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Basic mechanisms and translational approaches to understanding effort-based choice

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Basic mechanisms and translational approaches to understanding effort-based choice

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in Psychology

by

Evan Hart

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ABSTRACT OF THE DISSERTATION

Basic mechanisms and translational approaches to understanding effort-based choice

by

Evan Hart

Doctor of Philosophy in Psychology

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Professor Alicia Izquierdo Edler, Chair

Effort is a cost that must be overcome to procure rewards, and organisms frequently make cost benefit decisions based on effort in choosing which options to pursue. The majority of previous work probing the neural mechanisms of effort-based choice has focused on the striatum and dopaminergic signaling therein, and less work has investigated behavioral or environmental factors that contribute. In all of the following experiments, an effort-based choice task was adopted from previous findings where rats could freely choose between a high effort, preferred option and a low effort, less preferred option. The high effort option was lever progressing on a progressive ratio schedule for sucrose pellets in which each successive reward became more difficult to earn. The low effort option was freely available lab chow concurrently available that had no work requirement, but was less palatable. Thus, rats could freely choose between these options and self-titrate the amount of effort they were willing to exert. A number of control tasks were used to control for changes in motoric ability, appetite, or food preference. By omitting the freely available lab chow and testing subjects for progressive ratio performance, any impairment in motoric ability,

memory, and general willingness to exert effort, rather than cost-benefit valuation per se, could be ruled out. To test whether manipulations had effects on appetite or food preference, rats were tested with both food options freely concurrently available where they did not have to work for either. In the case of manipulations that took place prior to behavioral testing, we recorded the number of sessions it took to reach stable performance to rule out impairments in learning.

In the second chapter of this dissertation, the basolateral amygdala (BLA) was tested for its effects in effort-based choice. In a series of experiments, we found that BLA pharmacological inactivations reduced high effort lever pressing specifically in the context of choice, without impairing progressive ration responding when it was the only option. These effects were also not due to changes in appetite or food preference. Chapter three details effects of anterior cingulate cortex (ACC) lesions. Much like BLA inactivations, lesions of ACC decreased choice lever pressing without affecting lever pressing ability, appetite, or food preference. In chapter four, we tested whether a behavioral manipulation, withdrawal from methamphetamine self-administration, had effects on effort based choice. Methamphetamine withdrawn animals exhibited decreased choice lever pressing, which was not due to general work aversion, learning impairments, changes in appetite, or changes in food preference. This decreased effort was accompanied by decreased activation of anterior cingulate, ventral striatum, and amygdala. In the last set of experiments, we replicated results from the ACC lesion experiments using a modern chemogenetic approach. Finally, all of the above findings are discussed with their relevance to future studies and broader implications of the basic science of effort.

The dissertation of Evan Hart is approved.

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2019

Dedicated to the memory of Dr. Thomas R Minor

Thank you, Tom, for the many important lessons both in and out of the lab

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Chapter 1: Introduction

Background and significance

Pursuit of rewards is critical for survival and propagation of species (Sherrington, 1909). This fundamental behavior occurs in the simplest organisms consisting of dozens of neurons (Ward, 1973) all the way up to mammals with brains containing billions of neurons (Craig, 1917). What is consistent in all these cases is that rewards are not always given freely. Of course, there are various costs associated with pursuing rewards, which may include time, risk and effort. Time is a cost in that organisms prefer rewards to be given as soon as possible and will steeply discount future rewards (Chung & Herrnstein, 1967). Risk imposes another cost- there may be an element of uncertainty about the availability of reward: what if the bananas hanging from the tree yesterday have been taken in the past few hours and are now gone? For prey species in particular, risk could even mean risk of death (Bolles & Fanselow, 1980). Leaving the burrow to forage for food or mates leaves a mouse vulnerable to being eaten by predators. Lastly, during this time-wagering and/or risk-taking, organisms must exert some sort of physical effort. Foraging through bushes requires physical effort expenditure, as do tree climbing and hunting. This physical effort exertion and decisions that depend on such costs are the focus of this dissertation.

While all of the experiments to follow were performed in laboratory animals and were aimed at determining basic mechanisms, it is important to note the translational impact of studies involving effort. To start, fatigue, which can be characterized as decreased effort, is one of the most common symptoms in all of general medicine (Demyttenaere *et al.*, 2005), and decreased effort occurs in many conditions. Indeed, depressed patients choose high effort options less than neurotypical controls (Treadway *et al.*, 2012a), and schizophrenics show abnormal effort-based computations (Fervaha *et al.*, 2013). Further, drugs addicts exert too much effort toward the pursuit

of drugs (Robinson *et al.*, 2013), and sobriety could be considered a high-effort choice, given the relative ease of acquiring drugs and getting high for reinforcement. Abnormal effort allocation also occurs in autism: individuals often engage in high effort, repetitive activities (Damiano *et al.*, 2012). Notably, there is a large amount of convergence between animal and human studies. Rodent effort-based choice tasks have been translated to humans, showing similar dopaminergic mechanisms (Wardle *et al.*, 2011; Treadway *et al.*, 2012b), as well as involvement of the amygdala (Chong *et al.*, 2017) and anterior cingulate cortex (Croxson *et al.*, 2009). Further convergence occurs in anti-depressant response to treatment: antidepressant drugs that block the dopamine transporter are especially effective in reducing effort-related impairments in both rats (Nunes *et al.*, 2013; Randall *et al.*, 2014a; Yohn *et al.*, 2015) and humans (Papakostas *et al.*, 2006; Cooper *et al.*, 2014), where antidepressants that block the serotonin transporter are ineffective. Given the breadth of conditions in which effort is implicated, as well as the promising ability of dopaminergic drugs and related manipulations to reverse deficits, studies of the basic mechanisms of effort could have implications for understanding and ultimately treating such conditions.

Effort as a construct

Motivation and effort are not new ideas in psychology: Wundt described humans as "motivated and thinking" (Wundt & Lindau, 1906), and William James included motivation as a subject in his textbooks (James, 1890). The word "drive" as a term to describe the energizing (i.e., effort) of behavior was first used by Woodworth (Woodworth, 1918) and these ideas were later expanded upon by behaviorists such as Hull and Spence. While the usefulness of the concept of drive has been questioned (Bolles, 1958), it was well known that changing the schedule of reinforcement (i.e., amount of effort) would change behavior (Ferster & Skinner, 1957).

Ecological factors contribute to effort output. It is particularly noteworthy that animals will perform at ratios up to 5,120, when that is the only constraint on feeding behavior (Collier *et al.*, 1972). This apparent limit on the amount of effort an animal will exert is not due to the work demand itself but rather the rate of feeding and mechanics of ingestion. This level of performance was much higher than what was previously reported, as most previous food-motivation studies were conducted in the context of an open economy. That is, animals received food outside of the testing situation. When food can only be obtained during the testing situation (i.e., closed economy), much higher levels of responding can be observed. However, in this experiment, only two out of three rats were able to meet such high ratio demands. Although the authors do not speculate on why this was the case, it presumably could have been due to a biological individual difference. Indeed, individual differences in dopamine transmission are correlated with several-fold differences in effort output (Randall *et al.*, 2012). Thus, it seems likely that *both* biological variables and ecological factors contribute to effort output.

Development of effort-based choice tasks

While the idea of effort as a construct is quite old, tasks assessing cost-benefit decisions based on effort are relatively new. A logical starting point to begin detailing such tasks is with the "dopamine hypothesis of reward", as this was a major driving force for developing modern behavioral paradigms.

The dopamine hypothesis of reward, in its earliest and simplest form, posits that brain dopamine, particularly in the ventral striatum, directly signals the pleasurable effects of appetitive stimuli. This idea was based on evidence dating back to the 1950s, when it was discovered that rats would lever press for intracranial stimulation of the medial forebrain bundle (Olds & Milner, 1954), to the point of starvation under certain conditions (Routtenberg & Lindy, 1965). Upon

discovery of dopamine as a neurotransmitter, and that medial forebrain bundle stimulation caused dopamine release (Wise, 2002), the idea that dopamine signals pleasure began to gain popularity. This idea was further supported by the finding that dopamine receptor antagonists suppress lever pressing for intracranial stimulation and food (Wise *et al.*, 1978a; Wise *et al.*, 1978b). Nevertheless, this idea eventually fell out of favor in light of the many functions of dopamine, including aversively motivated behaviors (McCullough *et al.*, 1993; Salamone, 1994), reward prediction errors (Schultz *et al.*, 1997; Niv *et al.*, 2005), and motivation (Salamone *et al.*, 1991; Salamone *et al.*, 1994a; Cousins *et al.*, 1996; Salamone & Correa, 2012). Further, it was eventually found that aversive experiences (Abercrombie *et al.*, 1989) and anxiogenic drugs (McCullough & Salamone, 1992) increase ventral striatal dopamine, and that dopamine release to appetitive stimuli such as food was attenuated with experience (Salamone *et al.*, 1994b). Thus, the dopamine hypothesis of reward, at least in its strongest form, had to be rethought, at least in a subset of the scientific community, and certainly not in the public at large.

With rethinking the dopamine hypothesis of reward, an alternative had to be offered. In the first published example of an effort-based choice task, John Salamone and colleagues (Salamone *et al.*, 1991) exposed rats to a procedure where they could freely choose between lever pressing on a fixed-ratio 5 schedule of reinforcement for highly palatable Bioserv pellets and consuming concurrently freely available, though less palatable, lab chow. They found that ventral striatal dopamine D2 receptor blockade or dopamine depletion made rats less willing to exert high levels of effort for the preferred food, and rats' consumption of the freely available less preferred alternative increased. The same manipulations had no effect on food preference when both food options were freely available; all animals showed a strong preference for the Bioserv pellets. This work challenged the idea that ventral striatal dopamine directly signaled the reinforcing properties

of appetitive stimuli: rats were simply *less willing* to work for reward, but their behavior was still directed toward the acquisition and consumption of food. Further, when they did not have to exert high levels of effort for the preferred food type, it was still apparent they had a strong preference for it as revealed by free-feeding tests. Ergo, several decades of studies aimed at determining the mechanisms of effort-based choice began, during which a number of different tasks for doing so were developed.

Tasks for assessing effort-based choice

There are now many different tasks used for assessing effort-based choice, which broadly fit into two categories: one where the options animals choose between vary in quality or identity, and another where the options vary in magnitude. In both cases, the qualitative or quantitatively preferred option has a higher effort requirement.

In tasks where subjects choose between qualitatively different options, animals can be presented with a choice between working for a preferred option versus selecting a low effort (freely-available), but less preferred option. This finding was first published in 1991, as discussed above. Since then, similar qualitative effort-based choice procedures have been adopted, primarily by varying the schedule of reinforcement. Others have reinforced the preferred food type on progressive ratio (PR) schedules (Randall *et al.*, 2012; Randall *et al.*, 2014a; Randall *et al.*, 2014b; Yohn *et al.*, 2016; Hart *et al.*, 2017; Hart & Izquierdo, 2017; Thompson *et al.*, 2017) or random ratio schedules with higher work requirements than FR5, such as random ratio 15 (Trifilieff *et al.*, 2013; Bailey *et al.*, 2016; Bailey *et al.*, 2018). Notably, while FR5 and RR15 schedules generate near uniform lever pressing behavior, animals on PR schedules exhibit large individual differences.

In all of the following experiments in this dissertation rats were subjected to a PR schedule where the required number of presses for each pellet increased according to the formula:

$$n_i = 5e^{(i/5)} - 5$$

where n_i is equal to the number of presses required on the ith ratio, rounded to the nearest whole number (Richardson & Roberts, 1996), after 5 successive schedule completions. Rats were tested on the PR schedule until they earned at least 30 pellets on any given day (~5 d) or until stable performance above this level was achieved. Upon either criteria, a ceramic ramekin containing 18 g of lab chow was introduced during testing. Rats were then free to select between consuming freely-available but less preferred chow or lever pressing for preferred sucrose pellets.

In a similar way that palatable high carbohydrate pellets are preferable to lab chow, more of a food option is preferable to less of the same food option to hungry animals. Subjects must exert more effort to get to more food in this case. The first such procedure made use of a T-maze apparatus where rats could choose between two arms of a maze. One arm was reinforced by two highly palatable pellets that rats could simply approach without difficulty. The other arm was reinforced by four pellets, for which rats had to climb a tall barrier to procure (Salamone *et al.*, 1994a). There are variations to this maze task. For example, in an earlier study from our lab we assessed such effortful choices between a high- and low-magnitude reward on a t-maze (Experiment 1, (Ostrander *et al.*, 2011)) and effortful choices when a cue signaled changes in that reward magnitude (Experiment 2), requiring more flexibility than the average effort-choice paradigm. Unlike the quality effort-based choice tasks, in such quantity effort-based choice t-maze tasks rats are required to first learn about the reward values associated with each arm of the maze (discrimination training with free sampling phase). In this learning phase, one goal arm is baited with a high magnitude reward (HR) such as 2 cereal loops, and the other with a low magnitude

reward (LR), such as a ½ cereal loop. The rat is allowed to sample freely from both arms at the beginning of testing. In such paradigms, HR and LR arm designations are counterbalanced among rats but remain constant for each rat for the duration of testing. Also for these t-maze paradigms, there are typically forced-choice trials, where HR and LR arms are blocked, 'forcing' the animal to experience the reward contingencies/values, which serve as reminders throughout testing. These forced-choice trials typically occur on no more than 2 trials within a session, and during all other trials, the rat is allowed to freely select either the HR or LR arm.

Other automated quantity effort-based choice tasks have since been developed. A frequently used paradigm involves rats selecting between two different levers, each of which earns either a small magnitude (2) pellets or a large magnitude (4) of the same food (Floresco *et al.*, 2008). While the low effort/low reward lever requires only a single press, the high effort/high reward lever requires either 5, 10, or 20 presses, presented often in discrete trial blocks, in ascending order.

Mechanisms: a brief review

The primary motivating force behind the development of effort-based choice tasks was to further understand the behavioral functions of ventral striatal dopamine. As such, there is overwhelming evidence implicating these in effort (Sokolowski & Salamone, 1998; Aberman & Salamone, 1999; Nowend *et al.*, 2001; Salamone *et al.*, 2001; Salamone *et al.*, 2002) that is reviewed elsewhere (Salamone & Correa, 2012; Salamone *et al.*, 2012). Far less work has probed other regions, which will be discussed here.

The idea that ventral striatum is not the only part of the brain involved in cost-benefit analyses involving effort took approximately 10 years to gain popularity following publication of the first effort-based choice tasks. Much of this research focused on the anterior cingulate cortex

(ACC) based on its anatomical connectivity with the striatum (Berendse *et al.*, 1992; Brog *et al.*, 1993) and role in motivated behavior generally (Paus, 2001). The first work to probe ACC in effort adopted the aforementioned T-maze quantity effort-based choice task in rats with extensive lesions encompassing ACC, prelimbic, and infralimbic cortex (Walton *et al.*, 2002), which was soon after followed with smaller lesions targeting ACC (Walton *et al.*, 2003). In both of these cases, lesions decreased choice of the high effort/high reward option. Barrier climbing ability and reward magnitude discrimination were unaffected under conditions where both arms were impeded by a barrier (i.e. equal work).

These first two quantity-based findings were soon replicated and expanded upon by exposing ACC lesioned rats to a qualitative choice task where rats chose between lever pressing for sucrose pellets on a progressive ratio and consuming freely available lab chow (Schweimer & Hauber, 2005). Interestingly, although the ACC quantitative choice task findings replicated those assessed via a T-maze task, Schweimer & Hauber (2005) found no group differences when the same animals were subjected to the progressive ratio qualitative choice task. It is conceivable that this negative finding was due to the animals having had pre-training lesions and therefore more recovery time, as well as previous behavioral testing experience in a different task. This will be discussed in Chapter 3.

Regions of the amygdala, particularly the basolateral complex (BLA) project to both the striatum and frontal cortex (McDonald, 1991), and it was not long until an experimental line of evidence probing BLA in effort-based choice was initiated. The first of which made use of a T-maze quantity-based choice task. Floresco and colleagues found that BLA inactivations, like ACC lesions and VS manipulations, decrease choice of the high effort option without affecting motoric ability or reward discrimination (Floresco & Ghods-Sharifi, 2007). This effect was later replicated

in a similar T-maze quantitative choice task with BLA lesions (Ostrander *et al.*, 2011). Replication of the T-maze findings in a lever-based quantitative choice task confirmed that effects of BLA manipulations on effort-based choice were not due to delay discounting (Ghods-Sharifi *et al.*, 2009).

Although not explicitly examining the role of each of the aforementioned regions alone, disconnection studies provide considerable insight into the mechanisms of effort-based choice. By unilaterally inactivating or lesioning one structure and doing the same to another structure in the contralateral hemisphere, one can disrupt serial communication between two structures, thus determining the role of this communication in effort-based choice. Consistent with the idea that ventral striatum, ACC, and BLA act as part of a circuit that regulates effort-related functions (Salamone *et al.*, 2007), both disconnection of ACC and VS (Hauber & Sommer, 2009) as well as BLA and ACC (Floresco & Ghods-Sharifi, 2007) have similar effects as interfering with any of these regions alone.

Based on the above, three patterns emerge: 1. By far, the majority of the work investigating effort-based choice has involved dopamine signaling, particularly in the striatum. 2. While some studies have assessed other brain regions, they have only made use of quantity effort-based choice tasks. 3. Very few studies, save two exceptions (Shafiei *et al.*, 2012; Bryce & Floresco, 2016), have probed effects of behavioral manipulations on effort-based choice behavior. Several questions remain. While the amygdala was shown to be necessary for high effort choice in T-maze and lever-press quantitative tasks, does it play a similar role in qualitative effort-based choice? The same can be asked of anterior cingulate. Lastly, while stress was shown to have effects, what about other factors, such as exposure to drugs of abuse? These questions will be the focus of the experiments detailed in this dissertation.

Dissertation overview

Here I will answer four questions:

Q1: What is the contribution of BLA to effort-based choice where subjects choose between qualitatively different options?

Q2: What is the contribution of ACC to effort-based choice where subjects choose between qualitatively different options?

Q3: What is the effect of withdrawal from methamphetamine self-administration on effort-based choice where subjects choose between qualitatively different options?

Q4: Can effects of ACC lesions on effort-based choice be replicated using a modern technical approach?

The conclusion aims to apply findings from these four studies toward future studies of basic mechanisms as well as translation approaches to studying effort-based choice.

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Chapter 2: BLA in quality effort-based choice task

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Abstract

The basolateral amygdala (BLA) is known to be involved in appetitive behavior, yet its role in cost-benefit choice of qualitatively different rewards (more/less preferred), beyond magnitude differences (larger/smaller), is poorly understood. We assessed the effects of BLA inactivations on effortful choice behavior. Rats were implanted with cannulae in BLA and trained to stable lever pressing for sucrose pellets on a progressive ratio schedule. Rats were then introduced to a choice: chow was concurrently available while they could work for the preferred sucrose pellets. Rats were infused with either vehicle control (aCSF) or baclofen/muscimol prior to test. BLA inactivations produced a significant decrease in lever presses for sucrose pellets compared to vehicle, and chow consumption was unaffected. Inactivation had no effect on sucrose pellet preference when both options were freely available. Critically, when lab chow was not concurrently available, BLA inactivations had no effect on the number of lever presses for sucrose pellets, indicating that primary motivation in the absence of choice remains intact with BLA offline. After a test under specific satiety for sucrose pellets, BLA inactivation rendered animals less sensitive to devaluation relative to control. The effects of BLA inactivations in our task are not mediated by decreased appetite, an inability to perform the task, a change in food preference, or decrements in primary motivation. Taken together, BLA supports the specific value and effortful choice of a preferred option.

Introduction

Common barriers separating organisms from rewards include time, risk, and effort costs. Much of the previous work investigating mechanisms involved in overcoming effort costs to acquire rewards has implicated dopamine signaling in the ventral striatum (Salamone *et al*., 1991, 1994; Cousins & Salamone, 1994; Cousins *et al*., 1996; Nowend *et al*., 2001; Salamone & Correa, 2012). However, the striatum does not act alone in modulating such complex behaviors, and other regions including the anterior cingulate cortex and amygdala have both long been known to be involved in appetitive, effortful behaviors (Salamone *et al*., 2007).

Until somewhat recently, there have been far fewer investigations probing regions beyond the striatum in effortful choice. In a T-maze effortful choice procedure that involved choosing between climbing a barrier for a large magnitude reward or foregoing the barrier in pursuit of a less costly alternative, it was shown that anterior cingulate cortex lesions and D1 receptor blockade bias animals' behavior toward lower effort choices (Schweimer & Hauber, 2005, 2006). In an effort discounting task that involved choosing between a lever that required one press for two pellets or several presses (up to 20) for four pellets, inactivation of the basolateral amygdala (BLA) induced a work-averse phenotype, decreasing selection of the high effort lever when the work requirement was relatively high (Ghods-Sharifi *et al*., 2009). Similarly, inactivation or lesions of the BLA decrease selection of the high effort choice in a T-maze barrier climbing task (Floresco & Ghods-Sharifi, 2007; Ostrander *et al*., 2011). Disconnection studies have shown that communication between the striatum and anterior cingulate cortex (Hauber & Sommer, 2009) as well as the BLA and anterior cingulate cortex (Floresco & Ghods-Sharifi, 2007) is important in effortful choice. Thus, the anterior cingulate, striatum, and BLA are part of an interconnected

system involved in calculating how much work to exert for greater-magnitude rewards given concurrently available but less costly, lower magnitude rewards.

Several questions remain, however. Much of the previous work investigating the role of the BLA in effortful choice made use of T-maze or effort discounting tasks in operant chambers in which the magnitude of reward varied. It remains unknown if BLA exerts a similar function in choosing between qualitatively different reinforcers (preferred but costly vs. less preferred but freely available). Moreover, to our knowledge, no studies have systematically investigated the role of BLA in initial learning vs. later performance of effortful choice, given that most investigations allow animals several days of choice testing prior to manipulations. The BLA is also well-known to be involved in appetitive behaviors, and has been shown to be important for using specific outcome values to guide choice behavior in both rats and monkeys (Hatfield *et al*., 1996; Malkova *et al*., 1997; Izquierdo & Murray, 2007; Wassum & Izquierdo, 2015). It has not been reported whether BLA is important in using outcome values to guide behavior on a task where animals can select between two qualitatively different outcomes, each associated with different effort costs.

To address these questions, we tested the effects of BLA inactivation in an effortful choice task that required animals to choose between working for a preferred reward and consuming a concurrently available, lower cost, less preferred reward. Given that all previous reports assessed manipulations that were introduced after choice behavior was learned, we assessed the role of BLA on (i) the early learning of our task (i.e., the first 4 days of the choice), and (ii) choices after performance on our task was stable. For the former, we employed a between-subjects design to determine how animals having never experienced the initial learning of the choice with BLA

online would compare with controls and for the latter, we made use of a within-subjects design to maintain consistency with the majority of previous reports with BLA offline after initial learning and stable choices had been achieved. We also tested the effects of BLA inactivation on sensitivity to devaluation via selective satiation. Given that BLA inactivations and lesions produce work-aversion for rewards of greater magnitude in previous studies, it was hypothesized that this manipulation would similarly decrease effort for the qualitatively preferred option in our task. It was also predicted that BLA inactivations would impair animals' ability to use a value representation (in this case, one of qualitative preference) to guide their effortful behavior.

Method

Subjects. A timeline of all procedures is shown in **Fig. 2-1**. Subjects were 26 adult male Long-Evans rats (Charles River Laboratories, Hollister, CA, USA), singly housed for all phases of experiments with the exception of the acclimation period and handling. All animals were handled for 10 min in pairs for 5 days after a brief acclimation period in the vivarium (see below for details). Rats weighed an average of 250 g at the beginning of the experiments. Seventeen rats were assessed for effects of BLA inactivation across the first 4 days of effortful choice (n = 9 inactivation, n = 8 vehicle control), using a between-subjects design. Nine rats were allowed to reach stable choice performance prior to testing, and were tested under both control and inactivation (n = 9) conditions, using a within-subject design. One of these rats was omitted due to a target miss confirmed by histological verification. Due to a cannula clog and unexpected death, two additional rats did not receive the behavioral tests that followed effortful choice testing. Thus, one rat was omitted from all data, and the behavioral tests that followed effortful choice testing were conducted on 23 (out of 26) subjects. All animals weighed between 300 and 310 g at the time

of euthanasia and brain collection. The vivarium was maintained under a 12/12 h reverse light cycle at 22 °C. All procedures were approved by the Chancellor's Animal Research Committee at the University of California, Los Angeles.

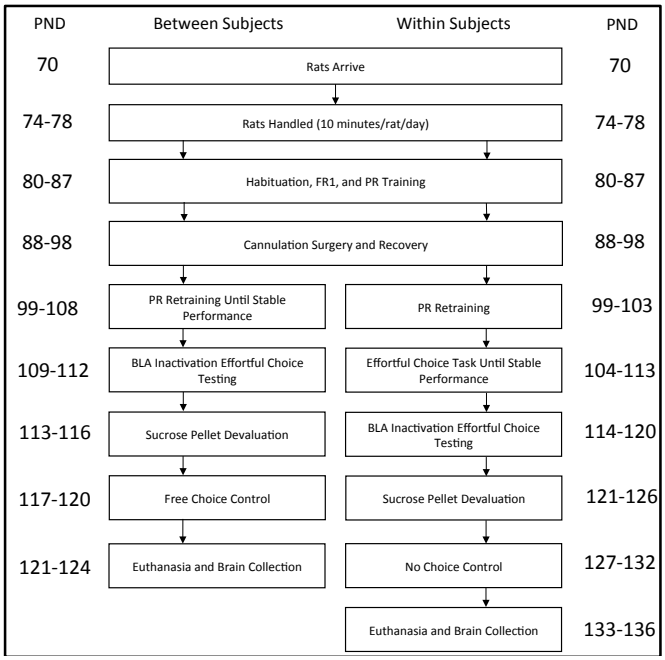

Figure 2-1. Timeline of events. Sequence of handling, surgery, testing, and euthanasia are depicted from top to bottom order. Between-subject (left) and within-subject (right) assessments were matched closely for postnatal day (PND).

Food restriction. Rats were pair housed and left undisturbed for 3 days after arrival to our facility to acclimate to the vivarium. All rats were food restricted to no < 85% of their free-feeding body weight to ensure motivation to work for food during behavioral testing, while water was always available ad libitum, except during the 30 min testing sessions. On the last day of handling prior to behavioral training, rats were fed ten sucrose pellets in their home cage to introduce them to those food rewards. Weights were monitored daily to ensure a healthy body weight, and rats were fed 12 g of food daily following behavioral testing as well as on non-testing days. Thus, on testing days animals consumed the freely available chow during testing (~6 g), the sucrose pellets that

they worked for, and 12 g of additional chow following testing. This allowed for modest weight gain throughout the course of testing, despite adherence to a food restriction regimen.

Apparatus. Training and testing were conducted in chambers outfitted with a house light, internal stimulus lights, food-delivery magazine, and two retractable levers positioned to the left and right of the chamber wall opposite the magazine. All hardware was controlled by a PC running Med-PC IV (Med-Associates, St. Albans, VT, USA). Training and testing were conducted during the early portion of the dark cycle (~8 to 11 am) and consisted of 30-min sessions. Food rewards obtained during training and testing were 45-mg dustless precision sucrose pellets (Bio-Serv, Frenchtown, NJ, USA). Experiments were conducted 5 days per week between the hours of 08:00 and 11:00 h, and animals were fed once daily on weekends.

Effortful choice training and testing

Habituation. On the first day of behavioral testing, rats were transferred to single housing. Five sucrose pellets were dropped into the magazine, and rats were then placed into the operant chambers with the house light on. The rats were left in the boxes for 15 min, at which point they were removed. If a rat had eaten all five pellets during that time it was allowed to advance to the next stage. If not, habituation was repeated daily until all the pellets were consumed.

Training. Rats were trained to press the lever on a fixed ratio schedule in which each lever response was reinforced with one sucrose pellet. Once a rat acquired 30 pellets within 30 min, it was shifted to the progressive ratio (PR) phase. In this stage, the required number of presses increased according to the formula:

$$n_i = 5e^{(i/5)} - 5$$

where n_i is equal to the number of presses required on the ith ratio, rounded to the nearest whole number, after five successive schedule completions. All rats were given five daily sessions of PR training prior to surgery. Rats were given further PR training after recovery from surgery (see below).

Surgery. Rats were anesthetized with isoflurane in oxygen at a rate of 2L/min (5% induction, 2–2.5% maintenance) and mounted on a stereotaxic apparatus (Model #963; Kopf Instruments, Tujunga, CA, USA). Respiratory rate and body temperature were monitored throughout the surgery. The head was shaved and skin was incised (anterior to posterior), retracted using hemostats, and the head position was adjusted to fit Bregma and Lambda on the same horizontal plane. Burr holes were drilled bilaterally on the skull for the placement of 22 gauge guide cannulae (PlasticsOne, Roanoke, VA, USA), after which four burr holes for anchor screws were drilled. The coordinates used for the guide cannulae targeting the BLA were adapted from previous reports (Ghods-Sharifi et al., 2009; Wassum et al., 2011): AP = −3.0 mm, ML = ±5.1 mm, DV = −7.0 mm from Bregma. Anchor screws were inserted, and guide cannulae were lowered and cemented in place using Bosworth Trim II dental acrylic. Stainless steel dummy cannulae were inserted through the guide cannulae until the time of infusion. The rats were placed on a heating pad and kept in recovery until ambulatory before being put back into the vivarium. Post-operative care consisted of five daily injections of carprofen (5 mg/kg, s.c.). Following a 7-days free-feeding recovery period, rats were put back on food restriction and PR training resumed.

PR retraining. Between-subjects animals were retrained on the PR schedule until stable performance was achieved (~10 daily sessions), defined as the number of lever presses across 3 days of testing falling between 85 and 115% of the mean of those 3 days. Once stable progressive

ratio performance was achieved, animals were counterbalanced for number of lever presses and assigned to either control (n = 7) or BLA inactivation (n = 8) conditions, according to a between-subject design. These animals were given infusions across the first 4 days of exposure to the choice procedure. The within-subject group (n = 9) was given 5 days of PR retraining, after which rats were introduced to the choice procedure and allowed to reach stable lever pressing performance in the choice task prior to infusion.

Effortful choice testing. Upon meeting criteria for stable PR performance, a ceramic ramekin containing 18 g of lab chow was introduced [modified from (Randall et al., 2012)]. Rats were free to choose between consuming freely available but less preferred chow or lever pressing for preferred sucrose pellets. The ramekin was located near the magazine in the opposite corner of the box from the lever to ensure that the two activities were mutually exclusive. After every 30-min testing session, the number of presses, the number of reinforcers, the highest ratio achieved and the amount of chow remaining (including chow dropped through the bars and collected in a tray) were recorded.

Infusion testing. Inactivation procedures were adapted from previous reports (Floresco & Ghods-Sharifi, 2007; Ghods-Sharifi et al., 2009). Inactivation of the BLA was achieved by microinfusion of a solution containing the GABAB agonist baclofen and the GABAA agonist muscimol (Sigma-Aldrich). Both drugs were dissolved in artificial cerebrospinal fluid (aCSF), mixed separately at a concentration of 500 ng/μL, and then combined in equal volumes so that the final concentration of each compound in solution was 250 ng/μL. Drugs or aCSF were infused at a volume of 0.5 μL so that the final dose of both baclofen and muscimol was 125 ng per side. Infusions of baclofen/muscimol or aCSF were administered bilaterally into the BLA via 28 gauge internal

cannulae (Plastics One, Roanoke, VA, USA) that protruded 1.0 mm past the end of the guide cannulae, at a rate of 0.25 μL/min by a microsyringe pump. Injection cannulae were left in place for an additional 1 min to allow for diffusion. Each rat remained in its home cage for an additional 10-min period prior to testing. Infusions were conducted 10 min prior to: each of the first 4 days of effortful choice testing (in the between-subjects design); effortful choice testing (in the within-subjects design); satiation procedures; free choice control and no choice control tests.

Sucrose pellet devaluation via specific satiety. To assess the effect of incentive state on effortful choice, and the role of BLA, all animals underwent a specific outcome devaluation task. After effortful choice testing on a separate test day, infusion of baclofen/muscimol or aCSF took place prior to allowing rats 30 min of unrestricted sucrose pellet availability in empty cages immediately prior to behavioral testing. Leftover pellets were weighed to ensure adequate devaluation could be achieved (at least 5 g consumed). To determine how sensitive animals were to the satiation procedure, data were analyzed by calculating difference scores (the session preceding devaluation testing – the devaluation testing session) for the number of lever presses and amount of chow consumed. One animal from the within-subjects group did not receive this test due to a cannula clog, and one animal from the between-subjects group also did not undergo this test, due to unexpected death.

Freely available choice (Free Choice Control). The between-subjects rats (n = 15) received a free choice control test. The within-subjects animals did not receive this test. This was done to limit the maximum number of infusions per animal to six. We conducted this test to assess potential effects of BLA inactivation on food preference, and to ensure that sucrose pellets would still be preferred over chow when both reinforcers had equal work requirements (i.e. when both were

freely available). Following devaluation testing and on a separate test day at least 48 h later, rats were infused with either aCSF (n = 7) or baclofen/muscimol (n = 8) prior to 30 min of free access to pre-weighed amounts of sucrose pellets and lab chow (~18 g each) in standard empty cages. Following the 30-min period, remaining food was collected and weighed to determine animals' food preferences when differential effort requirements were eliminated. One animal did not receive this test due to unexpected death.

No Chow available (No Choice Control). The within-subjects rats were given a no choice control test. The between-subjects animals did not receive this test. This was done to limit the maximum number of infusions per animal to six. This control experiment was conducted to rule out potential confounds mediating BLA effects on our effortful choice task-inability to lever press, impaired memory for lever pressing, or impairments in primary motivation to obtain sucrose pellets. Following devaluation testing and on a separate testing day at least 48 h later, rats were infused with aCSF (n = 8) or baclofen/muscimol (n = 8) 10 min prior to being placed in the operant chamber. During these tests, we omitted the ceramic ramekin with freely available lab chow to assess whether BLA inactivations decreased lever pressing in the absence of choice. One animal did not receive this test due to a cannula clog.

Cannula placement verification. Following behavioral testing, animals were humanely euthanized with 0.8 mL Euthasol (390 mg/mL pentobarbital, 50 mg/mL phenytoin; Virbac, Fort Worth, TX, USA). Animals were transcardially perfused with 100 mL isotonic saline followed by 100 mL 10% buffered formalin acetate (Fisher) at a flow rate of 10 mL/min. Brains were stored in 10% buffered formalin acetate for 2 days followed by 30% sucrose for 3 days, after which 50 micron coronal sections were taken using a cryostat and mounted on slides. After staining with

Cresyl Violet, slices were visualized with a BZ-X710 microscope (Keyence, Itasca, IL, USA) and analyzed with BZ-X Viewer software. Placements were determined by comparison with a standard rat brain atlas (Paxinos & Watson 1997) (**Fig. 2-7**).

Data analysis. All statistical tests were conducted using SPSS. Early effortful choice data were analyzed by repeated-measures ANOVA with condition as a between-subjects factor. Stable choice performance data were analyzed by separate paired t-tests comparing number of lever presses and amount of chow consumed on the control and BLA inactivation testing sessions. Devaluation data were analyzed by calculating difference scores (the session preceding devaluation testing – the devaluation testing session) for number of lever presses and amount of chow consumed. Unpaired t-tests (the between subjects experiment) or paired t-tests (the within subjects experiment) were used to compare control vs. BLA inactivation effects. Free choice control data were analyzed by two-way anova, and no choice control data were analyzed by paired t-test.

Results

Early effortful choice learning. There were no post-surgical (pre-infusion) differences, when rats stabilized on progressive ratio, prior to the introduction of choice (t_{14} = 0.529, P = 0.605; control = 1034.38 ± 150.90; inactivation = 950.75 ± 47.01). Animals then received either aCSF or baclofen/muscimol infusions across the first four sessions of choice exposure. Analysis of lever pressing data by repeated-measures anova revealed no effect of session ($F_{3,42}$ = 0.248, P = 0.862) and no significant session by condition interaction ($F_{3,42}$ = 0.671, P = 0.575). A significant between-subject effect of condition was found ($F_{1,14}$ = 8.746, P = 0.01; control = 342.38 ± 70.44; inactivation = 151.44 ± 25.50) such that animals in the BLA inactivation condition engaged in

significantly fewer lever presses for sucrose pellets (**Fig. 2-2A**). Conversely, analysis of chow consumed by repeated-measures anova revealed no effect of session ($F_{3,42} = 0.576$, P = 0.634), no significant session by condition interaction ($F_{3,42} = 1.297$, P = 0.288) or between-subjects effect of condition ($F_{1,14} = 0.094$, P = 0.764), (**Fig. 2-2B**).

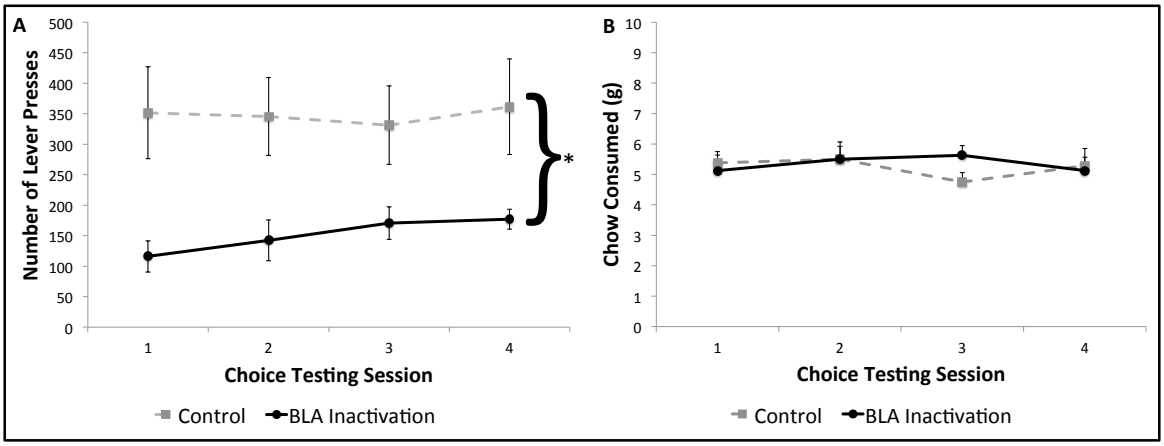

Figure 2-2. Between-subject effect of BLA inactivation early in effortful choice. (A) Number of lever presses on each of the first four sessions of the effortful choice task. BLA inactivation with baclofen/muscimol resulted in significantly fewer lever presses. (B) Amount of chow consumed on each of the first four sessions of the effortful choice task. BLA inactivation produced no difference in amount of chow consumed. Points denote mean ± SEM, *p<0.05

Stable effortful choice performance. Animals tested according to the within-subject design were first allowed several sessions of effortful choice testing until stable lever pressing performance was achieved (on average, 10 sessions), **Fig. 2-3A**. Chow consumption was also stable during this time, **Fig. 2-3B**. A significant difference in number of lever presses under control and BLA inactivation conditions was revealed by a paired t-test ($t_8 = 6.659$, P = 0.00016; control = 266.00 ± 38.92; inactivation = 168.67 ± 28.97) such that animals pressed significantly less in the BLA inactivation condition than in the control condition (**Fig. 2-3C**). Importantly, there

was no significant difference in the amount of chow consumed between these conditions (t_8 = 2.294, P = 0.051), (**Fig. 2-3D**).

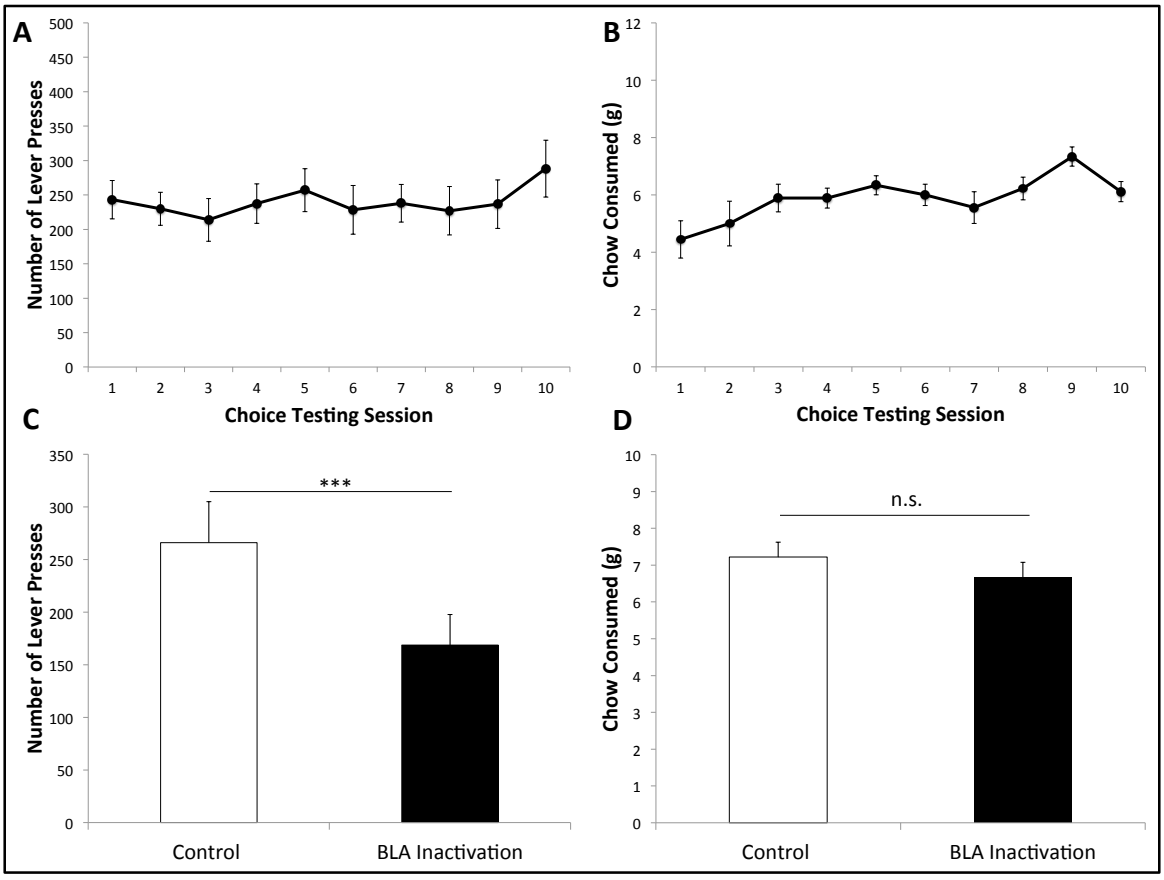

Figure 2-3. (A) Number of lever presses on the first ten sessions of the effortful choice task. (B) Amount of chow consumed on each of the first ten sessions of the effortful choice task. (C) Number of lever presses on vehicle versus BLA inactivation testing sessions. BLA inactivation with baclofen/muscimol resulted in significantly fewer lever presses (p<.001). (D) Amount of chow consumed on control versus BLA inactivation testing sessions. BLA inactivation produced no difference in amount of chow consumed. Bars denote mean ± SEM, ***p<0.001, n.s. nonsignificant.

Sucrose pellet devaluation. In the between-subjects design, devaluation effects were determined by calculating difference scores (number of lever presses on the session preceding devaluation minus number of lever presses on the devaluation test session). Analysis of difference scores by unpaired t-test revealed that control animals exhibited a larger difference between pre-post devaluation (t_{13} = 3.093, P = 0.009; control = 303.14 ± 77.19; inactivation = 68.88 ± 22.39), (**Fig. 2-**

4A). Difference scores were also calculated for chow consumption as well. An unpaired t-test revealed that difference scores for chow consumption were not different between control and BLA inactivation (t_{13} = −0.295, P = 0.773), (**Fig. 2-4B**). Following satiety with sucrose pellets, BLA

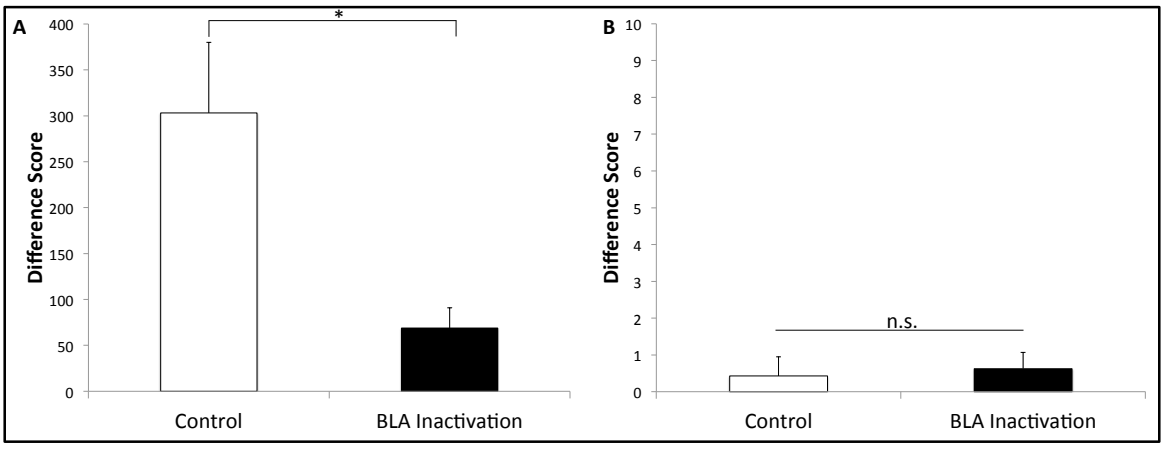

Figure 2-4. Between-subject effect of BLA inactivation on sucrose pellet devaluation testing. (A) Difference scores were calculated by subtracting the number of lever presses on the day of devaluation testing from the session preceding devaluation testing for the vehicle control and BLA inactivation (baclofen/muscimol) group. Difference scores were significantly higher in the control animals than the inactivation group. (B) Difference scores were calculated by subtracting the amount of chow consumed on the day of devaluation testing from the session preceding devaluation testing for the vehicle control and BLA inactivation group. Groups did not differ in the amount of chow they consumed on the devaluation test relative to the preceding testing session. Bars denote mean ± SEM, *p<0.05, n.s. nonsignificant.

inactivation animals pressed more than control animals (t_{13} = 2.627, P = 0.021; control = 58.29 ± 12.69; inactivation = 108.1 ± 13.19).

In the within-subjects design, analysis of difference scores by paired t-test revealed that animals tested under the vehicle control condition, relative to their BLA inactivation phase, exhibited a larger difference between pre-post satiety with sucrose pellets (t_7 = 5.567, P = 0.0008; control = 147.25 ± 25.22; inactivation = 114.13 ± 25.87), (**Fig. 2-5A**). Difference scores were also calculated for chow consumption. A paired t-test revealed that difference scores for chow

consumption were not different between the control and BLA inactivation conditions (t_7 = 1.426, P = 0.197), (**Fig. 2-5B**). During the test following pre-feeding with sucrose pellets,

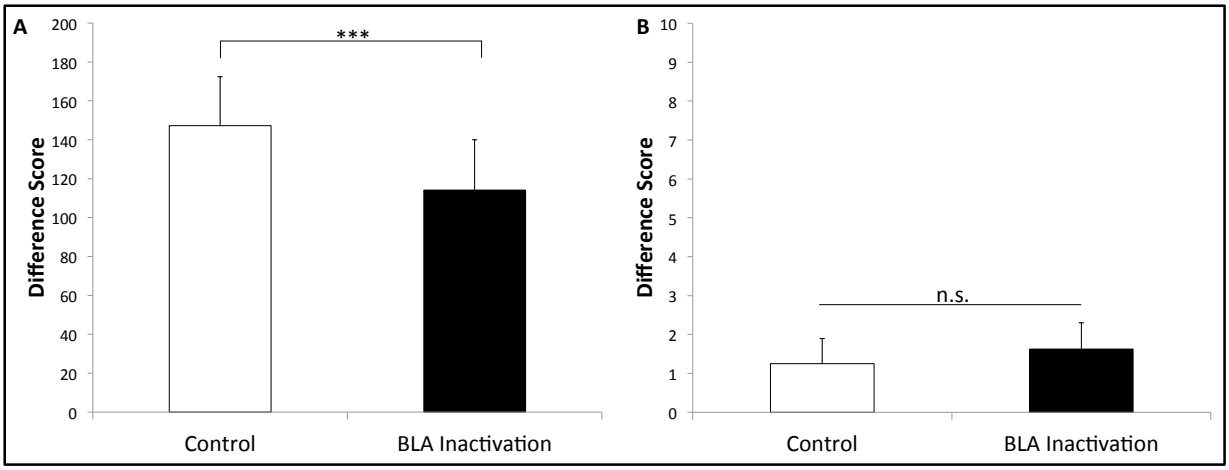

Figure 2-5. Within-subject effect of BLA inactivation on response to sucrose pellet devaluation. (A) Difference scores were calculated by subtracting the number of lever presses on the day of devaluation testing from the session preceding devaluation testing for both the vehicle control and BLA inactivation (baclofen/muscimol) devaluation tests. Difference scores were significantly higher in the vehicle control infusion test. (B) Difference scores were calculated by subtracting the amount of chow consumed on the day of devaluation testing from the session preceding devaluation testing for both the vehicle control and BLA inactivation (baclofen/muscimol) devaluation tests. In both conditions, rats did not differ in the amount of chow they consumed on the devaluation test relative to the preceding testing session. Bars denote mean ± SEM, ***p<0.001, n.s. nonsignificant.

animals pressed more in the BLA inactivation condition than in the control condition (t7 = 5.567, P = 0.0008; control = 87.63 ± 15.11; inactivation = 121.75 ± 19.06).

Free choice control. Analysis of amount of food consumed (g) using a two-way anova with food type (sucrose pellet, chow) and condition (control, inactivation) as fixed factors revealed a significant effect of food type ($F_{1,26}$ = 24.291, P = 0.0001; sucrose = 7.71 ± 0.62; chow = 3.57 ± 0.26). No significant food type by condition interaction ($F_{1,26}$ = 1.222, P = 0.2791), or effect of condition ($F_{1,26}$ = 0.089, P = 0.767) was found, (**Fig. 2-6A**).

No choice control. A control task was conducted wherein animals were infused with either vehicle or baclofen/muscimol and tested on the progressive ratio without concurrently available chow. A

paired t-test revealed no significant difference between the conditions (t_7 = 0.217, P = 0.835), (**Fig. 2-6B**).

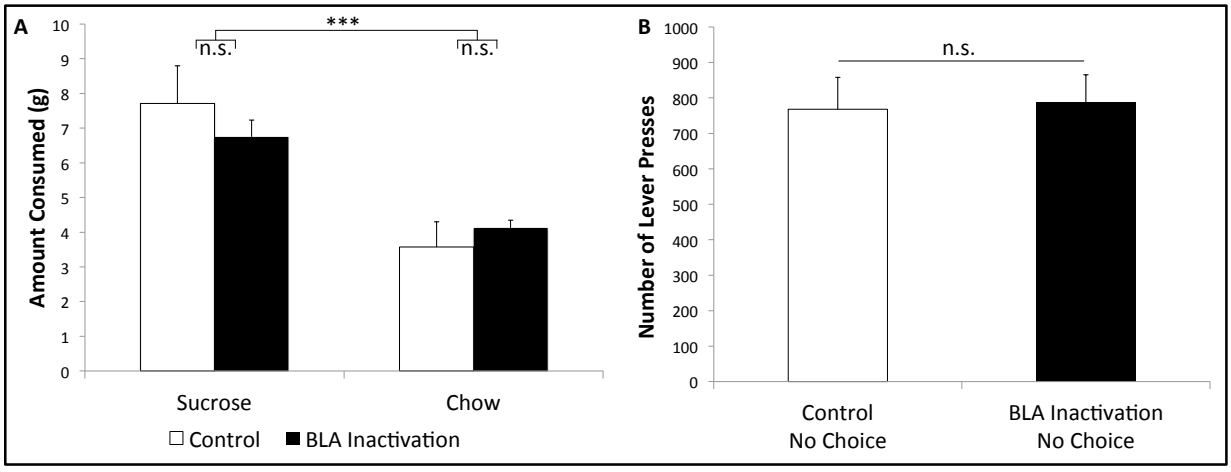

Figure 2-6. Free choice and no choice control tasks. (A) The between-subject animals received a test wherein both sucrose pellets and chow were freely available. There was a main effect of food type, but no effect of control or inactivation condition, and no food type x condition interaction. (B) The within-subject animals received a test wherein freely-available chow was not concurrently available. There was no significant difference in number of lever presses in the vehicle control and BLA (baclofen/muscimol) inactivation conditions. Bars denote mean ± SEM, ***p<0.001, n.s. nonsignificant.

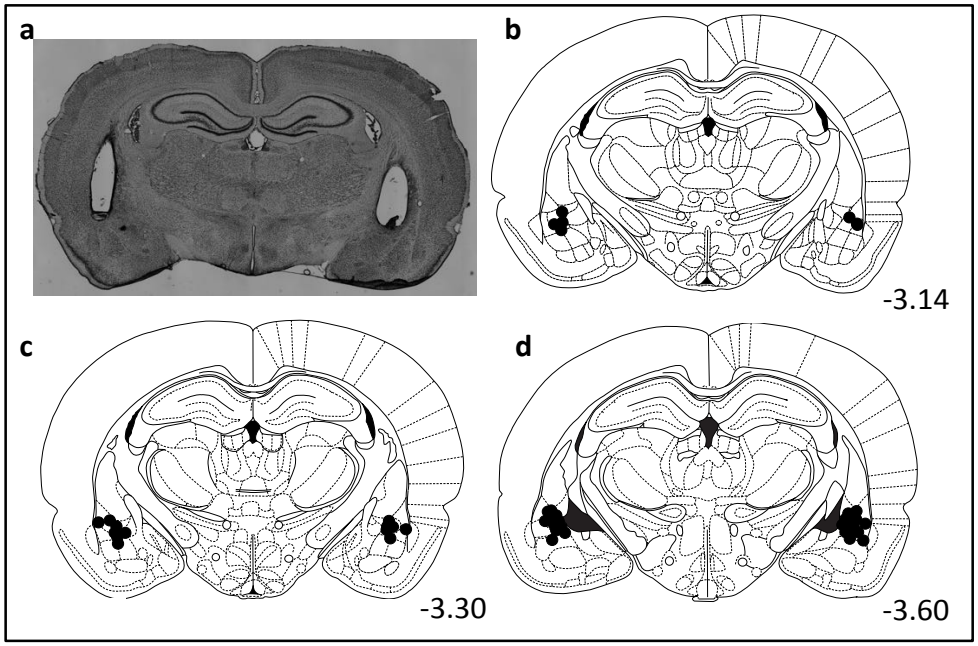

Figure 2-7. Reconstructions of infusion sites. (a) Representative photomicrograph of coronal section showing cannula tracks. Injectors extended 1 mm below the cannula track. (b-d) Depictions of coronal sections adapted from Paxinos & Watson (1997). Sites of infusions arranged from anterior to posterior where numerals on the lower right of each matched section represent the anterior-posterior distance

Discussion

BLA in learning and performance of effortful choice

Given that BLA exerts an important role in appetitive learning particularly during early experience (Wassum & Izquierdo, 2015), we investigated the role of BLA during both the initial phase of our effortful choice task as well as during well-established choice performance. Previous studies of the mechanisms of effortful choice have consistently allowed animals several days of testing to reach stable performance prior to any brain manipulation (Nowend *et al*., 2001; Floresco & Ghods-Sharifi, 2007; Ghods-Sharifi *et al*., 2009; Nunes *et al*., 2013), thus it was important to understand the role of initial learning vs. later performance in our task. We found that BLA inactivation resulted in significantly fewer lever presses on the first four choice testing days but also after extensive experience with the choice. This suggests that BLA is generally important in supporting the cost calculations involved in working for a qualitatively better option.

Basolateral Amygdala inactivation had no effect on the consumption of freely available lab chow during effortful choice testing, providing evidence that this manipulation did not reduce appetite. Indeed, appetite suppressants in similar tasks reduce both lever pressing and chow consumption (Salamone *et al*., 2002; Randall *et al*., 2012). Although we did not observe a concomitant increase in chow consumption, these findings are consistent with previous reports using a similar task (Randall *et al*., 2012): Impairments in work output on a progressive ratio choice task are often not accompanied by compensatory increases in chow consumption due to the fact that these animals are already close to the ceiling for chow consumption during a 30-min period (Randall *et al*., 2010, 2012).

We conducted several control tasks to ascertain that BLA effects on lever pressing in our task were due to alterations in effortful choice rather than alterations in preference or discrimination of different type of rewards. Because the amygdala is known to be involved in feeding behavior (Gosnell, 1987; Cooper, 2005), one potential explanation for our findings was that BLA inactivation simply decreased preference for the sucrose pellets. In a free-feeding choice task, we found a significant main effect of food type (sucrose pellets preferred over chow) and no interaction, indicating that BLA inactivation did not change food preferences when effort requirements were equal. We also conducted a task to ensure that BLA inactivation did not produce a general work aversion, memory impairment, or inability to lever press. In support of previous work in monkeys (Aggleton & Passingham, 1982; Parkinson *et al*., 2001) and a specific effect on cost-benefit evaluation, we found that omitting the freely available lab chow as an option resulted in an equal number of lever presses on control and BLA inactivation test sessions.

BLA in quality and quantity choices

Previous studies investigating the role of the BLA in effortful choice have used tasks that vary the reward magnitude (Floresco & Ghods-Sharifi, 2007; Ghods-Sharifi *et al*., 2009; Ostrander *et al*., 2011). However, both rodents and primates are often required to make decisions about *qualitatively* different rewards. Consistent with previous findings that BLA is important in choosing to work for a quantitatively preferred (i.e. higher magnitude) reward (Ghods-Sharifi *et al*., 2009; Ostrander *et al*., 2011), we report the first evidence that BLA is necessary for choosing to exert effort for a qualitatively preferred reward. This paradigm may be more relevant to real-world human and animal behavior where option space often includes several acceptable choices (e.g. cooking dinner vs. choosing fast food; (Sugrue *et al*., 2005).

However, similar to other choice tasks, our effortful progressive ratio requirement overlaps with a time cost: as the work difficulty for each successive reward increases, the amount of time to each successive reward also increases. Given that BLA has a putative role in delay discounting wherein lesions produce a time-cost averse phenotype (Winstanley *et al*., 2004), it is possible that our effects may be partially mediated by a decreased tolerance of time. However, previous studies that had an equal delay-to-reward with differential effort requirements showed that BLA inactivations still resulted in increased selection of the lower effort option (Ghods-Sharifi *et al*., 2009), indicating that the effects of BLA on effort discounting are at least partially dissociable from effects on time discounting. Therefore, the BLA is involved in cost-benefit analyses involving both time and effort, and future experiments should resolve these costs.

BLA and subjective value

We found that difference scores – a measure of how well animals are able to use a change in motivational state to guide effortful behavior – to be significantly greater in the control condition than in the BLA inactivation condition. This supports the idea that BLA inactivation renders animals less able to use changes in motivational state to appropriately guide their choices. In our task, freely available chow consumption was unaffected by sucrose pellet devaluation, indicating that the devaluation procedure had a more selective effect on choice of the preferred reward.

Though the present experiment was not designed to be a replication of prior devaluation studies, our results are generally consistent with previous reports using similar procedures: both primate amygdala (Malkova *et al*., 1997; Izquierdo & Murray, 2007) and rodent BLA (Balleine *et al*., 2003) have been shown to be important in using outcome value to guide choice behavior. We found the same pattern of effects in both our between- and within-subject

assessments: BLA inactivation produced a less-pronounced decrease in effortful performance following satiety with sucrose pellets than during the control condition.

The present study is one of the first assessments of effortful choice following a satiation procedure (Mai *et al*., 2012). Mai *et al*. (2012) studied effort-based choice on a T-maze following ventral striatal 6-OHDA lesions and found that rats were still sensitive to the effects of satiation, unlike our present finding of animals' reduced sensitivity to devaluation of sucrose pellets following BLA inactivation. This supports the idea, as recently suggested (Wassum & Izquierdo, 2015) that BLA dynamically encodes value with a cost parameter, akin to a 'subjective value,' that may be used by the striatum to contribute to action. Given the pattern of results we report here together with Mai *et al*. (2012), we can infer that BLA encoding of this subjective value precedes any striatal input in this process, likely by conferring the preferred option with specific value within the space of concurrently available options. Indeed, if the freely available lab chow is not presented concurrently for choice, BLA inactivations have no effect on the number of lever presses for the preferred reward. Our data also indicate that measures of primary motivation, such as progressive ratio responding for reward in the absence of choice, remain intact with BLA offline, consistent with previous reports in non-human primates (Aggleton & Passingham, 1982; Parkinson *et al*., 2001).

There are limitations to the present work. An alternative explanation for our effects after devaluation could be that BLA inactivation pushed animals closer to floor-level responding-leaving less room for change. We think this is not likely: In the within-subjects animals and during the BLA inactivation test, the average number of lever presses was well above what would be considered very low-level responding. This left BLA inactivation animals with sufficient room to

suppress lever pressing further. Moreover, following devaluation of sucrose pellets BLA inactivation animals lever pressed more, not less, than in the vehicle condition. Given these observations, it seems unlikely that any sort of floor effect mediated the present findings.

Another issue lies with the interpretation of our results in the context of goal-directed and/or habitual responding. It is well-understood that with extensive overtraining, behaviors can shift from a flexible, goal-directed strategy to one that is automated and inflexible, or a habit (Dickinson, 1985; Balleine & Dickinson, 1998). Furthermore, through reinforcer devaluation a response that is goal-directed would be attenuated, but a response that is habitual would not (Dickinson, 1985; Balleine & Ostlund, 2007). Critically, this seminal work typically featured manipulanda for which experimenters could omit the outcome during devaluation testing, yet this was not a possibility in the present experiment due to one of our outcomes being freely available. For this reason, we were unable to conduct devaluation testing in extinction, as in the cited work. Therefore, any conclusions regarding whether the behaviors we assess here are truly goal-directed or habitual cannot be made convincingly as animals continued to receive feedback from receipt of the now-devalued outcome. However, our findings that BLA inactivation rendered animals less able to adapt their responses following a change in their motivational state (induced by satiety) are broadly consistent with previous work supporting a role of BLA in encoding specific outcome value.

Conclusion

We found that BLA is necessary for choosing to exert effort for a preferred option as well as updating value to guide effortful choice behavior. Individuals encounter decisions based on effort demands daily, and disturbances in effort occur in a wide range of conditions, including

depression (Treadway *et al*., 2012), schizophrenia (Fervaha *et al*., 2013; Gold *et al*., 2013), Parkinson's disease (Friedman *et al*., 2010), multiple sclerosis (Lapierre & Hum, 2007), and autism (Damiano *et al*., 2012). Historically, the striatum has been a key focus in the study of disorders of motivation. More investigation of BLA in cost-benefit decision making may also prove fruitful in understanding these disorders.

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Chapter 3: ACC in quality effort-based choice task

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Abstract

The anterior cingulate cortex (ACC) is known to be involved in effortful choice, yet its role in cost-benefit evaluation of qualitatively different rewards (more/less preferred), beyond magnitude differences (larger/smaller), is poorly understood. Selecting between qualitatively different options is a decision type commonly faced by humans. Here, we assessed the role of ACC on a task that has primarily been used to probe striatal function in motivation. Rats were trained to stable performance on a progressive ratio schedule for sucrose pellets and were then given sham surgeries (control) or excitotoxic NMDA lesions of ACC. Subsequently, a choice was introduced: chow was concurrently available while animals could work for the preferred sucrose pellets. ACC lesions produced a significant decrease in lever presses for sucrose pellets compared to control, whereas chow consumption was unaffected. Lesions had no effect on sucrose pellet preference when both options were freely available. When laboratory chow was not concurrently available, ACC-lesioned rats exhibited similar lever pressing as controls. During a test under specific satiety for sucrose pellets, ACC-lesioned rats also showed intact devaluation effects. The effects of ACC lesions in our task are not mediated by decreased appetite, a change in food preference, a failure to update value or a learning deficit. Taken together, we found that ACC lesions decreased effort for a qualitatively preferred option. These results are discussed with reference to effects of striatal manipulations and our recent report of a role for basolateral amygdala in effortful choice.

Introduction

Organisms must frequently overcome physical effort costs in the pursuit of rewards. The striatum has been identified as a key region in regulating effort-related functions (Salamone et al., 1991, 2001, 2007; Nowend et al., 2001; Nunes et al., 2013). The basolateral amygdala (Floresco & Ghods-Sharifi, 2007) and cortical regions, namely the medial frontal cortex and specifically the anterior cingulate cortex (ACC; Walton et al., 2002, 2003, 2007; Rudebeck et al., 2006), have also been assigned important roles in decisions involving different effort costs when choosing between whether to work for more reward or to forego the effort cost and select the lower magnitude reward instead.

In seminal work by Walton et al. (2002), it was found that lesions encompassing prelimbic cortex, infralimbic cortex and ACC (i.e. the medial wall) biased animals' choices towards the low effort (low magnitude) option in a T-maze task. This effect was not mediated by an inability to physically perform the task, a memory impairment for the arm-reward magnitude assignment (i.e. animals chose the higher magnitude rewards when effort costs were equivalent) or a reduction in pursuing the rewards when they were freely available. In a subsequent study by this same group, lesions were specific to PL, IL or ACC. Lesions of PL and IL failed to produce any effect on effort-related choice behavior, and ACC lesions reproduced the effects of the large lesions administered in the earlier study (Walton et al., 2003). Other work replicated this effect and further probed the neurochemical signaling and circuitry mediating this effect: Schweimer & Hauber (2006) found that blockade of dopamine D1-like receptors in the ACC impaired effort in a barrier climbing task and that disconnection of the ventral striatum and ACC similarly resulted in the work-averse phenotype following either ACC or ventral striatal lesions alone (Hauber & Sommer, 2009). Similarly, disconnection of the basolateral amygdala and ACC impairs effort allocation (Floresco & Ghods-Sharifi, 2007). Thus, these regions have already been identified as critical components

of the brain circuitry that regulates effort in the context of choice between options of different magnitudes.

Previous work investigating the role of ACC in effortful choice in rodents made use of T-maze tasks in which the rat selected among different magnitudes of the same reward identity (more vs. less). We previously reported that basolateral amygdala supports the maintenance of value and effortful choice of a qualitatively preferred option (working for pellets vs. freely available chow; Hart & Izquierdo, 2017). It remains unknown whether ACC has a similar function in choosing between qualitatively different reinforcers. A task that involves qualitatively different reinforcers may more closely match that of the human condition where we are faced with different options that are more/less preferred, not differences in magnitude of the same reward. Additionally, previous studies probing regions other than the striatum (e.g. amygdala, ACC) in physical effort have primarily used T-maze tasks. Therefore, a thorough investigation of ACC effects in a task where animals can self-titrate the amount of effort they are willing to exert allows direct comparison with striatal manipulations and further understanding of the circuitry that regulates effort.

To address this question, we tested the effects of ACC lesions in the same effortful choice task as (Hart & Izquierdo, 2017) that required animals to choose between working for a preferred reward vs. consuming a concurrently available, lower cost, but less preferred reward. We assessed the role of ACC on (i) progressive ratio (PR) pressing for sucrose pellets (i.e. general motivation), and (ii) PR pressing in the presence of a freely available alternative (i.e. effortful decision-making: choosing between working for sucrose pellets vs. concurrently available laboratory chow). We also tested the effects of ACC on the choice between sucrose pellets vs. chow when these

reinforcers were both freely available. And finally, we assessed sensitivity to devaluation via satiety with the preferred sucrose pellet reward. Given that ACC lesions produced work aversion for rewards of greater magnitude and effort cost in previous studies, it was hypothesized that this manipulation would similarly decrease effort for the qualitatively preferred option in our task. It was also predicted that ACC lesions would not impair sensitivity to devaluation, consistent with a previous report in monkeys (Chudasama et al., 2013).

Method

Subjects. A timeline of all procedures is shown in **Fig. 3-1**. Subjects were 16 (n = 8 sham, n = 8 lesion) adult male Long–Evans rats (Charles River Laboratories, Hollister, CA), singly housed for all phases of experiments with the exception of the acclimation period and handling. All rats had previous experience in a behavioral radial arm maze task in an undergraduate laboratory class prior to experiments. All animals were handled for 10 min in pairs for 5 days after a brief acclimation period in the vivarium. Rats weighed an average of 300 g at the beginning of the experiments. One lesioned rat was omitted from analysis due to a target miss confirmed by histological verification. All animals weighed between 300 and 350 g at the time of euthanasia and brain collection. The vivarium was maintained under a 12/12-h reverse light cycle at 22 °C. All procedures were approved by the Chancellor's Animal Research Committee at the University of California, Los Angeles.

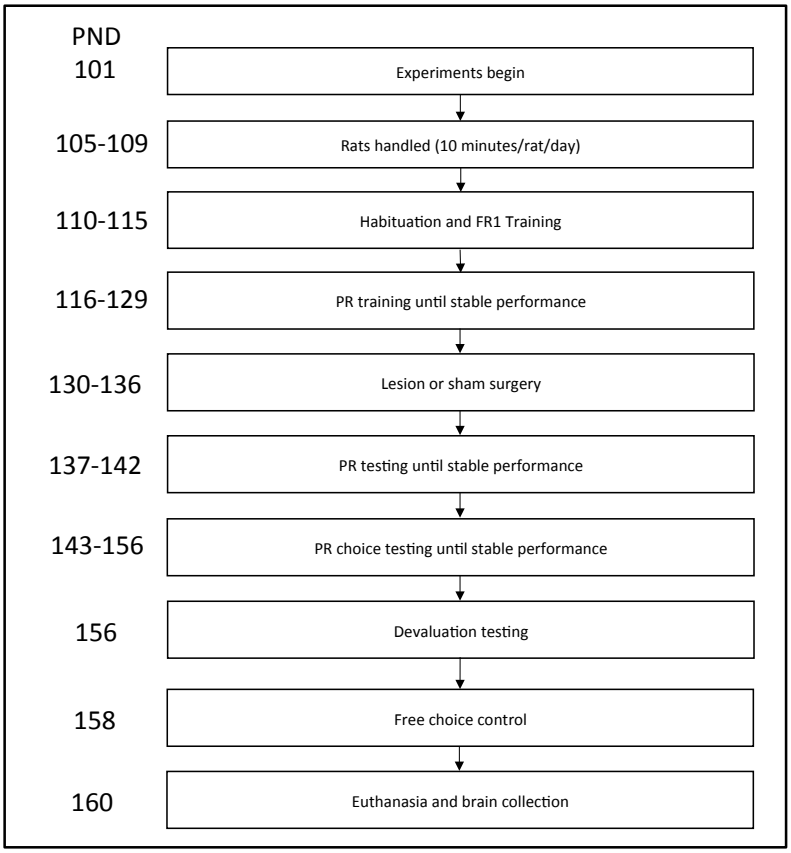

Figure 3-1. Timeline of events. Sequence of handling, surgery, testing and euthanasia are depicted from top to bottom order.

Progressive ratio training and surgery. Rats were handled, food restricted and given magazine training in Med-Associate operant chambers with hardware controlled by a PC running Med-PC IV (Med-Associates, St. Albans, VT, USA), as previously reported (Hart & Izquierdo, 2017). After this training, 30-min behavioral sessions were administered wherein rats learned to press on a PR schedule for 45 mg sucrose pellets (Bioserv, Frenchtown, NJ, USA) where the required number of presses increased according to the formula $n_i = 5e^{(i/5)} - 5$, where n_i is equal to the number of presses required on the ith ratio, rounded to the nearest whole number, after five successive schedule completions (Hart & Izquierdo, 2017). General surgical procedures were the same as recently published (Hart & Izquierdo, 2017). Animals were anesthetized with isoflurane (5% induction, 2% maintenance in 2 L/min O2). Burr holes were drilled bilaterally on the skull for insertion of 22-gauge guide cannulae (Plastics One, Roanoke, VA, USA), after which 28-gauge internal cannulae

(Plastics One) were inserted. Sham (control) surgery animals were infused with 0.5 μL saline at a flow rate of 0.25 μL/min after which injectors were left in place for one additional minute to allow for diffusion of solution. Lesion surgery animals were infused with 0.5 μL of 20 mg/mL NMDA (Sigma, St. Louis, MO, USA) dissolved in saline. The coordinates used for the guide cannulae targeting ACC were as follows: AP = +2.0 mm, ML = ±0.7 mm, DV = −1.9 mm from Bregma. Injectors extended 1 mm beyond the tip of the cannula. Following the 1-min diffusion time, the cannulae and injectors were removed, incisions were stapled closed, and the rats were placed on a heating pad and kept in recovery until ambulatory before being returned to the vivarium. Post-operative care consisted of five daily injections of carprofen (5 mg/kg, s.c.). Following a 7-days free-feeding recovery period, rats were put back on food restriction and PR training resumed. Following surgery, all animals were allowed to reach stable PR performance (85–115% of mean lever pressing across three consecutive days) as in Ref. Hart & Izquierdo (2017). Data from this phase of testing were used to assess the effects of ACC lesions on general willingness to work or primary motivation (**Fig. 3-2B**).

Effortful choice testing and devaluation. Upon meeting criteria for stable PR performance, effortful choice testing with concurrently freely available laboratory chow was conducted and was administered until stable lever pressing performance was achieved. Subsequently, to assess the effect of incentive state and the role of ACC on effortful choice, all animals underwent a satiation procedure for sucrose pellets following previously published methods (Hart & Izquierdo, 2017). To determine how sensitive animals were to this procedure, data were analyzed by calculating difference scores (the session preceding devaluation testing – the devaluation testing session) for the number of lever presses and amount of chow (g) consumed.

Freely available choice. We also conducted a test wherein both sucrose pellets and chow were freely available to assess potential effects of ACC lesions on food preference, and to ensure that sucrose pellets would still be preferred over chow when both rewards had equal work

requirements. Following devaluation testing and on a separate test day at least 48 h later, rats were given this test as previously described (Hart & Izquierdo, 2017).

Histology. Following behavioral testing, animals were killed by pentobarbital overdose (Euthasol, 0.8 mL, 390 mg/mL pentobarbital, 50 mg/mL phenytoin; Virbac, Fort Worth, TX, USA) and transcardial perfusion (Hart & Izquierdo, 2017). Brains were post-fixed in 10% buffered formalin acetate for 24 h followed by 30% sucrose for 5 days. 50-μm sections were stained for NeuN, visualized using a bz-x710 microscope (Keyence, Itasca, IL, USA) and analyzed with bz-x Viewer software. Lesions were determined by comparison with a standard rat brain atlas (Paxinos & Watson 1997). NeuN staining was performed by incubation for 24 h at 4 °C in a primary antibody consisting of 1 : 1000 rabbit polyclonal to NeuN (Abcam, Cambridge, MA, USA), 10% normal goat serum (Abcam) and 0.5% Triton X (Sigma) in PBS, followed by three 5-min washes in PBS. Secondary antibody incubations were performed for 2 h at 20 °C in the same solution with 1 : 500 goat anti-rabbit Alexa 594 (Abcam) replaced for the primary antibody, followed by three 5-min washes in PBS. Slides were subsequently mounted and cover-slipped with DAPI mounting medium (Abcam). Reconstructions of lesions are shown in **Fig. 4**.

Data analyses. Data were analyzed using spss (IBM Corp, Chicago, IL, USA) and plotted using graphpad prism (La Jolla, CA, USA). An alpha level for significance was set to $P < 0.05$. Two-way repeated-measures anova was used to compare number of lever presses and amount of chow consumed on the first four choice testing sessions. Unpaired *t*-tests on data from the last of the three stable sessions were conducted to analyze group differences on PR and on the choice task, and two-way ANOVA for group differences on food preference and number of sessions to stable performance. Devaluation effects were determined by calculating difference scores (number of lever presses on the session preceding devaluation minus number of lever presses on the devaluation test session), and analyzed with unpaired *t*-tests to probe group differences.

Results

Progressive ratio. There were no pre-surgical differences when rats stabilized on PR prior to surgery (t_{13} = 0.7835, P = 0.447; control = 913 ± 127.8; lesion = 749.3 ± 169.3; **Fig. 3-2A**). Following surgery, after allowing several days of PR testing until stable performance was reached, lesioned animals showed a slight tendency towards reduced lever pressing for sucrose pellets, although this did not reach statistical significance (t_{13} = 1.689, P = 0.115; control = 1072 ± 125.1; lesion = 740.1 ± 153.7; **Fig. 3-2B**). Animals were allowed several sessions of effortful choice testing until stable lever pressing performance was achieved.

Effortful choice testing. Group differences in effort appeared from the start of exposure to the choice task. A two-way repeated-measures anova on number of lever presses during each of the first four choice sessions revealed a significant main effect of condition ($F_{1,13}$ = 5.006, P = 0.0434), significant main effect of session ($F_{3,39}$ = 6.361, P = 0.0013) and no significant session-by-condition interaction ($F_{3,39}$ = 0.4987, P = 0.6854; **Fig. 3-2C**). We found no group differences in amount of chow consumed during the first four choice sessions: there was no effect of condition ($F_{1,13}$ = 0.6833, P = 0.4234), session ($F_{3,39}$ = 0.3982, P = 0.7550) or session-by-condition interaction ($F_{3,39}$ = 1.027, P = 0.3912; **Fig. 3-2D**). These differences in effortful choice persisted once all animals reached stable levels of performance: a significant difference in number of lever presses in the ACC-lesioned animals was revealed by an unpaired t-test (t_{13} = 2.616, P = 0.021; control = 492.3 ± 77.89; lesion = 255.1 ± 37.52) – animals pressed significantly less in the ACC lesion condition than in the control condition (**Fig. 3-2E**). Importantly, there was no significant difference in the amount of chow consumed between these conditions (t_{13} = 0.0736, P = 0.9425; control = 7.5 ± 0.7319; lesion = 7.571 ± 0.6117; **Fig. 3-2F**). Groups did not differ in the number of sessions required to reach stable performance during PR testing. A two-way anova with session

type (PR, choice) and condition (control, lesion) as fixed factors revealed no significant effect of session type ($F_{1,26}$ = 3.788, P = 0.0625; PR = 4.60 ± 0.190; choice = 5.933 ± 0.621), no effect of condition ($F_{1,26}$ = 0.0029, P = 0.9573) and no significant session type-by-condition interaction ($F_{1,26}$ = 1.169, P = 0.2895; **Fig. 3-2G**).

Freely available choice. A test wherein both sucrose pellets and laboratory chow were concurrently freely available was conducted to confirm that ACC lesion effects on PR responding were not due to decreased preference for sucrose. Analysis of amount of food consumed (g) using a two-way anova with food type (sucrose pellet, chow) and condition (control, lesion) as fixed factors revealed a significant effect of food type ($F_{1,26}$ = 38.79, P = 0.0001; sucrose = 8.727 ± 0.501; chow = 4.194 ± 0.484). No significant food type-by-condition interaction ($F_{1,26}$ = 0.4226, P = 0.5213) or effect of condition ($F_{1,26}$ = 0.5394, P = 0.4693) was found (**Fig. 3-2H**).

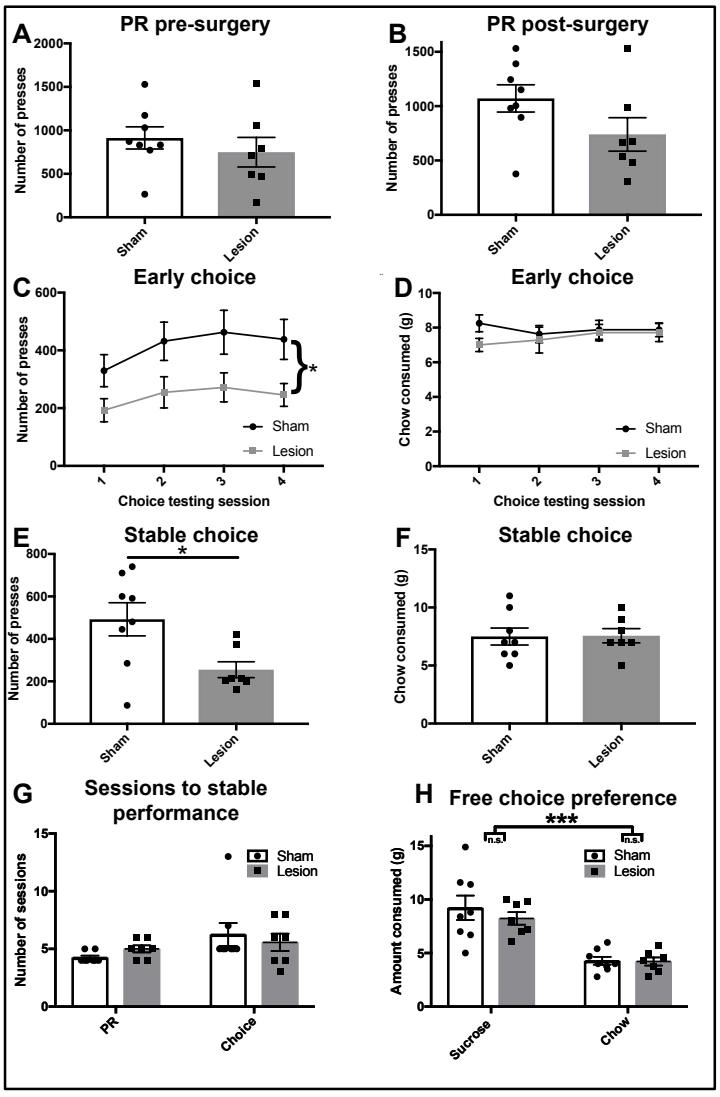

Figure 3-2. Effect of ACC lesions on PR responding, effortful choice, number of sessions to stable performance and food preference. (A) Number of lever presses on the last of the three stable PR testing sessions prior to surgery. There were no pre-existing differences in effort output. (B) Number of lever presses on the last of the three stable PR testing sessions post-surgery. ACC-lesioned animals showed a slight tendency towards attenuated lever pressing, although the difference did not reach statistical significance. (C) Number of lever presses on first four choice testing sessions, in the presence of freely available chow. ACC-lesioned animals showed significantly fewer lever presses. (D) Amount of chow consumed on the first four choice testing sessions. Groups did not differ in the amount of chow they consumed. (E) Number of lever presses on the last of the three stable choice testing sessions, in the presence of freely available chow. ACC-lesioned animals showed significantly fewer lever presses. (F) Amount of chow consumed on the last of the three stable choice testing sessions. Groups did not differ in the amount of chow they consumed. (G) Number of sessions required to reach stable PR performance and stable choice performance. Groups did not differ in the number of sessions to reach criterion. (H) In the free choice test where both sucrose pellets and chow were freely available (equivalent effort), there was a main effect of food type, but no effect of sham or lesion condition and no food type × condition interaction. Bars denote mean ± SEM, $*P < 0.05$, $***P < 0.001$.

Devaluation. Analysis of difference scores by unpaired t-test revealed that there was no significant difference between groups pre-post devaluation (t_{13} = 1.739, P = 0.1056; control = 332 ± 74.48; lesion = 179.3 ± 38.88; **Fig. 3-3A**). Difference scores were calculated for chow consumption as well. An unpaired t-test revealed that difference scores for chow consumption were not different between control and ACC lesion conditions (t_{13} = 1.104, P = 0.2896; control = 2.5 ± 0.6268; lesion = 1.429 ± 0.7514; Fig. 3-3B).

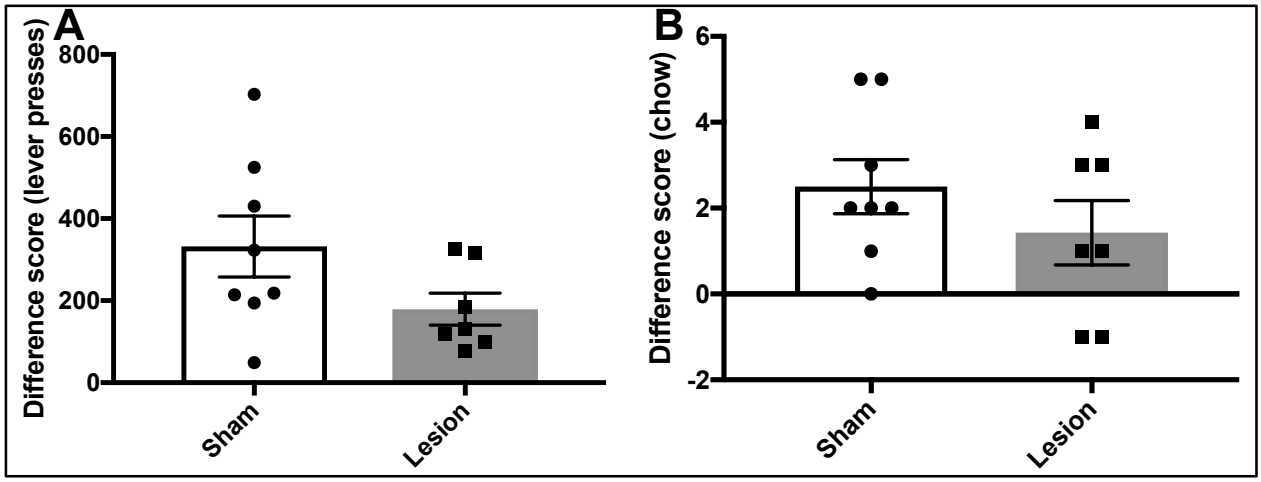

Figure 3-3. Effects of ACC lesions on sensitivity to sucrose pellet devaluation. (A) Difference scores were calculated by subtracting the number of lever presses on the day of devaluation testing from the session preceding devaluation testing for the control and ACC lesion groups. Difference scores were not significantly different between the two groups. (B) Difference scores were calculated by subtracting the amount of chow consumed on the day of devaluation testing from the session preceding devaluation testing for the control and ACC lesion groups. Groups did not differ in the amount of chow they consumed on the devaluation test relative to the preceding testing session. Bars denote mean ± SEM.

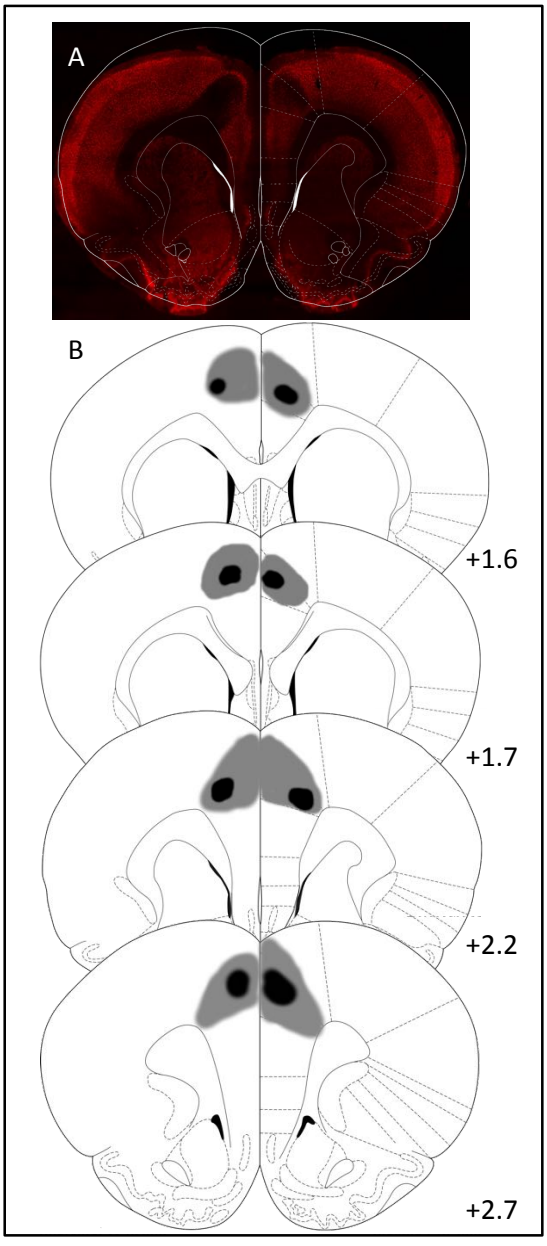

Figure 3-4. Reconstructions of lesions. (A) Representative photomicrograph of NeuN stained coronal section showing ACC lesions (AP +2.0 mm). (B) Depictions of coronal sections adapted from Paxinos & Watson (1997). Lesion areas arranged from anterior to posterior where numerals on the lower right of each matched section represent the anterior–posterior distance (mm) from Bregma. Grey and black represent maximum and minimum lesion area, respectively.

Discussion

We found that ACC lesions resulted in fewer lever presses on the PR schedule when laboratory chow was presented as a competing choice, an effect which was apparent from the beginning of choice testing that persisted even when performance was stable. This suggests that ACC is generally important in supporting the cost calculation involved in choosing how much effort to exert for a reward when a less costly alternative is concurrently available. ACC lesions had no effect on the consumption of freely available laboratory chow during effortful choice testing, providing evidence that this manipulation did not reduce appetite.

We conducted several control measures to rule out factors that could have accounted for ACC effects on lever pressing. One potential explanation for our findings was that ACC lesions simply decreased preference for the sucrose pellets. In a free-feeding choice task, we found a significant main effect of food type (sucrose pellets preferred over chow) and no interaction, indicating that ACC lesions did not change food preferences when effort requirements were equal. We also allowed animals to stabilize on the PR requirement prior to introduction of choice to investigate ACC effects on general willingness to exert effort. In our recent study of task performance after basolateral amygdala inactivations (Hart & Izquierdo, 2017), we saw a clearer dissociation between PR and choice (reduced lever pressing only in the latter condition following basolateral amygdala inactivation). Here, with ACC lesions we saw a tendency for reduced lever pressing in post-surgery PR, but with group differences only reaching significance in both early and stable choice performance. This indicates that ACC may have a broader role than basolateral amygdala in exertion to reward. Groups did not differ in the number of days required to reach stable performance on PR or the choice task, indicating that none of our effects were due to a learning deficit.

In support of related investigations of effort costs (Walton *et al*., 2002, 2003, 2007; Floresco & Ghods-Sharifi, 2007), here we also report effects of ACC lesions on effortful choice. The pattern of effects we obtained resembles that of D1 or D2 receptor antagonism (Randall *et al*., 2012) or dopamine depletion (Randall *et al*., 2014). Given that ACC sends excitatory projections to ventral striatum, it is possible that these projections mediate our effects. Indeed, disconnection of ACC and ventral striatum also reduces choice of the high-effort option in a T-maze task (Hauber & Sommer, 2009). However, another group has previously reported a null effect on PR performance when chow was freely available (Schweimer & Hauber, 2005). One potential explanation is that the experiment by Schweimer & Hauber (2005) assessed animals that had prior experience in another effort task, which may have allowed enough time and/or experience to improve performance. Indeed, recovery of function has been demonstrated after cortical lesions wherein the behavioral effects resolved spontaneously, leading to full recovery presumably by adaptations or 'repurposing' of unaffected circuits (Otchy *et al*., 2015). Such a consideration is a limitation for direct comparison of our present work (NMDA lesions) with recent work (baclofen/muscimol inactivations, Hart & Izquierdo, 2017). However, the finding that ACC-lesioned rats showed significantly reduced lever pressing in effortful choice over 2 weeks after surgery argues against this recovery of function.

Given the nature of PR, where each successive reinforcer requires more effort in addition to more time to earn, it is plausible that our effects on lever pressing could be due to a time-averse phenotype. This is likely not the case, as ACC lesions similarly impaired selection of a high-effort option in T-maze tasks where the time to reward delivery is more equivalent for both options (Walton *et al*., 2003; Schweimer & Hauber, 2006).

One recently proposed idea is that both cognitive and physical effort discounting share overlapping neurocomputational signatures, particularly those coding reward value (Chong *et al*., 2017), but that the substrates may segregate depending on the cost type (whether physical or cognitive; Hosking *et al*., 2014). We found that difference scores in a test of sucrose devaluation – a measure of how well animals are able to use a change in motivational state to guide effortful behavior – to be unaffected by ACC lesions, similar to a previous report in monkeys (Chudasama *et al*., 2013). These stand in contrast to effects of basolateral amygdala, which had an impact on both the valuation and effortful choice of the preferred option (Hart & Izquierdo, 2017). Taken together, our results support the idea that ACC, unlike basolateral amygdala, supports a more general effort to reward, without an update to value (i.e. valued vs. devalued responses were intact after ACC lesion). One possibility is that once preference is established through pre-surgery exposure with both preferred and non-preferred foods, ACC may be involved in supporting effort allocation using only, or *primarily*, reward identity information (sucrose pellets vs. chow), not value. Recent proposals suggest this may be similar to the role of other cortical regions, namely orbitofrontal cortex (Stalnaker *et al*., 2014). A question for future research using both rodent and primate effort paradigms would be to determine the conditions in which ACC and orbitofrontal cortex support action value in effortful choice, given that both cortical regions have now been ascribed roles in this process (Kolling *et al*., 2016; Winstanley & Floresco, 2016; Fiuzat *et al*., 2017).

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Chapter 4: Stimulant withdrawal effects in quality effort-based choice task

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Abstract

Exposure to drugs of abuse produces maladaptive changes in cost-benefit decision-making, including the evaluation of time and risk. Studies probing the effects of drug exposure on such evaluations have primarily used experimenter-administered drug regimens. Similarly, while much is known about the neural bases of effort, there have been relatively fewer investigations of the effects of drug experience on effort-based choices. We recently reported that experimenter-administered methamphetamine(meth) resulted in steeper discounting of effort for food rewards in rats, when assessed in protracted withdrawal. Here, we studied rats that underwent withdrawal from weeks of meth intravenous self-administration that later could freely select between a high effort, preferred option (progressive ratio lever pressing for sucrose pellets) versus a low effort, less preferred option (freely-available lab chow). We found decreased effort for the preferred reward and changes in a behavioral economic index demonstrating an increased sensitivity to effort in meth-experienced rats. Critically, the decreased effort for the preferred option was only present in the context of a competing option, not when it was the only option. We also confirmed rats preferred sucrose pellets over chow when both were freely available. These long-lasting changes were accompanied by decreased c-Fos activation in ventral striatum and basolateral amygdala, regions known to be important in effort-based choices. Taken together with our previous observations, these results suggest a robust and enduring effect of meth on value-based decision-making, and point to the underlying neural mechanisms that support the evaluation of an effort cost.

Introduction

Withdrawal from drugs of abuse leads to changes in motivated behavior and decision-making (Groman et al., 2012, Lee et al., 2009, Olausson et al., 2006, Stolyarova et al., 2015, Thompson et al., 2015, Thompson et al., 2017) A prominent feature of withdrawal from psychostimulants is fatigue (Lago and Kosten, 1994, McGregor et al., 2005), which may be related to mechanisms of decreased effort or altered cost-benefit decision-making involving effort (Salamone and Correa, 2012). The neural basis of effort has been well studied in rodent models and in humans, but fewer investigations have probed drug withdrawal experiences that may also contribute to changes in effort-based choice. Here we studied the long-term effects of psychostimulant withdrawal on choices involving *physical* effort for food rewards, to be distinguished from cognitive or attentional effort that is also probed in rats (Cocker et al., 2012, Hosking et al., 2014, Hosking et al., 2015, Hosking et al., 2016).

Recent work investigating motivated behavior following weeks of exposure to methamphetamine (meth) showed that rats exert *more* physical effort for natural rewards such as food (Stolyarova et al., 2015) and exercise (Thompson et al., 2015) in acute and protracted withdrawal from drug. Moreover, others have similarly reported increased lever pressing for grain pellets following withdrawal from psychostimulants like nicotine, MDMA, cocaine, and amphetamine (Olausson et al., 2006). Importantly, the effects mentioned above occur in the context of a single reward-type and/or only one response option. However, organisms face environments where there are qualitatively different options to choose from, each with different effort requirements. To model self-paced, free choice between qualitatively different options, we adopted an effort-based cost benefit decision-making task (Randall et al., 2012) where rats can select between a high effort, preferred reward (progressive ratio lever pressing for sucrose pellets)

and a low effort, less preferred alternative (freely, concurrently available lab chow). We previously reported that rats given experimenter-administered meth and tested in protracted withdrawal exert less effort for a preferred sucrose reward over freely-available chow (Thompson et al., 2017). This altered cost-benefit decision-making (i.e. increased sensitivity to effort costs) was not due to decreased reward sensitivity, anhedonia, or decreased preference for the preferred reinforcer, as determined by control experiments.

Studies investigating the lasting effects of drug experience on effort-based decision-making often use experimenter-administered methods (Floresco and Whelan, 2009, Kosheleff et al., 2012a, Olausson et al., 2006, Thompson et al., 2017). Additionally, studies that have probed related behaviors, such as delay discounting (Floresco and Whelan, 2009) or reversal learning (Groman et al., 2012, Izquierdo et al., 2010, Jentsch et al., 2002, Kosheleff et al., 2012b) have also frequently used experimenter-administered drug. Yet, several groups have also examined self-administered drug effects on delay discounting (Mendez et al., 2010) and other measures of cognitive flexibility(Cox et al., 2016). However, it is presently unknown if withdrawal from meth self-administration results in long-lasting alterations in effort-based decision making involving food rewards.

Behavioral economic indices can be used to assess drug taking and seeking similarly in humans and in experimental animals (Bentzley et al., 2013, Hursh, 1980, Hursh and Silberberg, 2008) and may have high translational value. Using these economic models, demand curves are generated that allow us to estimate consumption at the lowest effort cost (Q_0), the rate of decline in consumption as a function of increasing effort cost (α), and an index of demand inelasticity derived from α, essential value (EV). These measures have been shown to predict addiction-like behaviors (Bentzley and Aston-Jones, 2015, Galuska et al., 2011, Gray and MacKillop,

2014, Murphy et al., 2009, Petry, 2001) and decreased food demand (Galuska et al., 2011). For these reasons, we fit an exponential model (Hursh and Silberberg, 2008) to our effort-based choice data.

The present experiment also sought to investigate the effects of intravenous self-administration (IVSA) of meth and activation of brain regions that are known to be important in effortful choice. We assessed c-Fos in anterior cingulate cortex (ACC), ventral striatum (VS), dorsal striatum (DS), and basolateral amygdala(BLA) approximately 30 days following their last drug (or yoked saline) infusion. We predicted that rats withdrawn from meth IVSA would exhibit reduced lever pressing for sucrose, similar to what we have recently shown following experimenter-administered drug (Thompson et al., 2017). We also expected this effect would be accompanied by changes in activation of brain regions previously shown to support this behavior. And finally, we hypothesized that steeper discounting of the more effortful, preferred option would also be revealed as a long-lasting change in demand elasticity (α).

Method

Subjects. A timeline of all procedures is shown in **Fig. 4-1**. Subjects were 32 (n = 19 meth IVSA, n = 13 yoked saline) adult male Long-Evans rats (Charles River Laboratories, Hollister, CA), singly-housed for all phases of experiments with the exception of the acclimation period and handling. A subset of these rats (n = 14; n = 8 meth and n = 6 saline) were used for c-Fos experiments (details below). All animals were handled for 10 min in pairs for 5 d after a brief acclimation period (3 d). Rats weighed an average of 300 g at the beginning of the experiment. One subject was not included in the final data analyses due to premature death, and three others were omitted due to early (i.e., less than 7 IVSA sessions) catheter patency loss. The vivarium was

maintained under a 12/12 h reverse light cycle at 22 °C, and lab chow and water were available ad libitum prior to behavioral testing. Training and testing were conducted during the early portion of the dark cycle (~0800–1200 h). Experiments were conducted 5–7 d per week, and animals were fed once daily on weekends when testing was not conducted. All procedures were approved by the Chancellor's Animal Research Committee at the University of California, Los Angeles.

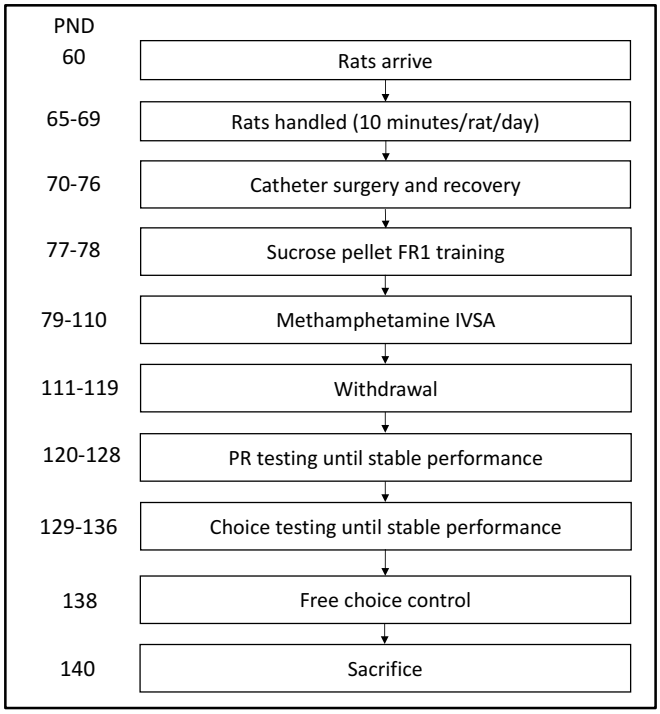

Figure 4-1. Timeline of events. Sequence of handling, surgery, testing, and euthanasia are depicted from top to bottom order in postnatal day (PND).

Food restriction. One day before behavioral testing began, the amount of chow given to each rat was reduced to 12 g/d, and rats were given ~10 sucrose pellets (45-mg dustless precision sucrose pellets; Bio-Serv, Frenchtown NJ) in their home cage to acclimate them to the food rewards. Following the initial 2 d of sucrose pellet training, rats were switched to free feeding for the duration of self-administration and withdrawal. Prior to the beginning of the progressive ratio

phase of training, rats were restricted to 12 g of chow per d. Finally, rats performing the choice task were given 8 g of chow per d, in addition to the food they consumed during testing. At the time of euthanasia, rats weighed on average ~350 g.

Surgery and FR1 sucrose pellet training. Implantation of chronic indwelling intravenous jugular catheters took place prior to all behavioral testing. Catheters were custom made and consisted of a 14 cm Silastic tubing fit to a 28-gauge 11 mm cannula (PlasticsOne, Roanoke, VA) bent to 90° and encased in dental cement with a mesh base. Rats were anesthetized with isoflurane (5% induction, 2% maintenance in 2 L/min O2), after which incisions were made on the right dorsolateral surface of the back and the right anterolateral surface of the neck. The cannula of the catheter was inserted through a small incision on the dorsal surface of the back between the scapulae through the back incision. The catheter tubing was passed subcutaneously from the dorsal surface of the back to the ventral incision, the jugular vein was isolated, and the catheter tip was inserted and secured with surgical silk sutures. Following a patency test with heparinized (30 USP units/mL) saline, incisions were sutured closed, and the rats were placed on a heating pad and kept in recovery until ambulatory. Post-operative care consisted of five daily injections of carprofen (5 mg/kg, s. c.). Following a 7-d free-feeding recovery period, rats were restricted to 12 g of food daily following testing and given 2 d of active lever pressing on a fixed ratio-1 schedule for sucrose pellets (45 mg, Bioserv, Frenchtown, NJ). Sessions lasted until 30 min had elapsed or 30 pellets had been earned. The first session was conducted untethered, and the second session was conducted with the steel leash attached to the catheter to acclimate animals to this procedure. Following surgery, catheters were flushed daily with 0.1 mL heparinized saline and for the duration of IVSA.

Apparatus. All behavioral testing was conducted in chambers outfitted with a house light, internal stimulus lights, a food-delivery magazine, 2 retractable levers positioned to the left and right of the chamber wall opposite the magazine, a syringe pump, and a liquid swivel attached to a steel leash for drug self-administration. All hardware was controlled by a PC running Med-PC IV (Med-Associates, St. Albans, VT).

Meth intravenous self-administration (IVSA) and withdrawal. Rats were given food ad-libitum for the duration of the 27 sessions of IVSA. Catheter patency was tested the day prior to beginning IVSA with 0.2 mL propofol solution (PropoFlo, Zoetis, Parsippany-Troy Hills, NJ). Rats were allowed to self-administer meth hydrochloride (Sigma, St. Louis, MO) for 2-h sessions 6–7 d per week, during which active and inactive lever presses were recorded. The active lever was the opposite from that used during the initial sucrose pellet training. Each infusion consisted of 0.1 mg/kg freebase meth/0.0875 mL saline and lasted for 5 s with a 20 s timeout between infusions. Catheters were flushed before and after sessions with 0.05 mL heparinized saline. Rats were given 6 sessions of FR1 IVSA, 14 sessions of FR3 IVSA, and 7 sessions of FR5 IVSA where 1, 3, or 5 active lever presses were required to earn a single infusion, respectively. Pressing of the inactive lever had no programmed consequences. Saline animals were yoked to meth animals to receive an equal number and volume of saline infusions. Following IVSA, rats were left undisturbed for 9 d to withdraw from the drug prior to beginning progressive ratio testing.

Progressive ratio and effortful choice testing. Following withdrawal, rats were restricted to 12 g food daily (to no less than 85% free-feeding weight) following testing on the PR schedule for sucrose pellets. The lever that earned pellets was opposite that of the active IVSA lever. The required number of presses increased according to the formula $\mathbf{n_i = 5e^{(i/5)}-5}$, where $\mathbf{n_i}$ is equal to the number of presses required on the ith ratio, rounded to the nearest whole number, after 5

successive schedule completions. No timeout was imposed. Rats were tested on the PR schedule until stable performance was achieved (~6 sessions), defined as the number of lever presses across three days of testing falling between 80% and 120% of the mean of those three days. This testing was performed to assess the willingness to work for sucrose pellets in the absence of freely available chow. Upon meeting criteria for stable PR performance, a ceramic ramekin containing 18 g of lab chow was introduced (modified from (Randall et al., 2012)) during testing. Rats were free to choose between consuming freely-available but less preferred chow or lever pressing for preferred sucrose pellets and tested until stable lever pressing performance was achieved as described above. Detailed methods have been published recently (Hart et al., 2017, Hart and Izquierdo, 2017).

Freely-available choice. Following effortful choice testing, we conducted a test wherein both sucrose pellets and chow were freely-available. Rats were given 30 min of free access to pre-weighed amounts of sucrose pellets and lab chow (~18 g each) in standard empty cages, but different from their home cages. Following the 30-min period, remaining food was collected and weighed to determine rats' food preferences.

Euthanasia. Rats in both pretreatment groups (meth and saline) were given a final effortful choice testing session. One hour following this session, animals were humanely euthanized by Euthasol overdose (Euthasol, 0.8 mL, 390 mg/mL pentobarbital, 50 mg/mL phenytoin; Virbac, Fort Worth, TX), and brains were removed for histological processing.

Histological processing and immunohistochemistry. Following removal, brains were fixed in 10% buffered formalin acetate for 24 h followed by 30% sucrose for 5 d 50 mm sections of ACC, striatum, and BLA were collected and stained for c-Fos. Staining was performed by

incubation for 24 h at 4 °C in a primary antibody consisting of 1:2000 rabbit polyclonal to c-Fos (Abcam, Cambridge, MA), 3% normal goat serum (Abcam, Cambridge, MA), and 0.5% Triton-X (Sigma, St. Louis, MO) in PBS, followed by four 5-min washes in PBS. Secondary antibody incubations were 2 h at 20 °C in the 0.5% Triton-X/5% normal goat serum/PBS solution with 1:500 goat anti-rabbit Alexa 488 (Abcam, Cambridge, MA) replaced for the primary antibody, followed by four 5-min washes in PBS. Slides were subsequently mounted and cover-slipped with fluoroshield DAPI mounting medium (Abcam, Cambridge, MA). Slices were visualized using a BZ-X710 microscope (Keyence, Itasca, IL), and analyzed with BZ-X Viewer and analysis software. Images for quantification of c-Fos immunoreactivity were taken with a 20× objective with a 724 μm by 543 μm field of view and converted to cells per millimeter squared. For each region, three images were taken from two or three separate slices from both hemispheres at the same approximate AP coordinate (ACC +2.0 mm; VS +2.0 mm; DS +1.6 mm; BLA -3.0 mm), and final cell counts were based on the averages of the three images.

Data analyses. Data were analyzed using GraphPad Prism v.7 (La Jolla, CA) and SPSS v.25 (Armonk, NY). An alpha level for significance was set to 0.05. Two-way repeated measures ANOVA was used to compare the number of active and inactive lever presses across the 27 IVSA sessions. Independent samples t-tests (reported as means ± SEM) on data from the last of the 3 stable sessions were conducted to analyze group differences on PR and on the choice task. Multivariate ANOVAs with Pillai's Trace corrections were conducted to analyze group differences on the density of c-Fos immunoreactive cells as well as group differences on behavioral economic indices (described below). A two-way ANOVA was used to test for group differences on food preference, and number of sessions to stable performance. Behavioral economic indices were generated by fitting an exponential model (Hursh and Silberberg, 2008) to our data:

$$Q = Q_0 * 10^{k(e^{-\alpha Q_0 C}-1)}$$

where Q = consumption at a given price C, or cost (FR value), Q_0 = demand intensity, consumption at lowest price, k = constant parameter reflecting the range of consumption values in log10units, α = demand elasticity, or the derived demand parameter reflecting the rate of consumption decline associated with increasing price. Q_0 and α were calculated based on the number of pellets earned at each ratio. Because the number of pellets earned at each ratio in our task is necessarily restricted to five, differences in α would be due to animals reaching higher/lower ratios or earning more/fewer pellets at a given ratio. For each rat, we also calculated Essential Value (EV) to quantify the 'strength' of the reinforcer (i.e. sucrose pellets) (Kearns et al., 2017), based on the value of the exponential rate constant. As indicated above, the value of α is the rate of consumption decline and *k* is the consumption range for each rat in the following:

$$\text{EV} = 1/(100*\alpha*k^{1.5})$$

All behavioral economic indices were correlated with brain activation data, and multiple linear regression analyses were conducted to predict indices from c-Fos immunoreactivity.

Results

IVSA. Rats acquired and maintained meth self-administration and increased active lever pressing as a function of increased work requirement. There was a significant main effect of lever ($F_{(1,28)} = 22.99$ $p < 0.001$), significant main effect of session ($F_{(26,728)} = 4.71$ $p < 0.0001$), and significant lever by session interaction ($F_{(26,728)} = 4.89$ $p < 0.001$) (**Fig. 4-2A**). These press rates yielded relatively stable levels of meth intake across the 27 sessions (**Fig. 4-2B**), average dose of 1.16 mg/kg/session. Yoked saline controls had no preference for the active lever over the inactive lever. There was no significant main effect of lever ($F_{(1,24)} = 0.83$, $p = 0.37$). There was a significant

main effect of session due to extinction (rats had previously received 2 d of FR1 for sucrose pellets) ($F_{(26,624)} = 4.76$, $p < 0.001$) and no significant lever by session interaction ($F_{(26,624)} = 1.46$, $p = 0.07$) (**data not shown**).

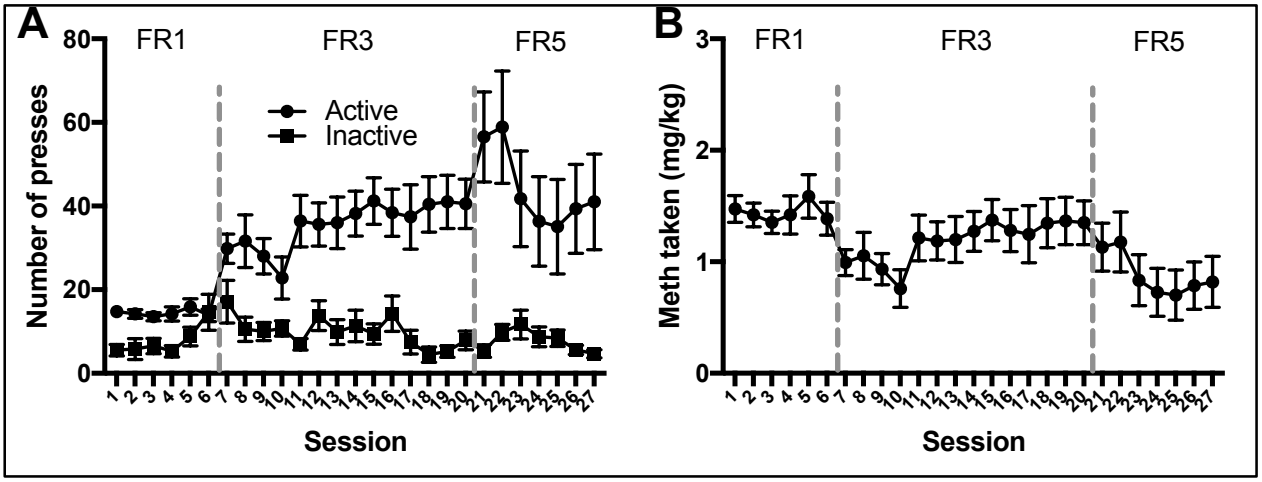

Figure 4-2. Meth IVSA. (A) Number of active and inactive lever presses on each of the 27 IVSA sessions that had FR1, FR3, and FR5 work requirements for each infusion. Animals showed a preference for the active lever over the inactive lever ($p<0.0001$). (B) Amount of meth taken (mg/kg) on each of the 27 IVSA sessions. Points denote mean ± SEM.

Progressive Ratio. There were no group differences in the number of lever presses on the PR schedule for sucrose pellets. An independent samples *t*-test on the number of lever presses on the last of the three stable days of PR testing revealed no significant difference between groups ($t_{(26)} = 0.91$ $p = 0.37$; saline = 582.3 ± 68.8; meth = 480.5 ± 85.29) (**Fig. 4-3A**).

Effortful Choice Testing. After allowing several sessions to achieve stable performance, a significant difference in number of lever presses on the last of the three stable sessions was revealed by an independent samples *t*-test ($t_{(26)} = 2.74$ $p = 0.01$; saline = 227.4 ± 21.00; meth = 130.00 ± 27.59): meth-experienced rats lever pressed significantly less for sucrose pellets than yoked saline controls (**Fig. 4-3B**). There was no significant difference in the amount of chow

consumed between these conditions ($t_{(26)} = 0.36$ $p = 0.72$; saline = 8.8 ± 0.35; meth = 8.63 ± 0.31) (**Fig. 4-3C**). Groups did not differ in the number of sessions required to reach stable performance on the PR or choice task. A two-way ANOVA conducted on the number of sessions to stable performance in the PR and choice tasks revealed no significant effect of session type ($F_{(1,52)} = 3.54$ $p = 0.07$), no significant effect of condition ($F_{(1,52)} = 0.769$ $p = 0.38$), and no session type by condition interaction ($F_{(1,52)} = 0.21$ $p = 0.64$) (**Fig. 4-3D**).

Freely-available Choice. A test wherein sucrose pellets and lab chow were concurrently freely-available was conducted to confirm that meth withdrawal effects on PR effortful choice were not due to decreased preference for sucrose. Analysis of amount of food consumed (g) using a two-way ANOVA with food type (sucrose pellet, chow) and condition (saline, meth) as fixed factors revealed a significant effect of food type ($F_{(1,52)} = 94.14$ $p < 0.001$; sucrose = 11.36 ± 0.68; chow = 3.55 ± 0.40). No significant food type by condition interaction ($F_{(1,52)} = 0.51$ $p = 0.47$), or effect of condition ($F_{(1,52)} = 0.09$ $p = 0.76$) was found (**Fig. 4-3E**).

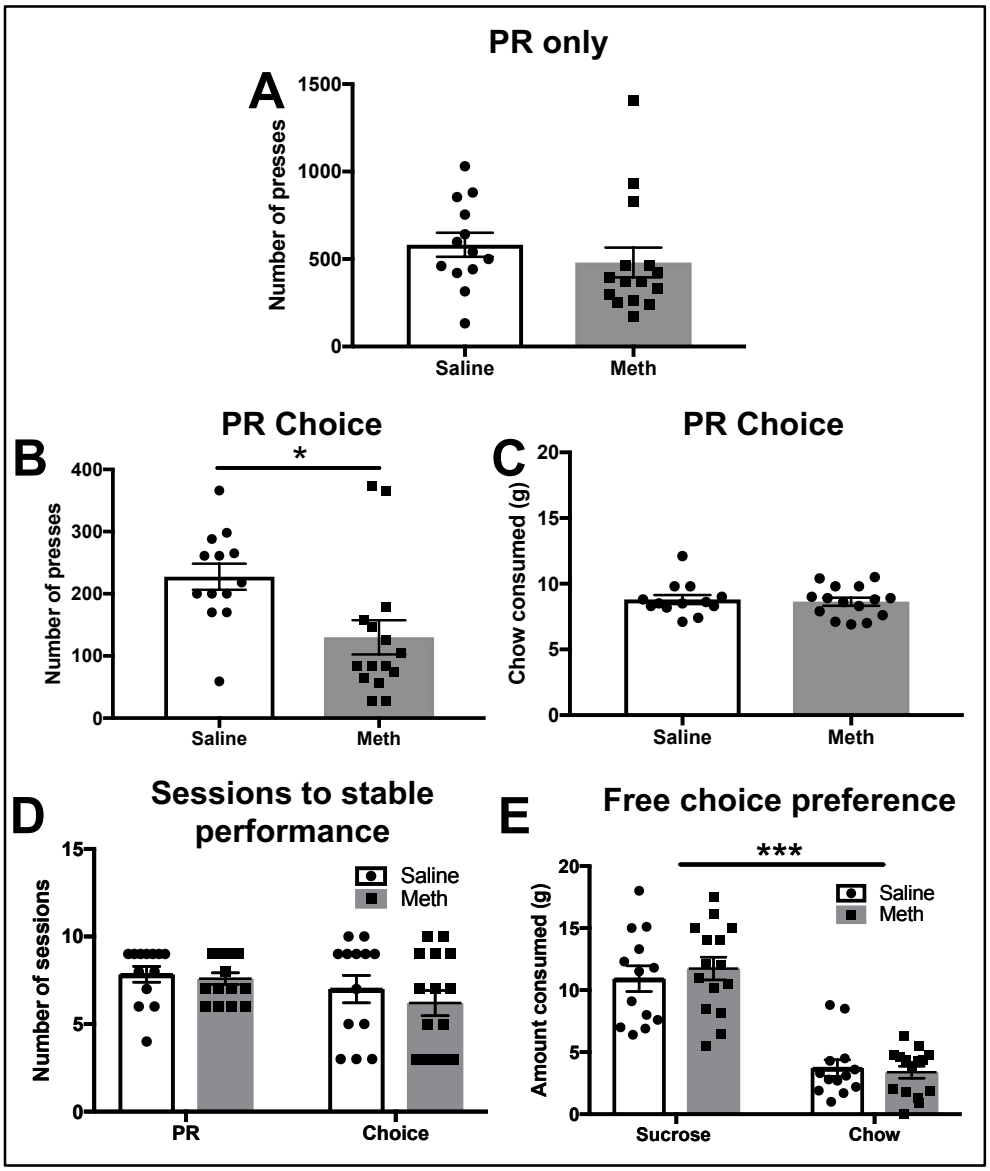

Figure 4-3. Effects of meth IVSA withdrawal on effort for food rewards. (A) Number of lever presses for sucrose pellets on the last of the three stable PR testing sessions in yoked saline and meth IVSA groups. Groups did not differ in the number of lever presses on the PR schedule. (B) Number of lever presses for sucrose pellets on the last of the three stable choice testing sessions in yoked saline and meth IVSA groups. Meth exposed animals emitted fewer lever presses compared to yoked saline controls (p<.05). (C) Amount of chow consumed on the last of the three stable choice testing sessions in yoked saline and meth IVSA groups. Groups did not differ in the amount of chow they consumed. (D) Number of sessions required to reach stable performance on the PR and choice tasks in yoked saline and meth IVSA animals. Groups did not differ in the number of sessions required to reach stable performance on either schedule. (E) In the free choice control task where both options were freely available, both groups preferred the sucrose pellets to the lab chow (p<0.001). Points denote mean ± SEM, *p<0.05, ***p<0.001.

Behavioral economic indices. Because we detected no group differences on progressive ratio testing in the absence of freely available chow, behavioral economic measures were taken from the last session of choice testing. An independent samples *t*-test on the number of pellets earned on the last of the three stable sessions revealed a significant difference between yoked saline and meth IVSA groups ($t_{(26)} = 3.27$ $p < 0.01$; saline = 32.92 ± 1.42; meth = 24.4 ± 2.09) (**Fig. 4-4A**). A similar pattern of effects was observed for the highest ratio achieved, or breakpoint: yoked saline animals reached a higher breakpoint than meth animals ($t_{(26)} = 2.58$ $p = 0.02$; saline = 15.38 ± 1.12; meth = 10.53 ± 1.46) (**Fig. 4-4B**). After fitting Hursh's exponential model (Hursh and Silberberg, 2008) to the number of pellets consumed at each ratio and generating individual demand curves Q_0 and α values for each subject, a multivariate ANOVA with Pillai's Trace correction revealed a significant effect of group ($F_{(3,24)} = 3.39$, $p = 0.03$). Specifically, meth IVSA animals consumed fewer pellets at higher ratios, as indicated by significantly higher α values than yoked saline controls ($p = 0.02$; saline = 3.9e−4 ± 5.5 e−5; meth = 6.8 e−4 ± 9.4 e−5) (Fig. 4C). Q_0 and EV values did not differ between groups ($p = 0.75$ and $p = 0.13$, respectively) (data not shown**).** Individual demand curves are shown in **Fig. 4-4D**.

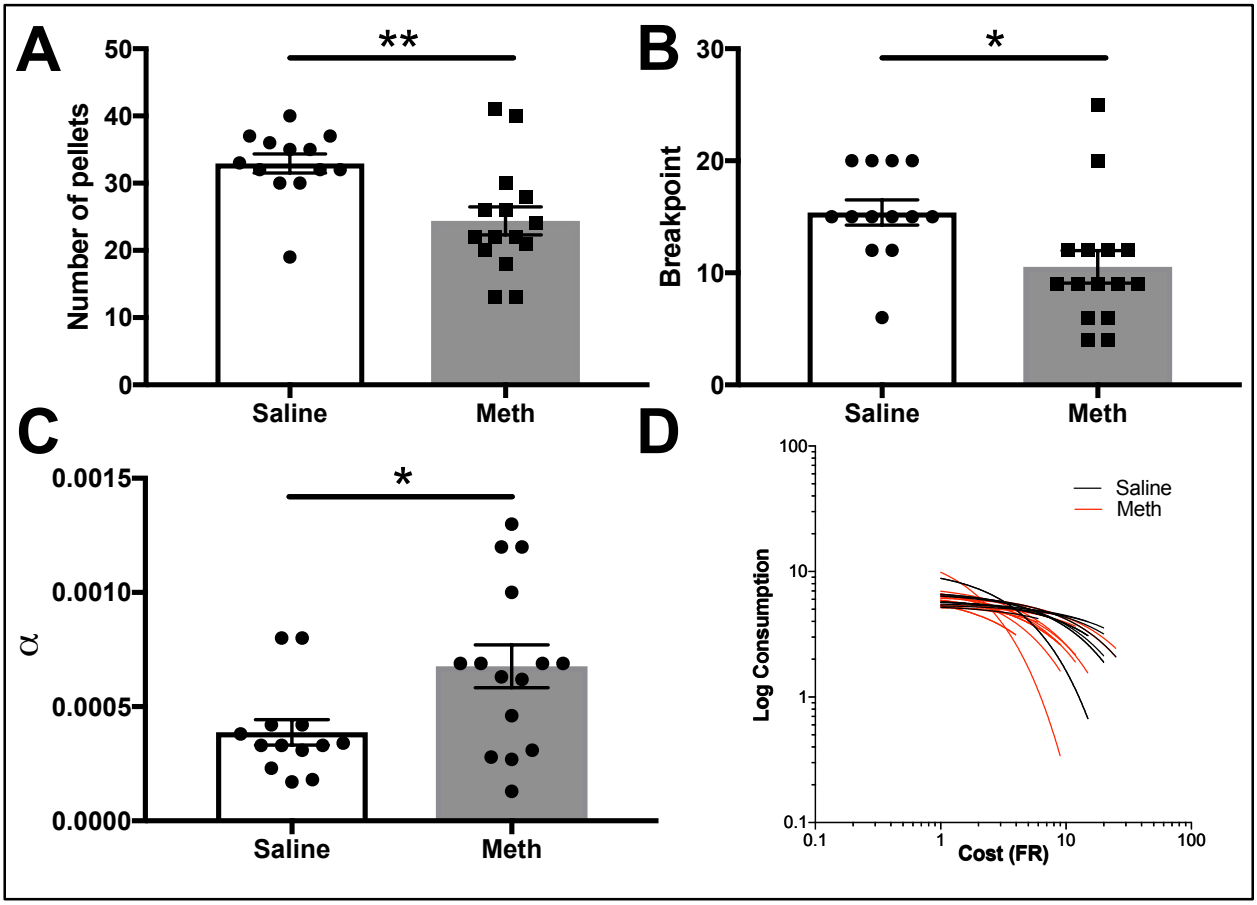

Figure 4-4 (A) Number of pellets earned on the last of the three stable PR testing sessions in yoked saline and meth IVSA groups. Meth exposed animals earned fewer pellets compared to yoked saline controls (p<.01). (B) Breakpoint (highest ratio achieved) on the last of the three stable choice testing sessions in yoked saline and meth IVSA groups. Meth exposed animals reached lower ratios compared to yoked saline controls (p<.05). (C) α values calculated from Hursh and Silberberg's exponential model. Meth exposed animals had steeper rates of decline, as indicated by higher α (p<.05). (D) Individual demand curves generated from Hursh and Silberberg's exponential model. Points denote mean ± SEM, *p<0.05, **p<0.01.

c-Fos immunoreactivity. Following the final effortful choice test, brains were collected 1 h after the termination of testing and processed for c-Fos immunoreactivity. A multivariate ANOVA with Pillai's Trace correction revealed a significant effect of group ($F_{(4,9)} = 8.403$, $p < 0.01$) In VS, there was a significant difference in the number of c-Fos immunoreactive cells with meth animals exhibiting reduced expression ($p = 0.04$; saline = 66.7 ± 9.68; meth = 40.53 ± 6.69) (**Fig. 4-5A**). In BLA, there was also a significant difference in the number of c-Fos immunoreactive cells with

meth animals exhibiting reduced expression ($p = 0.02$; saline = 67.27 ± 6.23; meth = 46.83 ± 4.43) (**Fig. 4-5B**). In ACC, there was a trend toward a significant decrease in the number of c-Fos immunoreactive cells in the meth rats ($p = 0.09$; saline = 64.28 ± 8.96; meth = 44.37 ± 6.52) (**Fig. 4-5C**). In DS, there was no significant difference in the number of c-Fos immunoreactive cells ($p = 0.39$; saline = 76.37 ± 15.15; meth = 57.39 ± 14.39) (**Fig. 4-5D**).

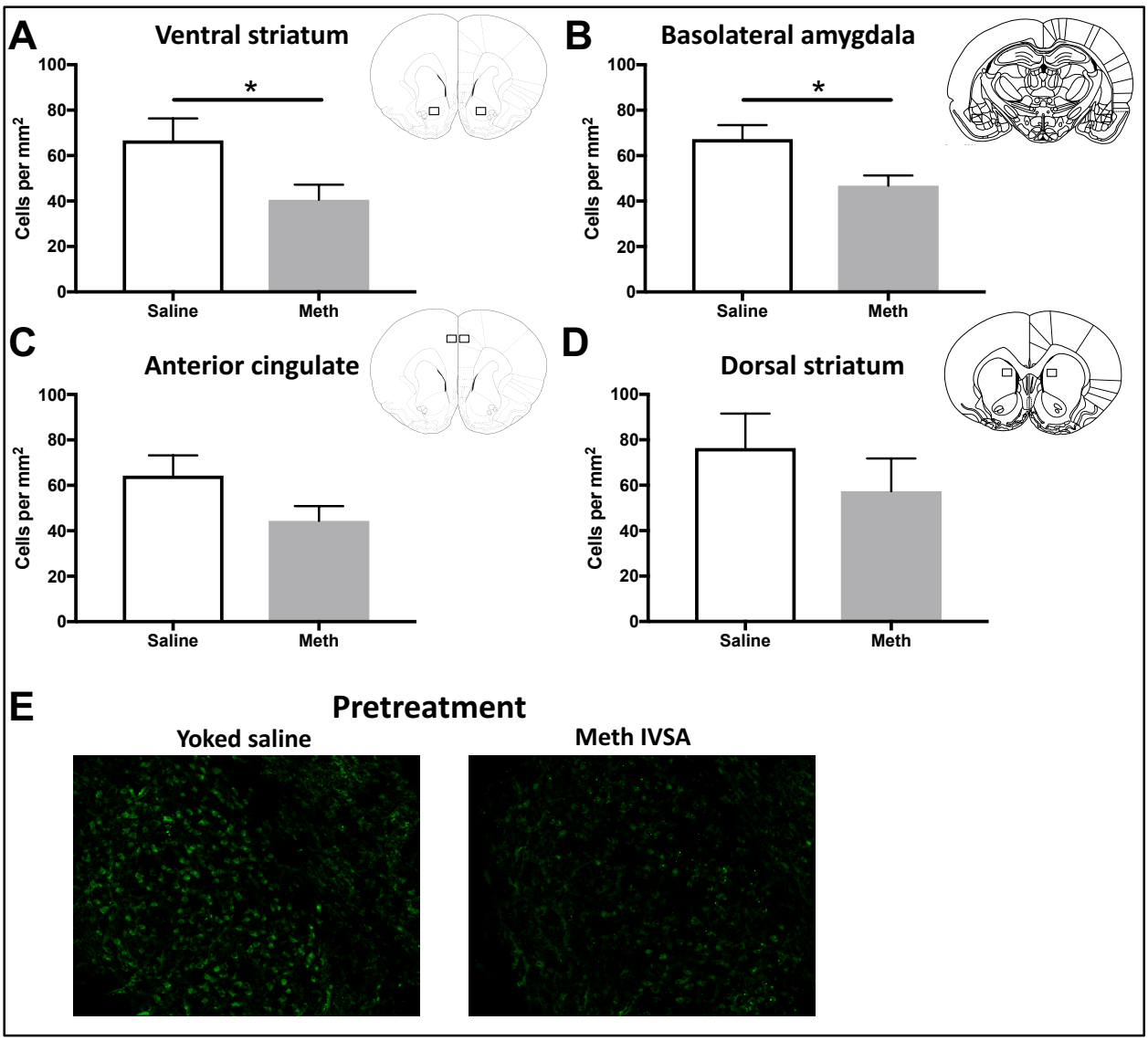

Figure 4-5. c-Fos activity following behavior (A) Number c-Fos positive cells/mm^2 in VS. Meth IVSA pretreatment decreased c-Fos immunoreactivity ($p<0.05$). (B) Number c-Fos positive cells/mm^2 in BLA. Meth IVSA pretreatment decreased c-Fos immunoreactivity ($p<0.05$). (C) Number c-Fos positive cells/mm^2 in ACC. Meth IVSA pretreatment had a marginal effect on c-Fos immunoreactivity (p=0.09). (D) Number c-Fos positive cells/mm^2 in DS. Meth IVSA pretreatment had no effect on c-Fos immunoreactivity in this region. (E) Representative photomicrographs of c-Fos immunoreactivity taken at 20x in BLA. Insets represent ROIs for image capture. Points denote mean ± SEM, *p<0.05.

A Pearson r-correlation matrix of behavioral economic indices and brain activation (c-Fos) data for all animals is shown in Table 1. Interestingly, only VS c-Fos was found to be significantly correlated with Q_0 and α. As might be expected, α and Q_0 were significantly correlated, as were α and EV. Multiple linear regression analyses were conducted to predict behavioral economic indices based on ROI c-Fos immunoreactivity. Based on the results of our correlation matrix, we employed a forward selection model with VS entered first, followed by BLA, ACC, and DS c-Fos. We found that only VS c-Fos significantly predicted α ($F_{(1,12)} = 5.244$, $p = 0.04$, $R^2 = 0.304$) and Q_0 ($F_{(1,12)} = 7.480$, $p = 0.018$, $R^2 = 0.384$). Results were the same if BLA c-Fos was entered in the model first.

	1.	2.	3.	4.	5.	6.	7.
1. Q_0							
2. α	**0.684****						
3. EV	-0.341	**-0.770****					
4. ACC cFos	0.059	-0.097	-0.145				
5. VS cFos	**-0.620***	**-0.551***	0.226	-0.276			
6. DS cFos	-0.005	-0.279	0.226	0.141	0.155		
7. BLA cFos	0.012	-0.104	-0.007	0.345	0.234	0.178	

Table 4-1. Correlation matrix of behavioral economic indices and regional c-Fos immunoreactivity. Demand elasticity (α) and demand intensity at lowest cost (Q_0) were significantly correlated, as were α and essential value (EV). Only VS cFos was negatively correlated with Q_0 and α. *p<0.05, **p<0.01.

Discussion

Exposure to drugs of abuse produces marked changes in cost-benefit decision-making, often increasing discounting of time and effort costs (Mendez et al., 2010, Thompson et al., 2017). Here we show that IVSA of meth and subsequent withdrawal also results in steeper effort

discounting. Importantly, these changes are accompanied by long-term adaptations in VS and BLA, regions known to be important in effort-based choice. We also demonstrate that meth withdrawal results in a persistent change in the demand elasticity (α), without affecting the demand intensity (Q_0) or essential value (EV) of a preferred sucrose reward.

Meth experience produces long-lasting changes in relative reward value

Our group previously showed decreased effort following binge exposure to meth assessed in a T-maze task (Kosheleff et al., 2012a). Critically, that pattern of dosing is likely not as relevant to the human condition and is well outside the dose range that rats self-administer. In the present experiment, rats readily acquired and maintained IVSA of relatively low sensitizing doses (~1 mg/kg) (Frey et al., 1997), consistent with doses others report (Winkler et al., 2018). We also did not observe escalating doses, likely due to the short 2-h sessions (Orio et al., 2010). Nevertheless, we replicate here what we have previously demonstrated following experimenter-administered escalating meth: steeper effort discounting in the presence of a lower cost option that is not due to reduced reward sensitivity or changes in food preference (Thompson et al., 2017). Importantly, rats' steep effort discounting for a preferred reward was similarly not due to a general lack of motivation, learning or memory impairment, or changes in the hedonic value of the preferred reward. Taken together, the finding that both experimenter- and self-administered meth produce the same long-lasting effects on (relative) food reward value points to a robust drug effect.

Our group previously showed meth treated animals exert more effort in the form of wheel running (Thompson et al., 2015) and will choose to climb a higher barrier to earn more food reward (Stolyarova et al., 2015) in withdrawal, and others have found increased progressive ratio lever pressing for food in amphetamine withdrawal (Olausson et al., 2006). Task differences and effects

of stimulants on delay discounting (Hoffman et al., 2006) may explain this potential discrepancy. Those tasks all imposed a single response option (i.e. running, barrier climbing, lever pressing) rather than providing animals a choice of actions. Given that exposure to meth increases delay discounting (Hoffman et al., 2006) it is plausible that, when faced with a single option, sensitivity to delay costs exerts stronger effects on behavior than sensitivity to physical effort costs, and drug-treated animals will maximize the number of rewards acquired per unit of time by responding more vigorously. Ostensibly, when an option is introduced that is less costly in terms of *both* time and effort, it becomes the preferred choice in meth-experienced animals, despite being of lesser hedonic value. Indeed, we found that meth-experienced rats reached lower stable levels of lever pressing when exposed to the choice phase, and we found no effect on PR responding in the absence of an alternative option, though deficits in effort were revealed when a lower cost, concurrently available option was introduced.

Meth experience increases sensitivity to effort cost in withdrawal

Behavioral economic analysis of our data yielded several interesting results. Consistent with previous reports, we found that exposure to meth IVSA decreased food demand (Galuska et al., 2011). Although those authors found slight effects on Q_0 as well as α, our task differences may explain the divergent effects. Rather than increasing ratios (costs) across sessions, our task progressively increases the cost every five reinforcers within a single session. Therefore, it is not surprising that Q_0 was not different between groups: consumption is necessarily equal at the lowest cost, as all animals earned more than five reinforcers. Thus, although we report Q_0 values, their interpretation in the context of a progressive ratio task is unclear, and further clarification would require a traditional behavioral economics paradigm (Bentzley et al., 2013, Cox et al., 2017, Galuska et al., 2011). Yet the lack of effect on EV, together with our control tasks involving

free-choice between sucrose pellets vs. chow, does also provide a point of convergence: rats express intact hedonic valuation of the preferred sucrose reinforcer. Critically, we observed effects of meth IVSA and withdrawal on α: rats exhibited greater elasticity of demand for food and were less willing to work for sucrose pellets, particularly at higher ratios, consistent with effects others report (Bentzley et al., 2013, Galuska et al., 2011). Recent behavioral economic analysis of meth seeking found that oxytocin decreases meth demand, and this effect is accompanied by decreased VS activation (Cox et al., 2017). Future work should determine whether meth and food demand are mediated by distinct mechanisms. Indeed, moving this research area forward are several interesting studies directly comparing the elasticity of demand between drug and non-drug reinforcers (Kearns et al., 2011, Kearns et al., 2017).

On the last day of behavioral testing we collected brains to determine whether there were regional brain adaptations following prolonged meth experience and withdrawal. The pattern of effects we observed in our c-Fos expression points to clear roles of VS and BLA in withdrawal effects on effort. We found that meth-withdrawn animals showed reduced c-Fos expression in BLA and VS, not DS and ACC. Our results are consistent with others showing greater immediate early gene expression in VS in animals that lever press more for high carbohydrate pellets (Randall et al., 2012). These findings are also consistent with a general role of VS (Ghods-Sharifi and Floresco, 2010) and BLA (Ghods-Sharifi et al., 2009, Hart and Izquierdo, 2017) in effort-based choice. Interestingly, though group differences did not reach statistical significance ($p = 0.09$), there was a trend for a decrease in c-Fos expression in ACC (Cai and Padoa-Schioppa, 2012, Hart et al., 2017, Klein-Flugge et al., 2016, Rudebeck et al., 2008, Schweimer and Hauber, 2005, Walton et al., 2003).

Different models of meth exposure produce varying effects on brain and behavior (Kosheleff et al., 2012b). The chronic IVSA regimen here resulted in the same behavioral effects as chronic escalating doses previously administered in our laboratory (Thompson et al., 2017). However, neither of these regimens results in neurotoxicity (Belcher et al., 2008); it is therefore unlikely the reduced c-Fos expression we observe here is due to fewer cells overall.

Analyses revealed only VS activation correlated significantly with Q_0 and α. These findings are consistent with the wealth of evidence implicating VS in effort (Cousins and Salamone, 1994, Ghods-Sharifi and Floresco, 2010, Nunes et al., 2013). Although BLA and ACC activation did not correlate with behavioral economic indices, we did find reduced activation in BLA and a trend in ACC in meth withdrawn animals, providing support for the idea that BLA, ACC, and VS interact in the regulation of effort (Floresco and Ghods-Sharifi, 2007, Hart et al., 2017, Hart and Izquierdo, 2017, Hauber and Sommer, 2009, Salamone et al., 2007). Further, the lack of effect of withdrawal on DS activation is consistent with several studies showing DS dopamine receptor blockade and related manipulations have no effects on effort-based choice (Farrar et al., 2010, Font et al., 2008, Nunes et al., 2013). While we did not examine orbitofrontal cortex (OFC), its role in effort should be further elucidated. While OFC neurons do encode economic value and choice between options (Padoa-Schioppa and Assad, 2006), OFC lesions do not result in impaired effort in a T-maze task (Ostrander et al., 2011, Rudebeck et al., 2008). Notably, there is a recent report of OFC lesions and pharmacological inhibition actually increasing progressive ratio responding and selection of a high effort choice (Munster and Hauber, 2017). However, to our knowledge, OFC has not been probed in an effort-based choice task involving qualitatively different options and thus it would be useful to compare its role to that of other cortical regions, like ACC (Hart et al., 2017, Rudebeck et al., 2006, Walton et al., 2003).

Concluding remarks

Overall, we found that meth IVSA and withdrawal leads to long-term changes in effort-based choice, a behavior that is associated with several conditions, including depression (Treadway and Zald, 2011) Parkinson's disease (Friedman et al., 2010), and schizophrenia (Gold et al., 2013). These findings are consistent with the clinical literature showing decreased effort is one of the most prominent symptoms in amphetamine withdrawal (McGregor et al., 2005), even in the absence of depressed mood (Volkow et al., 2001), further supporting the idea that mechanisms underlying the motivation to obtain rewards versus the primary hedonic response to rewards are dissociable (Salamone and Correa, 2012, Treadway and Zald, 2011). Overall, the pattern is in keeping with an increased elasticity of demand for food reward (i.e. greater sensitivity to effort cost) that has been reported following neurotoxic dopamine lesions, dopamine depletion, and dopamine receptor blockade (Aberman and Salamone, 1999, Salamone et al., 2017). The behavioral effect we report here was accompanied by changes in activation of several regions known to be important in effortful decision making. This finding contributes to the understanding of persistent, value-based decision-making changes that occur in drug withdrawal, and that may subvert long-term effort toward sobriety.

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Chapter 5: Anterior cingulate chemogenetic modulation of effort-based choice behavior

In preparation for submission

Abstract

Effort is a cost that must be overcome to procure rewards, and we previously demonstrated a role of rat anterior cingulate cortex (ACC) in effort-based choice using classical lesion methods. Here rats were tested in an effort-based choice task where they could select between lever pressing on a progressive ratio schedule for sucrose pellets versus consuming freely-available standard lab chow. We sought to replicate and extend our previous findings following ACC lesions, using a chemogenetic approach. Separate groups of rats were infused with inhibitory (G_i) or excitatory (G_q) DREADDs under a CaMKIIα promoter in ACC. We found that either inhibition or stimulation decreased lever pressing in the context of choice. Importantly, these effects were not due to virus exposure, nonspecific effects of clozapine-N-oxide, an inability to lever press, decreased appetite, or changes in food preference. Slice electrophysiology confirmed DREADD effects. Taken together, these findings point to a role for ACC in calculating relative value between options, and that interfering with normal function (either by inhibition or excitation), disrupts choice of the high-value option.

Introduction

Real-world decisions are rarely as straightforward as choosing between clearly "good" vs. "bad" options. Often, options must be evaluated along multiple dimensions that incorporate an evaluation of the rewards themselves and the actions or efforts to procure them (Skvortsova *et al.*, 2014). For example, we typically make decisions between options in comparison, where one

outcome may be more costly (i.e. more effortful) yet more preferred than the other. Several neuropsychiatric conditions are characterized by aberrant evaluation of effort costs, including Major Depression, Schizophrenia, Autism (Treadway *et al.*, 2012a; Barch *et al.*, 2014; McCarthy *et al.*, 2016; Mosner *et al.*, 2017). Studies on effort-based choice also have important implications for understanding food and substance addictions (Salamone *et al.*, 2001; Salamone *et al.*, 2003; Salamone *et al.*, 2007; Salamone & Correa, 2012; Treadway *et al.*, 2012b; Salamone & Correa, 2013).

Outside of the striatum, which has been the major node of emphasis in such effort-based decision making studies (Salamone *et al.*, 1991; Salamone *et al.*, 1994; Cousins *et al.*, 1996; Nowend *et al.*, 2001; Salamone *et al.*, 2003; Salamone *et al.*, 2007; Ghods-Sharifi & Floresco, 2010), the anterior cingulate cortex (ACC) is also implicated in decisions involving both physical and cognitive effort cost evaluation (Walton *et al.*, 2003; Schweimer & Hauber, 2005; Floresco & Ghods-Sharifi, 2007; Hillman & Bilkey, 2010; Cowen *et al.*, 2012; Hillman & Bilkey, 2012; Hosking *et al.*, 2014; Winstanley & Floresco, 2016). Neurons in both rat and primate ACC signal value during economic decision-making (Lapish *et al.*, 2008; Azab & Hayden, 2017; Hunt & Hayden, 2017; Mashhoori *et al.*, 2018). Neural responses in this region also track trial-by-trial outcomes of choices (Procyk *et al.*, 2000; Shidara & Richmond, 2002; Seo & Lee, 2007) Akam et al., 2017 biorxiv 126292), reward history (Bernacchia *et al.*, 2011), reward prediction errors, RPEs (Hayden *et al.*, 2009; Kennerley *et al.*, 2011; Hyman *et al.*, 2017), and counterfactual options, or 'rewards not taken' (Hayden *et al.*, 2009; Mashhoori *et al.*, 2018). Indeed, both anatomical and functional evidence supports the idea that ACC functions as a key integrator of reward, cognitive, and action plans (Shenhav *et al.*, 2013; Heilbronner & Hayden, 2016).

In rats, effort t-maze and effort discounting tasks typically require the animal to select among different magnitudes of the same reward identity (i.e. more sucrose pellets vs. fewer sucrose pellets). However, a paradigm that involves selecting between qualitatively different reinforcers may also closely model human decisions where we encounter options that are more/less preferred, not more/less of the same reward (Salamone *et al.*, 1991; Cousins & Salamone, 1994; Nowend *et al.*, 2001; Salamone *et al.*, 2007; Randall *et al.*, 2012; Nunes *et al.*, 2013; Randall *et al.*, 2014a; Randall *et al.*, 2015; Yohn *et al.*, 2016a; Yohn *et al.*, 2016b; Yohn *et al.*, 2016c; Salamone *et al.*, 2017). The majority of the seminal rodent studies in effort-based choice (Walton *et al.*, 2002; Walton *et al.*, 2003; Floresco & Ghods-Sharifi, 2007; Hauber & Sommer, 2009; Winstanley & Floresco, 2016) have used traditional pharmacological and lesion approaches, so a fine-grained analysis involving viral-mediated targeting of ACC in effort choice has not yet been reported. Designer receptors exclusively activated by designer drugs (DREADDS) provide an ideal means to do so. With this approach, we used a viral vector to selectively transfect ACC excitatory neurons with synthetic inhibitory or excitatory receptors that have no endogenous ligand. By administering the otherwise biologically inert ligand clozapine-N-oxide (CNO) (Armbruster *et al.*, 2007; Roth, 2016), were able to temporally, discretely manipulate the activity of these populations of neurons. We chose to express synthetic receptors under a CaMKIIα promoter, selectively targeting excitatory neurons in cortex (Nathanson *et al.*, 2009; Wang *et al.*, 2013). This was due to the complex effects observed when targeting both excitatory and inhibitory neurons (Lopez *et al.*, 2016).

We tested the effects of inhibitory (Gi) and excitatory (Gq) DREADDs in ACC on the same effortful choice task that we previously probed following lesions (Hart *et al.*, 2017) and pharmacological inactivations (Hart & Izquierdo, 2017). Briefly, our task required animals to

choose between working for a preferred reward (sucrose) vs. consuming a concurrently and freely-available, but less preferred reward (standard chow). We assessed the role of ACC on (i) progressive ratio (PR) lever pressing for sucrose pellets (i.e. general motivation), and (ii) PR pressing in the presence of a freely available alternative (i.e. effortful decision-making: choosing between working for sucrose pellets vs. concurrently available laboratory chow). We also tested the effects of ACC inhibition and excitation on the choice between sucrose pellets vs. chow when these reinforcers were both freely available.

Method

Subjects. Subjects were N=40 adult male Long-Evans rats (n=12 G_i DREADD experiment, n=12 G_q DREADD experiment, n=10 GFP (null virus) control experiment, n=6 acute slice recording for validation of DREADDs). Rats were obtained from Charles River Laboratories (Hollister, CA), were PND 60 at the time of arrival to the UCLA vivarium, and were singly-housed for all phases of experiments with the exception of the acclimation period and handling, during which they were pair housed. All animals were handled for 10 minutes in pairs for 5 d after a brief acclimation period (3 d). Rats weighed an average of 309.1 g at the beginning of experiments. Two subjects were not included in the final data analyses (one from G_i experiment, one from G_q experiment) due to unilateral (not bilateral) viral expression. Results from histological processing are shown in **Fig. 5-1.** The vivarium was maintained under a 12/12 h reverse light cycle at 22°C, and lab chow and water were available ad libitum prior to behavioral testing. Rats were food restricted one day prior to behavioral testing to ensure motivation to work for rewards. Given the sensitivity of the behavioral tests on motivation, special care was taken to maintain consistent food rations throughout the experiment. This was 12 g/d at the beginning of testing, but then decreased to 8 g/d at the beginning of the choice phase (details below). Rats were monitored every other day for their

body weight, and were never allowed to drop below 85% free feeding weight. Training and testing were conducted during the early portion of the dark cycle (~0800 to 1200 H). Experiments were conducted 5-7 d per week, and animals were fed once daily on weekends (12 g) when testing was not conducted. All procedures were approved by the Chancellor's Animal Research Committee (ARC) at the University of California, Los Angeles.

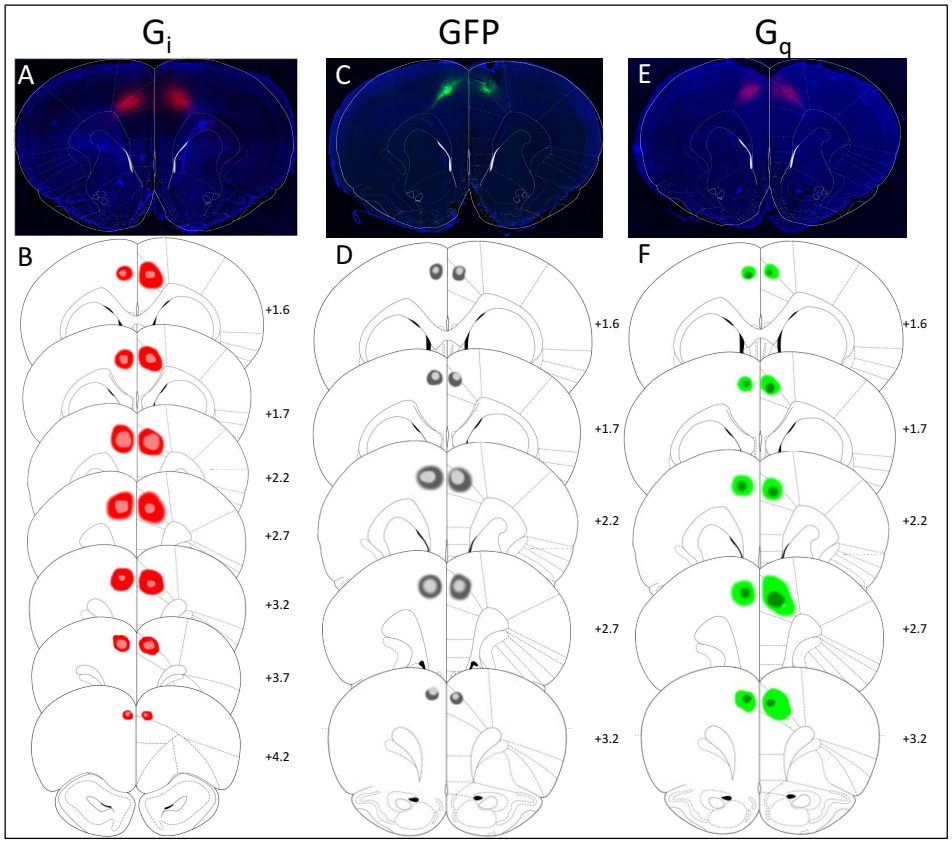

Figure 5-1. Histology. (A) Representative photomicrograph showing mCherry expression in ACC G_i experiment. **(B)** Schematic reconstruction of maximum (red) and minimum (pink) viral spread. **(C)** Representative photomicrograph showing GFP expression in ACC in GFP control experiment. **(D)** Schematic reconstruction of maximum (dark grey) and minimum (light grey) viral spread. **(E)** Representative photomicrograph showing mCherry expression in ACC G_q experiment. **(F)** Schematic reconstruction of maximum (light green) and minimum (dark green) viral spread.

Food restriction. One day before behavioral testing began, rats were singly housed, the amount of chow given to each rat was reduced to 12 g/d, and rats were given ~10 sucrose pellets (45-mg dustless precision sucrose pellets; Bio-Serv, Frenchtown NJ) in their home cage to acclimate them

to the food rewards. Rats were maintained on 12g of food daily for testing. Once rats progressed to the choice task they were given 8 g of chow per d, in addition to the food they consumed during testing. At the time of euthanasia, rats weighed an average of 356.5 g.

Stereotaxic Surgery. General surgical procedures were the same as those recently published (Hart & Izquierdo, 2017). Animals were anesthetized with isoflurane (5% induction, 2% maintenance in 2L/min O_2). Burr holes were drilled bilaterally on the skull for insertion of 26 gauge guide cannulae (PlasticsOne, Roanoke, VA), after which 33 gauge internal cannulae (PlasticsOne, Roanoke, VA) were inserted. Animals were infused with 0.5 μL of virus at a flow rate of 0.1 μL/minute after which injectors were left in place for 5 additional minutes to allow for diffusion of solution. In the G_i experiment, the virus used was AAV8- CaMKIIα -hM4D(G_i)-mCherry (Addgene, Cambridge, MA). In the G_q experiment, the virus used was AAV8- CaMKIIα -hM3D(G_q)-mCherry (Addgene, Cambridge, MA). In the GFP (null virus) control, the virus used was AAV8- CaMKIIα -eGFP (Addgene, Cambridge, MA). The coordinates used for the guide cannulae targeting ACC in the G_i, G_q, and GFP control experiments were: AP= +2.0 mm, ML= ±0.7 mm, DV= −1.9 mm from Bregma. Four of twelve animals in the G_i experiment received infusions at AP= +3.7 mm, ML= ±0.8 mm, DV= −1.6 mm from Bregma. Injectors extended 1 mm beyond the tip of the cannula. Following the 5-minute diffusion time, the cannulae and injectors were removed, incisions were stapled closed, and the rats were placed on a heating pad and kept in recovery until ambulatory before being returned to the vivarium.

Post-operative care for all animals consisted of five daily injections of carprofen (5mg/kg, s.c.) and oral sulfamethoxazole/trimethoprim solution. Rats were allowed a 5-d free feeding recovery period following viral infusion after which they were food restricted and behavioral testing began.

Apparatus. All behavioral testing was conducted in chambers outfitted with a house light, internal stimulus lights, a food-delivery magazine, and 2 retractable levers positioned to the left and right of the chamber wall opposite the magazine. All hardware was controlled by a PC running Med-PC IV (Med-Associates, St. Albans, VT).

FR1, initial progressive ratio (PR) and effortful choice testing. Rats were first given FR1 training where each lever pressed earned a single sucrose pellet (Bioserv, Frenchtown, NJ). They were kept on this schedule until they earned at least 30 pellets within 30 minutes. Following this, rats where shifted to a progressive ratio (PR) schedule where the required number of presses for each pellet increased according to the formula:

$$n_i = 5e^{(i/5)} - 5$$

where n_i is equal to the number of presses required on the ith ratio, rounded to the nearest whole number (Richardson & Roberts, 1996), after 5 successive schedule completions. No timeout was imposed. Rats were tested on the PR schedule until they earned at least 30 pellets on any given day (~5 d). Upon meeting this criterion, a ceramic ramekin containing 18 g of lab chow was introduced (modified from (Randall *et al.*, 2012)) during testing. Rats were free to choose between consuming freely-available but less preferred chow or lever pressing for preferred sucrose pellets. Rats (G_i, G_q, and GFP experiments) were given at least 5 choice testing sessions before injections began.

PR only control. During this test, we omitted the ceramic ramekin with freely available lab chow to assess whether manipulations decreased lever pressing in the absence of choice. This control experiment was conducted to rule out alternative explanations for our manipulations on effort, such as inability to lever press or impaired memory. Thus, in this phase, rats were still required to work for sucrose pellets, but there was no choice involved (i.e., no alternative food option offered).

Freely-available choice control. We conducted a test wherein both sucrose pellets and chow were freely-available. Rats were given 30 min of free access to pre-weighed amounts of sucrose pellets and lab chow (~18 g each) in standard empty cages, but different from their home cages. Following the 30-min period, remaining food was collected and weighed to determine rats' food preferences.

Drug testing. Animals in the G_i, G_q, and GFP control experiments were given either vehicle (95% saline, 5% DMSO) or CNO (3.0 mg/kg i.p. in 95% saline, 5% DMSO) (Tocris, Bristol, UK) 45 minutes prior to testing. Each of these conditions was administered in a choice testing session, a PR only testing session, and a free choice testing session, in that order. The order of vehicle versus CNO administration was counterbalanced based on the number of lever presses in the most recent choice testing session.

Euthanasia. Following behavioral testing, animals were sacrificed by sodium pentobarbital overdose (Euthasol, 0.8 mL, 390 mg/mL pentobarbital, 50 mg/mL phenytoin; Virbac, Fort Worth, TX) and perfused transcardially with 0.9% saline followed by 10% buffered formalin acetate. Brains were post-fixed in 10% buffered formalin acetate for 24 hours followed by 30% sucrose for 5 days. 50 µm sections were cover slipped with DAPI mounting medium (Prolong gold, Invitrogen, Carlsbad, CA), and visualized using a BZ-X710 microscope (Keyence, Itasca, IL).

Electrophysiological confirmation of DREADDs. Separate rats were prepared with ACC DREADDs using identical surgical procedures to the main experiments. Slice recordings did not begin until at least four weeks following surgery to allow sufficient hM receptor expression. Slice recording methods were similar to those previously published (Babiec, Jami, Guglietta, Chen, & O'Dell, 2017). Six rats were deeply anesthetized with isoflurane and decapitated. The brain was rapidly removed and submerged in ice-cold, oxygenated (95% O_2/5% CO_2) artificial cerebrospinal fluid (ACSF) containing (in mM) as follows: 124 NaCl, 4 KCl, 25 $NaHCO_3$, 1 NaH_2PO_4, 2 $CaCl_2$,

1.2 $MgSO_4$, and 10 glucose (Sigma-Aldrich). 400-μm-thick slices containing the ACC were then cut using a Campden 7000SMZ-2 vibratome. Slices from the site of viral infusion were used for validation. Expression of mCherry was confirmed post-hoc. Slices were maintained (at 30°C) in interface-type chambers that were continuously perfused (2–3 ml/min) with ACSF and allowed to recover for at least 2 hours before recordings. Following recovery, slices were perfused in a submerged slice recording chamber (2–3 ml/min) with ACSF containing 100 μM picrotoxin to block $GABA_A$ receptor-mediated inhibitory synaptic currents. A glass microelectrode filled with ACSF (resistance = 5–10 MΩ) was placed in layer 2/3 ACC to record field excitatory postsynaptic synaptic potentials and population spikes elicited by layer 1 stimulation delivered using a bipolar, nichrome-wire stimulating electrode placed near the medial wall in ACC. For inhibitory validation, stimulation intensity (0.2 msec duration pulses delivered at 0.33 Hz) was set to the minimum level required to induce reliable population spiking in ACC. Once reliable responses (measured as the area of postsynaptic responses over a 4 second interval) were detected, baseline measures were taken for at least 10 minutes, followed by a 20 minutes bath application of 10 μM CNO. Because all slices were silent without stimulation, slightly modified procedures were used for excitatory validation. Baseline measures without stimulation were taken for at least 10 minutes, followed by 20 minutes bath application of 10 μM CNO during which spontaneous activity was recorded. Input/output curves were generated before and after CNO application in cases where no spontaneous activity was observed. Unless noted otherwise, all chemicals were obtained from Sigma-Aldrich.

Data analyses. Behavioral data were analyzed using GraphPad Prism v.7 (La Jolla, CA) and SPSS v.25 (Armonk, NY). An alpha level for significance was set to 0.05. Paired samples t-tests (reported as means ± SEM) on data from the choice and PR-only tests were used to test for effects

of CNO. Two-way ANOVA was used to test for effects of CNO on food preference in the free choice test.

Results

Ex vivo electrophysiological validation of DREADDs. Application of CNO strongly suppressed FPs in G_i transfected slices (FP area was reduced to 13.4 ± 8% of baseline, n = 5 slices from 3 rats, $t_{(4)} = 11.333$, $p = 3.46 \times 10^{-3}$, paired t-test comparison to baseline) but had no effect on responses in non-transfected slices (FP area was 103.7 ± 7% of baseline, n = 4 slices from 3 rats, $t_{(3)} = 0.578$, $p = 0.604$) (**Fig. 5-2B)**. Application of CNO induced spontaneous bursting in four out of six G_q transfected slices (spontaneous activity rate was increased from 0 to 0.06 Hz ± 0.01 Hz, n=4 slices from 3 rats) (**Fig. 5-2C)**. In two other Gq transfected slices where no spontaneous bursting was observed, CNO application resulted in decreased threshold for stimulation induced postsynaptic responses (n=2 slices from 3 rats) (**Fig. 5-2D)**. CNO had no effect on responses in non-transfected slices (n=3 slices from 3 rats) (**Fig. 5-2E)**.

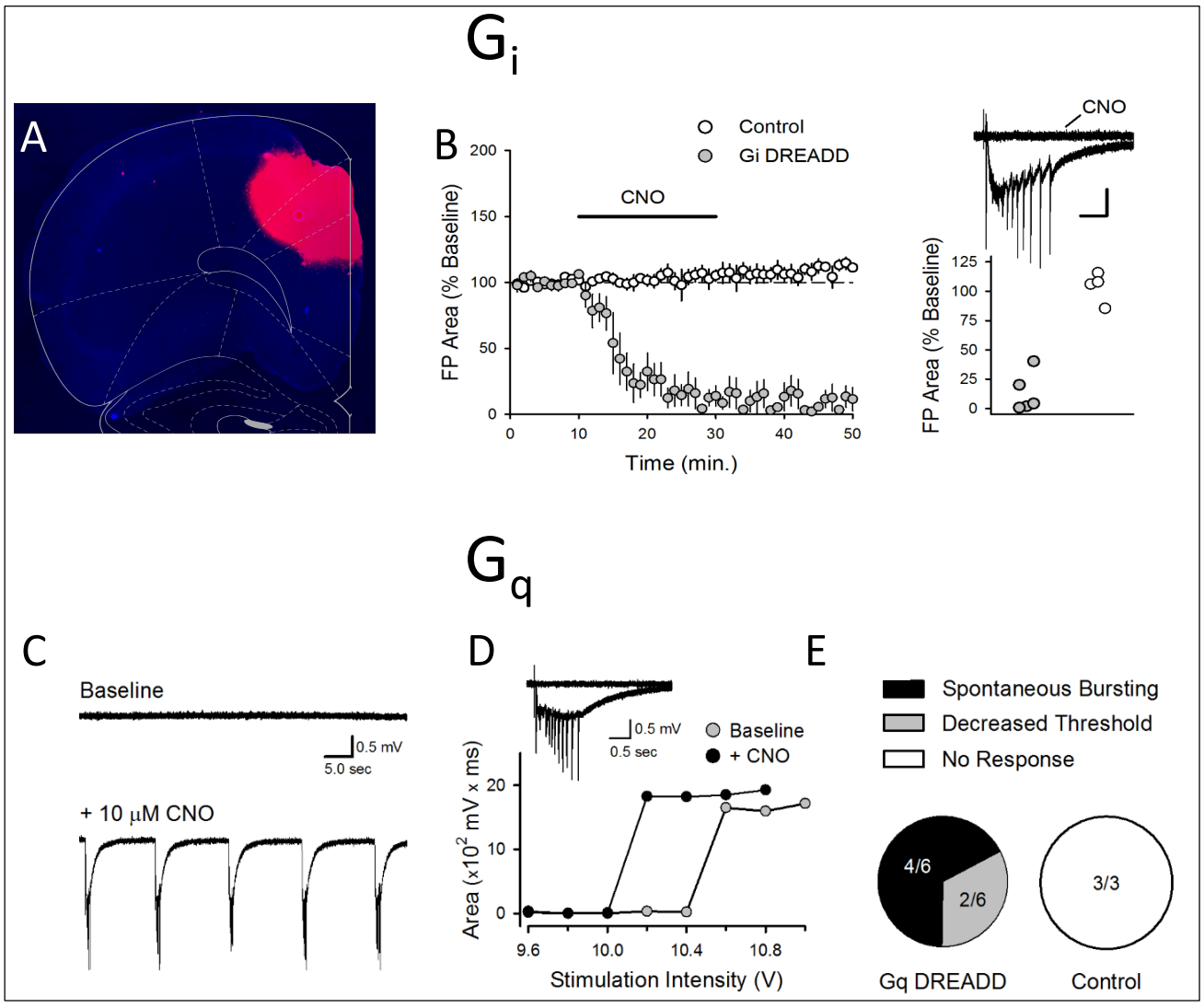

Figure 5-2. Electrophysiological effect of bath application of CNO in slice. **(A)** Representative photomicrograph showing mCherry expressing cells in ACC recording site of 400 µm slice. **(B)** Inhibition of field potentials (FP) elicited by layer I stimulation followed by bath application of CNO (10 µM, indicated by the bar). Traces show superimposed responses elicited before and after CNO application in a transfected slice. Calibration bars: 0.5 mV, 500 milliseconds. Bottom: Points show FP area (normalized to baseline) at end of CNO application (averaged over last 5 min) from individual slices. Application of CNO strongly suppressed FPs in transfected slices. **(C)** Spontaneous ACC activity in a Gq DREADD expressing slice before (top) and after a 20 minute bath application of 10 µM CNO (bottom). CNO-induced spontaneous bursting was seen in 4 out of 6 Gq DREADD-expressing slices from 3 animals. **(D)** In the two Gq DREADD-expressing slices that failed to show spontaneous bursting there was a decrease in the threshold for stimulation induced postsynaptic responses. Plot shows results from one of these slices before (baseline) and after CNO application. **(E)** Summary of responses to CNO in Gq DREADD-expressing and control slices. CNO did not elicit either spontaneous bursting or a decrease threshold for evoked responses in 3 control slices from 3 animals.

G_i experiment. A paired-samples t-test revealed CNO significantly reduced lever pressing during choice testing ($t_{(10)}$=3.047 p=0.01; VEH = 216.1 ± 52.85; CNO = 173.2 ± 40.27) **(Fig. 5-3A)**. Chow consumption was not affected by CNO treatment ($t_{(10)}$=1.048 p=0.32; VEH = 7.79 ± 0.33; CNO = 7.50 ± 0.36) **(Fig. 5-3B)**. CNO had no effect on PR responding in the absence of freely available chow ($t_{(10)}$=0.26 p=0.80; VEH = 905.1 ± 102.00; CNO = 893.9 ± 128.3) **(Fig. 5-3C)**. A two-way ANOVA on the amount of sucrose and chow consumed during free choice testing revealed a significant main effect of food type ($F_{(1,10)}$=12.02 p=0.006; sucrose = 8.59 ± 0.83; chow = 4.19 ± 0.34). No significant food type by condition interaction ($F_{(1,10)}$=1.415 p=0.26), or effect of condition ($F_{(1,10)}$=0.01 p=0.91) was found **(Fig. 5-3D)**.

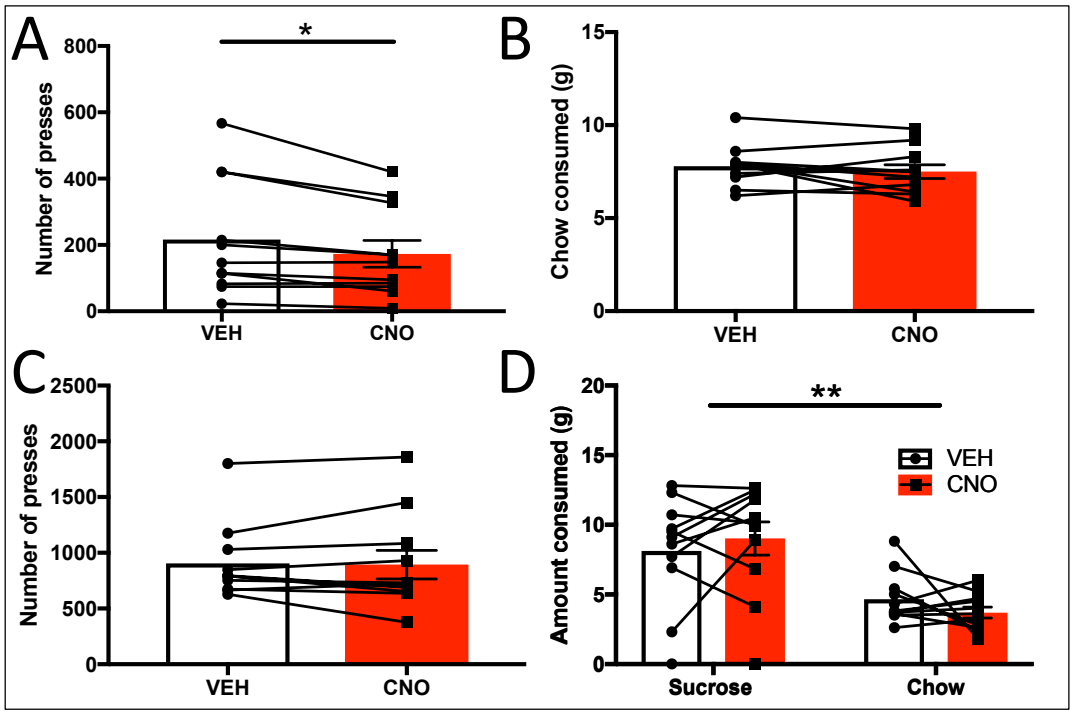

Figure 5-3. G_i effects. (A) Number of lever presses during choice testing under vehicle and CNO. CNO significantly reduced the number of lever presses. **(B)** Amount of chow consumed during choice testing under vehicle and CNO. **(C)** Number of lever presses during PR testing under vehicle and CNO, when there was no chow available as an alternative option. **(D)** Amount of sucrose and chow consumed during free choice testing under vehicle and CNO. * p<0.05, ** p<0.01

G_q experiment. A paired-samples t-test revealed CNO significantly reduced lever pressing during choice testing ($t_{(10)}$=2.31 p=0.04; VEH = 210.7 ± 49.44; CNO = 166.6 ± 40.4) **(Fig. 5-4A)**. Chow

consumption was not affected by CNO treatment ($t_{(10)}$=0.39 p=0.71; VEH = 7.71 ± 0.36; CNO = 7.81 ± 0.42) **(Fig. 5-4B)**. CNO had no effect on PR responding in the absence of freely available chow ($t_{(10)}$=1.15 p=0.28; VEH = 1107.00 ± 99.66; CNO = 1209.00 ± 156.3) **(Fig. 5-4C)**. A two-way ANOVA on the amount of sucrose and chow consumed during free choice testing revealed a significant main effect of food type ($F_{(1,10)}$=47.63 p<0.001; sucrose = 9.68 ± 0.47; chow = 5.10 ±

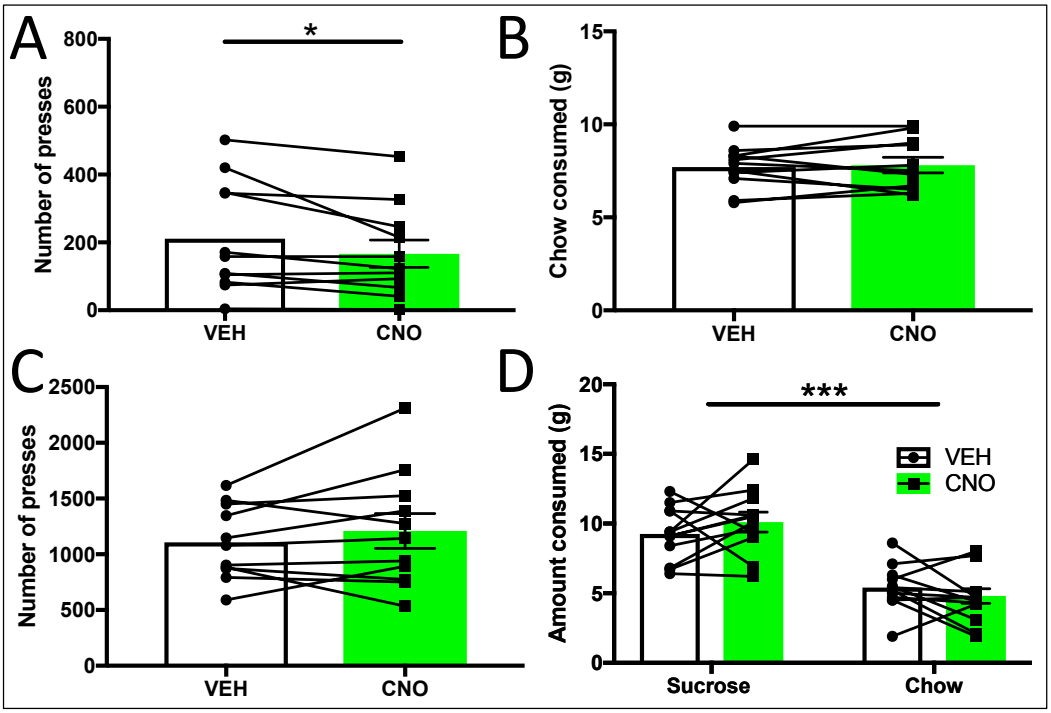

Figure 5-4. G_q effects. (A) Number of lever presses during choice testing under vehicle and CNO. CNO significantly reduced the number of lever presses. **(B)** Amount of chow consumed during choice testing under vehicle and CNO, when there was no chow available as an alternative option. **(C)** Number of lever presses during PR testing under vehicle and CNO. **(D)** Amount of sucrose and chow consumed during free choice testing under vehicle and CNO. * p<0.05, *** p<0.001

0.37). No significant food type by condition interaction ($F_{(1,10)}$=2.27 p=0.16), or effect of condition ($F_{(1,10)}$=0.06 p=0.81) was found **(Fig. 5-4D)**.

GFP (null virus) control experiment. A paired-samples t-test revealed no effect of CNO on lever pressing during choice testing ($t_{(9)}$=0.48 p=0.64; VEH = 314.9 ± 57.34; CNO = 302.7 ± 59.66) **(Fig. 5-5A)**. Chow consumption was not affected by CNO treatment ($t_{(9)}$=0.26 p=0.80; VEH = 6.65 ± 0.99; CNO = 6.85 ± 0.34) **(Fig. 5-5B)**. CNO had no effect on PR responding in the absence

of freely available chow ($t_{(10)}$=0.73 p=0.48; VEH = 1001.00 ± 109.4; CNO = 1043.00 ± 120.00) **(Fig. 5-5C)**. A two-way ANOVA on the amount of sucrose and chow consumed during free choice testing revealed a significant main effect of food type ($F_{(1,9)}$=80.63 p<0.001; sucrose = 8.37 ± 0.60; chow = 3.19 ± 0.35). No significant food type by condition interaction ($F_{(1,9)}$=1.27 p=0.29), or effect of condition ($F_{(1,9)}$=0.97 p=0.35) was found **(Fig. 5-5A)**.

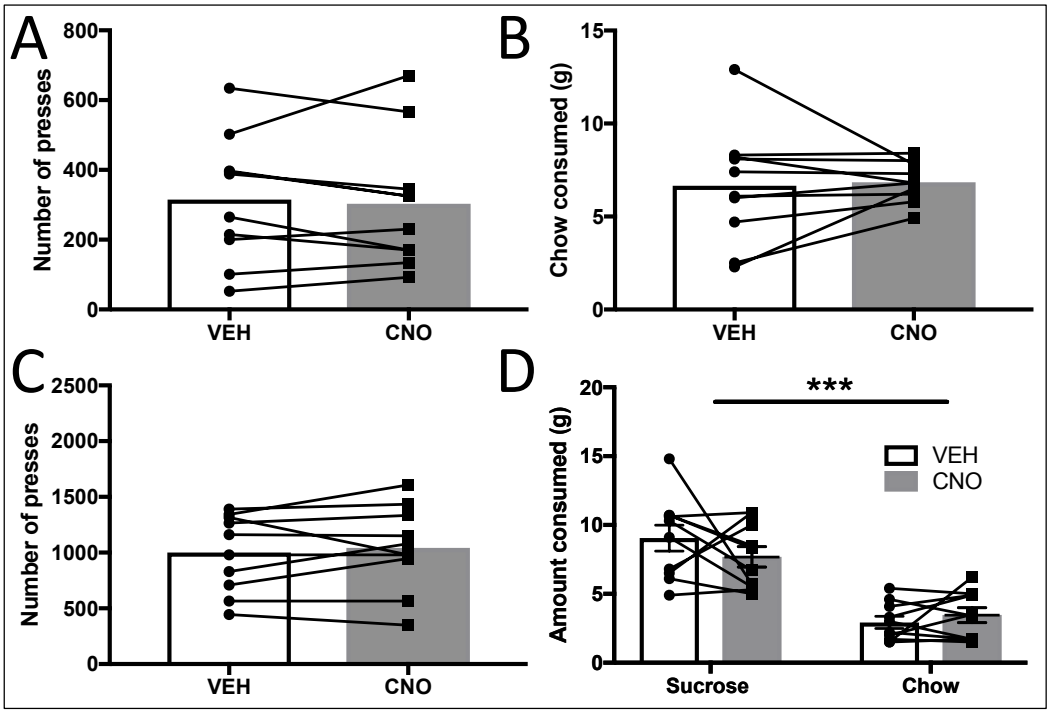

Figure 5-5. GFP (null virus) controls. (A) Number of lever presses during choice testing under vehicle and CNO. **(B)** Amount of chow consumed during choice testing under vehicle and CNO. **(C)** Number of lever presses during PR testing under vehicle and CNO, when there was no chow available as an alternative option. **(D)** Amount of sucrose and chow consumed during free choice testing under vehicle and CNO. *** p<0.001

Discussion

We found that chemogenetic silencing and stimulation of ACC excitatory neurons resulted in decreased PR lever pressing only when a concurrently available, lower effort alternative was available. These same manipulations had no effects on ability to lever press or food preference. CNO administration had no effects in animals lacking active hM4D-G_i or hM3D-G_q receptors.

Slice electrophysiology confirmed robust inhibition and excitation in hM4D-G_i and hM3D-G_q transfected slices.

ACC chemogenetic silencing

The earliest studies probing rat ACC in effort-based choice made use of T-maze tasks where rats chose between the same food option in different magnitudes (Walton *et al.*, 2002; Walton *et al.*, 2003; Schweimer & Hauber, 2006). The first test of rat ACC in a task where rats chose between qualitatively different options produced negative results (Schweimer & Hauber, 2005); it is possible this was due to pre-training lesions as well as prior behavioral testing, as later work from our lab found that ACC lesions resulted in decreased PR lever pressing in the context of choice (Hart *et al.*, 2017).

We replicated and expanded upon those results. Both hM3D and hM4D receptors were expressed under a CaMKIIα promoter, thus targeting primarily excitatory pyramidal neurons (Nathanson *et al.*, 2009; Wang *et al.*, 2013), where lesions or inactivations destroy or silence all neural activity. It therefore seems likely that ACC exerts its effects via projections to downstream targets, the densest of which are to dorsal striatum and mediodorsal thalamus (Vogt & Paxinos, 2014), though ACC also sends sparser efferents to ventral striatum and amygdala (Gabbott *et al.*, 2005). A recent report showed a role for dorsal striatal dopamine in effort-based choice (Bailey *et al.*, 2018). Interestingly, the role of dorsal striatum in effort-based choice is relatively understudied and could be a ripe avenue for future study given the strong cortical afferentation from ACC. Nevertheless, results from the G_i experiment provide replication of findings from lesion and inactivation studies using a modern, cell-type specific approach.

ACC inhibition versus stimulation

Effort-based choice could be a useful model of motivational symptoms that occur in a wide range of conditions, particularly depression (Nunes *et al.*, 2013). Ergo a major focus has been on reversing deficits in effort as well as increasing effort output in otherwise normal subjects. Indeed, impairments in effort induced by dopamine receptor blockade or dopamine depletion can be reversed by several manipulations that enhance dopamine transmission or block adenosine transmission (Farrar *et al.*, 2010; Nunes *et al.*, 2013; Randall *et al.*, 2014a; Yohn *et al.*, 2015a; Yohn *et al.*, 2015b). Further, enhancing dopamine transmission with major psychostimulants (Yohn *et al.*, 2016c), dopamine transporter blockers (Randall *et al.*, 2014b; Yohn *et al.*, 2016a), adenosine A2A receptor antagonists (Randall *et al.*, 2012), or 5-HT2C ligands (Bailey *et al.*, 2016; Bailey *et al.*, 2018) can increase effort output in otherwise untreated animals. We tested whether ACC chemogenetic stimulation would similarly enhance responding. This was not the case. Though there is overwhelming evidence that effort in similar paradigms is bidirectionally modulated by dopamine and related signaling mechanisms, the evidence suggests this does not occur in cortex. Blocking inhibitory transmission, which presumably increases local excitation, in orbitofrontal cortex resulted in decreased PR responding (Munster & Hauber, 2017). Others reported a similar effect of local infusion of the GABA-A antagonist bicuculline in prelimbic cortex: decreased high-effort choice in a two-lever operant task (Piantadosi *et al.*, 2016). We found a very similar pattern of effects here. The contributions of ACC and other cortical regions to effort are complex and likely involve ideal excitatory/inhibitory balance in computing relative cost-benefit analyses and sending appropriate output to downstream targets. It is possible that the G_q stimulation used here enhanced off-task neural activity, interfered with normal recruitment of ACC during choice lever pressing, or even produced local inhibition in-vivo that we were unable to detect in slices. The results of ACC stimulation here are consistent with our manipulation simply

introducing noise in otherwise normal neural computation (Mainen & Sejnowski, 1995; Stein *et al.*, 2005), thus impairing behavior.

Concluding remarks

We successfully replicated findings using classical techniques with a modern chemogenetic approach. Notably, the magnitude of effect observed here was much lesser than what was observed with lesions (Hart *et al.*, 2017). This could be due to several factors: fewer neurons were transfected with hM receptors than would be destroyed by lesions, DREADD receptors do not completely suppress neuronal firing in-vivo, interfering with output neurons does not have the same effects as interfering with all neurons, or some combination of these. Future research should come closer to a ground-truth validation of DREADD effects and should determine the role of ACC in effort-based choice in real-time using in-vivo recording approaches, especially imaging where single cells can be tracked across sessions (Cai *et al.*, 2016). This would allow a comparison between the pattern of ACC activity in sessions where lever pressing is the only food option versus where it is a choice. Given that all of our manipulations only affected lever pressing during choice testing, it seems likely that neural coding of high effort lever pressing behavior is vastly different depending on the organism's option space. This remains an empirical question.

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Chapter 6: Conclusions

Most studies aimed at understanding the mechanisms of effort-based choice focused on dopamine signaling in the striatum. Where other parts of the brain were probed, subjects chose between options that varied in quantity rather than quality. Very few studies have tested whether behavioral factors such as stress or exposure to drugs of abuse have effects. Here, we tested whether BLA and ACC are involved in effort-based choice where subjects chose between options that varied in quality, and we also tested the effects of withdrawal from methamphetamine intravenous self-administration.

In the first set of studies, we performed standard pharmacological inactivations of BLA and tested rats in our effort-based choice task. Animals were less willing to exert high levels of effort only when there was a concurrently available "easier" food option. Critically, this decreased high effort choice did not occur when rats were tested for PR performance in the absence of the free lab chow. The same manipulations had no effect on free-feeding food preference. Thus, BLA is involved selectively in relative cost-benefit analyses involving effort. The next set of studies tested whether ACC lesions had similar effects. We found that this manipulation, like BLA inactivations, decreased PR responding only in the context of choice; PR responding was intact when animals did not have freely available lab chow during testing. These effects were also not due to any change in food preference or learning impairment. We tested whether withdrawal from methamphetamine self-administration had effects in our task. We found that withdrawn animals exhibited a phenotype very similar to that of BLA inactivation or ACC lesion: decreased choice lever pressing, intact PR responding, and intact food preference. Somewhat unsurprisingly, this effect was accompanied by decreased activation of BLA and ACC. Lastly, we replicated ACC lesion effects using a modern chemogenetic approach. All of the manipulations tested had

remarkably similar effects: selective "laziness" for the preferred option that did not occur if there were no competing option. This is not the case with all brain and drug manipulations, as discussed later.

Implications of studies involving effort

To be successful at foraging, organisms must evaluate the cost of reward (i.e., risk, delay, effort) and often appreciate this cost in relative terms (i.e. in comparison with other options that may be concurrently available). This ability is of interest to a variety of fields including psychology, neuroscience, behavioral ecology, and ethology (Dayan and Daw 2008). Effort, delay, and risk discounting paradigms generally do not access how animals gather information of the options in unknown or changing reward environments (i.e. learning) but instead test how well they maximize or exploit rewards in well-known conditions (i.e. performance). As mentioned above, one important exception is that the rat must learn about and adapt to the decreasing value of the more effortful option, if food rewards are involved. When we consider a hierarchy of costs, some are given priority consideration: for example, risk of punishment or predation is weighed more heavily than physical effort costs, delay costs, or risk of non-reward. Similarly, the opportunity to exploit a resource often comes at the expense of exploring another (Addicott et al. 2017), known as the explore-exploit tradeoff. This is likely not a factor in environments where resources are not depleting, and where reinforcement contingencies are known to the organism. Physical effort has underpinnings deeply embedded in the mesocorticolimbic system. Indeed, we have found similarities with inhibition of normal functioning of ACC (Hart et al. 2017), BLA (Hart and Izquierdo 2017), and chronic methamphetamine experience, whether experimenter- or self-administered (Thompson et al. 2017; Hart, Gerson, and Izquierdo 2018).

Overcoming effort costs to acquire food is necessary for survival in humans and other animals. Of course, other appetitive events, such as exercise, sex, or drugs are often impeded by effort costs, and behavior directed toward any one of these events may or may not be adaptive depending on several situational factors. For example, it might be maladaptive for an obese rat to pursue more food at the cost of other rewards. It may be similarly maladaptive for addicted individuals to pursue drugs. Growing evidence implicates similar underlying mechanisms in effort for food as those in willingness to exercise (Correa et al. 2016; Lopez-Cruz et al. 2018) or to seek drugs (Kavanagh et al. 2015). Future experiments should test whether the circuitry that is known to regulate effort to acquire food similarly affects other rewards. This line of work is important because effort tasks have high translational value (Treadway et al. 2009; Wardle et al. 2011; Treadway and Zald 2011; Treadway et al. 2012): effort is implicated in many conditions, including drug withdrawal (Thompson et al. 2017; Hart, Gerson, and Izquierdo 2018), depression (Treadway et al. 2012), and schizophrenia (Gold et al. 2013; Treadway et al. 2015). Further, fatigue, which can be characterized as decreased effort, is one the most common symptoms in general medicine (Demyttenaere, De Fruyt, and Stahl 2005), and tasks assessing effort could be useful in modeling effort-related symptoms that occur in depression and other conditions (Nunes et al. 2013).

All of the manipulations used in this dissertation produced the same phenotype (**Fig. 6-1)**, which leaves many research questions open for study. Deficits in effort based choice that are induced by dopamine receptor blockade or dopamine depletion can be reversed by both major psychostimulants that increase dopaminergic transmission (Yohn *et al.*, 2016c), antidepressant drugs that block the dopamine transporter (Nunes *et al.*, 2013; Randall *et al.*, 2014; Yohn *et al.*, 2016a; Yohn *et al.*, 2016b), and adenosine A2A receptor antagonists that converge on dopamine-dependent signal transduction (Farrar *et al.*, 2010; Nunes *et al.*, 2010; Nunes *et al.*, 2013; Randall

et al., 2014; Yohn *et al.*, 2015). This raises the question: are the deficits in effort-based choice we observed here equally reversible?

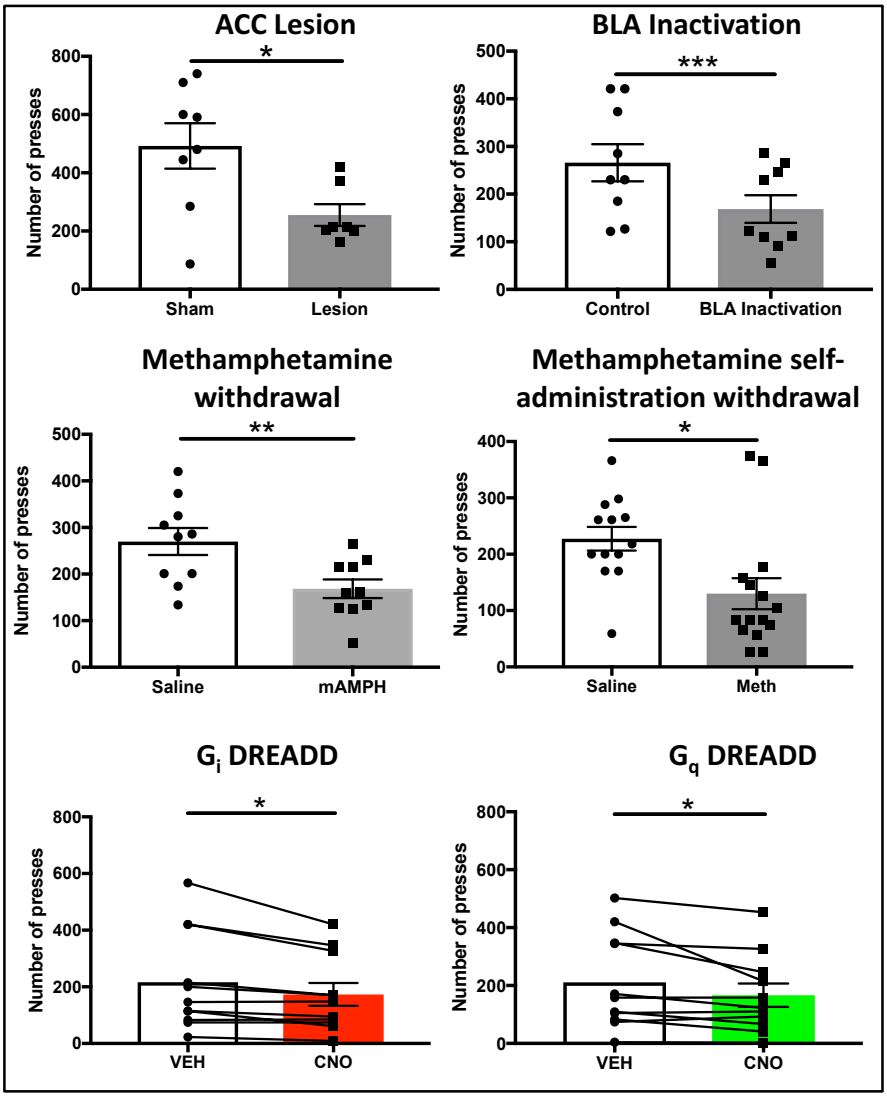

Figure 6-1. Comparison of different manipulations on the same quality-based effort paradigm. (Top left) ACC lesions decreased lever pressing in the context of a competing, low effort reinforcer. (Top right) BLA inactivations decreased high effort lever pressing in the context of choice. (Middle left) Withdrawal from experimenter-administered methamphetamine decreased choice lever pressing. (Middle right) Withdrawal from methamphetamine intravenous self-administration decreased choice lever pressing. (Bottom left and right) ACC G_i and G_q DREADD both decreased choice lever pressing. Data are adapted from Hart et al. (2017), Hart & Izquierdo (2017), Thompson et al. (2017), and Hart et al. (2018).

There is some evidence. A recent report showed that the dopaminergic stimulant methylphenidate attenuates deficits in effort output induced by manipulations of medial

orbitofrontal cortex (Munster & Hauber, 2017). Future experiments should determine whether this is also the case for deficits in effort induced by anterior cingulate and amygdalar manipulations as well as in stimulant withdrawal. This seems likely, given that BLA, ACC, and striatum act as part of a network involved in the regulation of effort (Salamone *et al.*, 2007). If deficits in effort induced by many different manipulations are equally reversible, these studies may have high translational value. Effort-based choice in humans shows correlates in cingulate cortex (Massar *et al.*, 2015), and amygdala (Chong *et al.*, 2017). Therefore, it is likely that aberrations in effort allocation which occur in many conditions due to diffuse pathology may be responsive to dopaminergic medication.

Future directions and final notes

As mentioned before, exerting effort (or not) toward any particular stimulus can be adaptive or maladaptive depending on many factors. Future experiments should seek to find manipulations that control effort allocation toward different classes of stimuli independently. Some such experiments have already been done. Indeed, subthalamic nucleus lesions increase PR responding for food but decrease PR responding for drugs (Baunez *et al.*, 2002; Baunez *et al.*, 2005), and stimulation of adenosine receptors can attenuate cocaine seeking without having any effects on food seeking (Bachtell & Self, 2009; O'Neill *et al.*, 2012). If novel medications and treatments are to be developed, it is desirable that they have properties such that they control effort output for any given stimulus independently.

Not every manipulation produces deficits in effort-based choice. In a separate experiment (unpublished) rats were exposed to chronic intermittent ethanol exposure (CIE) or water vapor control for six weeks. Both groups were allowed to reach stable performance as in the ACC experiments. While groups did not differ in the number of lever presses once performance was stable, they did differ in the number of sessions required to reach stable performance. Given that

opiate abuse is currently a major public health concern in the United States, future experiments should test the effects of opiate withdrawal as well as other drugs of abuse.

While studies probing how behavior is impaired following manipulations are necessary, studies testing whether behavior can be enhanced are equally important. Some such experiments have been performed. Major psychostimulants (Wardle *et al.*, 2011; Yohn *et al.*, 2016c), dopamine transporter blockers (Randall *et al.*, 2015; Yohn *et al.*, 2016a), and adenosine A2A receptor antagonists (Randall *et al.*, 2012) all increase progressive ratio responding in otherwise neurotypical subjects. Because all of the manipulations tested here resulted in decreased effort, we also tested whether we could replicate the effects of dopamine transporter blockade. This was the case: 40 mg/kg bupropion administered i.p. 30 min prior to testing significantly increased PR responding during choice testing and decreased the amount of chow consumed (**Fig. 6-2**). Thus, it is not the case that we can only produce "lazy" rats.

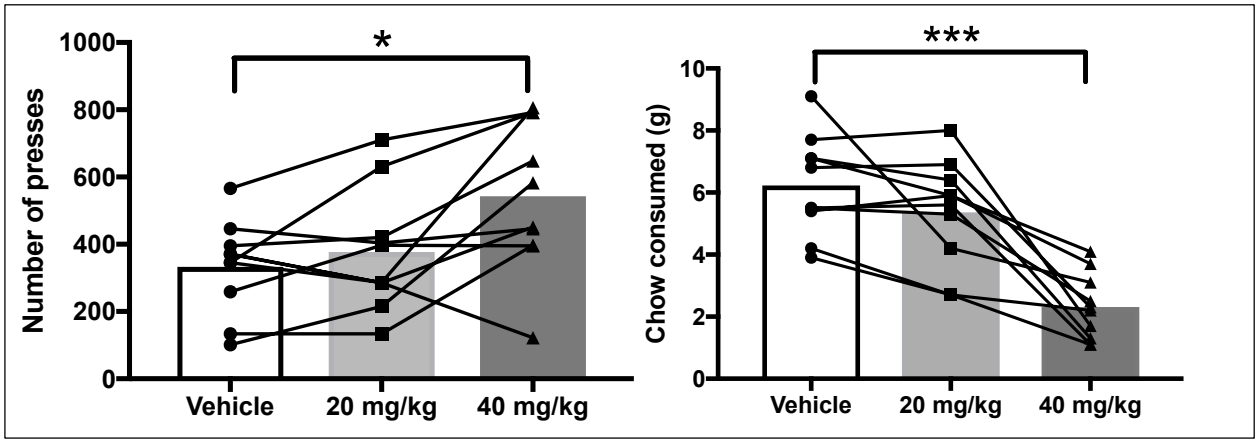

Figure 6-2 (Left) 40 mg/kg bupropion significantly increased the number of lever presses during choice testing. (Right) Bupropion 40 mg/kg significantly decreased the amount of chow consumed during choice testing. *, *** Significantly different from vehicle, $p<0.05$, $p<0.001$

Psychological science and other fields are currently undergoing a major reproducibility crisis (Estimating the reproducibility of psychological science, 2015), thus stressing the need for incremental, replicable science. In two chapters of this dissertation, brain regions that were known

to be important in effort-based choice where subjects choose between quantitatively different options were shown to have similar roles in qualitative choice, using classical interference methods. A known effect of stimulant withdrawal in humans was described in rats, with underlying correlates found. We further expanded upon our original findings using a modern, cell-type specific approach with rigorous validation of the technology. These studies provide a clear, converging line of evidence implicating several structures and manipulations, thus laying the groundwork for broad implications of research involving the basic mechanisms of effort-based choice.

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