CNO from bath caused a partial recovery of spike frequency. Scale bars are 20 mV and 100 ms, and stimulation was 250 pA and 350 pA for 1 second.



Chapter 4: Cerebellar Crus I/II involvement in actions and habits

Rodent cerebellar Crus I/II is involved S-R component of minimally and extensively trained actions, but not habits

Shipman, M. L., Thomas, C., & Green, J. T.

Abstract

The cerebellum has long been established as a mediator of motor coordination. However, human research has implicated the lateral cerebellum in cognitive functions normally associated with the medial prefrontal cortex including executive function, such as cognitive flexibility, working memory, and inhibition. Distinct anatomical connections from the lateral cerebellum to the prefrontal cortex via the thalamus (in comparison to the motor loop to the primary motor cortex) may support these prefrontal cortex-dependent functions. A handful of studies have shown that the lateral cerebellum may play a role in stimulus-guided operant (instrumental) behavior and/or in the representation of goals/outcomes, functions that are mediated by the medial prefrontal cortex. In experiment 1, we investigated a role for the lateral cerebellum in minimally and extensively trained goal-directed operant responding. We found that inactivation of Crus I/II, a lateral region of the cerebellar cortex implicated in human executive function, at time of test attenuated responding in the outcome devalued groups on both minimally and extensively trained responses. Residual responding after outcome devaluation is habitual. Therefore, in experiment 2, we extensively trained responding to be entirely habitual (i.e., no effect of outcome devaluation on responding) and found that Crus I/II inactivation had no effect. We therefore concluded that Crus I/II of the cerebellum may play a role during both minimally and extensively trained goal-directed responding by modulating habit expression.

Introduction

The human cerebellum has been implicated in "cognitive" performance for at least the past forty years, particularly in behaviors known to be prefrontal cortex-dependent (Bodranghien et al., 2016; Caligiore et al., 2017; Koziol et al., 2014; Leiner, Leiner, & Dow, 1986, 1989; Schmahmann, 1991). However, despite a vast array of findings in patient studies, non-human primate studies, and most recently, fMRI and transcranial magnetic stimulation (TMS) studies, the rodent characterization of these phenomena has lagged. Though the rodent literature has strongly implicated the lateral cerebellum in cognitive flexibility and spatial navigation, a few recent studies have suggested that it may also be important for the representation of goals and/or the development of habitual behavior (Shipman & Green, in press). Additionally, habit development is arguably an automatization of a behavior, and given the role of the cerebellum in automatizing motor coordination, it may be that a different region of the cerebellum projecting to a "cognitive" region can use the same cellular organization to coordinate a more "cognitive" automatization (Ramnani, 2006).

Anatomically, there is strong human and non-human primate evidence for a means by which the cerebellum could exert influence on the prefrontal cortex. Bernard et al. (2014) used resting-state functional connectivity magnetic resonance imaging (fcMRI) to investigate a "motor" network and "cognitive" network from the cerebellum to different areas of the cerebral cortex. They found that the dorsal dentate nucleus was linked to motor regions of the cerebral cortex and cerebellar lobule I-VI and Crus I, while the ventral dentate nucleus was associated with lobules VI and VIIb and Crus II as well

as the anterior cingulate cortex and thalamus. These separate connections imply distinct functionality of the dorsal and ventral dentate nucleus networks. Similarly, non-human primate studies have utilized transneuronal tracers and found projections from cerebellar lobules IV-VI via the ventral lateral thalamic nucleus to the primary motor cortex that are distinct from output from cerebellar Crus II (Kelly & Strick, 2003) which travel via the medial dorsal nucleus (Middleton & Strick, 2001) to the dorsolateral prefrontal cortex, an area involved in working memory and higher cognitive function (Barbey et al., 2013). These studies provide anatomical evidence for separate "motor" and "cognitive" loops and suggests that there are separate pathways by which cerebellar efferents communicate with motor areas and the prefrontal cortex (Barbey et al., 2013; Bernard et al., 2014; Kelly & Strick, 2003; Middleton & Strick, 2001).

In rodents, projections from the mPFC to the cerebellum (via pontine nuclei) have been well-established (Runyan, 2004; Siegel et al., 2015; Watson et al., 2009), but significantly less anatomical work has been done in examining cerebellar outputs to the mPFC. Stimulation of the prelimbic cortex (a region of the mPFC) both evoked field potentials in lobule VII along the vermis of the cerebellar cortex and caused complex spikes in Purkinje cells in the same area (Watson et al., 2009). This means that prelimbic activation caused neuronal signaling via the inferior olive and climbing fibers, since climbing fiber activity causes complex spiking in Purkinje cells. Conversely, electrophysiological data suggests that stimulation of the deep cerebellar fastigial nucleus elicits local field potentials in the rat prelimbic cortex (Watson et al., 2014). Additionally, stimulation of dentate nuclei resulted in dopamine efflux in the prelimbic cortex of mice

(Mittleman et al., 2008; Rogers et al., 2011; Rogers et al., 2013). These studies suggest that there is a functional connectedness between the rodent cerebellum and the rodent mPFC. The cerebellum sends many projections to the thalamus (Voogd, 2004) and at least some projections from the dentate nuclei colocalize with other cortical areas (posterior parietal cortex; anterior cingulate) in the ventrolateral thalamic nuclei (Giannetti & Molinari, 2002; Parker, Narayanan, & Andreasen, 2014). The mediodorsal nucleus of the thalamus also receives projections from the cerebellum and sends projections to the PL, and has been implicated in behaviors similar to those mediated by the PL (Corbit et al., 2003; Ostlund & Balleine, 2008; Uylings et al., 2003).

Operant behavior involves learning that a particular response produces a particular outcome (reinforcer). One example of this is a rat learning to lever-press for a sucrose pellet. Behavior is categorized as either goal-directed, i.e. sensitive to reinforcer devaluation and promoted by response-outcome (R-O) associations, or habitual, meaning that behavior is insensitive to reinforcer devaluation and promoted by stimulus-response (S-R) associations. The prelimbic cortex of the mPFC has been implicated in goal-directed behavior (Corbit & Balleine, 2003; Killcross & Coutureau, 2003; Shipman et al., 2018; Tran-Tu-Yen et al., 2009) and the infralimbic cortex of the mPFC has been implicated in habit (Coutureau & Killcross, 2003; Killcross & Coutureau, 2003; but see Shipman et al., 2018). Since the cerebellar cortex and these prefrontal regions may be disynaptically connected, and overlap in some executive functions that they support, it is reasonable to think that tasks that are dependent on these prefrontal regions may also be dependent on the cerebellum.

We have shown previously that minimally training one response in one context and extensively training a second response in a second context, results in the maintenance of goal-directed responding of both responses (Shipman et al., 2018). Interestingly, both inactivation of the prelimbic cortex and infralimbic cortex resulted in an attenuation of responding in the groups that had not received devaluation (unpaired) for the minimally and extensively trained responding (respectively) at test. This indicates that expression of the minimally-trained response is dependent on the prelimbic cortex, while expression of the extensively-trained response is dependent on the infralimbic cortex (Shipman et al., 2018). Therefore, if the cerebellum is functionally linked to either prelimbic cortex or infralimbic cortex, this method provides a means to detect that linkage.

Additionally, a recent study implicated cerebellar granule cells in reinforcer tracking, which would be a crucial component of R-O associations inherent in goal-directed behavior. Mice who expressed fluorescent indicators in granule cells were head-fixed and trained to perform a task while two-photon images were taken of activity in lobules VI/VII (very near to Crus I/II). Over a span of days, they learned to push a manipulandum with a forelimb to earn a sucrose reward. Surprisingly, some granule cells began to respond in anticipation of reinforcers. Distinct cells also responded when reinforcers were omitted (Wagner et al., 2017). Therefore, we have some evidence that cerebellar Crus I/II may be involved in at least representing goals.

In Experiment 1, rats followed the procedure outlined in Shipman et al. (2018), where they were trained on two distinct operant responses, one extensively and one minimally, that were both expressed as goal-directed. We inactivated Crus I/II at time of

test. To our surprise, we found that Crus I/II inactivation attenuated both minimally-trained and extensively-trained responding selectively in the devalued group. Residual responding in devalued groups is indicative of habit, so these results suggest that Crus I/II inactivation disrupted habit expression.

Methods

All procedures were conducted in accordance with the University of Vermont IACUC standards and approved in IACUC 18-062.

Experiment 1:

Subjects. Subjects were 48 male Wistar rats purchased from Charles River Laboratories (St. Constance, Quebec). Rats were run in two cohorts, the first with 32 subjects and the second with 16. They arrived at the University of Vermont between 59 and 63 days old and were individually housed in a room maintained on a 12:12-h light: dark cycle. Experimentation took place during the light period of the cycle. Rats were food-deprived to 90% of their baseline body weight throughout the experiment.

Surgery. Following a minimum of four days of acclimation to the colony, rats were anesthetized with isoflurane and guide cannulae (22 gauge, Plastics One) were implanted in Crus I/II of the cerebellar cortex via stereotaxic surgery. Guide cannulae tip coordinates were -12.5 mm from bregma, ± 3.5 mm from midline, and -4.0 mm ventral from bregma. Rats were given injections of 0.1 ml/mg of carprofen for analgesia during surgery and one day post-operatively. During surgery, bupivacaine was also administered as a local anesthetic and 1 ml of lactated Ringers was administered for hydration. Rats

were given post-op checks during five days of recovery. After this time, a new baseline weight was taken and rats began food deprivation.

Apparatus. Two sets of four conditioning chambers housed in separate rooms of the laboratory served as the two contexts for the experiment. Each chamber was contained in its own sound attenuation chamber. All boxes were from Med Associates (model ENV-008-VP, St. Albans, VT) and measured 30.5 cm × 24.1 × 21.0 cm (l × w × h). A recessed food cup (measuring 5.1 cm × 5.1 cm) was centered in the front wall approximately 2.5 cm from the floor. A retractable lever (Med Associates model ENV-112CM) was positioned to the left of the food cup and protruded 1.9 cm into the chamber. The chain pull manipulandum (Med Associates model ENV-111C) was suspended to the right of the food cup from a micro switch mounted on top (outside) of the ceiling panel of each operant chamber. The chain hung 1.9 cm from the front wall, 3 cm to the right of the food cup, and 6.2 cm above the grid floor. The chambers were lit by a 7.5-W incandescent bulb mounted to the ceiling of the sound attenuation chamber, approximately 34.9 cm from the grid floor at the front wall of the chamber. Ventilation fans provided background noise of 65 dBA.

Each set of boxes had distinct visual, tactile, and scent features to create discernable differences between contexts. In one set, the side walls and ceiling were made of clear acrylic plastic, while the front and rear walls were made of brushed aluminum. The floor consisted of stainless steel grids (0.48 cm diameter) staggered such that odd- and even-numbered grids were mounted in two separate planes, one 0.5 cm above the other. This set of boxes had no distinctive visual cues on the walls or ceilings

of the chambers. A dish containing 5 ml of lemon Pine-Sol (Clorox Co., Oakland, CA) was placed outside of each chamber near the front wall.

The second set of boxes were much like the lemon-scented boxes except for the following features. In each box, one side wall had black diagonal stripes, 3.8 cm wide and 3.8 cm apart, and the ceiling had similarly spaced stripes oriented in the same direction. The grids of the floor were mounted on the same plane and were spaced 1.6 cm apart (center-to-center). A piney odor was continuously presented by placing 5 ml of Pine-Sol (Clorox Co., Oakland, CA) in a dish outside the chamber.

The reinforcer in both contexts was a 45-mg sucrose-based food pellet (5-TUT: 1811251, TestDiet, Richmond, IN, USA) that was delivered to the magazine. The apparatus was controlled by computer equipment located in an adjacent room.

Procedure. The experimental design was the same as that utilized in Shipman et al. (2018).

Magazine Training. On the first day of the experiment, all rats were assigned to a box within each set of chambers. They then received one 30-min session of magazine training in Context A. On the same day, the animals also received a second 30-min session of magazine training in Context B. Half the animals were trained first in Context A, and half were trained first in Context B. The sessions were separated by approximately 1 hr. Once all animals were placed in their respective chambers, a two-minute delay was imposed before the start of the session. In each session, approximately 60 reinforcers

were delivered freely on a random time 30-s (RT 30-s) schedule. Manipulanda were not present during magazine training.

R1 Acquisition. All rats were trained in Context A on response 1 (R1) for 24 total sessions. These sessions were 30 minutes long and occurred twice daily. Throughout the sessions, R1 responding delivered reinforcers on a variable interval 30-s (VI 30-s) schedule of reinforcement. No hand shaping was necessary. Contexts A and B and R1 and R2 were counterbalanced amongst subjects so that for half the animals R1 was the lever and for half it was the chain, and the opposite response the animal received as R1 became its R2. Similarly, half of the animals received the "pine" room as Context A and the other half received the "lemon" room, and vice versa for Context B.

R2 Acquisition. On the last four days of training, rats additionally received 30-min training sessions (1 per day) on response 2 (R2) in Context B following two sessions of R1 training. R2 was the chain for animals whose R1 was the lever and vice versa. As before, R2 responding delivered reinforcers on a VI 30-s schedule of reinforcement and no hand shaping was necessary. These daily sessions occurred after the final R1 acquisition session on days 9 – 12 of training.

Reinforcer Devaluation. Over the next 12 days, animals were given 6 two-day reinforcer devaluation cycles (3 in each context, alternating; see Trask & Bouton, 2014). Half the rats received the contexts in the order of AABBAABBAABB, and half received them in the order of BBAABBAABBAA. On the first day of each cycle, rats were all given an injection of 20 mg/kg .15 M lithium chloride (LiCl) following time in the acquisition context. For half the animals, Group Paired-Muscimol and Group Paired-

Vehicle, LiCl injections were given following exposure to the sucrose reinforcer presented on a random time 30-s (RT 30-s) schedule into the magazine. For the other half, Group Unpaired-Muscimol and Group Unpaired-Vehicle, no reinforcer presentations occurred prior to LiCl injections. On the second day of each cycle, rats were given no injection following time in the appropriate context. Then, Group Paired received no reinforcers and Group Unpaired received an equivalent number of reinforcers as had been consumed by a yoked animal in Group Paired the day before. On the first cycle, rats in Group Paired were given 30 reinforcers. On subsequent cycles, they were given the amount that they had consumed on the last cycle.

Muscimol Infusions. On the final day of the experiment, rats were given a bilateral infusion into the cerebellar cortex via Hamilton syringes of sterile phosphate-buffered saline (PBS), or muscimol (2.0 mM; Sigma Aldrich, St Louis, MO), dissolved in PBS, to temporarily inactivate Crus I/II. Internal cannulae (28 gauge, Plastics One) were inserted bilaterally into guide cannulae. Internal cannula tips protruded 1 mm below the guide cannula tip. An infusion of 0.5 µL per side was delivered at a rate of 0.25 µL per minute using a microinfusion pump. Following completion of the infusion, the internal cannulae were left in place for 1 min to allow diffusion of the drug or saline away from the cannula tips. Internal cannulae were then removed and dummy cannulae replaced. Each rat was then placed in the transportation container. Time between the end of infusion and the start of testing was 15-30 minutes.

Test. Following infusions, all rats were given two 10-min extinction tests, one in Context A (where R1 was tested) and one in Context B (where R2 was tested).

Responding did not produce any pellets. Testing order was counterbalanced such that half the animals in each group were tested first in Context A and half were tested first in Context B. There was a delay of 30 min between tests for each animal.

Consumption Test. On the next day, animals all received 10 reinforcers delivered freely to the magazine on an RT 30-s schedule in each context (order counterbalanced) and pellet consumption was recorded.

Reacquisition Test. Following the consumption test, all animals were then given one 10-min reacquisition session in each context (with its respective response) in which reinforcers were delivered contingent on responding on a VI 30-s schedule. Half the animals were tested first with R1 and the other half were tested first with R2.

Histology. Following experiments, rats were injected with a lethal dose of sodium pentobarbital (150 mg/kg, i.p.) and transcardially perfused with 0.9% physiological saline followed by 10% formalin. Prior to removal of the brain, an insulated stainless steel insect pin (.3 mm diameter) was inserted into the cannulae so that the uninsulated tip protruded 1 mm below the bottom of the cannulae, at the site of infusion. Direct current (100 μA) was passed through the insect pin for 10 seconds. Brains were removed and postfixed. Brains were transferred to a 30% sucrose/10% formalin solution prior to embedding. After sinking, brains were embedded in albumin and sat in glutaraldehyde for one-hour prior to freezing. The cerebellum was sliced to include cannulae at 70 μm and slices were directly mounted to pre-subbed slides. Following drying, sections were stained with Prussian Blue to identify the marking lesion and run through a cresyl-violet

procedure. Cover slips were mounted to slides with mounting medium. Slices were examined under a microscope to confirm cannula placement.

Statistical Data Analysis. All data were subjected to analysis of variance. The rejection criterion was set at $p < .05$. Training data for each response were analyzed with three-way ANOVAs that included dummy factors of drug and devaluation, in addition to session. Devaluation (consumption) data were analyzed with two-way ANOVAs that included the dummy factor of drug, in addition to session. Test data were analyzed with three-way ANOVAs that included the factors of drug, devaluation, and response. Reacquisition data were analyzed with three-way ANOVAs that included the dummy factor of drug, as well as devaluation and minute.

Results

One lever broke during testing, so the rat who had been in that box was excluded from analysis. One rat lost a headcap during acquisition training and was removed from analysis. Two rats were removed from analysis due to being outliers on at least one test performance ($Z = 2.7$ on R1 baseline score for one rat and $Z=2.1$ on R2 baseline score). Eleven rats were excluded from analysis for having at least one cannula fall outside of the Crus I/II region, because cannulae couldn't be located, or because cannulae had caused extensive damage outside of the Crus I/II region (see Figure 5). In total, 15 rats were excluded from analysis. Group n's were as follows: Paired-Muscimol (n=9), Unpaired-Muscimol (n=9), Paired-Vehicle (n=9), Unpaired-Vehicle (n=6).

R1 Acquisition. All animals increased responses across R1 acquisition sessions, indicating that they had learned to make the extensively-trained response (see Figure 1

for R1 and R2 acquisition). This was confirmed by a 2 (Drug: Muscimol vs. Vehicle) x 2 (LiCl: Paired vs. Unpaired) x 24 (Session) ANOVA that revealed a main effect of session, F (23, 460) = 27.58, MSE = 66.19, $p < .001$. We found no other main effects or interactions, largest $F = 0.59$, $p = .93$.

R2 Acquisition. All rats showed an increase in responding across training sessions for R2 as well. This was confirmed by a 2 (Drug: Muscimol vs. Vehicle) x 2 (LiCl: Paired vs. Unpaired) x 4 (Session) ANOVA that revealed a main effect of session, F (3,87) = 101.79, MSE = 9.91, $p < .001$. We found no other main effects or interactions, largest $F = 1.30$, $p = .28$.

Devaluation. Animals in paired groups ate fewer pellets as devaluation progressed (see Figure 2). This was confirmed by a 2 (Drug: Muscimol vs. Vehicle) x 6 (Session) ANOVA which revealed a main effect of session, F (5, 80) = 125.74, MSE = 26.18, $p < .001$. There were no other main effects or interactions, Fs < 1.

Test. Response rates were expressed as a proportion (see also Killcross & Coutureau, 2003; Shipman et al., 2018) of the final rates achieved in the last session of acquisition. The extensively-trained response was goal-directed, as was the minimally trained response, as we had predicted based on the paradigm that we utilized and the results of Shipman et al. (2018). Furthermore, Crus I/II inactivation reduced expression of both responses in the paired but not the unpaired group.

A 2 (Drug: Muscimol vs. Vehicle) x 2 (LiCl: Paired vs. Unpaired) x 2 (Response: R1 vs. R2) ANOVA yielded a main effect of lithium chloride, F (1, 29) = 27.85, MSE = .20, $p < .001$. This also yielded a significant drug by LiCl interaction, F (1, 29) = 5.52,

MSE = .04, p = .03, but no other main effects or interactions, largest F = 1.24, p = .28 (see Figure 3). For the extensively trained R1, pairwise comparisons revealed a reduction in Group Paired-Muscimol compared to Group Unpaired-Muscimol, F (1, 29) = 16.30, MSE = .11, p <.001. They also revealed no significant difference between Group Paired-Muscimol and Group Paired-Vehicle, F (1, 29) = 2.72, MSE = .02, p =.11. For the minimally trained R2, planned pairwise comparisons revealed a reduction in Group Paired-Muscimol compared to Group Unpaired-Muscimol, F (1, 29) =19.48, MSE = .13, p < .001 as well as a reduction in Group Paired-Muscimol compared to Group Paired-Vehicle, F(1, 29) =4.80, MSE = .03, p = .037.

Further, we analyzed test responding including just rats with both cannulae in Crus I since some recent studies have suggested may be the most important cerebellar region in rodents for "cognition" (Deverett et al., 2018; Stoodley et al., 2017). Group n's were: Paired-Muscimol (n=6), Unpaired-Muscimol (n=7), Paired-Vehicle (n=9), Unpaired-Vehicle (n=6). We found the same pattern of responding, with attenuation in Paired-Muscimol groups on both R1 and R2.

A 2 (Drug: Muscimol vs. Vehicle) x 2 (LiCl: Paired vs. Unpaired) x 2 (Response: R1 vs. R2) ANOVA was conducted to assess R1 and R2 responding (% baseline as analyzed above) during the test. This yielded a main effect of LiCl, F (1, 24) = 23.38, MSE = .20, p < .001. This also yielded a significant drug by LiCl by response interaction, F (1, 24) = 5.37, MSE = .05, p = .03, but no other main effects or interactions, largest F = 1.65, p = .21. Again, for the extensively trained response, R1, planned pairwise comparisons yielded a difference between Groups Unpaired-Muscimol and Paired-

Muscimol, F (1, 24) = 11.77, MSE = .01, p = .002, showing that the Paired-Muscimol group reduced responding. Unlike in the Crus I/II analysis, there was also a marginally significant effect when the Unpaired-Muscimol and Unpaired-Vehicle groups were compared, F (1, 24) = 3.72, MSE = .01, p = .066. On the minimally trained response, R2, planned pairwise comparisons yielded a difference between the Paired-Muscimol and Paired-Vehicle Groups, F(1, 24) =4.448, MSE = .01, p=.046, as well as between Groups Unpaired-Muscimol and Paired-Muscimol groups, F (1, 24) =17.306, MSE = .01, p < .001. Thus, the Paired-Muscimol group responded less than all other groups on R2.

Consumption Test. Devaluation was effective, as rats in the paired group who received Muscimol ate an average of 0.11 pellets in Context A and 0.22 pellets in Context B. The rats in the paired vehicle group ate an average of .22 pellets in Context A and 0 pellets in Context B. All rats in the unpaired groups ate all pellets in both contexts.

R1 Reacquisition. Rats that underwent devaluation showed reduced lever-pressing across reacquisition while unpaired rats increased lever-pressing across trials. A 2 (Drug: Muscimol vs. Vehicle) x 2 (LiCl: Paired vs. Unpaired) x 10 (Minute) ANOVA was conducted to examine R1 responding (Figure 4). This revealed a main effect of minute, F (9, 261) = 8.29, MSE = 43.99, p < .001, and a main effect of LiCl, F (1, 29) = 37.10, MSE = 928.54, p < .001. These effects were qualified by a minute by LiCl interaction, F (9, 261) = 5.52, MSE = 43.99, p < .001. No other main effects or interactions were significant, largest F < 1. Follow-up independent samples T-tests comparing paired and unpaired groups at each minute of reacquisition revealed

significantly higher responding by unpaired groups at each time point (p was never above <.001).

R2 Reacquisition. Rats that underwent devaluation also showed reduced responding on R2 in comparison to unpaired rats. A 2 (Drug: Muscimol vs. Vehicle) x 2 (Devaluation: LiCl vs. Vehicle) x 10 (Minute) ANOVA was conducted to examine R2 responding (Figure 4). This revealed a main effect of minute, F (9, 261) = 7.78, MSE = 25.33, $p < .001$, and a main effect of LiCl, F (1, 29) = 65.52, MSE = 378.59, $p < .001$. These effects were qualified by a minute by LiCl interaction, F (9, 261) = 9.61, MSE = 25.33, $p < .001$. Follow-up independent-samples T-tests comparing paired and unpaired groups at each minute of reacquisition revealed significantly higher responding by unpaired groups at each minute (p range: <.001 to .005). Interestingly, a main effect of drug was also observed, F (1, 29) = 8.92, MSE = 378.59, $p = .006$. No other main effects or interactions were significant, largest $F = 2.58$.

Histology. Brain slices were examined to determine that cannulae were in the brain region of interest. Crus I/II was identified by atlas (Paxinos & Watson, 2006) by coordinate, slice shape, and with landmarks such as deep nuclei, brainstem, flocculi, and ventricles.

Experiment 2:

Experiment 1 found that Crus I/II inactivation attenuated responding in the paired (devalued) group. Because the outcome has been devalued in this group, any remaining responding is, by definition, habitual. A few studies have already hinted at a role for the cerebellum in habitual responding. In humans, Liljeholm et al. (2015) modified a human

working memory task to increase the likelihood of either habitual or goal-directed responding and examined fMRI activity during acquisition and following reward devaluation. This allowed for both the manipulation and assessment of habitual behavior without the difficulty of overtraining in a scanner. They found that stronger cerebellar activation in the habit group during the first two blocks of instrumental learning was predictive of an increase in responding on devalued trials, or more explicitly, an insensitivity to devaluation. Similarly, Watson, van Wingen, and de Wit (2018) trained multiple responses in participants in an fMRI scanner. Following devaluation of some responses, they used a "conflict" task, in which time pressure promotes fallback on habitual responses. They found that goal-directed performance during the test negatively correlated with cerebellar activity during acquisition. These studies suggest a relationship between cerebellar activity and habitual responding.

In rats, only one study has implicated the cerebellum in habitual responding. Callu et al. (2007) overtrained rats on a discriminated operant lever-press response. Rats with deep cerebellar interpositus nuclei lesions demonstrated a sensitivity to reward outcome following reward devaluation, meaning that unlike controls, they did not develop habitual lever-pressing. However, reinforcers did not appear to be completely devalued based on consumption test data, and the discriminated operant parameters that they used may be less-than-optimal for promoting habits (Thrailkill et al., 2018). Thus, much more work needs to be done to clarify a cerebellar role in habits. Our aim for experiment 2 was therefore to confirm if Crus I/II plays a role in habit expression. To investigate this, we extensively trained a single response and then inactivated Crus I/II prior to test. We found

that despite its role in habit expression in experiment 1, the cerebellar cortex was not involved in habit expression in experiment 2.

Methods

Procedures were exactly as those in the first experiment, but with a few differences. Rats were run in two cohorts of 32, for a total of 64 rats. The main procedural difference was that rats were extensively trained on one response, a lever-press. We did this so that we could test a cerebellar role in habit, since training of two responses concurrently appears to promote goal-directed responding of even extensively-trained responses. This single response was trained exactly as R1 in Experiment 1, for 24 acquisition sessions, except that there was a 2-minute time-out period prior to lever insertion at the beginning of each session. Devaluation, infusion prior to test, consumption, and reacquisition, proceeded exactly as in experiment 1, though devaluation only required 5 cycles for rats to reject all pellets. Data analysis reflected the fact that only a single response was trained, rather than two responses.

Results

One rat developed an infection around its headcap and had to be euthanized during acquisition; he was excluded from further analysis. Two rats were removed from further analysis because they were significant outliers during test responding ($Z = 2.19$; $Z=2.22$). Two rats were outliers in eating pellets during the consumption test ($Z > 3$) and were removed from all analysis because it was presumed that they had not undergone adequate reinforcer devaluation. Again, all rats without both cannulae in Crus I/II were removed (See Figure 10). This resulted in the removal of 11 rats, and 16 in total in

combination with the other removed rats (failure to show devaluation at consumption test or outlier as described in other results sections). Groups had the following n's: Paired-Muscimol (n = 13), Unpaired-Muscimol (n = 11), Paired-Vehicle (n = 10), Unpaired-Vehicle (n = 12).

Acquisition. Responding increased across acquisition sessions (see Figure 6), as indicated by a 2 (Drug: Muscimol vs. Vehicle) x 2 (LiCl: Paired vs. Unpaired) x 24 (Session) ANOVA that revealed a main effect of session, F (23, 966) = 71.08, MSE = 69.10, p <. 001. There were no other main effects or interactions as expected because no manipulations had yet occurred, largest F<1.

Devaluation. Animals in paired groups ate fewer pellets as devaluation progressed (see Figure 7). This was confirmed by a 2 (Drug: Muscimol vs. Vehicle) x 5 (Session) ANOVA which revealed a main effect of session, F (4, 84) = 119.08, MSE = 30.94, p < .001. There were no other main effects or interactions, Fs < 1.

Test. There was no difference in responding between paired and unpaired groups, indicating a habit. There was also no effect of Crus I/II inactivation on responding. Again, behavior was analyzed as test rate divided by response rate during the last acquisition session, as in the previous experiment. A 2 (Drug: Muscimol vs. Vehicle) x 2 (Devaluation: LiCl vs. Vehicle) ANOVA yielded no significant main effects or interactions, though there was a main effect of lithium chloride that approached statistical significance (largest F = 3.42, p =.07). Because this effect was not significant, this indicates that behavior was habitual, as there was no difference between devalued and non-devalued groups (see Figure 8). However, because a p value of .07 can be

categorized as marginally significant, this may also indicate that this behavior is not a "complete" habit. It also indicates that there were no drug effects, as muscimol infusion had no effect on responding. The same pattern of no effects was also observed when raw response rates were analyzed.

We again decided to examine Crus I placements only (Paired-Muscimol (n = 9), Unpaired-Muscimol (n = 8), Paired-Vehicle (n = 10), Unpaired-Vehicle (n = 9)) and found that just inactivating Crus I resulted in a similar pattern of responding to that of Crus I/II combined: A 2 (Drug: Muscimol vs. Vehicle) x 2 (Devaluation: LiCl vs. Vehicle) ANOVA yielded no significant main effects or interactions, largest F = 3.26, *p* =.08. Responding was habitual, as there was no difference between devalued and non-devalued groups and there were no drug effects, as muscimol infusion had no effect on responding.

Consumption test. Rats in the paired group that had received muscimol ate an average of .08 pellets. Rats who were in the paired vehicle group ate an average of 0.1 pellets. All rats who did not receive paired devaluation ate all pellets.

Reacquisition test. Overall, rats that had received paired LiCl treatment responded less than rats that received unpaired treatment. A 2 (Drug: Muscimol vs. Vehicle) x 2 (LiCl: Paired vs. Unpaired) x 10 (Minute) ANOVA was conducted to examine responding. This revealed a main effect of LiCl, F (1, 42) = 28.42, MSE = 2162.85, $p < .001$, but no main effect of minute ($p = .084$). These effects were qualified by a minute by LiCl interaction, F (9, 378) = 2.95, MSE = 80.80, $p = .002$. Follow-up independent samples T-tests at each minute between paired and unpaired groups showed

that paired groups responded less (p range: <.001 to .01). No other main effects or interactions were significant, largest $F = 1.83$. (See Figure 9).

Histology. Cannulae placement was confirmed by utilizing a brain atlas (Paxinos & Watson, 2006) and examining Prussian blue staining of marking lesions to determine where infusions were located. Again coordinates, slice shape, deep nuclei, brainstem, flocculi, and ventricles were used as landmarks to determine accurate cannulae location.

Discussion

In Experiment 1, we found that inactivation of cerebellar Crus I/II attenuated responding specifically for the group that had received devaluation, on both the minimally-trained and extensively-trained responses. In Experiment 2, we found that there was no effect of Crus I/II inactivation on the expression of a habit. Thus, the cerebellar cortex may play a role in the expression of habitual responding when two responses are trained, but not in the expression of habitual responding when one response is trained. Despite the cerebellum's role in motor coordination, we have reason to believe that we are not seeing motor effects. For one, inactivation had selective behavioral effects, sometimes not affecting behavior at all. If inactivation resulted in motor impairment, we would expect to see reduced responding in all groups. Further, cannulae from all groups seemed to be relatively evenly dispersed. We also saw no overt motor symptoms accompanying cerebellar inactivation.

This pattern of Crus I/II involvement in these two experiments is unexpected. In Experiment 1, responding in the paired groups reflects only habitual responding so Crus I/II inactivation appears to have reduced habitual responding. However, there should also

be an element of habit in the unpaired group too, yet there was no difference in responding between the unpaired muscimol and unpaired vehicle groups. Furthermore, in Experiment 2, habitual responding was unaffected by Crus I/II inactivation. Despite the difference in the status of behaviors across Experiments 1 and 2 (goal-directed vs. habitual, respectively), our findings that a habit element is only affected by Crus I/II inactivation when a goal-directed component of behavior is eliminated, but not when behavior is purely habitual, may indicate that the difference in paradigm (two contexts/responses vs. one, respectively) is engaging Crus I/II.

One interpretation of these results is that Crus I/II inactivation is reducing a particular element of habitual responding that is involved in paradigms with two responses. This could potentially be a hierarchical context-(S-R) association, which might explain the involvement of Crus I/II in paired group responding selectively in Experiment 1, with two responses and contexts involved, but not in Experiment 2. It may be that this hierarchical association is only involved when two separate contexts and/or two separate responses are involved in training; rats might use the context to distinguish manipulanda (S) – response associations when learning a second response. By contrast, with single response training, habits might involve simpler context-R and/or S-R associations. The argument then would be that Crus I/II is important for hierarchical context-(S-R) associations but not simpler, context-R or S-R, associations. This might be tested in a design like that of Trask and Bouton (2014), in which at least goal-directed behavior can only be governed by hierarchical associations. In this design, in context A, R1-O1 and R2-O2 are trained while in context B, R1-O2 and R2-O1 are trained. Devaluation of O2

selectively reduces R2 in context A and R1 in context B, indicating that each context controls associations between a specific response and specific outcome. Residual R2 in context A and R1 in context B would be indicative of a remaining habit component to behavior. Inactivation of Crus I/II should attenuate R2 in context A and R1 in context B. However, the possibility would seem to remain that this would be an attenuation of a context A-R2 association and a context B-R1 association.

Crus I/II inactivation seems to be affecting S-R components of responding, but only when a goal-directed component of behavior is first eliminated. Typically, when a brain region associated with habit is inactivated, behavior is maintained as goal-directed while controls respond habitually. For instance, Yin et al. (2004) found that responding in the paired group was attenuated after a dorsal striatal lesion. They lesioned the dorsolateral striatum (DLS) and trained behavior to a habit in controls. The DLS-lesioned group expressed behavior as goal-directed, however, and this was largely driven by an attenuation in responding in the devalued-lesioned group.

However, a maintenance of goal-directed behavior by lesion/inactivation of habit regions isn't always driven by a reduction in responding by the paired group, since an increase in responding by the non-devalued group can also increase the difference between paired and unpaired responding (i.e. sensitivity to devaluation, the operational definition of an action). Indeed, this is the pattern seen with IL lesion (Coutureau & Killcross, 2003; Killcross & Coutureau, 2003). Unlike these studies, our controls in Experiment 1 did not respond in a habitual manner, but rather in a goal-directed manner; behavior was made "even more" goal-directed (i.e., loss of some of the residual habit

component of responding) by Crus I/II inactivation, but when behavior was entirely habitual in Experiment 2, there was no effect of inactivation. Much like in Shipman et al. (2018), this may indicate overlap in action and habit circuitries.

Another interpretation for these results is that Crus I/II is involved in early habit expression (prior to full habit development). Different regions involved in action and habit come "online" at different times during training (Corbit, 2018; Lingawi et al., 2016). For instance, the prelimbic cortex is only important early in training (Killcross & Coutureau, 2003; Shipman et al., 2018). In habit formation, the DLS is involved once behavior is habitual, but it is unknown if the DLS is also active earlier in training when behavior is still an action. The IL has been examined throughout training and is only important during extensive training (both goal-directed and habitual) and not minimal training (Killcross & Coutureau, 2003; Shipman et al., 2018). Yet, there is early evidence of S-R associations (Dickinson et al., 1995) which could potentially be driven by Crus I/II activity. Based on our knowledge of the PL and IL, there is a precedent for brain regions to be online only at particular points during training (Killcross & Coutureau, 2003; Shipman et al., 2018). An experiment to test this is to minimally train one response and determine if Crus I/II inactivation still attenuates paired responding. If it does, this may support the involvement of the cerebellar cortex in a habitual component of responding present during the early stages of goal-directed responding. If it does not, then Crus I/II may only be involved in more complex behavior, like learning two responses in two contexts. To further understand this behavior, we could then train an action and a habit in separate contexts (two responses) and see if Crus I/II is involved in habit when

two contexts are involved. Preliminary work in the Bouton lab has shown that if extensively-trained responses are not intermixed with minimally-trained responses (as in the paradigm we used) and are instead trained sequentially (all sessions of R1 followed by all sessions of R2) then R1 responding is habitual and R2 responding is goal-directed. If we conducted this experiment and inactivated Crus I/II at test, we would expect to selectively reduce the minimally-trained response if Crus I/II is only involved in early S-R associations, as there would be no effect of inactivation specifically in habit expression. Though studies have suggested a role for the cerebellum in a mixture of habit (Callu et al., 2007; Liljeholm et al., 2015; Watson et al., 2018) and action (Fermin et al., 2016; Wagner et al., 2017), this disagreement may explain our findings of why the cerebellum appears to be involved in habitual responding but only when it is a component of goal-directed behavior.

One alternative explanation for the reduction of responding in the paired group with Crus I/II inactivation is that the memory of taste aversion is being affected. However, we did some post-hoc tests on rats in Experiment 2 in which we inactivated Crus I/II prior to consumption or reacquisition and found that behavior was unaffected. This indicates that taste aversion is not being affected by Crus I/II inactivation. We do not report these results here since we did not find effects of inactivation on responding in Experiment 2, though the lack of effect on taste aversion learning informs our interpretation of our Experiment 1 results.

One difficulty in interpreting Experiment 2, is that there is a borderline ($p < 0.07$) significant effect of LiCl, indicating that behavior may not be entirely habitual. Indeed,

this points to a larger issue in the way that habits specifically are identified based upon a null effect. For one, behavior is likely a spectrum of goal-directed to habitual behavior with increasing strength of S-R associations driving responding across training. However, we are forced to dichotomize behavior overall as either an action or a habit based on whether there is a difference between valued and devalued responding. Therefore, it is difficult to interpret marginally significant results. Additionally, devaluation only allows for the reduction of responding associated with that outcome, and thus the action component of behavior is removed, and the remainder of responding is interpreted as habitual without a means of directly manipulating habitual components of responding. One puzzling pattern of responding emerged in reacquisition of the minimally-trained response in Experiment 1. While there was a clear split, as expected, between non-devalued and devalued rats as they again encountered the pellets after the response, there was also an attenuation of responding in the non-devalued group that had received muscimol inactivation at time of test. In reacquisition, there was no drug infusion, and we saw an effect that hadn't been apparent during test when Crus I/II was inactivated. One possibility is that a single inactivation might impair acquisition, though it may require experience with the reinforcer again for this to become apparent. This could explain why the effect occurs in the minimally-trained and not the extensively-trained groups in Experiments 1 and 2: acquisition has not yet reached asymptote. It is possible that we might see a very different pattern of impairment if we inactivated prior to acquisition sessions rather than prior to test. Future studies should examine a role of the Crus I/II in acquisition vs. expression.

In conclusion, we found that inactivation of Crus I/II of the cerebellar cortex affected expression of habitual responding when two responses had been trained but not when one response had been trained. Additional studies will be needed to delineate the differences between two-response and one-response training in order to fully interpret this pattern of results.

References

Barbey, A. K., Koenigs, M., & Grafman, J. (2013). Dorsolateral prefrontal contributions to human working memory. *Cortex; a journal devoted to the study of the nervous system and behavior*, *49*(5), 1195–205.

Bernard, J. A., Peltier, S. J., Benson, B. L., Wiggins, J. L., Jaeggi, S. M., Buschkuehl, M., Jonides, J., et al. (2014). Dissociable functional networks of the human dentate nucleus. *Cerebral cortex (New York, N.Y. : 1991)*, *24*(8), 2151–9.

Bodranghien, F., Bastian, A., Casali, C., Hallett, M., Louis, E. D., Manto, M., . . . Dun, K. v. (2016). Consensus paper: Revisitng the symptoms and signs of cerebellar syndrome. *Cerebellum, 15*, 369-391.

Caligiore, D., Pezzulo, G., Baldassarre, G., Bostan, A. C., Strick, P. L., Doya, K., . . . Herreros, I. (2017). Consensus paper: Towards a systems-level view of cerebellum function: the interplay between cerebellum, basal ganglia, and cortex. *Cerebellum, 16*, 203-229.

Callu, D., Puget, S., Faure, A., Guegan, M., & Massioui, N. (2007). Habit learning dissociation in rats with lesions to the vermis and the interpositus of the cerebellum. *Neurobiology of disease*, *27*(2), 228–37.

Corbit, L. H. (2018). Understanding the balance between goal-directed and habitual behavioral control. *Current opinion in behavioral sciences*, *20*, 161-168.

Corbit, L. H., Muir, J. L., & Balleine, B. W. (2003). Lesions of mediodorsal thalamus and anterior thalamic nuclei produce dissociable effects on instrumental conditioning in rats. *The European journal of neuroscience*, *18*(5), 1286–94.

Coutureau, E., & Killcross, S. (2003). Inactivation of the infralimbic prefrontal cortex reinstates goal-directed responding in overtrained rats. *Behavioural brain research*, *146*(1–2), 167–74.

Deverett, B., Koay, S. A., Oostland, M., & Wang, S. S. (2018). Cerebellar involvement in an evidence-accumulation decision-making task. *Elife*, *7*, e36781.

Dickinson, A., Balleine, B., Watt, A., Gonzalez, F., & Boakes, R. A. (1995). Motivational control after extended instrumental training. *Animal Learning & Behavior*, *23*(2), 197-206.

Fermin, A. S., Yoshida, T., Yoshimoto, J., Ito, M., Tanaka, S. C., & Doya, K. (2016). Model-based action planning involves cortico-cerebellar and basal ganglia networks. *Scientific reports*, *6*, 31378.

Giannetti, S., & Molinari, M. (2002). Cerebellar input to the posterior parietal cortex in the rat. *Brain Research Bulletin, 58*, 481-489.

Kelly, R. M., & Strick, P. L. (2003). Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *23*(23), 8432–44.

Killcross, S., & Coutureau, E. (2003). Coordination of actions and habits in the medial prefrontal cortex of rats. *Cerebral cortex (New York, N.Y. : 1991), 13*(4), 400–8.

Koziol, L. F., Budding, D., Andreasen, N., D'Arrigo, S., Bulgheroni, S., Imamizu, H., . . . Yamazaki, T. (2014). Consensus paper: The cerebellum's role in movement and cognition. *Cerebellum, 13,* 151-177.

Leiner, H. C., Leiner, A. L., & Dow, R. S. (1986). Does the cerebellum contribute to mental skills? *Behavioral Neuroscience, 100,* 443-454.

Leiner, H. C., Leiner, A. L., & Dow, R. S. (1989). Reappraising cerebellum: What does the hindbrain contribute to the forebrain? *Behavioral Neuroscience, 103,* 998-1008.

Liljeholm, M., Dunne, S., & O'Doherty, J. P. (2015). Differentiating neural systems mediating the acquisition vs. expression of goal-directed and habitual behavioral control. *European Journal of Neuroscience, 41,* 1358-1371.

Lingawi, N. W., Dezfouli, A., & Balleine, B. W. (2016). The psychological and physiological mechanisms of habit formation. In R. A. Murphy & R. C. Honey (Ed) *The Wiley Handbook on the Cognitive Neuroscience of Learning,* 411-440.

Middleton, F., & Strick, P. (2001). Cerebellar projections to the prefrontal cortex of the primate. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *21*(2), 700–12.

Mittleman, G., Goldowitz, D., Heck, D. H., & Blaha, C. D. (2008). Cerebellar modulation of frontal cortex dopamine efflux in mince: Relevance to autism and schizophrenia. *Synapse, 62*, 544-550.

Ostlund, S. B., & Balleine, B. W. (2008). Differential involvement of the basolateral amygdala and mediodorsal thalamus in instrumental action selection. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *28*(17), 4398–405.

Parker, K. L., Narayanan, N. S., & Andreasen, N. C. (2014). The therapeutic potential of the cerebellum in schizophrenia. *Frontiers in Systems Neuroscience, 8*, Article 163.

Paxinos, G., & Watson, C. (2006). The rat brain in stereotaxic coordinates: hard cover edition. *Elsevier*.

Ramnani, N. (2006). The primate cortico-cerebellar system: anatomy and function. *Nature reviews. Neuroscience*, *7*(7), 511–22.

Rogers, T. D., Dickson, P. E., Heck, D. H., Goldowitz, D., Mittleman, G., & Blaha, C. D. (2011). Connecting the dots of the cerebro-cerebellar role in cognitive function: Neuronal pathways for cerebellar modulation of dopamine release in the prefrontal cortex. *Synapse, 65*, 1204-1212.

Rogers, T. D., Dickson, P. E., McKimm, E., Heck, D. H., Goldowitz, D., Blaha, C. D., & Mittleman, G. (2013). Reorganization of circuits underlying cerebellar

modulation of prefrontal cortical dopamine in mouse models of Autism Spectrum Disorder. *Cerebellum, 12*, 547-556.

Runyan, J. D., Moore, A. N., & Dash, P. K. (2004). A role for prefrontal cortex in memory storage for trace fear conditioning. *Journal of Neuroscience*, *24*(6), 1288-1295.

Schmahmann, J. D. (1991). An emerging concept: The cerebellar contribution to higher function. *Archives of Neurology, 48*, 1178-1187.

Shipman, M. L., & Green, J. T. (In press). Cerebellum and Cognition: Does the Rodent Cerebellum Participate in Cognitive Functions? *Neurobiology of learning and memory*.

Shipman, M. L., Trask, S., Bouton, M. E., & Green, J. T. (2018). Inactivation of prelimbic and infralimbic cortex respectively affects minimally-trained and extensively-trained goal-directed actions. *Neurobiology of Learning and Memory*, *155*, 164-172.

Siegel, J. J., Taylor, W., Gray, R., Kalmbach, B., Zemelman, B. V., Desai, N. S., . . . Chitwood, R. A. (2015). Trace eyeblink conditioning in mice is dependent upon the dorsal medial prefrontal cortex, cerebellum, and amgydala: Behavioral characterization and functional circuitry. *eNeuro, 2*, e0051-0014.2015.

Stoodley, C. J., D'Mello, A. M., Ellegood, J., Jakkamsetti, V., Liu, P., Nebel, M. B., ... & Pascual, J. M. (2017). Altered cerebellar connectivity in autism and cerebellar-

mediated rescue of autism-related behaviors in mice. *Nature neuroscience*, *20*(12), 1744.

Thrailkill, E. A., Trask, S., Vidal, P., Alcalá, J. A., & Bouton, M. E. (2018). Stimulus control of actions and habits: A role for reinforcer predictability and attention in the development of habitual behavior. *Journal of Experimental Psychology: Animal Learning and Cognition*, *44*(4), 370.

Tran-Tu-Yen, D. A., Marchand, A. R., Pape, J.-R. R., Scala, G., & Coutureau, E. (2009). Transient role of the rat prelimbic cortex in goal-directed behaviour. *The European journal of neuroscience*, *30*(3), 464–71.

Trask, S., & Bouton, M. E. (2014). Contextual control of operant behavior: evidence for hierarchical associations in instrumental learning. *Learning & behavior*, *42*(3), 281-288.

Uylings, H. B., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal cortex?. *Behavioural brain research*, *146*(1-2), 3-17.

Voogd, J. (2004). Cerebellum. In G. Paxinos (Ed.), *The Rat Nervous System* (3rd ed., pp. 205-242). Amsterdam: Elsevier Academic Press.

Wagner, M. J., Kim, T. H., Savall, J., Schnitzer, M. J., & Luo, L. (2017). Cerebellar granule cells encode the expectation of reward. *Nature, 544*, 96-100.

Watson, P., van Wingen, G., & de Wit, S. (2018). Conflicted between Goal-Directed and Habitual Control – an fMRI Investigation. *eNeuro*, *5*(4), ENEURO.0240-18.2018.

Watson, T. C., Becker, N., Apps, R., & Jones, M. W. (2014). Back to front: cerebellar connections and interactions with the prefrontal cortex. *Frontiers in systems neuroscience*, *8*, 4.

Watson, T. C., Jones, M. W., & Apps, R. (2009). Electrophysiological mapping of novel prefrontal - cerebellar pathways. *Frontiers in integrative neuroscience*, *3*, 18.

Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2004). Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *European journal of neuroscience*, *19*(1), 181-189.

Figures

Figure 1. Acquisition of R1 and R2 across 24 sessions.

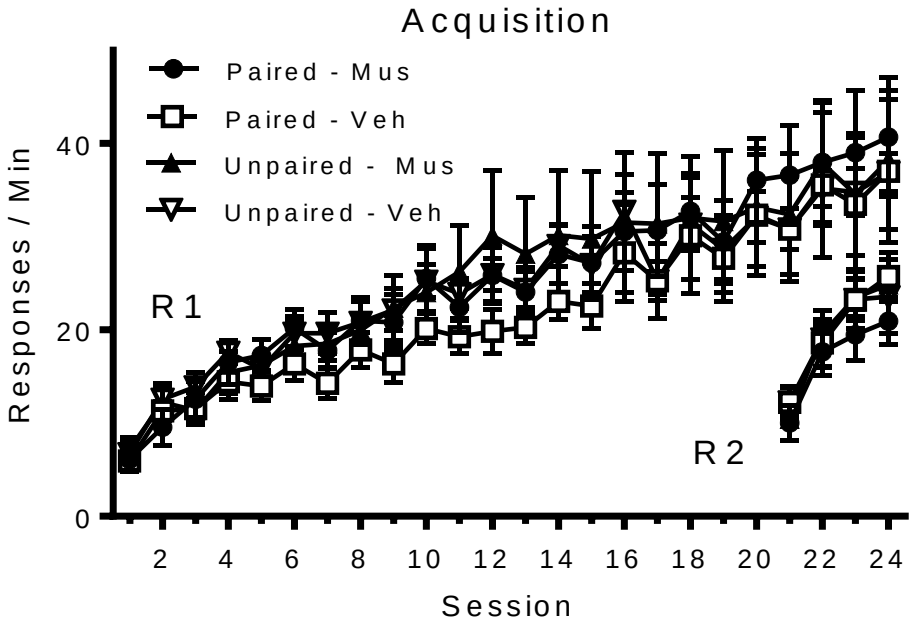


Figure 2. Reinforcer devaluation across six sessions.

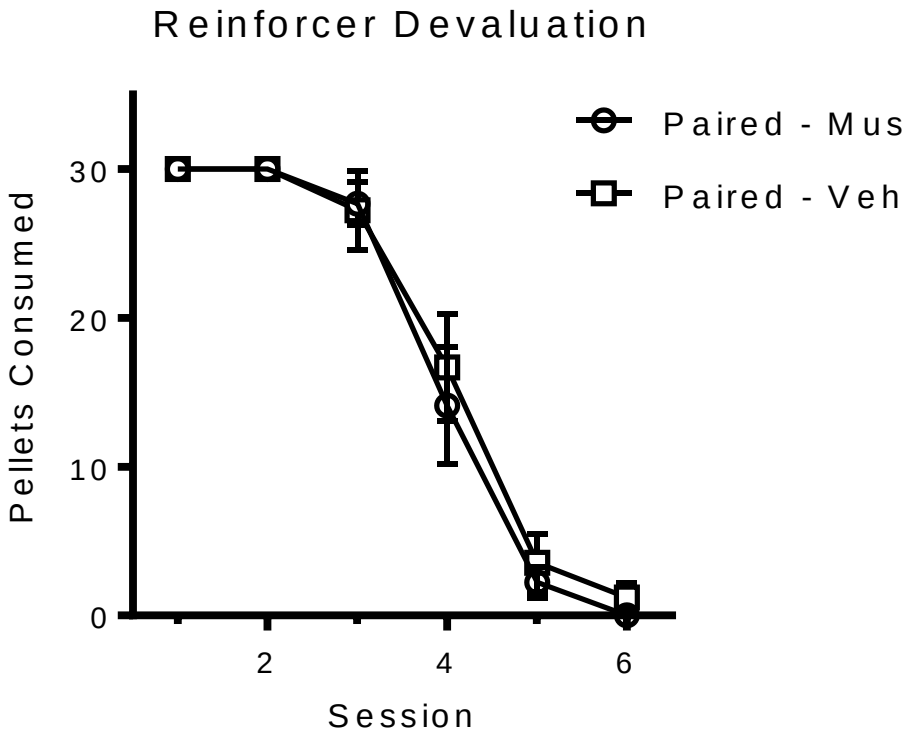


Figure 3. Responding (Responses per minute over response rate during the last acquisition session) during R1 (left) and R2 (right) tests in extinction for Crus I/II (above row) and just Crus I (below graphs).

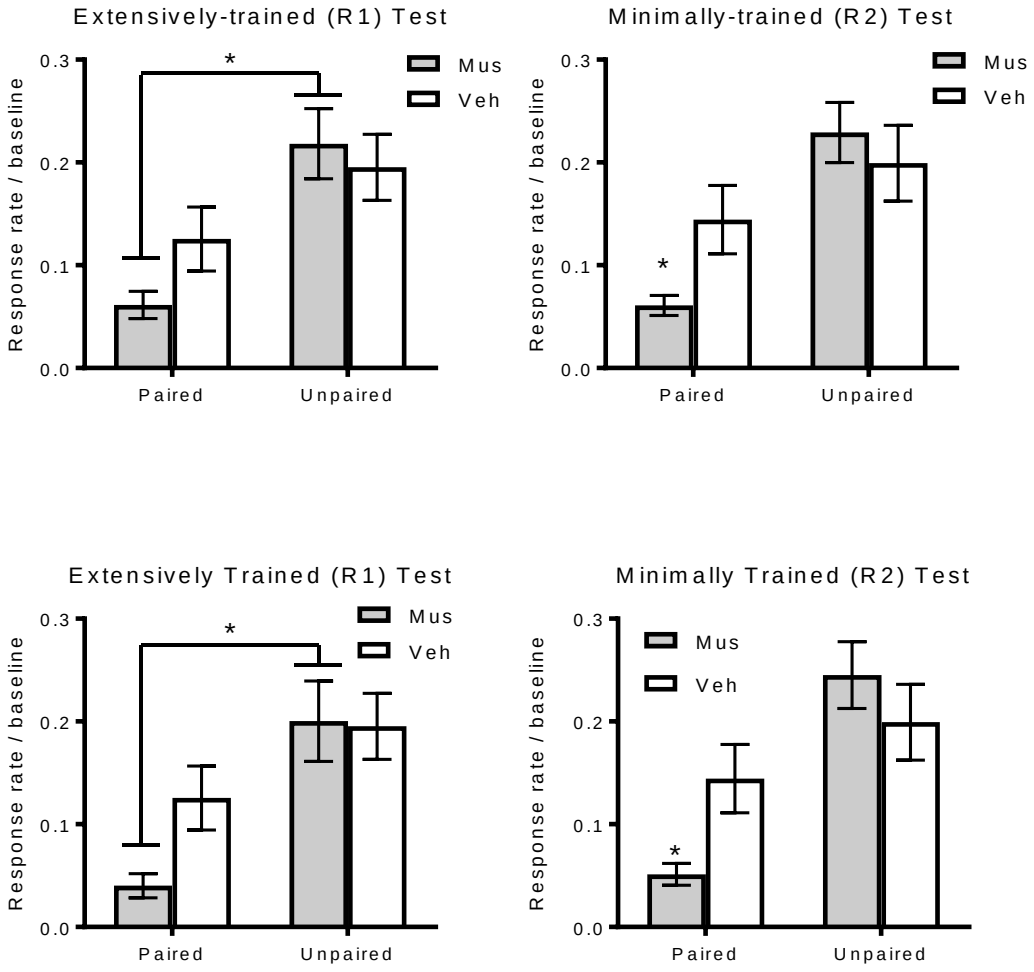


Figure 4. Responding during reacquisition on R1 (above) and R2 (below).

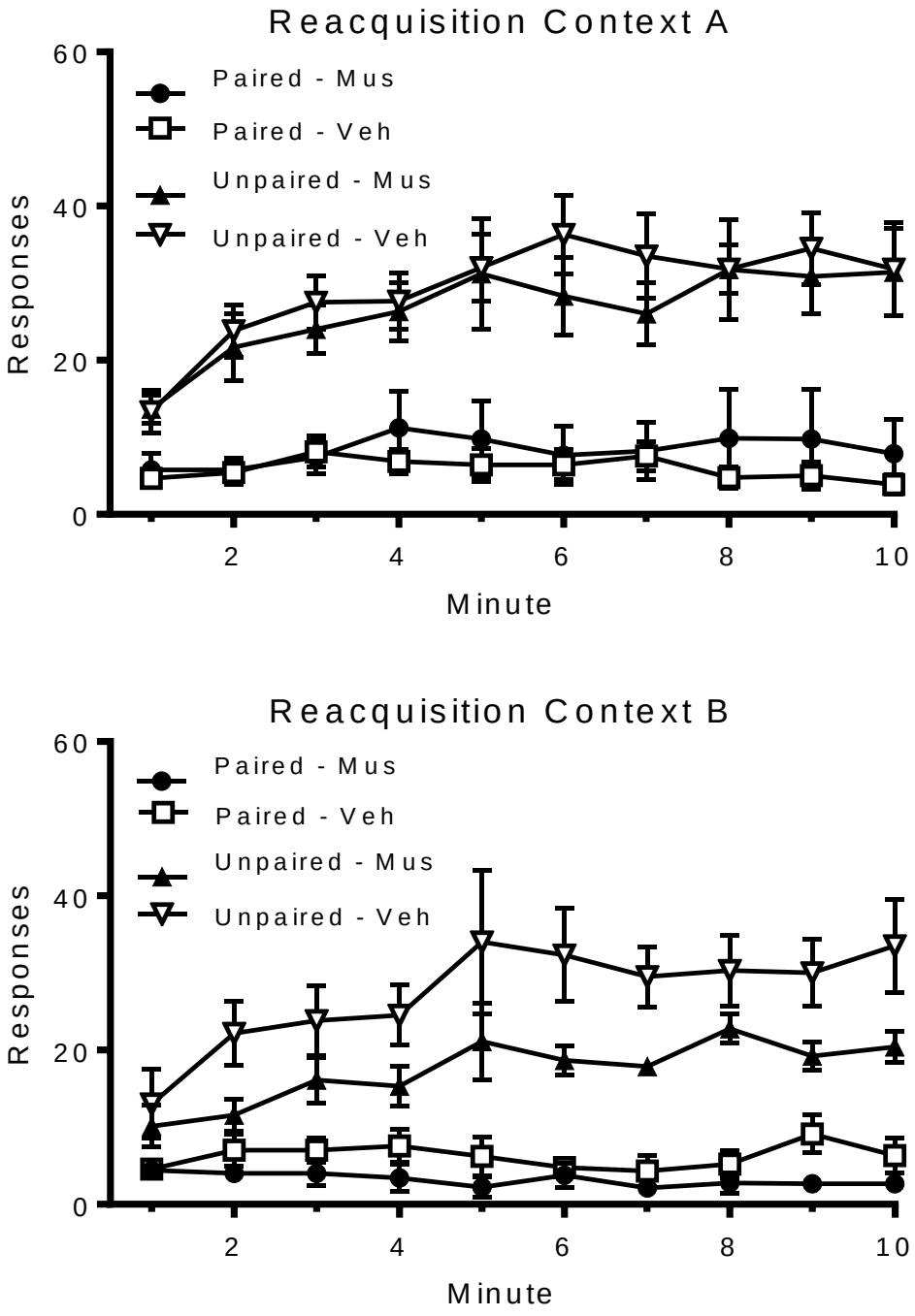


Figure 5. Cannula placements for included subjects in Crus I/II.

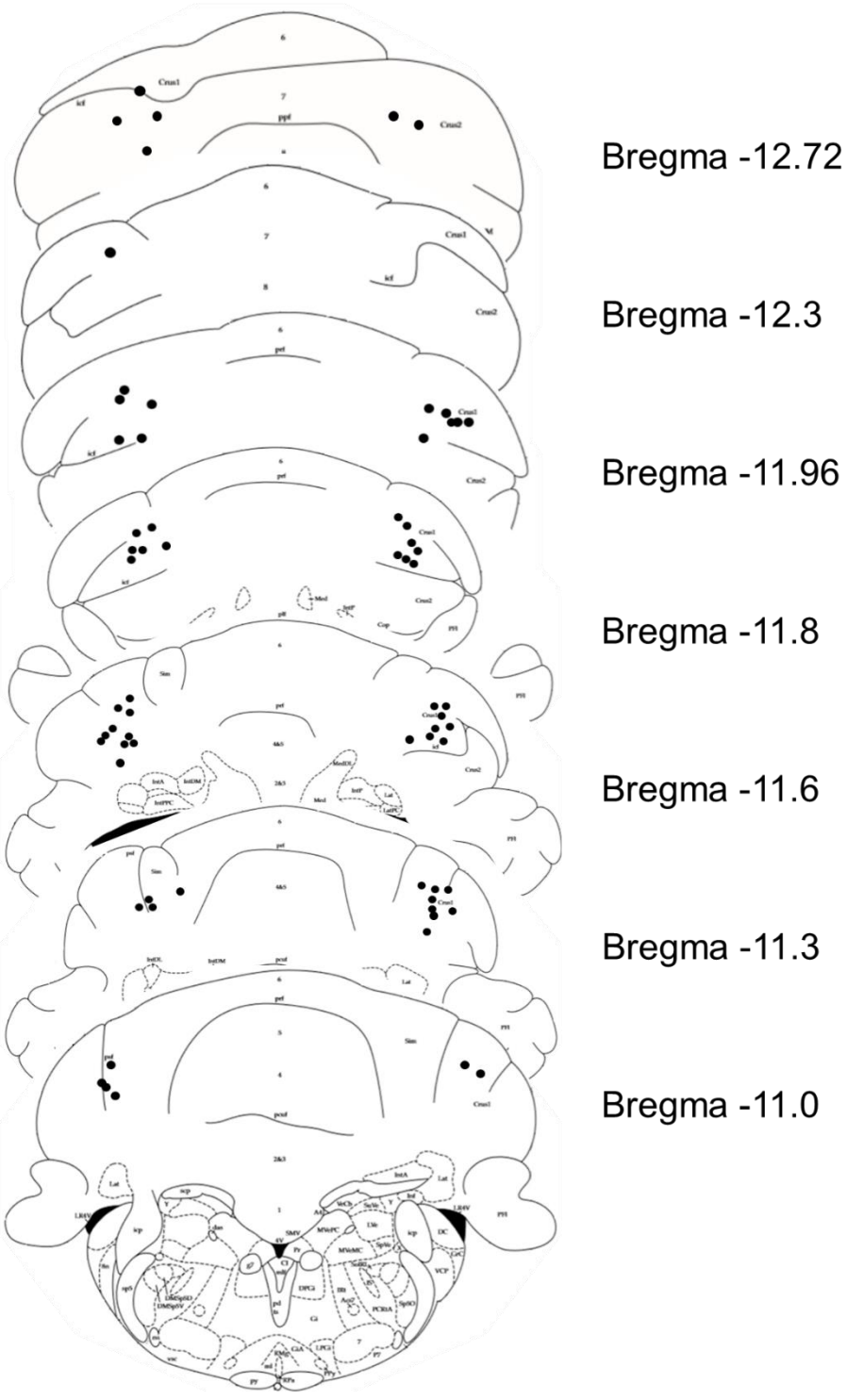


Figure 6. Acquisition of single-trained response across 24 training sessions.

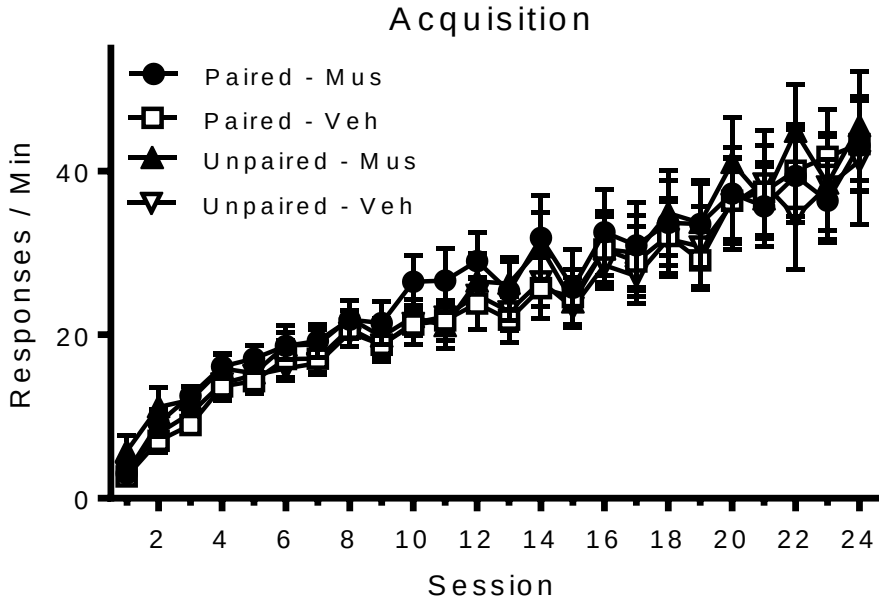


Figure 7. Reinforcer devaluation across 5 sessions.

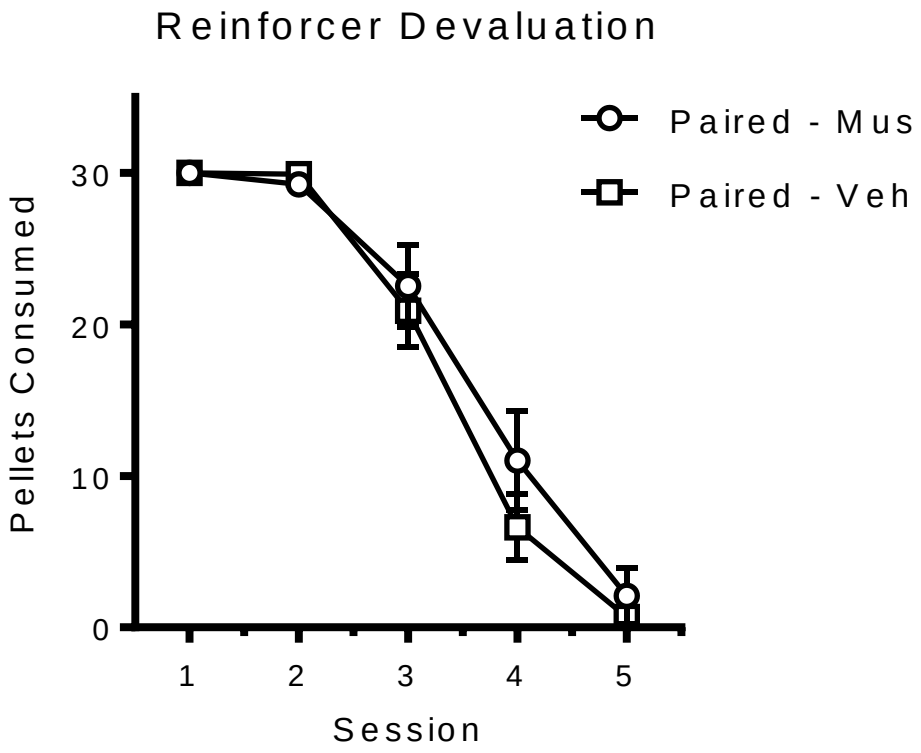


Figure 8. Responding on lever during ten-minute test. There was no significant differences in responding between groups. Test data for Crus I/II shown on the left and just Crus I on the right.

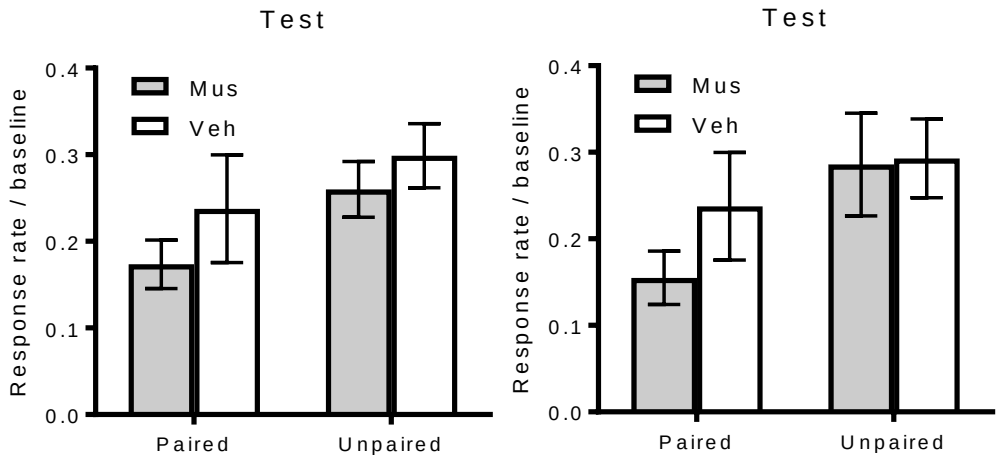


Figure 9. Reacquisition of responding across ten-minute session.

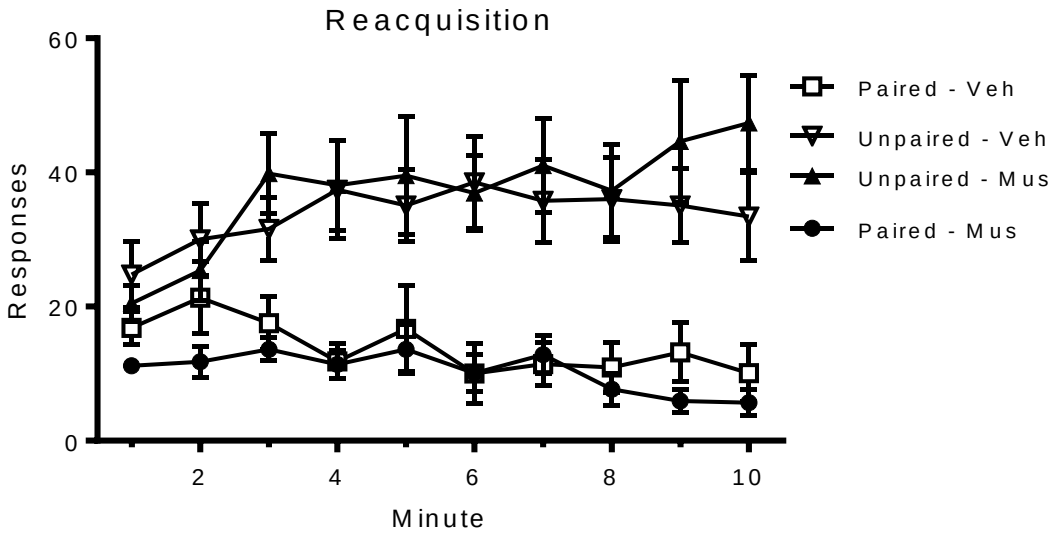
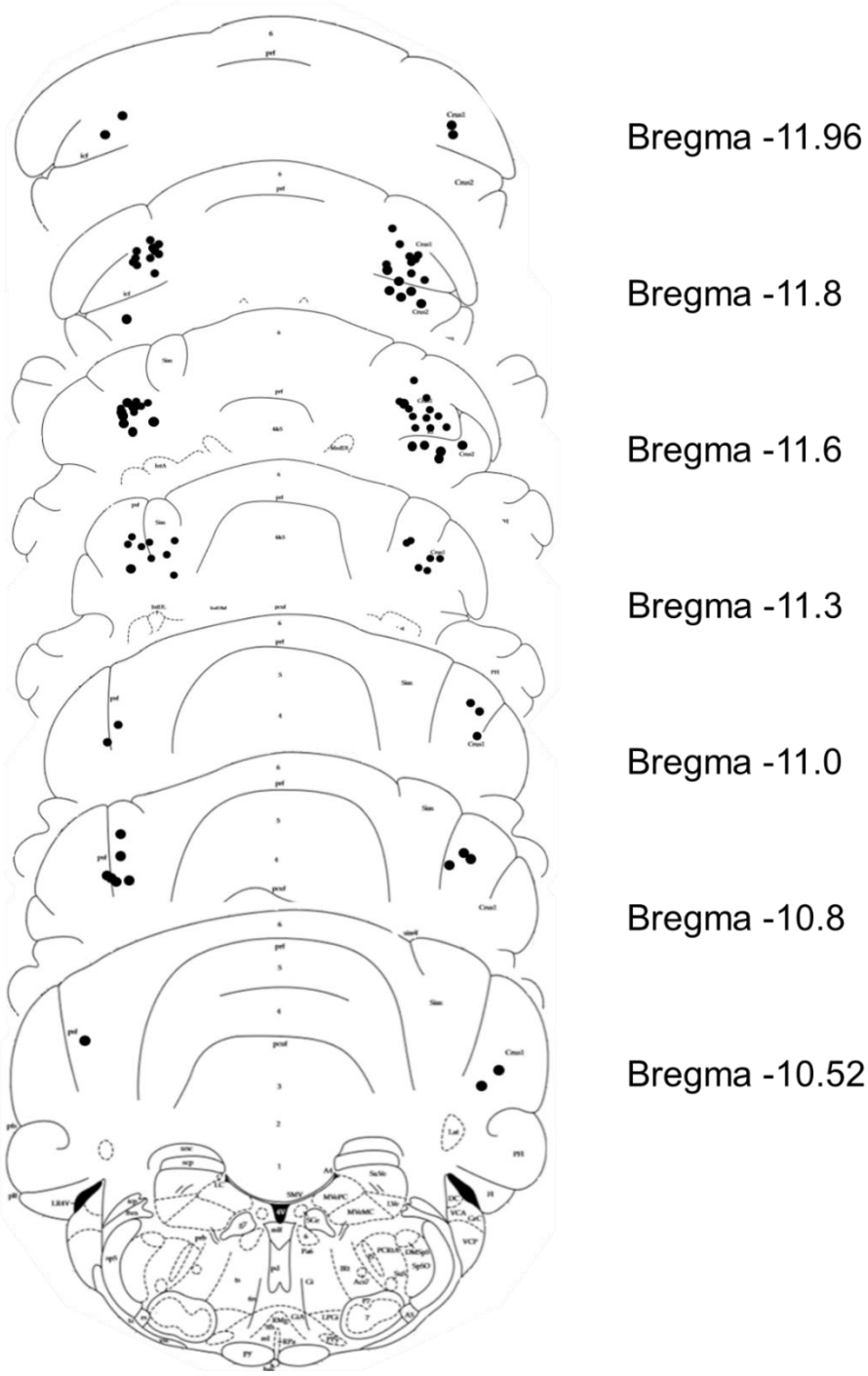


Figure 10. Cannula placements in Crus I/II for included rats.



Chapter 5: General Discussion

Brief Summary

This work has led to some novel findings. For one, we have shown that the prelimbic cortex is not involved in all types of goal-directed behaviors, as inactivation reduced minimally-trained but not extensively-trained goal-directed responding. We have also shown for the first time that the prelimbic cortex is involved in the expression and not just the acquisition of goal-directed responding. We found that the infralimbic cortex is involved in extensively-trained goal-directed responding and not just habit, as suggested by the literature. Additionally, this work has demonstrated with chemogenetic inactivation that projections from the prelimbic cortex to the dorsomedial striatum are important for the expression of minimally-trained operant responding. This was shown in a more anterior region of the dorsomedial striatum than the majority of research has examined in goal-directed behavior. Finally, we have shown that Crus I/II of the cerebellum is involved in expression of a habit component of responding when two responses are trained, but not habit when one response is trained. Thus, this dissertation has both expanded upon the circuitry known to be involved in goal-directed responding (infralimbic cortex) and habitual behavior (Crus I/II) and clarified some of the roles of the prelimbic and infralimbic cortices (minimally vs. extensively trained; expression of behavior).

Prelimbic cortex

Most notably, we have shown that the prelimbic cortex is not involved in all types of actions. The action/habit canon suggests that brain regions are involved in actions or

habits and that inactivation of a region involved in one (action or habit) results in performance of the opposing behavior (Corbit, 2018; Lingawi, Dezfouli, & Balleine, 2016). However, prior studies have not distinguished between habit (which is produced by extensive training) and extensively-trained behavior that is goal-directed. Indeed, research that has investigated the PL previously has either shown that it is not involved in habitual behavior (Killcross & Coutureau, 2003) or investigated its role in minimally-trained goal-directed responding only (Corbit & Balleine, 2003). Our results therefore do not necessarily challenge this one-or-the-other view of actions and habits but add important nuance to our understanding of the substrates of goal-directed behavior.

The paradigm that we utilized also raises other questions, as recent work in the Bouton lab suggests that adding a second minimally-trained response intermixed with a first extensively-trained response results in the first response being reverted from a habit to an action (see future directions for more on this). Our findings do not determine then whether there is a transition point prior to habitual responding where the prelimbic cortex is no longer involved, or if the PL is only no longer involved once an action is habitual but then reverted to an action. We know that a habit component to responding is present early on during training and seems to eventually suppress goal-directed behavior as more training occurs (Dickinson et al., 1995). Future work should determine the exact point in training at which the PL is no longer important in operant responding and whether this is dependent on the behavior being entirely habitual at some point during training.

One other way in which these results are novel is that they implicate the PL and the PL-to-DMS pathway in the *expression* of behavior rather than simply acquisition as

has previously been found (Ostlund & Balleine, 2005; Tran-Tu-Yen et al., 2009). This is important in understanding the contributions of different regions within the goal-directed circuit and differentiating regions involved in encoding R-O associations vs regions that store a memory of these associations. Current thinking is that the PL is unique because, unlike other goal-directed regions, it is involved in just acquisition. Ostlund and Balleine (2005) and Tran-Tu-Yen (2009) found that there was an effect of temporary or permanent PL lesion on goal-directed responding when lesions were made before acquisition sessions, but no effect of lesion/inactivation prior to only test. The lack of effects of PL lesion/inactivation on expression of goal-directed actions conflicts with our results, as we found an attenuation of operant responding with both a PL baclofen/muscimol temporary lesion at time of test and intracranial CNO infusion into the DMS onto PL projection neurons at time of test.

However, the null results of PL lesions on expression found by others may be explainable based on differences in paradigms. Ostlund and Balleine (2005) used 11 days of training where each of two responses was trained separately. One alternative explanation for their finding that pre-training medial prefrontal cortex lesions impaired responding while post-training lesions did not, is that lesioning after 11 training sessions may be late enough in training that the prelimbic cortex is no longer involved. Recall that we found that extensively-trained responses that are still goal-directed were not affected by prelimbic cortex inactivation. Ostlund and Balleine (2005) may therefore have missed the transitory period early in training in which the prelimbic cortex is involved in both acquisition and expression, though lesion prior to any learning likely impairs action

learning irreparably. It is also important to note that they lesioned the entire medial prefrontal cortex, which included some of the infralimbic cortex and some of the anterior cingulate cortex.

The other study that found a role for the PL in acquisition but not expression might also be explained by their test parameters. Tran-Tu-Yen (2009) utilized six training days. This amount of training is likely still "undertrained," especially since an earlier experiment of ours showed that inactivation of the prelimbic cortex after six sessions resulted in an attenuation of operant responding at time of test (Trask et al., 2017). However, one possible explanation of their null results from their single inactivation at time of test is that they utilized a longer test period. Tran-Tu-Yen et al. (2009) examined behavior across 15 minutes as lever presses per minute. We usually conduct tests that are a maximum of ten minutes because extinction during the test produces more variability in responding. Their test is longer, meaning that this analysis may not be an accurate representation of initial differences in responding. When testing the role of the PL in acquisition, they also infused baclofen/muscimol intracranially for six days, potentially resulting in receptor desensitization and/or tissue damage.

We also found that selective inactivation of the PL-DMS pathway resulted in an attenuation of minimally-trained operant responding. We sought to expand upon our prior research showing that PL inactivation at time of test reduces minimally-trained operant responding by examining a particular projection target of the PL. This result was as predicted based on previous work that found a role for the PL in the excitatory effect of acquisition context on operant responding (Trask et al., 2017) as well as a slew of

research that has implicated similar functions of the PL and DMS in minimally-trained, goal-directed behavior (Corbit & Balleine, 2003; Corbit & Janak, 2010; Killcross & Coutureau, 2003; Ostlund & Balleine, 2005; Shiflett, Brown, & Balleine, 2010; Shipman, Trask, Bouton, & Green, 2018; Tran-Tu-Yen, Marchand, Pape, Di Scala, & Coutureau, 2009; Trask, Shipman, Green, & Bouton, 2017; Yin, Ostlund, Knowlton, & Balleine, 2005; Yin, Knowlton, & Balleine, 2006). It also complements the work of Hart et al. (2018) who found that the bilateral PL-pDMS pathway is crucial for the acquisition of goal-directed responding. Our findings both expand the breadth of this circuit (involvement of the aDMS) as well as the time during learning and memory in which this projection is important (expression as well as acquisition).

Our results implicate the PL-*anterior* DMS pathway in minimally-trained operant behavior. Yin et al. (2005) found that pretraining lesions, post-training lesions, and pharmacological inactivation of the pDMS resulted in an inability to express goal-directed behavior. However, they found that lesions of the aDMS did not impair goal-directed responding. An extensive body of work that continued from the Balleine lab cited this study as a reason for further investigating the pDMS, and its connections' involvement in actions, rather than the aDMS. However, a study from the Janak lab found that both the aDMS and pDMS are important for the expression of goal-directed behavior (Corbit & Janak, 2010). Further work out of her lab has continued to find a role for the aDMS much like the pDMS in actions (Corbit, Nie, & Janak, 2012). We chose to investigate the role of the PL-aDMS in the expression of minimally-trained operant behavior, because in addition to the findings by Corbit and Janak (2010), anatomical

studies have found that the densest connection from the PL to the DMS arrives in the aDMS as opposed to pDMS (Hunnicutt et al., 2016; Mailly et al., 2013). Our results support the findings of the Janak lab, in showing that the aDMS (and specifically projections to the aDMS from the PL) is involved in early operant behavior, and likely, goal-directed responding.

Infralimbic Cortex

We found that IL inactivation resulted in attenuation of an extensively-trained, goal-directed response. Much like our PL findings, these results are novel in that we have found a brain region (IL) that doesn't adhere to the strict action/habit circuitry dichotomy, as brain regions implicated in goal-directed behavior and habits have historically been suggested to be involved in one type of behavior or the other (Corbit, 2018; Lingawi & Balleine, 2012). A future direction to pursue is why extensively-trained behavior that is goal-directed is engaging a region typically associated with habits (IL) and not a region associated with actions (PL). It may be that "habit" regions are really tracking something involved with extensive training, but since habits develop across training, differences in habit vs extensive training haven't been dissected. It may also be the case that our two-response paradigm is returning a habit to an action by the addition of a concurrent second response (but see the future directions section for more on this). In that case, perhaps, once a behavior becomes entirely a habit then it is stored within habit regions but can still act as part of the goal-directed circuitry. To examine this, we could extensively train a single behavior with a ratio schedule to maintain behavior as goal-directed (Dickinson, Nicholas, & Adams, 1983) and see if the IL is still involved. If it is, this may indicate that

the IL comes online prior to habit development, and that it may also be involved in an aspect of extensive training rather than habit. Further research could also examine other known habit regions such as the DLS and seeing if extensively-trained behavior that is still goal-directed requires the DLS, or instead, the DMS, a region involved in action. Alternatively, this flexibility to be involved in both actions and habits could be unique to the mPFC.

A related literature in the cognitive sciences may predict a role for the IL that isn't modeled in the animal behavioral literature. Reinforcement learning utilizes computational models to investigate brain systems and their involvement in optimal action control. Model-based behavior utilizes a chain of predictions about actions in a sequence, allowing for immediate feedback on the consequence of each action at each step. Though model-based processing differs in some components from goal-directed behavior, it is also relatively timely, effortful, flexible, and sensitive to changes in outcome. Model-free behavior is modeled on the "caching" that occurs during learning with dopaminergic neurons, where firing initially occurs in response to reinforcers but ultimately transfers to the stimuli that predict them. Like the rodent model of habitual responding, this system is not concerned with value outcomes and is relatively inflexible (Daw, Niv, & Dayan, 2005). Researchers in this area have examined the interactions of the two circuits to greater degrees than in the animal literature, dealing with the issue of "exploration vs. exploitation", meaning competing systems that balance exploring the environment for more/more efficient ways of earning rewards vs. engaging in behaviors that result in known rewards (Ludwig, Bellemare, & Pearson, 2011). In a typical

paradigm, human participants will have to interact with a variety of responses without initial information about their associated reinforcement schedules. How participants allocate their times and how model-based vs model-free systems and their brain systems predict these behaviors to maximize rewards is the subject of much reinforcement learning research. One model of these interactions proposes that a system receives input from both circuitries, model-based and model-free. Because the system can make a determination, at any point of learning, which model is most advantageous, there must be an arbitrator that can switch behavior back and forth from model-based and model-free, inhibiting the system that is not in use (Lingawi et al. 2016). We show here that the IL has some involvement in goal-directed responding, namely when behavior is extensively-trained. Smith et al. (2012) also demonstrated that IL perturbation prevented habit expression in a maze running task. However, interestingly, when they let the new goal-directed behavior develop into a habit, IL perturbation then returned behavior to the initial habit that had been blocked. These results may indicate that the IL is an arbitrator between the action and habit circuitry. A future study in our lab could extensively train an operant response (R1) and utilize the same two-response, two-context paradigm we have used before, in which adding a second response maintains R1 as goal directed. Inactivation of the IL should attenuate responding, thus maintaining behavior as "habitual." We could then inactivate the IL in a follow-up test the next day. Assuming behavior would still be habitual, if this returns the extensively-trained behavior to goal-directed, then this is further evidence that the IL can toggle behavior between the two systems. Further, this could explain why the IL has been implicated in the seemingly

opposing behaviors of driving habit and extinction: the IL drives the most adaptive behavior and inactivation switches behavior.

Crus I/II

We examined the cerebellar cortex in minimally-trained goal-directed responding, extensively-trained goal-directed responding, and extensively-trained habitual responding. The pattern of our results suggests that Crus I/II is important for expression of a habitual component of responding when two responses are trained but not when one response is trained. However, additional research is needed to test this interpretation. Intriguingly, behavior was attenuated by Crus I/II inactivation specifically in the devalued group (Paired-Muscimol) and not in the non-devalued group (Unpaired-Muscimol) when two responses were trained, a pattern that differs from what we observed after either PL or IL inactivation. PL and IL inactivation reduced the margin of goal-directed responding (difference between non-devalued and devalued groups) on minimally and extensively trained responses, respectively, while Crus I/II inactivation increased this margin. This pattern of responding is normally seen when parts of the habit circuitry are inactivated; however, there was no effect of Crus I/II inactivation on habitual responding when just one response was extensively trained to habit. This could mean that Crus I/II is only involved in the early development of S-R associations. Alternatively, this inactivation could be suppressing a specific aspect of habit circuitry engaged when two responses are trained concurrently, each in its own context. We elaborate more specifically on each of these hypotheses below.

Early S-R hypothesis

In two-response training, when both responses were expressed as actions, a reduction of responding in the paired group (where residual responding can only be based on habit) after Crus I/II inactivation served to make responding appear more goal-directed (as would inactivation of a habit region when behavior is expressed as a habit). Therefore, under normal conditions (i.e. no inactivation) Crus I/II may promote expression of S-R associations, though only prior to when behavior is entirely habitual. Since both R-O and S-R associations develop in parallel, it may be that different contributors to the habit system, such as the IL and Crus I/II, are active at different times and in communication with the habit center, the DLS. In this case, the cerebellum would drive initial S-R learning and expression but would no longer be needed once habit circuitry controls behavior, and much like the PL, is only involved in initial learning (though unlike the PL involved in S-R rather than R-O).

This hypothesis, that Crus I/II is important early on in training when a goal-directed component is removed from responding, could be tested by minimally training a single response so that behavior is goal-directed, devaluing the reinforcer, and then inactivating Crus I/II at time of test. If Crus I/II is important for the initial development or expression of S-R associations then despite only one context and response being involved, the Paired-Muscimol group should show attenuated responding. However, this does not explain why responding wouldn't be dampened by inactivation in the unpaired group, whose responding should theoretically contain some element of habitual responding.

Hierarchical hypothesis

Alternatively, Crus I/II may be involved in hierarchical contextual-S-R associations. One confound in our examination of the role of the cerebellar cortex in actions and habits is that our action paradigm has two responses and two contexts, thus perhaps leading to a different associative structure controlling behavior and/or requiring different circuitry than when only one response is learned in one context. A potential interpretation of our results is that Crus I/II inactivation reduced responding in the two-response paired group (where residual responding is driven exclusively by S-R association) by impairing context-S-R associations, which might be a larger component of the residual S-R responding with more than one context. This does not explain why there was no attenuation in the unpaired group, a group where responding should be driven by both R-O and S-R associations. It may be that R-O related systems (i.e. goal-directed regions) are able to compensate for this silencing of an S-R system.

One way that we might test for a Crus I/II role in expression of hierarchical associations is to use a paradigm such as that utilized by Trask and Bouton (2014). In their experiment, they trained R1-O1 and R2-O2 in Context A and in Context B trained R1-O2 and R2-O1. They then devalued O2 and found that R2 was reduced in Context A and R1 in Context B. This indicates context-(R-O) associations supporting responding. Residual responding in the devalued group may also indicate the presence of a context-S-R association. If our hypothesis that Crus I/II is involved in context-S-R associations is supported, then we would expect to see a further reduction of responding on R2 in Context A and R1 in Context B. However, residual responding in the devalued group in

this paradigm could still be due to separate context-R associations, rather than hierarchical context-(S-R) associations.

We could also utilize a paradigm in which one goal-directed behavior and one habit is trained to see if inactivation affects responding when one response is demonstrably an action and the other is a habit. If Crus I/II is important for context-(S-R) associations that form when two responses are trained, then we should look at two different behaviors (trained in different contexts) to determine the extent of Crus I/II involvement. If responding is only affected in the goal-directed group, then our hypothesis would not be supported. Preliminary data from the Bouton lab suggests that training of an extensively-trained response in one context followed by training a minimally-trained response in a second context (and thus not intermixing training of the two) results in goal-directed responding on the minimally-trained response and habitual responding on the extensively-trained response. If Crus I/II is involved in context-(S-R) associations then we would expect inactivation to result in reduced responding in the paired groups when both an action and a habit is expressed. This crucially would show that the status of the behavior (action or habit) or amount of training is not the determinant for Crus I/II involvement.

Crus I/II inactivation did not suppress responding in the unpaired group in our two-response experiment. One possible explanation for this finding is that the R-O circuitry compensates for a loss of contextual-S-R input. To test this idea, we could train a response and then institute a context change. Thrailkill and Bouton (2015) showed that S-R components of responding are sensitive to context switches, whereas R-O

components are not. Therefore, if R-O circuitry is compensatory then we should see no context switch effect in animals that receive Crus I/II muscimol in comparison to animals that receive vehicle (See Figure 1 of the Introduction for a summary of these associations). This would indicate that context-(S-R) (or even just context-R) associations are not part of the control of responding that is occurring in unpaired animals after Crus I/II inactivation.

Future directions

One major direction for future experiments is to determine why our two-response paradigm maintained extensively-trained behavior as goal-directed. We have noted this phenomenon in PL, IL, and cerebellar experiments, finding that quite robustly, behavior was always goal-directed despite extensive training of a second response, as long as training of the second response was intermixed with the first. Moreover, the same amount of extensive training of one response resulted in a habit (experiment 2 of chapter 4). This concords with prior research by Colwill and Rescorla, who were unable to promote habit expression in rats despite overtraining, when more than one response was trained (Colwill & Rescorla, 1985). However, co-trained responses on different reinforcement schedules have resulted in the formation of an action and a habit in mice (Gremel & Costa, 2013). Preliminary data has also suggested that behavior is habitual on the extensively-trained response prior to the introduction of a second response, and that even introducing non-contingent pellets in a second context can revert R1 to an action. Behavioral work needs to be done to determine exactly what is happening that causes habit disruption with intermixing of a second response/outcome. One theory is that when

a second response (or even non-contingent pellets) is introduced, attention is then drawn to the outcome and initial response again. Habits can be disrupted if they prove to not be advantageous, such as during extinction (Dezfouli, Lingawi, & Balleine, 2016), and attention may be a second means by which goal-directed circuitry can re-gain control (Thrailkill, Trask, Vidal, Alcala, & Bouton, 2018).

The pathway-specific silencing capabilities of DREADDs also open a number of future experimental possibilities. For one, it is not well understood how the goal-directed circuitry interacts with the habit circuitry. Since the mPFC is believed to be the cognitive switch between the two, it has been suggested that the IL inhibits the PL during the development of habits and that reciprocally the PL can inhibit the IL if habits are no longer useful. Indeed, the IL does not directly project to the DLS, implying that their interactions during habit expression must go through another brain region (Vertes, 2004). We could utilize DREADDs to silence IL-PL projection following extensive training to see if this may be how the separate action and habit circuits are interacting. It would also be interesting to do this using the same paradigm that we utilized in aim 1, in which we believe that behavior is reverted from a habit to an action during the addition of a second response. Since there is no effect of PL inactivation at time of test on the extensively-trained response, this might mean silencing the IL-PL pathway during secondary response training could maintain habitual responding. One caveat to this experiment is that anatomically the PL and IL are located very close together and the IL is directly ventral to the IL. This proximity may make silencing this particular pathway much more

difficult as it would be hard to prevent DREADD vector infusion spread and CNO infusion spread down cannulae into adjacent regions.

Final conclusions

In conclusion, we have found that minimally-trained behavior is mediated by the PL-aDMS pathway, and this is likely goal-directed. We have found that the prelimbic cortex is not involved in extensively-trained goal-directed behavior, and instead, that extensively-trained goal-directed behavior is mediated by the infralimbic cortex. The IL has previously only been linked to habitual responding. Finally, the Crus I/II region of cerebellar cortex plays a role in both minimally-trained and extensively-trained goal-directed behaviors, though it mediates responding in the opposite direction, as inactivation makes behaviors appear even more goal-directed by reducing habitual responding. Thus, this work adds to the body of literature surrounding actions and habits by beginning to expand our knowledge of goal-directed circuitry to a new brain region (Crus I/II of the cerebellum), demonstrating that there is a neural difference in minimally and extensively-trained goal-directed behaviors, and delineating a PL-to-anterior DMS projection as important for expression of minimally-trained operant behavior.

References

Bergstrom, H. C., Lipkin, A. M., Lieberman, A. G., Pinard, C. R., Gunduz-Cinar, O., Brockway, E. T., ... & Rubio, F. J. (2018). Dorsolateral striatum engagement interferes with early discrimination learning. *Cell reports*, *23*(8), 2264-2272.

Colwill, R. M., & Rescorla, R. A. (1985). Instrumental responding remains sensitive to reinforcer devaluation after extensive training. *Journal of Experimental Psychology: Animal Behavior Processes*, *11*, 520-536.

Corbit, L. H. (2018). Understanding the balance between goal-directed and habitual behavioral control. *Current opinion in behavioral sciences*, *20*, 161-168.

Corbit, L. H., & Balleine, B. W. (2005). Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *25*(4), 962–70.

Corbit, L. H., & Janak, P. H. (2010). Posterior dorsomedial striatum is critical for both selective instrumental and Pavlovian reward learning. *The European journal of neuroscience*, *31*(7), 1312–21.

Corbit, L. H., Muir, J. L., & Balleine, B. W. (2003). Lesions of mediodorsal thalamus and anterior thalamic nuclei produce dissociable effects on instrumental conditioning in rats. *The European journal of neuroscience*, *18*(5), 1286–94.

Corbit, L. H., Nie, H., & Janak, P. H. (2012). Habitual Alcohol Seeking: Time Course and the Contribution of Subregions of the Dorsal Striatum. *Biological Psychiatry, 72*(5), 389–395.

Daw, N. D., Niv, Y., & Dayan, P. (2005). Uncertainty-based competition between prefrontal and dorsolateral striatal systems for behavioral control. *Nature neuroscience, 8*(12), 1704.

Dickinson, A., Balleine, B., Watt, A., Gonzalez, F., & Boakes, R. A. (1995). Motivational control after extended instrumental training. *Animal Learning & Behavior*, *23*(2), 197-206.

Dickinson, A., Nicholas, D. J., & Adams, C. D. (1983). The effect of the instrumental contingency on susceptibility to reinforcer devaluation. Quarterly Journal of Experimental Psychology, 35B, 35–51.

Gremel, C. M., & Costa, R. M. (2013). Premotor cortex is critical for goal-directed actions. *Frontiers in computational neuroscience, 7*, 110.

Hart, G., Bradfield, L. A., Fok, S. Y., Chieng, B., & Balleine, B. W. (2018). The Bilateral Prefronto-striatal Pathway Is Necessary for Learning New Goal-Directed Actions. *Current Biology*, *28*.

Hunnicutt, B. J., Jongbloets, B. C., Birdsong, W. T., Gertz, K. J., Zhong, H., & Mao, T. (2016). A comprehensive excitatory input map of the striatum reveals novel functional organization. *eLife*, *5*.

Killcross, S., & Coutureau, E. (2003). Coordination of actions and habits in the medial prefrontal cortex of rats. *Cerebral cortex (New York, N.Y. : 1991), 13*(4), 400–8.

Lingawi, N. W., Dezfouli, A., & Balleine, B. W. (2016). The psychological and physiological mechanisms of habit formation. In R. A. Murphy & R. C. Honey (Ed) *The Wiley Handbook on the Cognitive Neuroscience of Learning,* 411-440.

Ludvig, E. A., Bellemare, M. G., & Pearson, K. G. (2011). A primer on reinforcement learning in the brain: Psychological, computational, and neural perspectives. In E. Alonso, & E. Mondragon (Eds.), Computational neuroscience for advancing artificial intelligence: Models, methods and applications (pp. 111–144). Hershey, PA: IGI Global.

Mailly, P., Aliane, V., Groenewegen, H. J., Haber, S. N., & Deniau, J. M. (2013). The rat prefrontostriatal system analyzed in 3D: evidence for multiple interacting functional units. *Journal of Neuroscience*, *33*(13), 5718-5727.

Ostlund, S. B., & Balleine, B. W. (2005). Lesions of medial prefrontal cortex disrupt the acquisition but not the expression of goal-directed learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *25*(34), 7763–70.

Ostlund, S. B., & Balleine, B. W. (2008). Differential involvement of the basolateral amygdala and mediodorsal thalamus in instrumental action selection. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *28*(17), 4398–405.

Shiflett, M. W., Brown, R. A., & Balleine, B. W. (2010). Acquisition and Performance of Goal-Directed Instrumental Actions Depends on ERK Signaling in Distinct Regions of Dorsal Striatum in Rats. *The Journal of Neuroscience*, *30*(8), 2951–2959.

Shipman, M. L., Trask, S., Bouton, M. E., & Green, J. T. (2018). Inactivation of prelimbic and infralimbic cortex respectively affects minimally-trained and extensively-trained goal-directed actions. *Neurobiology of Learning and Memory*.

Smith, K. S., & Graybiel, A. M. (2013). A dual operator view of habitual behavior reflecting cortical and striatal dynamics. *Neuron*, *79*(2), 361–74.

Thrailkill, E. A., & Bouton, M. E. (2015). Contextual control of instrumental actions and habits. *Journal of Experimental Psychology: Animal Learning and Cognition*, *41*(1), 69.

Thrailkill, E. A., Trask, S., Vidal, P., Alcalá, J. A., & Bouton, M. E. (2018). Stimulus control of actions and habits: A role for reinforcer predictability and attention in the development of habitual behavior. *Journal of Experimental Psychology: Animal Learning and Cognition*, *44*(4), 370.

Tran-Tu-Yen, D. A., Marchand, A. R., Pape, J.-R. R., Scala, G., & Coutureau, E. (2009). Transient role of the rat prelimbic cortex in goal-directed behaviour. *The European journal of neuroscience*, *30*(3), 464–71.

Trask, S., Shipman, M. L., Green, J. T., & Bouton, M. E. (2017). Inactivation of the Prelimbic Cortex Attenuates Context-Dependent Operant Responding. *The Journal of Neuroscience*, *37*(9), 2317–2324.

Uylings, H. B., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal cortex?. *Behavioural brain research*, *146*(1-2), 3-17.

Vertes, R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse (New York, N.Y.)*, *51*(1), 32–58.

Xiao, L., Bornmann, C., Hatstatt-Burklé, L., & Scheiffele, P. (2018). Regulation of striatal cells and goal-directed behavior by cerebellar outputs. *Nature Communications*, *9*(1), 3133.

Yin, H. H., Ostlund, S. B., Knowlton, B. J., & Balleine, B. W. (2005). The role of the dorsomedial striatum in instrumental conditioning. *The European journal of neuroscience*, *22*(2), 513–23.

Comprehensive Bibliography

Balleine, B. W., Killcross, A., & Dickinson, A. (2003). The effect of lesions of the basolateral amygdala on instrumental conditioning. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *23*(2), 666–75.

Balleine, B. W., & O'Doherty, J. P. (2010). Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, *35*(1), 48–69.

Balsters, J. H., Laird, A. R., Fox, P. T., & Eickhoff, S. B. (2014). Bridging the gap between functional and anatomical features of cortico-cerebellar circuits using meta-analytic connectivity modeling. *Human Brain Mapping, 35*, 3152-3169.

Balsters, J. H., Whelan, C. D., Robertson, I. H., & Ramnani, N. (2013). Cerebellum and cognition: Evidence for the encoding of higher order rules. Cerebral Cortex, 23, 1433-1443.

Barbey, A. K., Koenigs, M., & Grafman, J. (2013). Dorsolateral prefrontal contributions to human working memory. *Cortex, 49*, 1195-1205.

Barker, J. M., Taylor, J. R., & Chandler, L. (2014). A unifying model of the role of the infralimbic cortex in extinction and habits. *Learning & memory (Cold Spring Harbor, N.Y.)*, *21*(9), 441–8.

Bergstrom, H. C., Lipkin, A. M., Lieberman, A. G., Pinard, C. R., Gunduz-Cinar, O., Brockway, E. T., ... & Rubio, F. J. (2018). Dorsolateral Striatum Engagement Interferes with Early Discrimination Learning. *Cell reports*, *23*(8), 2264-2272.

Bernard, J. A., Peltier, S. J., Benson, B. L., Wiggins, J. L., Jaeggi, S. M., Buschkuehl, M., . . . Seidler, R. D. (2014). Dissociable functional networks of the human dentate nucleus. *Cerebral Cortex, 24*, 2151-2159.

Berntson, G. G., & Torello, M. W. (1982). The paleocerebellum and the integration of behavioral function. *Physiological Psychology, 10*, 2-12.

Bloedel, J. R., & Bracha, V. (1997). Duality of cerebellar motor and cognitive functions. *International Review of Neurobiology, 41*, 613-634.

Bodranghien, F., Bastian, A., Casali, C., Hallett, M., Louis, E. D., Manto, M., . . . Dun, K. v. (2016). Consensus paper: Revisiting the symptoms and signs of cerebellar syndrome. *Cerebellum, 15*, 369-391.

Bossert, J. M., Stern, A. L., Theberge, F. R., Cifani, C., Koya, E., Hope, B. T., & Shaham, Y. (2011). Ventral medial prefrontal cortex neuronal ensembles mediate context-induced relapse to heroin. *Nature neuroscience*, *14*(4), 420.

Bostan, A. C., Dum, R. P., & Strick, P. L. (2013). Cerebellar networks with the cerebral cortex and basal ganglia. *Trends in Cognitive Sciences, 17*, 241-254.

Bouton, M. E., Todd, T. P., Vurbic, D., Winterbauer, N. E. (2011). Renewal after the extinction of free operant behavior. *Learning and Behavior, 39*, 57–67.

Bradfield, L. A., Bertran-Gonzalez, J., Chieng, B., & Balleine, B. W. (2013). The Thalamostriatal Pathway and Cholinergic Control of Goal-Directed Action: Interlacing New with Existing Learning in the Striatum. *Neuron*, *79*(1), 153–166.

Buckner, R. L. (2013). The cerebellum and cognitive function: 25 years of insight from anatomy and neuroimaging. *Neuron, 80*, 807-815.

Caligiore, D., Pezzulo, G., Baldassarre, G., Bostan, A. C., Strick, P. L., Doya, K., . . . Herreros, I. (2017). Consensus paper: Towards a systems-level view of cerebellum function: the interplay between cerebellum, basal ganglia, and cortex. *Cerebellum, 16*, 203-229.

Callu, D., Puget, S., Faure, A., Guegan, M., & Massioui, N. (2007). Habit learning dissociation in rats with lesions to the vermis and the interpositus of the cerebellum. *Neurobiology of disease*, *27*(2), 228–37.

Campbell, E. J., & Marchant, N. J. (2018). The use of chemogenetics in behavioural neuroscience: receptor variants, targeting approaches and caveats. *British journal of pharmacology*, *175*(7), 994-1003.

Colwill, R. M., & Rescorla, R. A. (1985). Instrumental responding remains sensitive to reinforcer devaluation after extensive training. *Journal of Experimental Psychology: Animal Behavior Processes, 11*, 520-536.

Colwill, R. M., & Rescorla, R. A. (1990). Effect of reinforcer devaluation on discriminative control of instrumental behavior. *Journal of Experimental Psychology: Animal Behavior Processes, 16*, 40-47.

Corbit, L. H. (2018). Understanding the balance between goal-directed and habitual behavioral control. *Current opinion in behavioral sciences*, *20*, 161-168.

Corbit, L. H., & Balleine, B. W. (2003). The role of prelimbic cortex in instrumental conditioning. *Behavioural brain research*, *146*(1–2), 145–57.

Corbit, L. H., & Balleine, B. W. (2005). Double dissociation of basolateral and central amygdala lesions on the general and outcome-specific forms of pavlovian-instrumental transfer. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *25*(4), 962–70.

Corbit, L. H., & Janak, P. H. (2010). Posterior dorsomedial striatum is critical for both selective instrumental and Pavlovian reward learning. *The European journal of neuroscience*, *31*(7), 1312–21.

Corbit, L. H., Leung, B. K., & Balleine, B. W. (2013). The role of the amygdala-striatal pathway in the acquisition and performance of goal-directed instrumental actions.

The Journal of neuroscience : the official journal of the Society for Neuroscience, *33*(45), 17682–90.

Corbit, L. H., Muir, J. L., & Balleine, B. W. (2003). Lesions of mediodorsal thalamus and anterior thalamic nuclei produce dissociable effects on instrumental conditioning in rats. *The European journal of neuroscience*, *18*(5), 1286–94.

Corbit, L. H., Nie, H., & Janak, P. H. (2012). Habitual Alcohol Seeking: Time Course and the Contribution of Subregions of the Dorsal Striatum. *Biological Psychiatry*, *72*(5), 389–395.

Coutureau, E., & Killcross, S. (2003). Inactivation of the infralimbic prefrontal cortex reinstates goal-directed responding in overtrained rats. *Behavioural brain research*, *146*(1–2), 167–74.

Daw, N. D., Niv, Y., & Dayan, P. (2005). Uncertainty-based competition between prefrontal and dorsolateral striatal systems for behavioral control. *Nature neuroscience*, *8*(12), 1704.

Desmond, J. E., Gabrieli, J. D. E., Wagner, A. D., Ginier, B. L., & Glover, G. H. (1997). Lobular patterns of cerebellar activation in verbal working-memory and finger-tapping tasks as revealed by functional MRI. *Journal of Neuroscience, 17*, 9675-9685.

Deverett, B., Koay, S. A., Oostland, M., & Wang, S. S. (2018). Cerebellar involvement in an evidence-accumulation decision-making task. *Elife*, *7*, e36781.

Dezfouli, A., Lingawi, N. W., & Balleine, B. W. (2014). Habits as action sequences: hierarchical action control and changes in outcome value. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, *369*(1655).

Dickinson, A. (1985). Actions and habits: the development of behavioural autonomy. *Philosophical Transactions of the Royal Society of London, 308*, 67-78.

Dickinson, A., Balleine, B., Watt, A., Gonzalez, F., & Boakes, R. A. (1995). Motivational control after extended instrumental training. *Animal Learning & Behavior*, *23*(2), 197-206.

Dickinson, A., Nicholas, D. J., & Adams, C. D. (1983). The effect of the instrumental contingency on susceptibility to reinforcer devaluation. *Quarterly Journal of Experimental Psychology*, 35B, 35–51.

Eddy, M. C., Todd, T. P., Bouton, M. E., & Green, J. T. (2016). Medial prefrontal cortex involvement in the expression of extinction and ABA renewal of instrumental behavior for a food reinforcer. *Neurobiology of learning and memory*, *128*, 33–9.

Fermin, A. S., Yoshida, T., Yoshimoto, J., Ito, M., Tanaka, S. C., & Doya, K. (2016). Model-based action planning involves cortico-cerebellar and basal ganglia networks. *Scientific reports, 6,* 31378.

Field, A. (2005). Discovering statistics using SPSS. Thousand Oaks, CA: Sage.

Floresco, S. B., Block, A. E., & Maric, T. L. (2008). Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. *Behavioural brain research, 190*(1), 85-96.

Fuchs, R. A., Evans, K. A., Ledford, C. C., Parker, M. P., Case, J. M., Mehta, R. H., & See, R. E. (2005). The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. *Neuropsychopharmacology 30,* 296-309.

Giannetti, S., & Molinari, M. (2002). Cerebellar input to the posterior parietal cortex in the rat. *Brain Research Bulletin, 58,* 481-489.

Glickstein, M. (1993). Motor skills but not cognitive tasks. *Trends in Neurosciences, 16,* 450-451.

Glickstein, M. (2006). Thinking about the cerebellum. *Brain, 129,* 288-292.

Glickstein, M. (2007). What does the cerebellum really do? *Current Biology, 17,* R824-R827.

Gomez, J. L., Bonaventura, J., Lesniak, W., Mathews, W. B., Sysa-Shah, P., Rodriguez, L. A., ... & Pomper, M. G. (2017). Chemogenetics revealed: DREADD occupancy and activation via converted clozapine. *Science*, *357*(6350), 503-507.

Gourley, S. L., & Taylor, J. R. (2016). Going and stopping: dichotomies in behavioral control by the prefrontal cortex. *Nature neuroscience*, *19*(6), 656–64.

Gremel, C. M., & Costa, R. M. (2013). Orbitofrontal and striatal circuits dynamically encode the shift between goal-directed and habitual actions. *Nature Communications*, *4*, 2264.

Groenewegen, H. J., & Uylings, H. B. (2010). Organization of prefrontal-striatal connections. In *Handbook of Behavioral Neuroscience,* 20, 353-365. Elsevier.

Hart, G., Bradfield, L. A., & Balleine, B. W. (2018). Prefrontal cortico-striatal disconnection blocks the acquisition of goal-directed action. *Journal of Neuroscience, 38,* 1311-1322.

Hart, G., Bradfield, L. A., Fok, S. Y., Chieng, B., & Balleine, B. W. (2018). The bilateral prefronto-striatal pathway is necessary for learning new goal-directed actions. *Current Biology, 28,* 1-12.

Houck, B. D., & Person, A. L. (2015). Cerebellar premotor output neurons collateralize to innervate the cerebellar cortex. *Journal of Comparative Neurology*, *523,* 2254-2271.

Hunnicutt, B. J., Jongbloets, B. C., Birdsong, W. T., Gertz, K. J., Zhong, H., & Mao, T. (2016). A comprehensive excitatory input map of the striatum reveals novel functional organization. *eLife, 5*.

Hutton, S. B. (2008). Cognitive control of saccadic eye movements. *Brain and Cognition, 68,* 327-340.

Ito, M. (2008). Control of mental activities by internal models in the cerebellum. *Nature Reviews Neuroscience, 9,* 304-313.

Johnson, A. W., Gallagher, M., & Holland, P. C. (2009). The basolateral amygdala is critical to the expression of pavlovian and instrumental outcome-specific reinforcer devaluation effects. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *29*(3), 696–704.

Jonkman, S., Kosaki, Y., Everitt, B. J., & Dickinson, A. (2010). The role of contextual conditioning in the effect of reinforcer devaluation on instrumental performance by rats. *Behavioural Processes, 83,* 276-281.

Katz, D. B., & Steinmetz, J. E. (2002). Psychological functions of the cerebellum. *Behavioral and Cognitive Neuroscience Reviews, 1,* 229-241.

Kelly, R. M., & Strick, P. L. (2003). Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *23*(23), 8432–44.

Keramati, M., Dezfouli, A., & Piray, P. (2011). Speed/accuracy trade-off between the habitual and the goal-directed processes. *PLoS computational biology*, *7*(5), e1002055.

Killcross, S., & Coutureau, E. (2003). Coordination of actions and habits in the medial prefrontal cortex of rats. *Cerebral cortex (New York, N.Y. : 1991)*, *13*(4), 400–8.

Kim, S. G., Uğurbil, K., & Strick, P. L. (1994). Activation of a cerebellar output nucleus during cognitive processing. *Science, 265,* 949-951.

Kosaki, Y., & Dickinson, A. (2010). Choice and contingency in the development of behavioral autonomy during instrumental conditioning. *Journal of Experimental Psychology: Animal Behavior Processes, 36,* 334-342.

Koziol, L. F., Budding, D., Andreasen, N., D'Arrigo, S., Bulgheroni, S., Imamizu, H., . . . Yamazaki, T. (2014). Consensus paper: The cerebellum's role in movement and cognition. *Cerebellum, 13,* 151-177.

Koziol, L. F., Budding, D. E., & Chidekel, D. (2012). From movement to thought: Executive function, embodied cognition, and the cerebellum. *Cerebellum, 11,* 505-525.

Kuper, M., Dimitrova, A., Thurling, M., Maderwald, S., Roths, J., Elles, H. G., . . . Timmann, D. (2011). Evidence of a motor and a non-motor domain in the human dentate nucleus: An fMRI study. *Neuroimage, 54,* 2612-2622.

Lalonde, R. (1994). Cerebellar contributions to instrumental learning. *Neuroscience and Biobehavioral Reviews, 18*, 161-170.

Lalonde, R., & Botez, M. I. (1990). The cerebellum and learning processes in animals. *Brain Research Reviews, 15*, 325-332.

LaLumiere, R. T., Niehoff, K. E., & Kalivas, P. W. (2010). The infralimbic cortex regulates the consolidation of extinction after cocaine self-administration. *Learning & memory (Cold Spring Harbor, N.Y.), 17*(4), 168–75.

Larsell, O. (1952). The morphogenesis and adult pattern of the lobules and fissures of the cerebellum of the white rat. *Journal of Comparative Neurology, 97*, 281-356.

Legg, C. R., Mercier, B., & Glickstein, M. (1989). Corticopontine projection in the rat: The distribution of labelled cortical cells after large injections of horseradish peroxidase in the pontine nuclei. *Journal of Comparative Neurology, 286*, 427-441.

Leiner, H. C., Leiner, A. L., & Dow, R. S. (1986). Does the cerebellum contribute to mental skills? *Behavioral Neuroscience, 100*, 443-454.

Leiner, H. C., Leiner, A. L., & Dow, R. S. (1989). Reappraising cerebellum: What does the hindbrain contribute to the forebrain? *Behavioral Neuroscience, 103*, 998-1008.

Lichtenberg, N. T., Pennington, Z. T., Holley, S. M., Greenfield, V. Y., Cepeda, C., Levine, M. S., & Wassum, K. M. (2017). Basolateral amygdala to orbitofrontal cortex projections enable cue-triggered reward expectations. *Journal of Neuroscience*, *37*(35), 8374-8384.

Liljeholm, M., Dunne, S., & O'Doherty, J. P. (2015). Differentiating neural systems mediating the acquisition vs. expression of goal-directed and habitual behavioral control. *European Journal of Neuroscience, 41*, 1358-1371.

Lingawi, N. W., Dezfouli, A., & Balleine, B. W. (2016). The psychological and physiological mechanisms of habit formation. In R. A. Murphy & R. C. Honey (Ed) *The Wiley Handbook on the Cognitive Neuroscience of Learning*, 411-440.

Lingawi, N. W., & Balleine, B. W. (2012). Amygdala central nucleus interacts with dorsolateral striatum to regulate the acquisition of habits. *The Journal of neuroscience : the official journal of the Society for Neuroscience, 32*(3), 1073–81.

Locke, T. M., Soden, M. E., Miller, S. M., Hunker, A., Knakal, C., Licholai, J. A., . . . Carlson, E. S. (2018). Dopamine D1 receptor-positive neurons in the lateral nucleus of the cerebellum contribute to cognitive behavior. *Biological Psychiatry*.

Ludvig, E. A., Bellemare, M. G., & Pearson, K. G. (2011). A primer on reinforcement learning in the brain: Psychological, computational, and neural perspectives. In E.

Alonso, & E. Mondragon (Eds.), Computational neuroscience for advancing artificial intelligence: Models, methods and applications (pp. 111–144). Hershey, PA: IGI Global.

Mahler, S. V., Vazey, E. M., Beckley, J. T., Keistler, C. R., McGlinchey, E. M., Kaufling, J., ... & Aston-Jones, G. (2014). Designer receptors show role for ventral pallidum input to ventral tegmental area in cocaine seeking. *Nature neuroscience*, *17*(4), 577.

Mailly, P., Aliane, V., Groenewegen, H. J., Haber, S. N., & Deniau, J. M. (2013). The rat prefrontostriatal system analyzed in 3D: evidence for multiple interacting functional units. *Journal of Neuroscience*, *33*(13), 5718-5727.

Middleton, F., & Strick, P. (2001). Cerebellar projections to the prefrontal cortex of the primate. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *21*(2), 700–12.

Mihailoff, G. A., Burne, R. A., Azizi, S. A., Norell, G., & Woodward, D. J. (1981). The pontocerebellar system in the rat: An HRP Study. II. Hemispheral components. *Journal of Comparative Neurology, 197*, 559-577.

Mittleman, G., Goldowitz, D., Heck, D. H., & Blaha, C. D. (2008). Cerebellar modulation of frontal cortex dopamine efflux in mince: Relevance to autism and schizophrenia. *Synapse, 62*, 544-550.

Moorman, D. E., & Aston-Jones, G. (2015). Prefrontal neurons encode context-based response execution and inhibition in reward seeking and extinction. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(30), 9472–7.

Ostlund, S. B., & Balleine, B. W. (2005). Lesions of medial prefrontal cortex disrupt the acquisition but not the expression of goal-directed learning. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *25*(34), 7763–70.

Ostlund, S. B., & Balleine, B. W. (2008). Differential involvement of the basolateral amygdala and mediodorsal thalamus in instrumental action selection. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, *28*(17), 4398–405.

Parker, K. L., Narayanan, N. S., & Andreasen, N. C. (2014). The therapeutic potential of the cerebellum in schizophrenia. *Frontiers in Systems Neuroscience, 8*, Article 163.

Paxinos, G., & Watson, C. (1998). The Rat Brain in Stereotaxic Coordinates, 4th edn Academic Press: New York.

Paxinos, G., & Watson, C. (2006). The rat brain in stereotaxic coordinates: hard cover edition. *Elsevier*.

Peak, J., Hart, G., & Balleine, B. W. (2018). From learning to action: the integration of dorsal striatal input and output pathways in instrumental conditioning. *The European journal of neuroscience*.

Peters, J., Kalivas, P. W., & Quirk, G. J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. *Learning & memory (Cold Spring Harbor, N.Y.), 16*(5), 279–88.

Peters, J., LaLumiere, R. T., & Kalivas, P. W. (2008). Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. *The Journal of Neuroscience, 28*(23), 6046-6053.

Popa, L. S., Hewitt, A. L., & Ebner, T. J. (2014). The cerebellum for jocks and nerds alike. *Frontiers in Systems Neuroscience, 8*, Article 113.

Ramnani, N. (2006). The primate cortico-cerebellar system: anatomy and function. *Nature reviews. Neuroscience*, *7*(7), 511–22.

Riedel, M. C., Ray, K. L., Dick, A. S., Sutherland, M. T., Hernandez, Z., Fox, P. M., . . . Laird, A. R. (2015). Meta-analytic connectivity and behavioral parcellation of the human cerebellum. *Neuroimage, 117*, 327-342.

Riga, D., Matos, M. R., Glas, A., Smit, A. B., Spijker, S., & den Oever, M. C. (2014). Optogenetic dissection of medial prefrontal cortex circuitry. *Frontiers in systems neuroscience, 8*, 230.

Rogan, S. C., & Roth, B. L. (2011). Remote control of neuronal signaling. *Pharmacological reviews, 63*(2), 291-315.

Rogers, T. D., Dickson, P. E., Heck, D. H., Goldowitz, D., Mittleman, G., & Blaha, C. D. (2011). Connecting the dots of the cerebro-cerebellar role in cognitive function: Neuronal pathways for cerebellar modulation of dopamine release in the prefrontal cortex. *Synapse, 65*, 1204-1212.

Rogers, T. D., Dickson, P. E., McKimm, E., Heck, D. H., Goldowitz, D., Blaha, C. D., & Mittleman, G. (2013). Reorganization of circuits underlying cerebellar modulation of prefrontal cortical dopamine in mouse models of Autism Spectrum Disorder. *Cerebellum, 12*, 547-556.

Runyan, J. D., Moore, A. N., & Dash, P. K. (2004). A role for prefrontal cortex in memory storage for trace fear conditioning. *Journal of Neuroscience, 24*(6), 1288-1295.

Schmahmann, J. D. (1991). An emerging concept: The cerebellar contribution to higher function. *Archives of Neurology, 48*, 1178-1187.

Schmahmann, J. D. (2004). Disorders of the cerebellum: Ataxia, Dysmetria of Thought, and the Cerebellar Cognitive Affective Syndrome. *Journal of Neuropsychiatry and Clinical Neurosciences, 16*, 367-378.

Schmahmann, J. D., Doyon, J., McDonald, D., Holmes, C., Lavoie, K., Hurwitz, A. S., . . . Petrides, M. (1999). Three-dimensional MRI atlas of the human cerebellum in proportional stereotaxic space. *Neuroimage, 10*, 233-260.

Schmahmann, J. D., & Sherman, J. C. (1998). The cerebellar cognitive affective syndrome. *Brain, 121*, 561-579.

Schmahmann, J. D., Weilburg, J. B., & Sherman, J. C. (2007). The neuropsychiatry of the cerebellum -- insights from the clinic. *Cerebellum, 6*, 254-267.

Seamans, J. K., Lapish, C. C., & Durstewitz, D. (2008). Comparing the prefrontal cortex of rats and primates: insights from electrophysiology. *Neurotoxicity research*, *14*(2–3), 249–62.

Sesack, S., Deutch, A., Roth, R., & Bunney, B. (1989). Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. *The Journal of comparative neurology*, *290*(2), 213–42.

Sharpe, M. J., & Killcross, S. (2018). Modulation of attention and action in the medial prefrontal cortex of rats. *Psychological review*, *125*(5), 822.

Shiflett, M. W., Brown, R. A., & Balleine, B. W. (2010). Acquisition and Performance of Goal-Directed Instrumental Actions Depends on ERK Signaling in Distinct Regions of Dorsal Striatum in Rats. *The Journal of Neuroscience*, *30*(8), 2951–2959.

Shipman, M. L., Trask, S., Bouton, M. E., & Green, J. T. (2018). Inactivation of prelimbic and infralimbic cortex respectively affects minimally-trained and extensively-trained goal-directed actions. *Neurobiology of Learning and Memory, 155*, 164-172.

Siegel, J. J., Taylor, W., Gray, R., Kalmbach, B., Zemelman, B. V., Desai, N. S., . . . Chitwood, R. A. (2015). Trace eyeblink conditioning in mice is dependent upon the dorsal medial prefrontal cortex, cerebellum, and amgydala: Behavioral characterization and functional circuitry. *eNeuro, 2*, e0051-0014.2015.

Smith K. S. & Graybiel, A. M. (2016). Habit formation. *Dialogues in Clinical Neuroscience*, *18*, 33–43.

Smith, K. S., & Graybiel, A. M. (2013). A dual operator view of habitual behavior reflecting cortical and striatal dynamics. *Neuron*, *79*(2), 361–74.

Smith, K. S., Virkud, A., Deisseroth, K., & Graybiel, A. M. (2012). Reversible online control of habitual behavior by optogenetic perturbation of medial prefrontal cortex. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(46), 18932–7.

Stachniak, T. J., Ghosh, A., & Sternson, S. M. (2014). Chemogenetic synaptic silencing of neural circuits localizes a hypothalamus→ midbrain pathway for feeding behavior. *Neuron, 82*(4), 797-808.

Steele, C. J., Anwander, A., Bazin, P.-L., Trampel, R., Schaefer, A., Turner, R., . . . Villringer, A. (2017). Human cerebellar sub-millimeter diffusion imaging reveals the motor and non-motor topography of the dentate nucleus. *Cerebral Cortex, 27*, 4537-4548.

Stefani, M. R., Groth, K., & Moghaddam, B. (2003). Glutamate receptors in the rat medial prefrontal cortex regulate set-shifting ability. *Behavioral neuroscience, 117*(4), 728.

Steinmetz, J. E., Logue, S. F., & Miller, D. P. (1993). Using signaled barpressing tasks to study the neural substrates of appetitive and aversive learning in rats: Behavioral manipulations and cerebellar lesions. *Behavioral Neuroscience, 107*, 941-954.

Stoodley, C. J., & Schmahmann, J. D. (2010). Evidence for topographic organization in the cerebellum of motor control versus cognitive and affective processing. *Cortex, 46*, 831-844.

Strick, P. L., Dum, R. P., & Fiez, J. A. (2009). Cerebellar and nonmotor function. *Annual Review of Neuroscience, 32*, 413-434.

Sugihara, I. (2018). Crus I in the rodent cerebellum: Its homology to Crus I and II in the primate cerebellum and its anatomical uniqueness among neighboring lobules. *Cerebellum, 17*, 49-55.

Suzuki, L., Coulon, P., Sabel-Goedknegt, E. H., & Ruigrok, T. J. H. (2012). Organization of cerebral projections to identified cerebellar zones in the posterior cerebellum in the rat. *Journal of Neuroscience, 32*, 10854-10869.

Thrailkill, E. A., & Bouton, M. E. (2015). Contextual control of instrumental actions and habits. Journal of Experimental Psychology: *Animal Learning and Cognition*, *41*, 69–80.

Thrailkill, E. A., Trask, S., Vidal, P., Alcalá, J. A., & Bouton, M. E. (2018). Stimulus control of actions and habits: A role for reinforcer predictability and attention in the development of habitual behavior. *Journal of Experimental Psychology: Animal Learning and Cognition*, *44*(4), 370.

Thurling, M., Hautzel, H., Kuper, M., Stefanescu, M. R., Maderwald, S., Ladd, M. E., & Timmann, D. (2012). Involvement of the cerebellar cortex and nuclei in verbal and visuospatial working memory: A 7T fMRI study. *Neuroimage, 62*, 1537-1550.

Tran-Tu-Yen, D. A., Marchand, A. R., Pape, J.-R. R., Scala, G., & Coutureau, E. (2009). Transient role of the rat prelimbic cortex in goal-directed behaviour. *The European journal of neuroscience, 30*(3), 464–71.

Trask, S., Shipman, M. L., Green, J. T., & Bouton, M. E. (2017). Inactivation of the prelimbic cortex attenuates context-dependent operant responding. *Journal of Neuroscience*, 3361-16.

Trask, S., & Bouton, M. E. (2014). Contextual control of operant behavior: evidence for hierarchical associations in instrumental learning. *Learning & Behavior 42*, 281–288.

Uylings, H. B., Groenewegen, H. J., & Kolb, B. (2003). Do rats have a prefrontal cortex?. *Behavioural brain research*, *146*(1-2), 3-17.

Vandaele Y., Pribut, H. J., & Janak, P. H. (2017). Lever insertion as a salient stimulus promoting insensitivity to outcome devaluation. *Frontiers in Integrative Neuroscience*, *11*, 1-23.

Vertes, R. P. (2004). Differential projections of the infralimbic and prelimbic cortex in the rat. *Synapse (New York, N.Y.)*, *51*(1), 32–58.

Voogd, J. (2004). Cerebellum. In G. Paxinos (Ed.), *The Rat Nervous System* (3rd ed., pp. 205-242). Amsterdam: Elsevier Academic Press.

Voogd, J., & Glickstein, M. (1998). The anatomy of the cerebellum. *Trends in neurosciences*, *21*(9), 370–5.

Wagner, M. J., Kim, T. H., Savall, J., Schnitzer, M. J., & Luo, L. (2017). Cerebellar granule cells encode the expectation of reward. *Nature, 544*, 96-100.

Watson, P. J. (1978). Nonmotor functions of the cerebellum. *Psychological Bulletin, 85*, 944-967.

Watson, P., van Wingen, G., & de Wit, S. (2018). Conflicted between goal-directed and habitual control, an fMRI investigation. *eNeuro;* ENEURO.0240-18.2018.

Watson, T. C., Becker, N., Apps, R., & Jones, M. W. (2014). Back to front: Cerebellar connections and interactions with the prefrontal cortex. *Frontiers in Systems Neuroscience*, *8*, Article 4.

Watson, T. C., Jones, M. W., & Apps, R. (2009). Electrophysiological mapping of novel prefrontal-cerebellar pathways. *Frontiers in Integrative Neuroscience, 3*, Article 18.

Wiesendanger, R., & Wiesendanger, M. (1982). The corticopontine system in the rat. II. The projection pattern. *Journal of Comparative Neurology, 208*, 227-238.

Willcocks, A. L., & McNally, G. P. (2013). The role of medial prefrontal cortex in extinction and reinstatement of alcohol-seeking in rats. *The European journal of neuroscience*, *37*(2), 259–68.

Xiao, L., Bornmann, C., Hatstatt-Burklé, L., & Scheiffele, P. (2018). Regulation of striatal cells and goal-directed behavior by cerebellar outputs. *Nature Communications*, *9*(1), 3133.

Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2005). Blockade of NMDA receptors in the dorsomedial striatum prevents action-outcome learning in instrumental conditioning. *The European journal of neuroscience*, *22*(2), 505–12.

Yin, H. H., Knowlton, B. J., & Balleine, B. W. (2006). Inactivation of dorsolateral striatum enhances sensitivity to changes in the action-outcome contingency in instrumental conditioning. *Behavioural brain research*, *166*(2), 189–96.

Yin, H. H., Ostlund, S. B., Knowlton, B. J., & Balleine, B. W. (2005). The role of the dorsomedial striatum in instrumental conditioning. *The European journal of neuroscience*, *22*(2), 513–23.

Zhu, H., & Roth, B. L. (2014). Silencing synapses with DREADDs. *Neuron*, *82*(4), 723-725.