# Investigating the Neurobiology Regulating Cognitive Effort Allocation Using a Rodent Model of Cost/Benefit Decision Making

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## Abstract

Choosing adaptively among candidate actions requires a cost-benefit analysis, in which potential rewards are considered against the costs required to obtain them. One cost frequently encountered by humans is the cost of cognitive effort, in which executive processes spanning attention, working memory, reasoning, and the like are taxed. This form of effort is in contrast to the physical effort costs that have generally been the focus of the cost/benefit decision-making literature. This thesis reviews the currently available literature investigating the brain regions and neurotransmitter systems guiding these respective forms of decision making, and then carries out a set of experiments to further characterize the neurobiology guiding cognitive effort allocation. These experiments utilize an animal model of decision making known as the rodent Cognitive Effort Task (rCET), in which subjects decide whether to exert more attention in pursuit of larger rewards, or to obtain smaller reward for comparatively less attentional demand. In experiment 1, I use chemogenetics to downregulate cholinergic neurons of the basal forebrain as rats perform the rCET, to determine whether this neuronal population is responsible for the previously ascribed role of acetylcholine in regulating decision making with cognitive effort costs. Experiments 2 and 3 use standard inactivation techniques to investigate striatal and orbitofrontal cortex contributions to this form of decision making, and Experiment 4 uses a disconnection procedure to assess whether BLA- ACC signaling regulates

willingness to apply cognitive effort. Collectively, the current findings complement existing work in the domain of physical effort allocation, and support the notion that these two forms of effort-based decision making are mediated by distinct, albeit overlapping circuitries. While this work fundamentally contributes to an understanding of how organisms navigate their environment, it also has practical utility. Indeed, work with the rCET and related cognitive effort paradigms may help identify behavioural or pharmacological therapies that can boost cognitive willingness. Alternatively, information gained may shed light on the aberrant processes underlying a blunted desire to achieve lucrative outcomes, as observed in disorders like depression, schizophrenia, and Parkinson's Disease.

## Lay Summary

As we interact with the world, we often face situations where we can decide to invest cognitive effort for rewarding outcomes. Take, for example, the student who strives to do well in school in hopes that it will lead to lucrative job prospects. In its purest form, cognitive effort allocation is a cost-benefit analysis, and it is valuable to know the neurotransmitters and brains regions guiding this process. This thesis uses a rodent model of cognitive effort allocation to experimentally assess how different neurotransmitters and brain areas regulate decision making with cognitive effort costs. The findings from this thesis identify a number of brain structures and neurotransmitters that interact to bias organisms to put in mental work for outcomes they want. Importantly, this information may shed light on how these processes are disrupted in disorders characterized by a blunted desire to achieve lucrative outcomes, such as depression, schizophrenia, and Parkinson's Disease.

## Preface

The candidate, Dr. Adams, and Dr. Winstanley designed Experiment 1 (Chapter 3). Sukhbir Kaur bred the rats and I assisted with genotyping. I conducted behavioural testing. Dr. Adams and I performed the viral transfection surgeries. I prepared and delivered the pharmacological challeneges. Emily Allin assisted with pharmacological challenges. I independently analyzed the data, interpreted the results, and wrote the chapter. Dr. Winstanley reviewed and revised the chapter prior to inclusion in the thesis.

Experiment 2 (Chapter 4) has been previously published in manuscript form: Silveira, M.M., Tremblay, M., & Winstanley, C.A. 2018. Dissociable contributions of dorsal and ventral striatal regions on a rodent cost/benefit decision-making task requiring cognitive effort. *Neuropharmacology, 137,* 322-331. The candidate and Dr. Winstanley were responsible for study concept and design. I collected the behavioural data, performed cannulation surgeries, and conducted the inactivations. Dr. Tremblay assisted with surgeries and inactivations. I performed data analyses and drafted the manuscript. Dr. Winstanley reviewed and revised the manuscript prior to manuscript submission.

The candidate and Dr. Winstanley designed Experiment 3 (Chapter 5). Sukhbir Kaur bred the rats and I assisted with genotyping. Sebastian Wittenkindt conducted behavioural testing and assisted with inactivations. I performed the cannulations and the inactivations. Sophie Ebsary sectioned brains for

histological analysis. I independently analyzed the data, interpreted the results, and wrote the chapter. Dr. Winstanley reviewed and revised the chapter prior to inclusion in the thesis.

The candidate and Dr. Winstanley designed Experiment 4 (Chapter 6). Leili Mortazavi conducted behavioural testing and assisted with inactivations. I performed the cannulations and the inactivations. Leili Mortazavi sectioned brains for histological analysis. I independently analyzed the data, interpreted the results, and wrote the chapter. Dr. Winstanley reviewed and revised the chapter prior to inclusion in the thesis.

The candidate and Dr. Winstanley designed Experiment 5 (Appendix 1). Sukhbir Kaur bred the rats and I assisted with genotyping. Sebastian Wittenkindt and Leili Mortazavi conducted behavioural testing. I prepared and delivered the drugs. Leili Mortazavi assisted with pharmacological challenges. I consulted Dr. Wendy Adams for advice on preparing the drugs. I independently analyzed the data, interpreted the results, and wrote the chapter. Dr. Winstanley reviewed and revised the chapter prior to inclusion in the thesis.

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All animal testing was performed in accordance with the Canadian Council on Animal Care (CCAC) and received ethical approval by the University of British Columbia Animal Care Committee, certificate numbers A13-0011 (behavioural protocol) and A15-0011 (breeding protocol). Adeno-associated virus delivery was

performed in accordance with approved standard operating procedure ACC WINSTANLEY CAW116. All procedures are standard for working with rAAVs classified as Risk Group 1, and have been approved by the UBC Biosafety Committee (protocol B15-0027).

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## List of Abbreviations

ACC – anterior cingulate cortex

Ach – acetylcholine

ANOVA- analysis of variance

BLA- basolateral amygdala

BOLD- blood-oxygen-level-dependent

CRF – corticotrophin releasing factor

DA – dopamine

DLS – dorsolateral striatum

DMS – dorsomedial striatum

EEfRT- Effort Expenditure for Rewards Task

FR- fixed ratio

HR – high cost/high reward

IL- infralimbic

LR- low cost/low reward

kg – kilogram

ITI- inter-trial interval

mg - milligram

mPFC- medial prefrontal cortex

NA – noradrenaline

NAcc- nucleus accumbens

OFC- orbitofrontal cortex

PFC- prefrontal cortex

PL – prelimbic

PR- progressive ratio

rCET- rodent Cognitive Effort Task

SEM- standard error of the mean

SV- subjective value

VMAT - vesicular monoamine transporter-2

VS- ventral striatum

VTA- ventral tegmental area

## Glossary

### Dopaminergic Drugs

7-OH-DPAT – $D_3$ agonist

A77 636 - $D_1$ agonist

Buproprion (Wellbutrin) - dopamine transporter (DAT) reuptake inhibitor

Cis-flupenthixol - nonspecific DA antagonist

Eticlopride - $D_2$ antagonist

GBR12909 - dopamine transporter (DAT) reuptake inhibitor

Haloperidol – $D_2$ antagonist

PRX-14040 - dopamine transporter (DAT) reuptake inhibitor

U-99194 – $D_3$ antagonist

SCH23390 - $D_1$ antagonist

SCH 39166 (ecicopam) - $D_1$ antagonist

SKF 38393 – $D_1$ agonist

SKF 81297 – $D_1$ agonist

Tetrabenazine - vesicular monoamine transporter-2

Tolcapone - catchecol-O-methyltransferase (COMT) inhibitor

### Adrenergic Drugs

Atomoxetine - norepinephrine transporter (NET) reuptake inhibitor

Desipramine - NET reuptake inhibitor

Yohimbine - a2-adrenergic antagonist

## Cholinergic Drugs

Mecamylamine – nonspecific nicotine antagonist

Nicotine – nonspecific nicotine receptor agonist

Oxotremorine – muscarinic agonist

Pilocarpine - muscarinic agonist

Scopolamine - muscarinic antagonist

## Cannabinoid Drugs

AM 251 - $CB_1$ receptor inverse agonist/antagonist

AM 4113 - neutral $CB_1$ antagonist

AM 630 - $CB_2$ antagonist

arachidonyl-2-chloroethylamide (ACEA) - synthetic $CB_1$ receptor agonist

Rimonabant - $CB_1$ receptor inverse agonist/antagonist

THC - $CB_1$ receptor agonist

URB 597 – fatty acid amide hydrolase inhibitor

WIN 55, 212-2 - $CB_1$ receptor agonist

## Adenosine Drugs

Caffeine – nonspecific adenosine antagonist

DPCPX - $A_1$ adenosine antagonist

GS21680 - $A_2$ agonist

KW6002 - $A_2$ adenosine antagonist

MSX-3 - $A_2$ adenosine antagonist

## Serotonergic Drugs

Fluoxetine - serotonin transporter (SERT) reuptake inhibitor

M100 907 – 5-$HT_{2A}$ antagonist

Ro-60-0175 - 5-$HT_{2C}$ agonist

SB 242, 084 – 5-$HT_{2C}$ antagonist

## Other Drugs

alpha-helical CRF - corticotrophin-releasing factor antagonist

DRN - ERβ agonist

Estradiol – nonspecific ER agonist

Ketamine – NMDA antagonist

PPT - estrogen receptor (ER)-α agonist

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# Chapter 1: General Introduction

Choosing adaptively among candidate actions requires a cost-benefit analysis, in which potential rewards are considered against the costs required to obtain them (Kahneman and Tversky, 1979; Winstanley and Floresco, 2016). Many decisions faced by humans involve costs resulting from the effects of action on the environment, including delays to reward receipt (Kable and Glimcher, 2007), risk of loss (Bechara *et al*, 1994; Kuhnen and Knutson, 2005), and social exclusion (Ruff and Fehr, 2014). However, one cost- specifically the cost of effort- is uniquely intrinsic to action itself (Kool *et al*, 2010a). It is well established that all things being equal, an organism will gradually learn to prefer options low in physical effort producing reinforcement (Hull, 1943). This *Law of Least Effort* has received extensive support, and for many years a number of researchers have studied the mechanisms guiding physical effort allocation (Salamone *et al*, 2009a; Walton *et al*, 2006). Only until recently, however, has a similar *Law of Least Mental Effort* been experimentally verified (Kool *et al*, 2010a). Work in this new area of research has demonstrated that humans generally avoid cognitively demanding tasks in domains spanning working memory, cognitive flexibility, response inhibition, and attention (Kool et al, 2010; Westbrook et al, 2013, Dixon and Christoff, 2012; Reddy et al, 2015, Chong et al, 2017). Indeed, the subjective value of rewarding outcomes is discounted by difficult cognitive challenge, and cognitive allocation towards harder tasks can be

biased by incentives, suggesting that like physical effort, organisms may decide between options in ways which simultaneously maximize gains while minimizing effort costs (Chong *et al*, 2017; Dixon and Christoff, 2012; Massar *et al*, 2015).

Thus, while there is evidence that both forms of effort appear to factor into value-based decision making, it is generally unknown whether overlapping or distinct brain processes mediate cognitive and physical effort allocation. To this end, a number of human paradigms and preclinical models have been developed to assess the ways in which organisms weigh potential rewards against the inherent effort costs required to obtain them. While the human literature has identified key neural substrates guiding effort allocation, preclinical models have also proven invaluable by allowing behavioural neuroscientists to address how different neurotransmitters and brains region causally regulate valuations of effort. In the sections that follow, I review the effort-based decision-making tasks developed for use in humans, and briefly summarize the main neurotransmitter and brain systems implicated in effort allocation on the basis of imaging and systemic pharmacology studies. This discussion then segues into an exhaustive review of: 1) animal models of effort allocation, 2) the results of systemic pharmacology completed using these tasks, as well as a 3) survey of the regions and circuits implicated in effort-based decision making on the basis of lesions or temporary inactivations. There are many findings for which data is available in the physical domain, but the equivalent work has not been modeled with cognitive effort, and so a thorough comparison of these processes is limited. I then propose a set of experiments in which I use a rodent model of cognitive

effort allocation to answer these outstanding questions. My goal is that the knowledge gained from these experiments will allow for a point-to-point comparison of the neural systems guiding effort in the physical and cognitive realm. The outline of these experiments and their objectives can be found in section 1.11.

## 1.1 Overview of the Behavioural Paradigms Used to Investigate Effort-Based Decision Making in Humans

### 1.1.1 Physical Effort Tasks

Physical effort-based paradigms generally fall into two categories: those that manipulate effort costs via repeated button pressing, and those that operationalize effort through forceful grip. A popular button pressing paradigm is the Effort Expenditure for Rewards Task (EEfRT) developed by Treadway and Colleagues (Treadway *et al*, 2009). In this task, subjects make repeated decisions between two levels of task difficulty. Successful completion of "hard-task" trials requires the subject to make 100 button presses using the nondominant little finger within 21 seconds, while successful completion of "easy-task" trials requires the subject to make 30 button presses, using the dominant index finger within 7 seconds (Treadway *et al*, 2009). Subjects can earn $1 for completing easy trials, but can earn higher amounts upon successful completion of hard trials, within a range of $1.24 – $4.30. Additionally, trials differ

in their probability of reward receipt, ranging from low (12.5%), medium (50%), and high (88%), allowing researchers to assess how effort-based decision making is modulated by reward magnitude, probability of reward receipt and expected value (Treadway *et al*, 2009). Healthy subjects normally increase effortful choice as probability of reward receipt increases (Treadway *et al*, 2009). A variant of the task has also been developed which titrates effort demands for each individual, which has proven useful when assessing patients suffering from motor impairments (Fervaha *et al*, 2013; Reddy *et al*, 2015). Similarly, in the Balloon Effort Task, subjects decide between two response alternatives in attempts to inflate a balloon on a computer screen until it bursts. For easy trials, subjects must alternately press game controller buttons 10 times to receive $1 hypothetical reward, whereas hard trials require 100 alternating buttons presses and are associated with 5 different levels of reward varying from $3 to $7 (Gold *et al*, 2013). In this paradigm, choice of the more effortful option increases as the associated reward levels increase. Other button pressing paradigms have been developed, but are generally similar to the tasks described above.

Forceful grip has similarly been used to vary effort costs in decision-making tasks. The individual parameters vary from study to study, but generally require subjects to decide between squeezing a hand-grip device with varying levels of intensity (Chong *et al*, 2017; Kurniawan *et al*, 2010, 2013; Prevost *et al*, 2010; Reddy *et al*, 2015; Schmidt *et al*, 2012). Easy trials require simply holding a hand-grip, or squeezing with less than 50% of maximal force for small monetary reward. In contrast, the high effort response alternative requires 80-

90% maximal force exertion, but is associated with larger payout. Force parameters are titrated based on an assessment of maximal force prior to testing, and feedback on the levels of force achieved in a single trial is provided by an image on-screen, such as a thermometer with rising mercury levels (Kurniawan *et al*, 2010, 2013; Prevost *et al*, 2010) or a ladder with rungs (Schmidt *et al*, 2012). The tasks have generally used fictive currency as reward, but at least one study has used erotic stimuli (Prevost *et al*, 2010), which is interesting given evidence that primary and secondary reward representations recruit distinct neural substrates (Sescousse *et al*, 2010).

Recently, a novel effortful grip task has been developed by Husain and colleagues, in which the decision is not between concurrent easy or hard grip alternatives, but whether subjects are willing to put in the effort for a specific stake (Chong *et al*, 2015). During the experiment, participants are shown cartoons of apple trees, and are told to collect as many apples as possible based on the combined stake and effort presented. Potential rewards are indicated by the number of apples on the tree (1, 3, 6, 9, 12, 15), while the associated effort is specified by the height of a yellow bar on the tree trunk, ranging over six levels as a function of participants' maximal force (60%, 70%, 80%, 90%, 100%, 110%) (Chong *et al*, 2015). An analysis of indifference points suggests that willingness to accept offers increases as stake level increases (Chong *et al*, 2015).

### 1.1.2 Cognitive Effort Tasks

Like physical effort, cognitive effort can take on many forms. It is important

to keep this in mind when discussing the cognitive effort literature, as the term may be too broad to reflect only the processes taxed in a single paradigm. As will be described below, a number of cognitive effort allocation paradigms have been developed, which probe processes ranging from working memory, attention, cognitive flexibility, and response inhibition. Kool and colleagues developed the first test of cognitive effort allocation, known as the Demand Selection Task (Kool *et al*, 2010b, 2013). In this paradigm, subjects choose between two options (card decks or cues in computerized versions) that differ in difficulty. Numbers ranging from 1 to 9 are presented in one of two colours that specify the mental activity required to successfully complete the trial. Depending on the color of the numeral (blue or yellow, for example), the participant must make a parity (odd/even) or magnitude (less/greater than five) judgment. The easy option requires little switching between colours and their corresponding rules (either no switching or 90% congruence between trials), while the hard option requires frequent rule switching (Kool *et al*, 2010a). Subjects readily avoid the more challenging option, suggesting that these trials are indeed more effortful. While the original task did not include reward incentives for choosing between options, subsequent studies have baited the hard option with larger fictive monetary rewards, and this works to bias choice towards the more demanding option (Reddy *et al*, 2015, 2017). Additionally, while the original version of the task did not label choices for difficulty, subsequent iterations have been developed for patient populations that explicitly label options as “easy” and “hard” (Gold *et al*, 2014; Kool *et al*, 2013; Reddy *et al*, 2015). Most recently, Wardle and Colleagues have adapted the

physical EEfRT task to model cognitive effort costs using the Demand Selection Task described above. Like the EEfRT task, participants choose to exert cognitive effort across trials varying in reward magnitude and probability of reward receipt. However, rather than manipulating effort via button pressing, the easy option requires no switching between colours and their corresponding rules (4 judgments in 7 s), while the hard option requires frequent rule switching (19 judgments in 21s) (Lopez-Gamundi and Wardle, 2018).

Another task that assesses effort allocation in the domain of working memory is the Cognitive Effort Discounting Paradigm (COG-ED) (Westbrook *et al*, 2013). In this paradigm participants choose whether to perform a low-effort *N-back* task for a small monetary reward, or a high-effort *N-back* task for a larger reward. Multiple choices are made, and the amount offered for the low-effort task is titrated until the offers are subjectively equivalent (Westbrook *et al*, 2013). The objective load of the high-effort task is varied parametrically, akin to the estimation of discounting across a range of delays in delay discounting paradigms (Kable and Glimcher, 2007). A similar task developed by Massar and Colleagues requires subjects to choose between low effort, low reward, and high effort, high reward alternatives, where the cognitive effort is operationalized by the number of words subjects are willing to type out backwards (Massar *et al*, 2015). Likewise, another group has developed behavioural paradigms in which participants decide between easy or difficult trials of the Stroop, Go/No-Go, and Wisconsin Card Sorting Tasks for small or large reward, respectively (Dixon and Christoff, 2012). In line with the other effort allocation paradigms described

above, offering incentives biases subjects away from their preference for automatic, pre-potent responding in these tasks (Dixon and Christoff, 2012).

In contrast to the tasks described above which probe the domains of working memory and cognitive flexibility, recent task development has focused on attentional effort. The Perceptual Effort Task is based on the rodent Cognitive Effort Task created by Winstanley and colleagues (Cocker *et al*, 2012b; Reddy *et al*, 2015) (discussed in detail in section 1.6.2 ). In this task, the objective is to correctly identify a faint stimulus in one of seven possible locations, and task difficulty is manipulated by the amount of gray-scale contrast between the stimulus and background. Individual perceptual thresholds are determined for each participant, so that easy stimuli are 98% of the participant's threshold for detection, while the hard stimuli have a gray-scale that is 101% of their perceptual threshold. Easy trials offer a consistent small reward of $.10, while the size of the large reward is either equal or higher ($.10, $.20, $.30, $.40). Another attentional effort task utilizes the rapid serial visual presentation paradigm, in which participants monitor one of two target streams to the left and right of a fixation point for a target number, and cognitive effort is parametrically varied over six levels by increasing the number of times attention has to be switched between streams from one to six (Apps *et al*, 2015; Chong *et al*, 2017). On each trial, participants choose between a fixed, low-effort/low-reward "baseline" option, and a variable high-effort/high-reward "offer". The increasing effort demands imposed by greater spatial attention are perceived as subjectively effortful, and act to devalue possible rewards (Apps *et al*, 2015)

## 1.2 Effort-Based Decision Making in Humans: Summary of Behavioural Findings, Relationship to Traits and Disease States

Most of the effort paradigms described above have either shown that subjects tend to avoid options as cognitive demand increases (Chong *et al*, 2017; Massar *et al*, 2015, 2016; Prevost *et al*, 2010; Reddy *et al*, 2015; Westbrook *et al*, 2013), or have demonstrated that increasing incentives can bias subjects towards otherwise costly cognitive expenditure (Chong *et al*, 2015; Dixon and Christoff, 2012; Massar *et al*, 2016; Reddy *et al*, 2015). Thus effort, whether in the physical or cognitive domain, is inherently costly, but these costs can be offset by potential lucrative outcomes. Subsequent work has assessed the different factors that may modulate effort allocation. For example, older adults show greater cognitive effort discounting relative to young adults on the COG-ED task, and willingness to exert cognitive effort is correlated with self-reported need for cognition (a trait measure of daily engagement with and enjoyment of cognitively demanding activities), but not intelligence (Lopez-Gamundi and Wardle, 2018; Westbrook *et al*, 2013). In the physical domain, men make more high effort choices on the EEfRT task relative to females, and social influence can bias effort allocation towards hard options, especially on low-reward probability trials (Gilman *et al*, 2015; Treadway *et al*, 2009). Trait anticipatory pleasure predicts effort exertion in the EEfRT task when reward receipt is not

guaranteed, while healthy individuals with elevated self-reports of both trait and state anhedonia exhibit a reduced willingness to make choices requiring greater effort (Geaney *et al*, 2015; Treadway *et al*, 2009).

This last finding, that trait anhedonia is negatively correlated with high effort choice, is particularly notable given that many psychiatric disorders characterized by anhedonia and avolition are also associated with an impaired willingness to exert effort, both in the physical and cognitive domain. Indeed, there a rich literature assessing effort-based decision making in schizophrenia, as these effort tasks may provide an objective measure of the negative symptoms characteristic of the disorder (Gold *et al*, 2015; Green *et al*, 2015). Negative symptoms can be divided into motivational negative symptoms (i.e avolition, anhedonia, and asocialty), and diminished expression (i.e. blunted affect and alogia), and are strong predictors of poor functional outcome (Green *et al*, 2009). Across tasks, individuals with schizophrenia regularly show impairments in both physical (Barch *et al*, 2014; Docx *et al*, 2015; Fervaha *et al*, 2013; Gold *et al*, 2013; McCarthy *et al*, 2016; Treadway *et al*, 2015) as well as cognitive effort allocation (Culbreth *et al*, 2016, but see Gold *et al*, 2014). Many of these studies reporting impaired effort allocation also find that these deficits are associated with negative symptoms (Barch *et al*, 2014; Culbreth *et al*, 2016b; Gold *et al*, 2013; Hartmann *et al*, 2015; Treadway *et al*, 2015; Wolf *et al*, 2014), while some studies have found no relationship (Docx *et al*, 2015; Fervaha *et al*, 2013; Gold *et al*, 2014), as well as positive relationships between negative symptoms and more effortful choices (McCarthy *et al*, 2016). Discrepancies in

the literature might be explained by recent findings suggesting that defeatist beliefs moderate the relationship between negative symptoms and effort-based decision making. Specifically, high negative symptoms are associated with impaired effort allocation, but only in individuals with high levels of defeatist beliefs (Reddy *et al*, 2017). In any case, effort-based decision-making tasks appear to hold great promise in providing objective measures of the negative symptoms inherent to schizophrenia.

Deficits in effort allocation are not just limited to schizophrenia, but to other disorders characterized by symptoms of anhedonia or apathy. For example, depressed individuals are less willing to exert effort than controls, and duration of current depressive episode is negatively associated with effort investment (Treadway *et al*, 2012a). Likewise, Parkinson's patients are less willing to accept bids to exert effort for potential rewards (Chong *et al*, 2015), Specifically, patients with concurrent apathy are less likely to exert effort for small rewards, while those off dopaminergic medication are less willing to exert high effort for high rewards (Le Heron *et al*, 2018). It must be said that deficits in effort allocation are not exclusive to disorders characterized by negative symptoms. For example, obesity has also been linked to reduced willingness to exert physical effort for larger reward outcomes, which is predictive of poor adherence to a weight loss program (Mata *et al*, 2017). When considered with the research linking negative symptoms to functional outcomes in schizophrenia described above, it appears that boosting effort allocation may be a promising therapeutic target across a number of conditions.

## 1.3 Neurotransmitter Systems Implicated in Effort Allocation from Human Studies

A handful of human experiments have uncovered key substrates guiding effort-based decision making. Most of this work has focused on dopamine (DA) and its links to physical effort allocation, given the wealth of preclinical research attributing a crucial role for DA signaling in valuations of physical effort (see section 1.8.1). In support of this link, acute administration of d-amphetamine (10, 20 mg) increases high effort choice in the EEfRT task, effects which are most pronounced in low and medium probability blocks where effort does not guarantee reward (Wardle *et al*, 2011). Using the same EEfRT task, DA sensitivity assessed via PET imaging is positively associated with high-effort choice on low probability trials in the left caudate nucleus, the left inferior temporal gyrus and ventrolateral prefrontal cortex, and ventromedial prefrontal cortex bilaterally (Treadway *et al*, 2012b). In contrast, DA sensitivity is negatively correlated with high effort choice across all trials in the anterior insula bilaterally, which is interesting given this area has been previously shown to process response costs (Kuhnen and Knutson, 2005; Prevost *et al*, 2010). Parkinson's patients are less likely to invest effort for larger rewards when off medication, but are motivated to invest more effort when on their dopaminergic medication (Chong *et al*, 2015; Le Heron *et al*, 2018). Together this work suggests that

dopamine acts to bias choice towards effortful, more lucrative outcomes. Indeed, the preclinical literature provides strong support for this conclusion. While theoretical accounts suggest DA likely plays a similar role in cognitive effort allocation (Westbrook and Braver, 2016), this has not been experimentally verified.

The cannabinoid system has also received due consideration in regards to effort allocation. Acute treatment with vaporized cannabis (8 mg) reduces the likelihood of high-effort choices in the EEfRT task (Lawn *et al*, 2016). Similarly, smoking a joint containing 3.58% Δ9-THC, the main psychoactive component in cannabis and a $CB_1/CB_2$ receptor agonist, reduces button pressing in a choice task where participants earn money by choosing between a lucrative progressive ratio (PR) option or a fixed-time schedule (Cherek *et al*, 2002). In line with this, evidence suggests that heavy smoking adolescent cannabis users are less likely to select the effortful, high reward option in an effort discounting task, but that adult cannabis dependent users are identical to controls in their choice of effortful, high reward options in the EEfRT task (Lane *et al*, 2005a; Lawn *et al*, 2016). Notably, the study showing a similar choice profile between controls and adult cannabis dependent users did find impaired reward learning processes in this group, which is in agreement with work in the risky decision-making literature suggesting that THC reduces sensitivity to win and loss outcomes (Lane *et al*, 2005b).

Two other neurotransmitters that have received comparatively less attention are adenosine and serotonin. Acute administration of caffeine (200 mg)

does not affect effort expenditure in the EEfRT task, except in a subgroup of individuals with a high cardiovascular response to caffeine, where the stimulant decreases their high-effort choice (Wardle *et al*, 2012). While serotonin has not been assessed explicitly in a cost/benefit decision-making framework, chronic escitalopram treatment increases physical grip for reward relative to placebo control (Meyniel *et al*, 2016).

Notably, all the studies described above focused on physical effort allocation, while no human study has attempted to delineate pharmacological regulation of effort allocation in the cognitive domain.

### 1.4 Human Imaging Studies on Effort Allocation

When reviewing the imaging literature, it is important to distinguish between tasks that present participants with concurrent options they must choose from, versus tasks presenting subjects with a cue denoting how much effort is to be invested in a subsequent trial. In the former, activity reflects the brain processes as subjects decide between options varying in their effort-cost/reward contingencies, while in the latter activity reflects how a lucrative outcome is discounted by the imposition of effort costs.

In response to cues denoting how much button pressing is required to obtain reward, blood-oxygen-level-dependent (BOLD) activity in the ventral striatum, ACC, and midbrain reflects the net value of the course of action, signaling the expected amount of reward discounted by the amount of effort to be

invested (Croxson *et al*, 2009). When presented with options differing in the amount of hand grip effort that must be invested for presentation of primary reward (erotic stimuli), and where subjects must choose how much effort to invest, anterior cingulate cortex and anterior insula activity represent the decreasing subjective value of the effortful option, in line with these regions coding the expected expense of energy (Prevost *et al*, 2010). Activity in the caudal dACC also represents the effort costs and benefits of a chosen relative to an alternative option, and integrates this into a subjective value signal that correlates with participants' choices between levels of forceful grip (Klein-Flugge et al., 2016). A similar study using fictive monetary rewards found that choosing to grip over choosing to passively hold a hand-grip device increases BOLD activity in the superior frontal gyrus, while activity in the caudate-putamen is higher in anticipation of low-effort relative to high-effort trials, akin to a net value signal (Kurniawan *et al*, 2010). Similarly, the ACC and dorsal striatum (dorsal putamen) signal the anticipation of physical effort independently of the prospect of winning or losing reward in a task modeling monetary gain, but also monetary loss (Kurniawan *et al*, 2013).

In regard to effort allocation in the cognitive domain, anticipation of an upcoming arithmetic task activates the bilateral striatum, right ACC, and left brainstem (Vassena *et al*, 2014). Notably, this finding is not in line with a value-related encoding, according to which anticipation of low effort should elicit a greater response. However, a number of studies have found evidence for valuation signals in anticipation of a cognitive challenge. Botvinick (2009) found

that ventral striatal BOLD signals track the net value of outcomes in the Demand Selection Task (reviewed in 1.1.2), where cognitive demand varies as a function of the number of rule switches necessary for correct performance. Notably, this study found that activity in the dACC predicts the subsequent VS bold signal, supporting the notion that the dACC may regulate the allocation of cognitive effort via interactions with striatal subregions (Botvinick *et al*, 2009). In another study, in which participants make decisions between exerting different levels of cognitive effort (i.e., spelling words backwords), subjective value of effortful options is encoded in a network comprising the anterior and posterior cingulate cortex, caudate nuclei, cerebellum, bilateral temporal and parietal cortex, and lateral OFC (Massar *et al*, 2015). Interestingly, this study found ACC activation is more strongly correlated to effort discounting relative to delay discounting (Massar *et al*, 2015).

To date, only two studies have investigated physical and cognitive effort allocation in the same subjects and scanning session, providing a powerful means to compare and contrast the circuitries guiding these two forms of decision making. In one study, participants alternated between exerting different levels of force and rule switching in order to obtain fictive monetary reward (Schmidt *et al*, 2012). Ventral striatal activity predicts expected reward in either form of effort, and psychophysical interaction (PPI) analysis revealed the VS is significantly connected with cognitive regions (mostly the caudate) when cognitive demand is high, and with motor regions (mostly the putamen) when physical effort is required. The authors take this as evidence that the VS may be

a common motivational node, prioritising cognitive circuits during mental effort and motor circuits during physical effort exertion (Schmidt *et al*, 2012). In a study directly assessing effort-based choice, reward devaluation by both forms of effort is observed in a network of structures including the dorsomedial and dorsolateral prefrontal cortex, the intraparietal sulcus, and the anterior insula (Chong *et al*, 2017). Notably, this study also found that the right amygdala processes the subjective value of rewards discounted by cognitive, but not effort, costs (Chong *et al*, 2017).

## 1.5 Limitations of the Current Human Research on Effort Allocation

While valuable, the interpretation of the imaging results described above must be considered in light of the limitations of imaging data, such as difficulties in ascribing causality or directionality to any specific set of findings. Likewise, our understanding of the neurotransmitter systems involved in effort-based decision making (described in section 1.3) is limited by ethical considerations, and is inferred from certain disorders characterized by aberrant neural processes, or by assessing patients on or off certain medications. Often, however, these phenomena are confounded. A powerful alternative to circumvent these issues is to develop animal models of cost/benefit decision making, which allow researchers to causally investigate the neural systems guiding effort allocation in ways not feasible in human studies. As will be discussed in the below, animal

models have offered behavioural neuroscientists unprecedented insights into the neural mechanisms guiding effort-based decision making.

## 1.6 Animal Models of Effort-Based Cost/Benefit Decision Making

Animal models have proven invaluable towards investigating the neurotransmitter systems and neural circuits guiding cost/benefit decision making (Winstanley and Floresco, 2016). Many models have been developed, but all are based on the premise that subjects will evaluate whether the cost investment associated with different options is “worth” the potential reward resulting from their choice. Most of these paradigms present rodents with a low-cost, low-reward response option that is available with minimal to no expense, while selection of the other response alternative results in more lucrative reward, but is discounted by greater price. These costs can vary, and researchers have been truly creative in the way they have modeled different forms of decision making under this basic framework. Indeed, models exist which probe cost/benefit decision making involving delays (Cardinal *et al*, 2001), uncertainty (Cardinal and Howes, 2005; Cocker *et al*, 2012a; St Onge and Floresco, 2009), avoidance (Friedman *et al*, 2015), risk of punishment (Simon *et al*, 2009), and of particular interest, effort. Notably, animal effort-based decision-making models inspired many of the human tasks described above, such as the EEfRT task, which is based on the early work of Salamone and colleagues (Cousins and Salamone,

1994), and recently the rodent Cognitive Effort task has been back-translated for use in humans by Young and colleagues (Reddy *et al*, 2015).The sections that follow describe the various effort tasks used by behavioural neuroscientists, and systematically review the neurotransmitter systems and neural regions causally implicated in effort-based cost/benefit decision making. Most of this work has focused on the physical effort costs that discount lucrative outcomes, but I also detail work our lab has carried out investigating the substrates guiding cognitive effort allocation in rodents.

### 1.6.1 Physical Effort Decision-Making Tasks

#### 1.6.1.1 Concurrent Choice Paradigms

A number of sophisticated animal models have been developed to probe the neural mechanisms guiding physical effort-based choice, the earliest of which was developed by Salamone and colleagues (1991). In this concurrent fixed ratio 5 (FR5)/chow feeding procedure, a rat is placed in an operant box and can decide whether to eat freely available chow placed in the corner of the box (typically 15-20g), or to lever press on a FR-5 schedule for a single sugar pellet (Salamone *et al*, 1991). Given their preference for the high carbohydrate sugar pellets, rats typically spend most of the task lever pressing and consume very little of the freely available chow. Manipulations that reduce effortful choice (such as $D_2$ receptor antagonism with haloperidol or 6-OHDA depletions; see section

1.8.1) decrease lever pressing and increase the amount of chow consumed, whereas appetite suppressants (such as cannabinoid receptor antagonist AM 251, see section 1.8.4) decrease both lever pressing and chow consumed (Cousins *et al*, 1994; Sink *et al*, 2008b). Notably, a variant of this task has rats choose between different concentrations of sucrose solution, where the choice is made between 7 lever presses for 5% sucrose or freely available 0.3% sucrose (Pardo *et al*, 2015). A subsequent iteration of the FR task, known as the progressive (PROG)/chow concurrent choice task, uses a progressive ratio schedule where the response requirements for a sugar pellet are increased by one each time 15 reinforcements are obtained (FR1×15, FR2×15, FR3×15,…) (Randall *et al*, 2012). In this task, animals repeatedly make within-session choices between lever pressing and chow intake under conditions in which the ratio requirement is gradually increasing. This procedure generates much more variability in behaviour between individuals compared to the concurrent FR-5 choice procedure, which is useful when studying the neural mechanisms responsible for individual differences in behaviour (Randall *et al*, 2012). Most recently a concurrent choice paradigm has been used by Izquierdo and colleagues, in which the required number of lever presses increases after five successive schedule completions, according to the formula $n_i = 5e^{(i/5)} - 5$, where $n_i$ is equal to the number of presses required on the *i*th ratio, rounded to the nearest whole number (Hart and Izquierdo, 2017).

The advantage of these concurrent choice tasks is that they probe the cost/benefit evaluations of qualitatively different rewards (more/less preferred),

instead of differences in magnitude (larger/smaller) of the same reward (a feature of all the physical effort choice tasks discussed below). The disadvantage inherent to these tasks is the confound of delay costs associated with increasing response requirements. As described above, the imposition of delays can discount future rewards, and so can be difficult to interpret whether the switch to chow consumption reflects an aversion to effort costs, or an unwillingness to wait for the preferred sugar reward, unless appropriate control experiments are also conducted.

#### 1.6.1.2 T-maze Choice Task

In the concurrent choice tasks described above, the responses required to obtain more-preferred sugar or less-preferred chow are quite distinct: lever pressing is an instrumental (or "preparatory") response whereas feeding on chow is a consummatory ("to complete") behaviour (Salamone *et al*, 1994; Salamone and Correa, 2012). While chow consumption also relies on simple responses such as locomotion to food, it is critical to evaluate cost/benefit decisions where explicit instrumental processes are compared. To address this, Salamone and colleagues developed a T-maze task in which rats decide between two arms varying in their reward/response cost ratios (Salamone *et al*, 1994). Subjects begin in a start arm and must choose between entering one of two opposing arms, where one arm end is associated with a small amount of freely available reward, whereas the other contains a higher magnitude of reward blocked by a scalable barrier. Typically, the low reward (LR) and high reward (HR) is set at two

and four pellets, respectively, while the scalable barrier has ranged in height from 25 cm (Schweimer, 2005), to the standard 30 cm used in most studies (Floresco and Ghods-Sharifi, 2007; Walton *et al*, 2002), up to 44 cm used in the original task (Salamone *et al*, 1994). At the beginning of each session rats typically receive two forced choice trials to expose them to the contingencies associated with each arm, and then receive 10-30 free choice trials where the number of high-effort/high reward arm entries or % HR choice is reported (Floresco and Ghods-Sharifi, 2007; Rudebeck *et al*, 2006; Salamone *et al*, 1994). Rats willingly scale the barrier for larger reward, and choice of the hard lever typically falls between 70-90% across studies. Alterations in choice behaviour due to motor, spatial memory, or discrimination deficits can be ruled out by equalizing response costs via insertion of an identical barrier in the LR arm, and sensitivity to reward magnitudes or effort costs can be assessed by altering the reward ratio differential (such as 5-1 versus 4-2) or changing the height of the barrier (e.g. from 30cm to 20 cm) (Walton *et al*, 2003a). A notable variation of the standard T-maze choice task employs a discounting procedure, where each time a rat chooses the HR arm, the amount of food in the arm is reduced by one pellet on the next trial (Bardgett *et al*, 2009). Such a procedure allows one to determine an indifference point for each rat, which provides a sensitive measure of reward value for each subject (Richards *et al*, 1997). In another variant of the T-maze task, Euston and colleagues have changed the cost parameter by replacing the scalable barrier with a weighted lever that rats must depress for larger sucrose reward (Holec *et al*, 2014). This is an interesting take on the traditional T-maze

task, allowing researchers to assess how manipulations affect allocation of distinctly effortful actions.

The T-maze choice task is the most commonly employed test of physical-effort based decision making, which has also been adapted for use with mice (Iodice *et al*, 2017; Pardo *et al*, 2012). However, training and testing is more involved than the operant box-based tasks described below, and again there is the confound of delay costs invariably imposed by scaling a barrier for reward. While some investigators have recorded latencies at different phases towards HR receipt (e.g. start to choice point, choice point to top of barrier, top of barrier to food) (Denk *et al*, 2005), few have factored latency data into their statistical analyses when discussing shifts in choice from HR to LR arms ( but see Yohn *et al*, 2015a).

#### 1.6.1.3 Lever-Based Effort Discounting Tasks

Two groups have developed lever-based operant tasks of physical effort allocation. The first, developed by Walton et al., (2006), requires rats to decide between two response alternatives, one of which involves investing effort by lever pressing on a high-fixed ratio to gain high reward (four food pellets, HR), while the other produces lower reward through completion of a less effortful FR requirement (two food pellets, LR) (Walton *et al*, 2006). Each session consists of repeating blocks of two-forced choice (one to each lever) and four free-choice trials, a protocol that allows sufficient assessment of decision-making behaviour while ensuring that rats sample each option at least once out of every six trials.

The FR requirement on the LR lever remains constant at FR4, while the FR requirements on the HR lever also remain constant within a session (typically set at FR12). The second operant task developed by Floresco and colleagues is similar, but within each session rats go through four blocks of trials where the fixed ratio requirement for the high reward (HR) systemically increases (Floresco *et al*, 2008b). Each block of trials begins with two forced choice trials (one for each lever), and is followed by ten free choice trials where rats can press a lever once for an immediate reward of two sugar pellets, or can complete a more effortful fixed ratio requirement on the other lever, resulting in four sugar pellets. The fixed ratio responses required to obtain the HR varies systematically across each block (starting at 2, then 5, 10 and 20 presses), resulting in a discounting curve showing how the value of HR reward is depreciated by increasing physical effort costs. An advantage of operant based paradigms is the ability to assess choice latencies, rates of responding (an index of response vigor), and the ability to equate the delay to obtain either reward. Indeed, a notable variant of the effort discounting task by Floresco et al, known as the “Effort Discounting Tasks with Equivalent Delays”, eliminates the confound of delay costs associated with increased responding on a lever, and is the only physical effort task to adequately control for time to reward receipt (Floresco *et al*, 2008b). Specifically, after responding on the LR lever two pellets are delivered after a delay equivalent to the time required for rats to complete the FR ratio on the HR lever. This delay to LR reward receipt increases across trial blocks, and is calculated based on the average time required for rats to press the lever 2,5,10, and 20 times. When

delays to receiving LR or HR are equalized, choice of HR significantly increases, suggesting that delays to reinforcement incurred when animals must respond on a lever multiple times influence the preference for these options above and beyond their inherent response costs (Floresco *et al*, 2008b).

### 1.6.2 Modelling Cognitive Effort Costs in Rodents: The Cognitive Effort Task

While a number of paradigms have been developed to model decision making with physical effort costs, only one animal model of cognitive effort allocation currently exists. Developed by Winstanley and colleagues, the rodent Cognitive Effort Task (rCET) is an adaptation of the well-established 5-choice serial reaction time task (5-CSRTT) (Cocker *et al*, 2012b). The 5-CSRTT is an animal model of sustained and divided attention, based on the human continuous performance task originally developed by Mirsky and Rosvold, which has recently been back-translated for use in humans (Voon *et al*, 2014). In the original 5-CSRTT, rats scan a visual array of five stimulus holes and must correctly identify the presentation of a brief visual stimulus (typically 0.5s in duration), which is rewarded with a single sucrose pellet. In the rCET, rats are presented with two levers at trial onset, allowing them to choose whether to engage in an easy or difficult attentional challenge. Choice of the easy, low reward option (LR) requires accurate detection of a 1s stimulus, and results in the delivery of one sucrose reward if correctly identified. Alternatively, rats can opt to initiate hard, high

reward trials (HR) where the stimulus is much briefer (0.2s), but can result in double the reward. HR trials are more difficult, as evidenced by lower accuracy rates relative to LR trials, but are ultimately the advantageous option if rats can successfully complete them. Indeed, while all rats can detect the HR stimulus successfully (~65-70% HR accuracy rates, on average), there is great individual variation in preference for the hard option. On average, choice of the HR lever is ~70%, and subjects are split into “workers” and “slackers” depending on whether they tend to choose the HR lever more or less than 70% during a session, respectively. Notably, “workers” and “slackers” do not differ in their accuracy to detect the difficult HR stimulus, nor do they differ in their rates of premature responding (ability to wait for the stimuli to appear), and so differences in cognitive effort allocation are not mediated by disparities in ability to perform the task.

Notably, choice patterns on the rCET are influenced by the cognitive effort costs associated with HR trials, and cannot simply be explained by sensitivity to differential rates of reinforcement. In a yoked-control rCET task, rats were trained on a control task where the reinforcement probabilities on both the LR and HR trials depended on a master animal’s performance on the rCET. The average accuracy on LR and HR trials was calculated for each rat performing the rCET at baseline, and these accuracies were used to determine the reward schedule for a yoked control rat. The structure of the control task was identical to that of the rCET, with the exception that the stimulus light remained illuminated until the rat made a response. Baseline choice behavior of cognitive effort and

control animals was directly compared, and these analyses revealed significant differences in choice behaviour between tasks. While workers and yoked-workers selected the HR option at similar rates, yoked-slackers chose HR trials more than slackers, suggesting that the imposition of a cognitive cost (increased attention) discounts selection of the HR reward in the standard task.

Like the original 5-CSRTT, the rCET provides a number of cursory measures to help interpret the effects produced by experimental manipulation (Robbins, 2002). LR and HR accuracies provide measures of attention for stimuli of differing durations, and the percent prematures for each lever indexes rats' willingness to withhold responding during the inter-trial-interval prior to stimulus presentation. This is identical to the premature responding measure in the 5-CSRTT, which is considered a sensitive assay of impulsive action (Robbins, 2002). Other measures recorded include choice omissions at the decision time point, response omissions following presentation of the stimulus, as well as latencies to make a decision, to correctly detect the visual stimulus, and to collect reward from the magazine tray. By taking into account the overall constellation of effects observed on these variables within a session, it is possible to disambiguate possible interpretations of the data. For example, increased choice, correct, and collect latencies along with increased omissions likely reflect sedative or motor effects, whereas a pronounced slowing in collect latency likely reflects a motivational impairment. Likewise, increases in response omissions that are accompanied by accuracy impairments, but not changes in collect latencies likely reflect attentional deficits.

## 1.7 Comparing Effort Allocation Tasks

As described above, a number of different tasks can be used to study effort allocation in rodents, and deciding which one to employ for a particular investigation will depend on a host of factors. For example, if individual differences are of primary interest, then the (PROG)/chow concurrent choice task and the rCET are advantageous because they generate greater variability in effort allocation, such that “high” versus “low” effort preference groups can be generated and included in subsequent analyses (Cocker *et al*, 2012b; Randall *et al*, 2012). If the primary interest is in modelling cognitive effort costs, then the rCET is the clear choice as it is currently the only task available. Importantly, the rCET manipulates cognitive effort by increasing attentional demand, while cognitive effort discounting has been observed in the domains of working memory, response inhibition, and cognitive flexibility as well (Dixon and Christoff, 2012; Kool *et al*, 2010a; Reddy *et al*, 2015; Westbrook *et al*, 2013). It is likely that different neural systems mediate the allocation of effort across these executive functions, and so discretion must be taken when extrapolating the results of the rCET to cognitive effort allocation as a general phenomenon. Likewise, physical effort is not a unitary construct, and the processes involved in scaling a barrier for larger reward may not be the same as those regulating lever pressing for lucrative outcomes (Holec *et al*, 2014). This must be kept in mind when

considering tasks that vastly differ in the instrumental responses defining the effort cost.

Some considerations are purely practical, such as equipment availability (operant box, T-maze) and the amount of resources that can be invested for behavioural training (e.g., rats must be trained for 6-8 months on the rCET before any experimental manipulations can begin). Other considerations, however, are more nuanced, and reflect subtleties in task design that may interact with a given manipulation. For example, consider the effects of nucleus accumbens (NAc) 6-OHDA depletions on the operant effort-discounting task developed by Walton et al., (2009), and the effect of NAc inactivations on the similar operant discounting task developed by Floresco et al., (2008). 6-OHDA depletions have no effect on choice in the former task, while NAc inactivations reduce choice of the effortful option in latter (Ghods-Sharifi and Floresco, 2010; Walton *et al*, 2009). Although the difference in NAc manipulation may explain these divergent results, it has been suggested that these disparate results are due to subtle differences in task design, interacting with the role of NAc dopamine in reward-seeking behaviour (Nicola, 2007, 2010). According to the Flexible Approach Hypothesis, NAc dopamine is recruited only when actions required to obtain reward are variable. As such, when an animal's starting location varies across trials, different actions are required to reach a fixed goal, and NAc dopamine is required for those actions to occur. In contrast, when the start and end locations are fixed across trials, identical actions can bring the animal to the goal, and NAc dopamine is not required. Notably, in the task reporting no effects on choice, rats start at the

exact same position on each trial, because a nosepoke in the magazine tray is required to extend the left and right lever. In the study reporting reduced choice of the HR lever following striatal inactivation, trial initiation is not contingent on a nosepoke in the magazine tray, and so approach towards a given lever in this task is necessarily flexible (Nicola, 2010). This is but one example of the factors a behavioural neuroscientist must consider when deciding between effort-based decision-making tasks. In the sections that follow, I review the neurotransmitter and neural systems that have been implicated in effort allocation using these behavioural paradigms.

## 1.8 Neurotransmitter Systems Implicated in Effort-Based Decision Making

A number of neurotransmitter systems have been implicated in decision making with effort costs. The research in this section is primarily based on the results of systemic pharmacology studies, in which a drug is administered intraperitoneally (I.P) or subcuteanously (S.C) before task onset. Site-specific targeting of a neurotransmitter system has also been achieved by intracerebral drug infusion, and more recent studies have used transgenic or knockout animals to similar effect. Most of this work has been conducted in rodents, but where applicable the results of nonhuman primate and human studies will be briefly described. A review of the literature suggests that while many neurotransmitter systems play similar roles across effort domains, others have more nuanced

roles in decision making with either physical or cognitive effort costs.

### 1.8.1 Dopaminergic Systems

#### 1.8.1.1 Physical Effort: Systemic Administration

Across a variety of tasks with physical effort costs, systemic administration of dopaminergic compounds overwhelmingly implicate the dopamine (DA) system in physical effort allocation.

In the concurrent (FR5)/chow feeding task, systemic administration of the nonspecific DA antagonist cis-flupenthixol (0.15,0.30 mg/kg)[1], and the $D_2$ receptor antagonists haloperidol (0.1-0.15 mg/kg), eticlopride (.05, 0.1 mg/kg) and sulpiride (100, 150 mg/kg) decrease lever pressing for sucrose and increase intake of freely available chow (Cousins *et al*, 1994; Salamone *et al*, 1991; Sink *et al*, 2008b). Initial studies revealed that the $D_1$ receptor antagonist SCH23390 (0.05, 0.1, 0.15 mg/kg) reduces lever pressing and increases chow intake, although this drug displays high binding affinity for 5-HT2A and 5-HT2C receptors (Alburges *et al*, 1992). Subsequent studies have employed the $D_1$ antagonist ecicopam (SCH 39166), which has high affinity for $D_1$ receptors and low affinity for 5-HT receptors (Alburges *et al*, 1992). Systemic administration of this compound (.05, 0.1, and 0.2 mg/kg) also decreases lever pressing and increases chow intake, effects which are partially reversed by the $D_1$ receptor agonists SKF 38393 (.05 mg/kg), SKF 81297 (.05 mg/kg), and A77 636 ( 0.5

[1] Doses listed in this section are effective doses that had effects on decision-making, not all doses used in a specific investigation

mg/kg) (Sink *et al*, 2008b; Yohn *et al*, 2015a). Another method of downregulating DA transmission has involved systemic administration of tetrabenazine, a vesicular monoamine transporter-2 (VMAT-2) inhibitor, which blocks the storage of monoamine and depletes their levels (Pettibone *et al*, 1984). Salamone and Colleagues have used this compound, in conjunction with effort-based decision-making tasks, to model the motivational symptoms of depression in animals (Nunes *et al*, 2013b). Administration of tetrabenazine (0.75, 1 mg/kg) decreases lever pressing and increases chow intake, effects which are attenuated by the dopamine transporter (DAT) reuptake inhibitors GBR12909 (2.5, 5 mg/kg), buproprion (10, 15 mg/kg) and PRX-14040 (2.5, 5,10 mg.kg), but not by the norepinephrine transporter (NET) reuptake inhibitor desipramine, or by the serotonin transporter (SERT) reuptake inhibitor fluoxetine (Randall *et al*, 2014; Yohn *et al*, 2016b, 2016d). Notably, the effects of these dopaminergic agents are not restricted to concurrent choice of qualitatively different rewards: in a variant of the FR(5)/chow concurrent choice task, ecicopram (0.1, 0.2 mg/kg), haloperidol (0.05, 0.1 mg/kg), and tetrabenazine (0.75, 1.0 mg/kg) decrease lever pressing on a FR7 schedule for 5% sucrose solution, and increase selection of freely available 0.3% sucrose (Pardo *et al*, 2015).

When administered prior to the (PROG)/chow concurrent choice task, the $D_1$ receptor antagonist ecopipam (0.5, 1.0, 2.0 mg/kg) decreases lever pressing while concomitantly increasing chow intake (Randall *et al*, 2014). In contrast, eticlopride (.04, .08 mg/kg), haloperidol (0.1 mg/kg), and tetrabenazine (0.5, 0.75, 1.0 mg/lg) decrease lever pressing at increasing FR ratios for sucrose, but this is

not accompanied by increases in chow intake (Randall *et al*, 2012, 2014).This is unlikely to reflect a reduced motivation for food, as rats will still seek food in a session. Instead this likely reflects a ceiling effect, as normal chow consumption in the (FR5)/chow feeding task is relatively low (1-2 grams), whereas in the (PROG)/chow feeding task it is higher (7-8) grams, and approximates the maximum amount of food rats will consume within a 30 minute session without water (Randall *et al*, 2010). Tetrabenazine-induced declines in progressive ratio lever pressing for lucrative reward are attenuated by the DAT inhibitor and common antidepressant bupropion (Wellbutrin) (15 mg/kg), by the monoamine oxidase B inhibitor (MAOBi) deprenyl (5.0, 1.0 mg/kg), but not by the catchecol-O-methyltransferase (COMT) inhibitor tolcapone (Randall *et al*, 2014),

In the T-maze task, numerous investigators have demonstrated that doses of haloperidol ranging from 0.1 (Bardgett *et al*, 2009; Salamone *et al*, 1994) to 0.2 mg/kg (Denk *et al*, 2005; Walton *et al*, 2005) decrease willingness to scale the barrier for larger reward. A motor slowing, as indexed by increased trial latencies, routinely accompany these doses of haloperidol. Regardless, rats are still capable of scaling the barrier, because they resume selection of the high reward arm when costs in both arms are equated. Similar effects of haloperidol are observed in a mouse variant of the T-maze task (0.05, 0.1 mg/kg) (Pardo *et al*, 2012). The $D_1$ receptor antagonist ecicopram (0.1, 0.2 mg/kg) similarly decreases choice of the HR arm in rats, an effect which is reversed by the $D_1$ receptor agonist SKF 3893 (0.25, 0.5, 0.75 mg/kg) (Yohn *et al*, 2015a). In one of the few investigations targeting the $D_3$ receptor subtype, Bardgett et al., (2009)

administered the $D_3$ receptor agonist 7-OH-DPAT (0.1, 0.3 mg/kg) and $D_3$ receptor antagonist U-99194 (6.25 mg/kg) on the T-maze, and found no effects of either on the number of trials it took rats to switch preference to the low reward arm. In keeping with its effects on the concurrent choice tasks, tetrabenazine (0.75 mg/kg) decreases rats' willingness to scale the barrier for larger reward (Yohn *et al*, 2015b). These impairments in HR choice are attenuated by SKF 81297 (0.05 mg/kg), and by bupropion (10, 15 mg/kg) (Yohn *et al*, 2015b).

In reference to the lever-based operant effort discounting tasks, haloperidol (0.1 mg.kg) decreases choice of the high reward/ high FR (4 pellets/FR8) lever in lieu of increased selection of the low reward/ low FR (2 pellets/FR4) alternative (Walton *et al*, 2009). Cis-flupenthixol (0.25, 0.5 mg/kg), also decreases choice of the effortful option across trials blocks with increasing response costs, an effect which is observed even when the delays to receive LR and HR reward are matched (Floresco *et al*, 2008b). High dose eticlopride (0.06 mg/kg) and SCH 23390 (0.01 mg/kg) similarly increase effort discounting, but SCH 23390 only decreases choice of the HR lever when response requirements are high (20 lever presses) (Hosking *et al*, 2015b).

Most systemic work has focused on DA receptor antagonism, and few studies have targeted the DA receptor subtypes alone with selective DA agonists. In most cases, a hyperdopaminergic state has been achieved by administering DAT inhibitors. GBR 12909 (5, 10 mg/kg), bupropion (20, 40 mg/kg) and PRX-14040 (20, 40 mg/kg) increase lever pressing and decrease chow consumption in the (PROG)/chow concurrent choice task, suggesting that

excess DA may invigorate behaviour towards lucrative, yet costlier response alternatives (Randall *et al*, 2015; Yohn *et al*, 2016c, 2016d). Amphetamine has also served as a proxy for dopamine agonism, given substantial evidence that the drug acutely enhances extracellular levels of DA by blockade of DA reuptake (Hutson *et al*, 2014). In humans, amphetamine (10, 20 mg) increases willingness to expend effort for monetary reward, in a task where subjects can decide between easy (i.e. 30 button presses within 7s) or hard (i.e.100 presses with nondominant pinky finger in 21s) response costs (Wardle *et al*, 2011). Notably, this effect is most pronounced for low reward probability blocks, where fulfilling the effort requirements produces reward on 12.5 or 50% of trials (but not 88%). When administered to rodents, amphetamine (2-3 mg/kg) decreases lever pressing and chow consumption on the concurrent (FR5)/chow feeding task, although these doses are substantially higher than those used to modulate other forms of decision making in this species (Silveira *et al*, 2014). On the T-maze, a lower dose of amphetamine (.75 mg/kg) increases choice of the HR arm, an effect that is antagonized by both SCH23990 (0.0125 mg/kg) and haloperidol (0.1 mg/kg), indicating a DA receptor-mediated mechanism of action (Bardgett *et al*, 2009). Likewise, a low dose of amphetamine (.25 mg/kg) increases choice of the HR option in the operant effort-discounting task, but a higher dose (0.5 mg/kg) has the opposite effect, systematically decreasing preference for the HR lever as the response requirement increases across trials blocks (Floresco *et al*, 2008b). An interesting follow-up revealed that when the delays to receive LR and HR were equated, low dose amphetamine no longer biases choice towards the

effortful option, but the higher dose continues to amplify effort discounting (Floresco *et al*, 2008b). This work suggests that low doses of amphetamine may promote effort investment towards larger reward by reducing the impact of delay costs inevitably associated with most high-effort requirements.

When considered across physical effort tasks, the results of systemic administration studies overwhelmingly indicate that dopamine, acting primarily on D1 and D2 receptors, biases organisms to exert more physical effort for lucrative reward. As will be discussed below, these effects are likely mediated by dopaminergic transmission in the nucleus accumbens.

#### 1.8.1.2 Physical Effort: Intracerebral Infusions and 6-OHDA Lesions

Site-specific targeting of DA transmission has been investigated in two regions: the anterior cingulate cortex (ACC) and nucleus accumbens (NAcc) (see section 1.10.1 and 1.10.2 respectively for a general discussion of these areas). 6-hydroxydopamine (6-OHDA) lesions to the anterior cingulate decrease choice of the HR arm in the T-maze task, an effect that is mimicked by infusions of a $D_1$ (SCH 23390), but not a $D_2$ receptor (eticlopride) antagonist into this region (Schweimer *et al*, 2005; Schweimer and Hauber, 2006). This suggests DA in the ACC may be acting on $D_1$ receptors to bias behaviour towards high effort, high reward options. However, a similar study also found no effects of ACC 6-ODHA lesions on effort allocation in the T-maze task (Walton *et al*, 2005). A number of methodological differences might account for this discrepancy, including the doses of 6-OHDA used (the study reporting effects on choice infused 3 µg at 12

sites, whereas the one reporting null effects infused 2 μg at 8 sites), the time from surgery to retesting, as well as rodent strain used (Sprague versus Lister Hooded, respectively) (Schweimer *et al*, 2005).

6-OHDA lesions of the nucleus accumbens similarly disrupt lever pressing for sucrose when chow is concurrently available, but rats exhibit normal levels of pressing on alternate days when chow is not available (Cousins and Salamone, 1994). Although a significant correlation between DA levels and lever pressing was observed in this study, the shifts in choice cannot be attributed to motor impairments induced by depletions of NAcc DA, as rats readily lever press for sucrose when the chow is not available (Cousins and Salamone, 1994). This can be contrasted with DA depletions of the ventrolateral striatum, which produce severe deficits in motor ability that interfere with lever pressing and chow consumption (Cousins *et al*, 1993). The effects of accumbal DA depletions are likely driven by reduced DA transmission in the accumbens core, as selective DA lesions to this region, but not the shell, impair optimal response allocation in the concurrent choice task (Sokolowski and Salamone, 1998). Accordingly, infusion of the $D_1$ antagonist SCH 23990, $D_2$ antagonist raclopride, or the VMAT inhibitor tetrabenazine into the NAc mimic the effects of DA depletions to this area on the (FR5)/chow concurrent choice task (Nowend *et al*, 2001; Nunes *et al*, 2013b). NAc 6-OHDA lesions similarly decrease choice of the HR arm in the effort T-maze task, suggesting that mesoaccumbens DA invigorates responding to overcome work-related costs for preferred reward (Salamone *et al*, 1994).

While NAc DA signaling is clearly implicated in decision making with effort

costs, it is unclear exactly how mesolimbic DA contributes to the cost/benefit computation. DA release in the NAc appears to operate under two modes- a "phasic" rapid signaling driven by midbrain DA neuron burst firing, and an extrasynaptic "tonic" mode which changes on a slower timescale and reflects the overall number of active DA neurons (Floresco, 2015). It is well-established that phasic signaling codes a temporal reward prediction error reflecting the difference in value between a received versus predicted reward at each moment in time (Schultz, 2016; Schultz *et al*, 1997). However, this phasic signaling is also thought to convey the subjective value or utility attached to reward-predictive stimuli and actions (Schultz *et al*, 1997), such that "cached" action values may be used to guide future choice behaviour (Lee *et al*, 2012). Phasic DA has been shown to signal many of the economic attributes that might guide choice behaviour, including objective expected value (Fiorillo *et al*, 2003; Roesch *et al*, 2007; Tobler *et al*, 2005), as well as subjective preference for risky (Sugam *et al*, 2012) or delayed rewards (Day *et al*, 2010). However, a number of studies have demonstrated that the cost of effort is not faithfully encoded in the DA signal in either rodents (Gan *et al*, 2010; Hollon *et al*, 2014) or primates (Pasquereau and Turner, 2013). Rather than conveying a cached action value, it has been proposed that DA may modulate incoming inputs from other brain regions which themselves encode effort costs, such as the anterior cingulate cortex (Rudebeck *et al*, 2006), basolateral amygdala, and insula (Prevost *et al*, 2010). NAc DA may set the threshold for what is considered appropriate effort for a given reward, whereby action policies provided by cortical and limbic inputs are then modified

according to this DA tone (Hauber and Sommer, 2009; Phillips *et al*, 2007; Sesack and Grace, 2010). Specifically, high DA in the NAc may minimize response costs and increase willingness to exert effort for larger reward, while low DA tone (as observed following 6-OHDA lesions) reduces this willingness. This is then compared to a general utility threshold, where effortful options high in utility are selected, but those falling below this threshold are not (Phillips *et al*, 2007). This proposal does not differentiate between phasic and tonic signaling, but tonic DA may impose a steady state cost-benefit bias, whereas phasic signaling in response to unexpected rewards may provide an opportunistic window where higher effort might result in more reward (Phillips *et al*, 2007). Similar theories suggest that tonic DA may act as a running meter of available reward, which can increase response vigor in situations where average reward rate is high (Niv *et al*, 2007). In support of these theories, DAT knockout mice which exhibit increased tonic firing and elevated DA levels demonstrate increased lever pressing in a modified version of the FR/chow concurrent choice task (Cagniard *et al*, 2006).

#### 1.8.1.3 Cognitive Effort

In contrast, DA signaling does not appear to play a critical role in the processes guiding cognitive effort allocation, at least in rodents. When administered prior to performance of the rCET, the $D_1$ receptor antagonist SCH 233190 and the $D_2$ receptor antagonist eticlopride do not affect choice of the hard lever option, even though both drugs decrease the number of trials

completed and increase omission rates (Hosking *et al*, 2015b). In contrast, these dopaminergic agents reduce hard lever choice in an operant physical effort choice task (Hosking *et al*, 2015b). It is worth noting that the design of the rCET necessarily makes it a physically effortful task; in a typical session, rats complete approximately 130 trials in which they hit a lever, orient towards the stimulus array to detect the stimulus, then turn around to either collect reward or commence the next trial. However, the cognitively effortful options do not differ in their physical requirements, and so it is notable that dopamine antagonism does not affect choice, but does affect trials completed and omission rates. This might suggest DA is not involved in cognitive effort allocation *per se*, but may regulate the amount of physical effort recruited in a typical rCET session. While computational modeling studies have suggested a role for DA in allocation of cognitive effort, no human work has directly tested this hypothesis (Westbrook and Braver, 2016). It will be interesting to test a broader range of dopaminergic agents on the rCET, to assess whether DA involvement reflects a fundamental distinction between decision making with physical or cognitive effort costs.

### 1.8.2 Adrenergic Systems

The existing evidence suggests that noradrenaline (NA) is not involved in encoding of effort costs, or in the decision to engage in an effortful challenge, regardless of whether it is in the physical or cognitive domain. Enhancing noradrenergic tone with the NET inhibitors desipramine (2.5, 5.0, 10.0 mg/kg) or

atomoxetine (0.125, 0.25, 0.5, 1.0 mg/kg) fails to increase response output in the (PROG)/chow concurrent choice task, and actually decreases lever pressing at higher doses or when administered repeatedly (Yohn *et al*, 2016c). On the operant effort discounting task, atomoxetine also has no effect on rats' choice profile (0.1, 0.3, 1.0 mg/kg) (Hosking *et al*, 2014b). The a2-adrenergic receptor antagonist yohimbine (5 mg/kg) appears to have some minor effects on choice behavior, decreasing choice of the HR lever during the first two blocks when the response costs are two and ten lever presses, but this effect is not robust (Hosking *et al*, 2015b). Rather, NA is likely mobilized during the actual experience of physical effort, as the body recruits the necessary muscles and autonomic arousal processes necessary to fulfill an effortful challenge (Burnstock, 2009; Carter *et al*, 2010; Robbins, 1984). In line with this, Varazzani et al., (2015) showed that in response to cues signaling the anticipated effort level of an upcoming physical challenge, firing of dopamine neurons in the substantia nigra pars compacta (SNc) reflects both the size of expected reward and amount of effort required to obtain it. In contrast, modulation of LC activity reflects the difficulty of the task at hand, increasing with effort level during the action (Varazzani *et al*, 2015). This work suggests DA and NA may work cooperatively to regulate effort-based choice: DA may encode the expected value of an upcoming effortful challenge, whereas NA may react to information about a current challenge, and will mobilize the resources necessary to overcome it.

When administered on the rCET, atomoxetine (0.1, 0.3, 1.0 mg/kg) has no

effort on choice behavior, but increases choice latencies and choice omissions, and slightly impairs accuracy for easy trials in worker rats (Hosking *et al*, 2015b). Similarly, a high dose of yohimbine (5 mg/kg) does not affect choice, but impairs accuracy across all rats (Hosking *et al*, 2015b). This profile mirrors the contributions of NA to physical effort, where it does not affect the decision to engage, but does affect actual performance of the effortful challenge. Indeed, attentional effort is associated with autonomic arousal (Egeth and Kahneman, 1975; Howells *et al*, 2010), and NA appears to play a selective role in the 5-CRTT in novel or arousing circumstances (i.e variable ITI, random bursts of white noise), by potentially optimizing attentional performance in these situations (Carli *et al*, 1983; Cole and Robbins, 1987; Robbins, 2002).

### 1.8.3 Cholinergic Systems

A number of cholinergic drugs have been assessed on the rCET. These experiments suggest that acetylcholine has dissociable roles in regulating attention versus the decision to engage in an attentional challenge. Systemic administration of nicotine (1.0 mg/kg) decreases choice of the HR option in slacker rats, while concomitantly increasing their attentional accuracy on hard trials (Hosking *et al*, 2014c). Importantly, the improved accuracy in slackers following nicotine does not reflect amelioration of an attention deficit, as numerous studies have shown that workers and slackers do not differ in attentional ability (Cocker *et al*, 2012b). Rather, increasing neuronal activity via

nicotine receptor activation simultaneously improves ability while diminishing optimal choice, suggesting that cholinergic regulation of these processes is dissociable. Indeed, cholinergic regulation of attention versus decision making may map onto dissociable cholinergic neurons in the brain, such as the cortically projecting neurons of the basal forebrain, versus the locally acting striatal interneurons (Bubser *et al*, 2012). In contrast, systemic administration of the muscarinic receptor antagonist scopolamine (0.3 mg/kg) decreases choice of the HR option across all rats without affecting attentional accuracy, while the muscarinic receptor agonist oxotremorine and the nicotine antagonist mecamylamine are without effect (Hosking *et al*, 2014c). Collectively, these results suggest that perturbing cholinergic signaling may impair or improve decision making with cognitive effort costs, the results of which depend on individual propensity to work or slack at baseline. Additionally, these effects on choice behaviour are separable from the established role of acetylcholine in regulating attentional processes (Himmelheber *et al*, 2000; Mcgaughy *et al*, 2002; Passetti *et al*, 2000).

In comparison, relatively little work has focused on cholinergic contributions to physical effort-based decision making. The nonspecific muscarinic agonist pilocarpine (1.0, 2.0, 3.0 mg.kg) decreases lever pressing on the (FR5)/chow concurrent choice task, but does not mimic the increased chow consumption commonly observed following systemic administration of dopamine antagonists (Cousins *et al*, 1994). In contrast, direct infusions of pilocarpine into the NAc causes rats to reallocate their responding away from the lever and

towards the freely available chow, an effect which is antagonized by co-infusion of the antagonist scopolamine (Nunes *et al*, 2013a). Thus it appears cholinergic signaling does contribute to physical effort allocation, likely via interactions with DA signaling in the NAc, but this has not been directly investigated (Threlfell *et al*, 2012).

In order to further characterize the contribution of cortically projecting cholinergic neurons to decision making with cognitive effort costs, I carried out an experiment (Chapter 3) in which I used chemogenetics to selectively downregulate basal forebrain cholinergic neurons as rats performed the rCET.

### 1.8.4 Cannabinoid Systems

Studies in both humans and rodents suggest that pertubrations of the cannabinoid system can influence effort-based decision making (See section 1.3 for a discussion of the available human evidence). In rodents, $CB_1$ receptor inverse agonism/antagonism with AM 251 (2.0, 4.0, 8.0 mg/kg) decreases lever pressing in the FR5/chow concurrent choice task, but does not lead to a corresponding rise in chow consumption (Sink *et al*, 2008b). A similar effect of AM 251 (16.0) mg/kg is also observed in the (PROG)/chow concurrent choice task, and administration of the neutral $CB_1$ anatagonist AM 4113 (4.0, 8.0, 16.0 mg/kg) likewise reduces lever pressing and decreases chow consumption (Randall *et al*, 2014). These effects are line with the known role of $CB_1$ receptor antagonists as appetite suppressants, and their ability to reduce food-reinforced behavior (Salamone *et al*, 2007; Sink *et al*, 2008a). In contrast, direct infusions of

arachidonyl-2-chloroethylamide (ACEA), a potent synthetic CB1receptor agonist, into the ACC causes a robust shift in choice towards the small, unobstructed reward option, suggesting that rats are less willing to exert physical effort for preferred rewards (Khani et al., 2015). This shift to LR does not occur when the costs in reward arms are equalized, or when the antagonist AM 251 is microinjected into the ACC (Khani *et al*, 2015). Notably, intra-ACC ACEA infusions affect physical effort but not delay-based decision making, whereas infusions of ACEA into the OFC have the opposite effect (Khani *et al*, 2015). These results mimic the effects of lesions to the ACC and OFC on effort and delay-based decision making, respectively, and suggest that $CB_1$ receptors in distinct prefrontal regions are necessary when choosing between options varying in delay or effort (Rudebeck *et al*, 2006). Most recently, it has been demonstrated that microinjection of the $CB_1$ receptor agonist WIN 55, 212-2 (2, 10 and 50 μM) into the nucleus accumbens decreases HR choice in the T-maze task, an effect which is antagonized by co-infusion of AM 251 (45 μM) (Fatahi and Haghparast, 2018). Overall, it appears then that cannabionoid receptor activation, in both prefrontal and striatal areas, may bias choice away from rewards that are discounted by physically effortful endeavors.

In accordance with the available evidence from the physical effort literature, we have also shown that cannabinoid signaling regulates the willingness to exert cognitive effort. When administered on the rCET, THC (1.0, 2.0, 3.0 mg/kg) dose-dependently decreases choice of the difficult, high-reward option requiring accurate detection of a brief (0.2 s) light stimulus;

correspondingly, rats shift choice to the easier, low-reward option where the light stimulus is presented for a longer duration (1 s) (Silveira *et al*, 2016). Importantly, the lack of effect of THC on attentional accuracy suggests that the choice shift is not due to an inability to complete high-reward trials. Moreover, THC-induced choice impairments are correlated with $CB_1$ receptor density in the medial prefrontal cortex (mPFC), indicating that prefrontal $CB_1$ receptors may contribute to THC-induced alterations in effortful decision making. These findings complement those of Khani et al. (2015), where CB1receptor agonism in the ACC decreases physical effort-based decision making. Interestingly, and unlike the effects of THC, $CB_1$ agonism by administration of the synthetic cannabinoid WIN 55, 212-2 does not shift choice on the rCET (Silveira *et al*, 2016). These discrepancies are likely related to the distinct pharmacodynamic profiles of these drugs, and/or differences in the intracellular signalling pathways they recruit, with THC a potent recruiter of the arrestin-2 pathway, and WIN 55, 212-2 a recruiter of the classical G-protein $G\alpha_{i/o}$ and $G\beta\gamma$ pathways (Laprairie *et al*, 2014). These data emphasize that not all forms of cannabinoid receptor activation produce converging effects, and animal studies aiming to model the psychoactive effects of cannabis would do well to administer THC in lieu of its synthetic counterparts. In contrast, modulating endogenous cannabinoid tone, via $CB_1$ receptor inverse agonism with rimonabant, $CB_2$ antagonism with AM 630, or by inhibi tion of endogenous anandamide hydrolysis by FAAH with URB 597, does not affect choice on the rCET (Silveira *et al*, 2016). Together, this work suggests that endocannabinoid signaling does not tonically regulate effort-based decision

making with cognitive effort costs, but that endogenous activation of $CB_1$ receptors may temper selection of otherwise lurcrative, albeit cognitively demanding challenges (Silveira *et al*, 2017).

### 1.8.5 Adenosine Systems

An impressive body of work by Salamone and Colleagues has demonstrated an interesting role for the purine nucleoside adenosine in the allocation of physical effort. Systemic administration of the $A_2$ adenosine receptor antagonists MSX-3 (2 mg/kg) and KW6002 (0.5 mg/kg), the $A_1$ adenosine receptor antagonist DPCPX (0.75 mg/kg), and the nonspecific adenosine receptor antagonist caffeine (20 mg/kg) have no effect on response allocation in the (FR5)/chow concurrent choice task (Farrar *et al*, 2007; Salamone *et al*, 2009b). Similarly MSX-3 and DPCPX do not alter selection of the HR option in the T-maze effort task (Mott *et al*, 2009), but MSX-3 (2mg.kg) does increase lever pressing and decreases chow consumption in the (PROG)/chow concurrent choice task (Randall *et al*, 2012). $A_2$ receptor knockout mice also display a similar preference for the HR arm relative to wild types in the effort T-maze task (Pardo *et al*, 2012). Taken at face value, these studies suggest that under baseline conditions, adenosine signaling does not play a major role in the valuations guiding choice with physical effort costs.

However, adenosine does play a critical role in mediating the effects of dopamine signaling on physical effort allocation. The decline in lever pressing

and increased chow consumption observed following haloperidol (0.1 mg/kg) administration in the (FR5)/chow concurrent choice task is attenuated by the $A_2$ antagonists MSX-3 and KW6002 (0.125, 0.25, 0.5 mg/kg), and by the general adenosine antagonist caffeine (10, 20 mg/kg) (Salamone *et al*, 2009b). MSX-3 also attenuates the haloperidol-induced shifts to the LR arm in both rats (Mott *et al*, 2009) and mice (Pardo *et al*, 2012) on the T-maze. Similarly, choice deficits induced by the VMAT inhibitor tetrabenazine (0.75 mg/kg) in the effort T-maze and both the (FR5) and (PROG) concurrent choice tasks are reversed by MSX-3 (0.5, 1, 2 mg/kg) (Nunes *et al*, 2013b; Randall *et al*, 2014; Yohn *et al*, 2015b). These effects appear to be mediated by $A_2$ and $D_2$ receptor interactions, as MSX-3 (0.5, 1, 2 mg/kg) and KW6002 (0.25, 0.5 mg/kg) can fully attenuate the shift from lever pressing to chow consumption induced by the $D_2$ receptor antagonist eticlopride (0.8 mg/kg), but MSX-3 only partially attenuates the choice impairments produced by the $D_1$ antagonist SCH 39166 (0.2 mg/kg) (Nunes *et al*, 2010; Worden *et al*, 2009). In contrast, the $A_1$ receptor antagonist DPCPX does not reverse haloperidol's effects in the concurrent choice or T-maze tasks (Mott *et al*, 2009; Salamone *et al*, 2009b), and choice impairments produced by $D_1$ receptor antagonist ecicopam (.2 mg/kg) are not reversed by the $A_1$ receptor antagonists DPCPX or CPT (Nunes *et al*, 2010). As further evidence of $A_2$-$D_2$ interactions guiding effort allocation, $A_2$ receptor knockout mice are insensitive to the effects of haloperidol (0.05, 0.1 mg/kg) on effort T-maze performance (Pardo *et al*, 2012).

Notably, $A_2$ adeosine receptors are abundant in striatal zones, including

the nucleus accumbens, and are colocalized with $D_2$ receptors on medium spiny neurons (Jarvis and Williams, 1989). Stimulation of either receptor has opposing effects on cAMP signal transduction pathways, thus providing a potential mechanism by which $A_2$ receptors may mediate the effects of mesoaccumbens DA signaling on effort-based choice. In line with this, intra-NAcc infusions of the $A_2$ agonist GS21680 (24 ng/ .5 ul per side) mimic the effects of intra-NAcc DA antagonism on the (FR5)/chow concurrent choice task (Font *et al*, 2008), and systemic (1, 2 mg/kg) or intra-NAcc (1.25, 2.5, 5 ug/side) administration of MSX-3 blocks the shift from lever pressing to chow produced by intra-accumbal eticlopride infusion (2,4, ug/side) (Farrar *et al*, 2010). Collectively, these studies overwhelmingly suggest that $A_2$ receptors in the nucleus accumbens may mediate the effects of dopamine signaling on effort-based choice, via interactions with colocalized $D_2$ receptors in this region.

Like its effects on the physical effort tasks, adenosine receptor antagonism on the rCET does not have a pronounced role in cognitive effort allocation. Administration of caffeine does not affect HR choice across subjects, but has mild effects in worker rats, resulting in a trending decrease in HR choice at higher doses (10, 20 mg/kg) (Cocker *et al*, 2012b). Given that DA signaling does not play as prominent a role in rCET decision making (Hosking *et al*, 2015b), $A_2$-$D_2$ interactions in the nucleus accumbens are less likely to regulate effort in the cognitive domain, although this is an exciting avenue for future study.

### 1.8.6 Serotonergic Systems

In contrast to the neurotransmitter systems described earlier, serotonin does not appear to play a role in the mechanisms guiding physical effort allocation. In both the FR5 and PROG concurrent choice tasks, the SERT inhibitor fluoxetine decreases lever pressing (2.5, 5.0, 10.0 mg/kg) and either has no effect, or decreases consumption of freely available chow (Yohn *et al*, 2016b, 2016c). Denk et al., (2005) directly compared serotonergic contributions to delay and physical effort discounting, by administering systemic pCPA (300 mg/kg; 2 injections, administered 48h and 24h before testing) prior to testing on a T-maze in which the costs to HR were either physical (a 30 cm scalable barrier) or delay-based (15s). This dosing regimen reduces serotonin levels and its metabolite, 5-HT1AA, by more than 85% in the frontal cortex and hippocampus, for up to seven days (Hajós *et al*, 1998; Jakala *et al*, 1992). Serotonergic depletion increases delay discounting, causing more animals to opt for the small, immediate reward, but has no effect on physical effort allocation (Denk *et al*, 2005). These results are in accordance with the prominent role of the serotonergic system in mediating decisions with delay costs (Winstanley *et al*, 2003a, 2004a, 2004b) .

Currently no work has investigated serotonergic contributions to cognitive effort-based decision making. The neurotransmitter does not figure into current theories of cognitive effort allocation, and no drugs targeting serotonin have been investigated on the rCET (although see Appendix 1 for a summary of my recent attempts to study the role of serotonin in decision making with cognitive effort costs).

### 1.8.7 Other Neurotransmitter Systems: Glutamate and GABA

Few investigations have directly assessed glutamatergic and GABAergic signaling in the domain of effort-based decision making, but they will be briefly mentioned here for the sake of completeness. In an operant-based lever pressing task, the NMDA antagonist ketamine (5, 10 mg/kg) increases effort discounting across blocks with increasing effort requirements (Floresco *et al*, 2008b). However, when tested in an equivalent task with delays to reward receipt equalized across the low-effort and high-effort options, ketamine no longer results in effort aversion. Indeed, it appears that ketamine may specifically increase delay discounting, which explains why an effect is observed in the original task where the increased response requirements necessarily increase the time it take for rats to receive the larger reward (Floresco *et al*, 2008b). Interestingly, a recent study using a knockout mouse line demonstrated that glutamatergic transmission onto midbrain DA neurons is necessary for mice to lever press on a FR5 schedule for preferred sugary reward, instead of opting for freely available chow (Hutchison *et al*, 2017). This work highlights the complex neurotransmitter interactions that guide effort allocation, which to date have not received much attention.

In the concurrent choice task, the barbiturate sodium pentobarbital (10 mg.kg) decreases lever pressing and increases consumption of freely available chow, while a higher dose (15 mg.kg) decreases both lever pressing and chow consumption, likely reflecting sedative effects (Cousins *et al*, 1994). In contrast,

ethanol does not affect rats' willingness to exert cognitive effort for larger rewards on the rCET (Cocker *et al*, 2012b). Thus, drugs primarily acting to increase GABA in the brain do not necessarily decrease willingness to work for reward, although more work is needed in this area.

## 1.9 Individual Differences and Physiological Factors Mediating Effort Allocation

### 1.9.1 Individual and Sex Differences

The study of individual differences has been incorporated into a number of models. This work has demonstrated that basal willingness to exert effort is modulated by a number of neurotransmitters systems and brain areas. Randall and colleagues were the first to study individual differences as they relate to physical effort allocation, by using a median split to classify rats as high or low responders based on lever presses in the (PROG)/chow concurrent choice task (Randall *et al*, 2012). Notably, the progressive ratio variant of the concurrent choice task generates high response variability, with some rats pressing the lever less than 100 times for sucrose and instead preferring the freely available chow, while others press the lever more than 1000 times and consume small amounts of chow (Randall *et al*, 2012). High responders are more sensitive to the suppressive effects of haloperidol on lever pressing for preferred sucrose, and show increased pDARPP-32(Thr34) expression in the core region of the nucleus accumbens (Randall *et al*, 2012).

Likewise, in the cognitive effort domain, the rCET generates high variability in baseline willingness to exert attentional effort for lucrative rewards. In a typical rCET study, baseline choice of the high reward (HR) option falls within 65-70% across rats. Rats are categorized as “workers” if they choose HR on more than 70% of trials, while rats whose choice of HR is less than 70% are termed “slackers”. This subdivision is based on the mean split from the original rCET paper (Cocker et al, 2012), and has been used in subsequent studies to aid comparison of individual differences across studies using the task. Individual propensities to “work” or “slack” at baseline have been shown to mediate the effects of a number of pharmacological challenges (e.g. amphetamine (section 1.8.1), nicotine (section 1.8.3), as well as the effects of regional inactivations (e.g. the basolateral amygdala (section 1.10.3)).

Overwhelmingly, animal models of cost/benefit decision making have used male rodents as subjects. Only recently have attempts been made to assess females on tasks in which effort costs are manipulated. In the operant physical effort discounting task, baseline choice of the high effort, high reward (HR) lever does not fluctuate with the female estrous cycle, but ovariectomy reduces effort discounting (Uban *et al*, 2012). In contrast, estradiol (10ug) decreases choice of the HR lever 24 hours post-injection, suggesting that ovarian hormones appear to bias choice towards smaller, albeit more accessible rewards. Notably, co-administration of the selective estrogen receptor (ER)-α agonist PPT (10 ug) and the ERβ agonist DRN (10 ug) appear to mimic the effects of estradiol on effort discounting, but paradoxically each ligand administered alone increases effortful

choice (Uban *et al*, 2012). Estrous cycling has not been investigated on the rCET, but baseline choice approximates those of male subjects (personal observation). However, in line with previous 5-CSRTT literature, female rats generally display lower accuracy rates (Bayless *et al*, 2012), providing further evidence that willingness to engage in cognitive effort and cognitive performance itself are dissociable.

### 1.9.2 Stress, Inflammation, and Fatigue

A number of investigators have used animal effort models to probe the physiological processes, such as stress and inflammation, which may mediate valuations of effort. These have received due consideration because disorders characterized by anergia, such as depression and schizophrenia, are also associated with stress and biomarkers of inflammation (Felger and Treadway, 2017; Monroe and Harkness, 2005). In the operant physical effort-discounting task developed by Floresco and Colleagues (2008), one hour of acute restraint stress, but not systemic administration of corticosterone (1, 3 mg.kg), dramatically decreases choice of the HR option (Shafiei *et al*, 2012). This effort discounting is observed across all trial blocks with varying response costs (2, 5, 10, 20 lever presses), and is attenuated by the corticotrophin-releasing factor antagonist (CRF) alpha-helical CRF (Bryce and Floresco, 2016). Indeed, a similar effort discounting is observed following intra-ventricular or intra-ventral tegmental area (VTA) infusions of CRF, suggesting that the effects of acute stress are mediated by CRF-induced perturbations in DA transmission (Bryce

and Floresco, 2016). Similarly, systemic administration of the pro-inflammatory cytokines interleukin (IL) -1β (2, 4 mg/kg) and IL-6 (4,6,8 mg/kg) decrease lever pressing and increase chow consumption in the FR(5)/chow concurrent choice task, suggesting that like acute stress, inflammation reduces willingness to exert physical effort for lucrative rewards (Nunes *et al*, 2014; Yohn *et al*, 2016a). This is in agreement with recent human work showing that systemic administration of the bacterial endotoxin *E. coli* lipopolysaccharide (LPS) transiently increases IL-6 and decreases acceptance of high effort options in an accept/reject choice paradigm (Draper *et al*, 2017). Paradoxically, another human study found LPS-induced sleepiness increases high effort choice (Lasselin *et al*, 2017), in line with the notion that sickness might not generally decrease motivation, but may lead to a reorganization of priorities where individuals become more discerning in their effort allocation; at least one study in mice supports this (Vichaya *et al*, 2014).

Another physiological state which has received due consideration is fatigue. Until recently, it was unknown whether metabolic states produced by fatigue are factored in the cost-benefit analysis. Mice fatigued by running on a treadmill at 80% of their peak workload show a sharp choice reversal from HR to LR arms (Iodice *et al*, 2017). These effects are temporary and specific, as subjects resume selection of the HR option when tested at 60% peak workload or when the efforts costs in either arm are equated (Iodice *et al*, 2017). This work suggests that levels of fatigue are dynamically factored into the cost/benefit analysis, presumably by magnifying the cost of effort in relation to the benefit of expected reward.

## 1.10 Functional Neural Systems Mediating Effort-Based Decision Making

Animal models haven proven invaluable towards identifying the neural circuits guiding effort-based cost/benefit decision making. This research has generally implicated a cortical-limbic-striatal circuit in guiding choice processes. Below I review three regions that have been assessed for their contribution to physical and cognitive effort allocation: the prefrontal cortex, the striatum, and the basolateral amygdala. Most of these experiments have assessed performance on-task following irreversible lesion of a brain area, typically with quinolinic or ibotinic acid. Other studies have relied on temporary inactivation via intracerebral drug infusion, most commonly achieved via gaba agonism, but lidocaine has also been used. Recently, researchers have also begun using chemogenetic and optogenetic techniques to address similar questions, albeit with greater specificity in select cases.

### 1.10.1 Prefrontal Cortices

The utility of animal models to investigate the processes underlying effort-based decision making rests on the circuit homology between rats and primates. This becomes especially pertinent when comparing cortical regions across species, which show great variation. Indeed, the rat cortex is composed exclusively of agranular cortex. Subsequent studies have established that orbital and medial prefrontal areas are similar in relative position and connectivity

across species, suggesting these areas are comparable (Heidbreder and Groenewegen, 2003; Öngür and Price, 2000). The infralimbic cortex (IL) of rodents is most analogous to area 25 of the primate brain, based on their similar connectivity to the striatal “emotional processing network”, consisting of three well-conserved and easily identifiable striatal regions: the nucleus accumbens shell, hippocampal-striatal projections, and amygdala-striatal projections (Heilbronner *et al*, 2016) . Based on this shared corticostriatal projection, ventrolateral areas of the rodent PFC (medial orbital and ventrolateral orbital) correspond to primate OFC (medial orbital and centrolateral orbital), whereas the dorsomedial wall of the rodent PFC (prelimbic, ventral Cg1) is most akin to areas of the primate ACC (32, 24). Notably, corticostriatal projections across species share a dual pattern of focal and diffuse projections, and follow a similar topographic organization across limbic, associative, and sensorimotor striatal zones (Haber, 2016; Mailly *et al*, 2013). While the absence of a granular zone in rodent cortex makes it difficult to establish which areas in the rat brain are homologous to the primate dorsolateral prefrontal cortices, this region has been compared to rodent PL cortex based on functional similarities (Dalley *et al*, 2004; Granon and Poucet, 2000; Vertes, 2004). Thus circuit and functional homology across species suggest that questions of effort allocation are amenable to analysis with rodent models. Highlighted here are three areas that have been investigated in regard to effort allocation: the orbitofrontal cortex, the medial prefrontal cortex (encompassing prelimbic and infralimbic regions), and the anterior cingulate cortex.

#### 1.10.1.1 Orbitofrontal Cortex

The available evidence suggests that the orbitofrontal cortices do not play a critical role in the processes guiding physical effort allocation. Lesions or inactivations of this area do not affect rats' propensity to scale a barrier for large reward, but do affect their ability to withstand delays for larger reward (Mobini *et al*, 2002; Rudebeck *et al*, 2006; Winstanley, 2004; Zeeb *et al*, 2010). This is in stark contrast to lesions of the anterior cingulate, which bias choice away from the effortful T-maze option, but which also leave delay-based decision making intact (Rudebeck *et al*, 2006). In line with this, intra-OFC infusions of the potent $CB_1$ receptor agonist ACEA do not affect choice in the T maze effort task, but drastically reduce high effort, high reward choice when infused into the ACC (Khani *et al*, 2015). Such work is in agreement with the general assertion that dorsal and ventral areas of the prefrontal cortex appear to regulate different forms of decision making, based on the cost inherent to the choice at hand. While lesions or inactivations of the anterior cingulate cortex affect physical or cognitive effort-based valuations (Croxson *et al*, 2009; Hosking *et al*, 2014a; Rudebeck *et al*, 2006; Walton *et al*, 2003b), similar manipulations to the orbital frontal regions selectively affect decision making involving delay, risk, and probability costs (Barrus *et al*, 2017; Orsini *et al*, 2015a; Stopper *et al*, 2014; Winstanley, 2004; Winstanley and Floresco, 2016). Notably, dissociable roles for dorsal and ventral prefrontal areas in goal-directed choice are also observed in the human literature (Prevost *et al*, 2010; Rangel and Hare, 2010; Rushworth *et*

*al*, 2011a). Viewed another way, the OFC can be said to mediate decisions in which costs are inherent to the *outcome* (i.e. delay, risk), while costs incurred on the *individual* (i.e. physical, cognitive effort) appear to be mediated by dorsal prefrontal regions. In support of this distinction, lesions to either the OFC or ACC impair stimulus or action value learning, respectively (Camille *et al*, 2011; Rudebeck *et al*, 2008), and neurons in either area appear to preferentially encode information about stimulus or action value (Hayden and Platt, 2010; Padoa-Schioppa and Assad, 2006). Notably, one study has found increased metabolic activity in the rodent left orbitofrontal cortex following performance of a physical effort-discounting task, in addition to increased activity in the left ACC and PL cortex, and decreased metabolic activity in the IL region (Endepols *et al*, 2010). Thus, it appears that decisions requiring an assessment of physical effort investment for reward evoke metabolic changes in a variety of prefrontal regions, but some areas, such as the OFC, play a less critical role.

To date, OFC contributions to decision making with cognitive effort costs has not been empirically investigated. While the physical effort literature suggests the OFC plays a nominal role in effort discounting, work with the rCET has demonstrated that the two forms of effort recruit overlapping, yet distinct, areas of the PFC (such as the medial prefrontal cortex, discussed below in section 1.10.1.1.2). Chapter 4 addresses this gap in the cognitive effort literature by investigating the effects of OFC inactivation on rCET performance.

#### 1.10.1.2 Medial Prefrontal Cortex

Lesions to the medial prefrontal cortex (mPFC), encompassing prelimbic (PL) and infralimbic (IL) regions, do not affect choice behaviour on the T-maze effort task, suggesting a minimal role for this prefrontal area in the processes guiding physical effort allocation (Walton *et al*, 2003b). Notably, one study did find that unilateral lesion of the mPFC decreases HR choice in the physical T-maze task, effects which are greatest when rats have to choose to put in more work for reward when it is on the contralateral side of space to the lesion (Croxson *et al*, 2014). These lesions encompassed the ACC, and so the authors suggest the ACC may have a lateralised role in evaluating the costs and benefits of actions based on their spatial location. However, another study did find increased and decreased metabolic activity in the PL and IL cortex, respectively, in rats following performance of an operant lever pressing choice task (Endepols *et al*, 2010), and using a similar task lower $D_2$ mRNA expression in the IL cortex is associated with greater willingness to exert effort for reward (Simon *et al*, 2013). It is possible these discrepancies are related to the parameters of effort (maze running versus repeated lever pressing) under investigation, but this has not been systematically studied.

In contrast, temporary inactivations of the mPFC affect rats' willingness to exert cognitive effort, such that selectively targeting the PL or IL reduces choice of the HR option in the rCET (Hosking *et al*, 2015a). Notably, these reductions in effortful choice are accompanied by distinct performance deficits: inactivations of the PL cortex impair attentional accuracy and response omissions (an indirect index of attention), while inactivations of IL cortex spare accuracy but robustly

increase impulsive action as measured by rates of premature responding (Hosking *et al*, 2015a). Although it is difficult to interpret the directionality of these deficits, it is possible that with attentional and response control processes impaired, rats shift choice towards a low cost (i.e LR) strategy. Another possibility is that different networks in the mPFC simultaneously mediate decision making, attention, and impulsivity, and so inactivations of the mPFC impair these independent processes. Indeed, the involvement of the mPFC in the rCET reflects the demands of the task at hand, given the medial wall of the frontal cortex plays a critical role in regulating attention and impulsivity in the standard 5-CSRTT (Chudasama *et al*, 2003b; Muir *et al*, 1996; Passetti, 2002). These processes are likely mediated via serial flow of information to the striatum, and it is possible different corticostriatal “loops” regulate distinct facets of task performance (i.e., choice, attention, response inhibition), the resulting output of which can then bias motor output via palladal-thalamic-cortical pathways (Chudasama and Robbins, 2006; Haber and Behrens, 2014).

#### 1.10.1.3 Anterior Cingulate Cortex

As reviewed in section 1.4, a number of imaging studies have implicated the anterior cingulate cortex (ACC) in valuations of effort. Individuals with localized lesions of the ACC demonstrate impaired performance on tests of real world decision making and are unable to report conscious feelings of effort (Naccache *et al*, 2005). In contrast, direct stimulation of the ACC can elicit a feeling of “gearing up” for an effortful challenge (Parvizi *et al*, 2013). Individual

neurons in the ACC are sensitive to changes in reward magnitude (Akkal *et al*, 2002; Shima, 1998), and relative to other regions of the PFC, the ACC has a high proportion of neurons encoding the decision parameters (i.e. payoff, probability, physical effort cost) necessary for valuations of effort expenditure for lucrative outcomes (Cowen *et al*, 2012; Hillman and Bilkey, 2010; Kennerley *et al*, 2011). Individual ACC neurons have also been shown to encode progression through a series of steps toward reward, effectively monitoring the "effort" exerted towards a goal (Shidara & Richmond, 2002). Accordingly, lesions of the ACC decrease rats' willingness to scale a barrier for larger reward, but have no effect when costs for the low and hard reward alternatives are equated (Rudebeck *et al*, 2006; Walton *et al*, 2002, 2003b). Lesions to the ACC also decrease lever pressing on a variant of the (PROG)/chow concurrent choice task, and decrease lever pressing for the HR option in an operant decision-making task (Hart *et al*, 2017; Walton *et al*, 2009). These effects on effortful choice are likely mediated via interactions with the nucleus accumbens and basolateral amygdala, as functional disconnections of the ACC and either region decreases rats' willingness to scale the effortful barrier in the T-maze effort task (Floresco and Ghods-Sharifi, 2007; Hauber and Sommer, 2009) Collectively, this work suggests that the ACC is part of a larger cortico-limbic-striatal circuitry regulating allocation of physical effort.

However, a closer inspection of the literature suggests that the ACC does not play a ubiquitous role in decision making, especially when different manifestations of physical effort are considered. While a recent study found an

effect of NMDA ACC lesions on lever pressing in a progressive ratio/concurrent chow choice task (Hart *et al*, 2017), an earlier study found no effects in a similar task following quinolinic acid lesions to the ACC (Schweimer, 2005). Notably, the latter study reporting no effects trained rats on the concurrent choice task after being trained on the effort T-maze. Thus it is possible that this previous experience improved performance in the subsequent decision-making task, or enough time elapsed for other brain regions to compensate for the extent of the lesion (Hart *et al*, 2017). Notably, the decision-making deficit observed following ACC lesions on the effort T-maze task is also ameliorated if rats receive previous training with response costs equated on each arm (Rudebeck *et al*, 2006), perhaps suggesting that other areas (i.e. NAc, BLA) may also regulate effort allocation when cost-benefit contingencies are updated. In contrast, lesions to the ACC do not affect decision making when effort is operationalized as weighted lever pressing (Holec *et al*, 2014). Clearly, more work is needed to determine under what conditions the ACC is recruited in valuations of physical effort, as the available evidence indicates that it plays a more nuanced role than current consensus would suggest (Winstanley and Floresco, 2016).

The ACC has also been implicated in decision making with cognitive effort costs. Indeed, objective measures of task difficulty correlate with activity in the ACC, while lesions to this region can abolish conscious feelings of mental effort (McGuire and Botvinick, 2010a; Naccache *et al*, 2005). While the ACC has been ascribed a number of cognitive functions including error detection (MacDonald, 2000), conflict monitoring (Botvinick *et al*, 1999), and action selection (Posner *et*

*al*, 1988), a recent proposal suggests that these may all reflect a general role of the ACC in cognitive effort allocation, and specifically in computing the expected value of cognitive control (Shenhav *et al*, 2013). Under this proposal, the decision to engage in a cognitive episode and the intensity of this engagement is based on the expected value of the goal it corresponds to, which is discounted by the costs required to maintain on task (Shenhav *et al*, 2013; Westbrook A1, 2016). If expected value is high, ACC signals for increased cognitive control, likely mediated by adjacent dorsolateral prefrontal cortices and their interconnections with the striatum via corticostriatal loops (Frank *et al*, 2001; Haber and Behrens, 2014; O'Reilly *et al*, 2014). In line with this proposal, temporary inactivation of the ACC decreases choice of the high-effort, high-reward option on the rCET (Hosking *et al*, 2014a).

### 1.10.2 Striatum

The ventral striatum (VS) has figured prominently in most, if not all, models of cost/benefit decision making, which is perhaps unsurprising given the region is the nexus at which cortical, limbic, and midbrain dopaminergic afferents – regions critical in reward and cognitive processes- can influence behaviour (Mogenson *et al*, 1980; Sesack and Grace, 2010). A major focus of the physical effort literature has been on mesolimbic dopamine in the nucleus accumbens (reviewed in section 1.8.1.2), but lesions, as well as temporary inactivation, have been also been carried out. Lesions to the nucleus accumbens (NAcc) reduce rats' willingness to choose the HR arm in the T-maze effort task, an effect which

is reproduced following contralateral disconnection of the ACC and NAcc (Hauber and Sommer, 2009). This work suggests that ACC signals relayed to the NAcc may be a means to bias motor output towards more lucrative, albeit costly, options; a human imaging study using a physical effort task supports this interpretation (Croxson *et al*, 2009). The core region of the NAcc appears to be particularly involved in effort discounting, as temporary inactivation of this region, but not the lateral shell, reduces choice of the high-effort lever in the operant effort discounting task (Ghods-Sharifi and Floresco, 2010). In contrast, the dorsal striatum does not appear to play a role in decision making with physical effort costs. While no study has temporarily inactivated or lesioned the area, ventral striatal manipulations that affect valuations of physical effort allocation (e.g. intra-accumbens adenosine, acetylcholine, or dopamine neurotransmission manipulation) are ineffective when targeted toward a dorsal striatal site, suggesting that the contributions of this striatal zone to decision making with physical effort costs is minimal (Cousins *et al*, 1993; Farrar *et al*, 2010; Font *et al*, 2008; Nunes *et al*, 2013a).

In contrast, the role of the striatum has not yet been probed on the rCET. Given its role as an integrator of cortical and limibic inputs, it is likely this area is involved in rCET performance, as cortical and amygaldar regions guide choice on the rCET (Hosking *et al*, 2014a, 2015a). Indeed, a human imaging study has shown that NAcc activity is inversely related to cognitive demand, and this is preceded by coordinated activity in the ACC (Botvinick *et al*, 2009). Unlike the physical effort literature, however, there is reason to believe that cognitive effort

allocation may recruit more dorsal regions of the striatum. Indeed, human imaging data suggests that while the ventral striatum may be a common motivational node, cognitive and physical effort may preferentially recruit the dorsomedial and dorsolateral striatum, respectively (Schmidt *et al*, 2012). The dorsal striatum may play a particularly important role in decisions based on cognitive effort costs, given widespread areas of the cortex project to this "associative" area, and aberrant signaling in this region can induce deficits in a number of cognitive processes, such as attention, working memory, and behavioural flexibility across species (Bradfield *et al*, 2013; Cools *et al*, 2006; Ragozzino, 2007; Rogers *et al*, 2001). Chapter 5 assesses dorsal and ventral striatal contributions to cognitive effort allocation as probed by the rCET.

### 1.10.3 Basolateral Amygdala

The amygdala is traditionally viewed as a critical substrate for appetitive and aversive learning, but contemporary view of amygdalar function emphasizes its role in acquiring, monitoring, and updating value (Seymour and Dolan, 2008; Wassum and Izquierdo, 2015). Neurons in the primate amygdala track the positive and negative value of both conditioned and unconditioned stimuli, and single neurons in the human amygdala respond linearly to the monetary value assigned to individual items (Belova *et al*, 2007, 2008; Jenison *et al*, 2011; Paton *et al*, 2006). Additionally, neurons in the rodent amygdala anticipate reward encounter, respond during reward consumption, and differentiate between low and high reward magnitude (Pratt and Mizumori, 1998). Interestingly the rodent,

primate, and human studies described above recorded from neurons in the basolateral nucleus of the amygdala (BLA), an area that appears to make unique contributions to decision making with effort costs. Inactivation of the BLA decreases choice of the effortful HR option in the operant effort discounting task, an effect which persists even when the delays on the easy lever are matched to those necessarily incurred when fulfilling the response requirements on the hard lever (Ghods-Sharifi *et al*, 2009a). Similarly BLA inactivation, during initial learning or following stable baseline, decreases effortful choice in a variant of the (PROG)/chow concurrent choice task, but has no effect on lever pressing for sucrose when chow is no longer concurrently available (Hart and Izquierdo, 2017). This suggests that BLA inactivations leave primary motivational processes intact, but impair the valuations which bias choice towards the lucrative and effortful option (Hart *et al*, 2017). Notably, the BLA appears to guide physical effort-based choice via interactions with the ACC, as contralateral disconnection of these two areas decrease choice of the HR option in the T-maze effort task (Floresco and Ghods-Sharifi, 2007)

Similarly, the BLA appears to plays a critical, albeit more nuanced role, in the mechanisms guiding cognitive effort allocation. Whereas inactivation of the BLA in physical effort paradigms systematically decreases choice of the HR option, the effects of BLA inactivation on the rCET are dependent on rats' propensity to "work" or "slack" at baseline (Hosking *et al*, 2014a). Indeed, rats initially preferring the hard option at baseline decrease selection of this option, whereas rats opting for the easier of the two options at baseline now increase

hard lever choice following BLA inactivation (Hosking *et al*, 2014a). These nuances in BLA contributions to effortful choice are particularly interesting given recent imaging work in humans suggesting that activity in the right amygdala appears to process subjective value uniquely for rewards associated with cognitive, but not physical effort costs (Chong *et al*, 2017). In contrast to the amygdalostriatal circuitry that appears to guide physical effort-based decision making (Floresco and Ghods-Sharifi, 2007), this pathway has not been probed on the rCET. The goal of Chapter 6 is to assess whether communication between the ACC and BLA regulates cognitive effort allocation on the rCET.

## 1.11 Comparison of the Neurotransmitter and Neural Systems Guiding Physical and Cognitive Effort Allocation and Rationale for the Current Experiments

The available literature suggests that decision making characterized by physical or cognitive effort costs is subserved by overlapping, yet distinct, neural mechanisms. For example, cannabinoid signaling appears to play a similar role across domains of effort, while the physical effort literature has established a critical role for DA and adenosine signaling in such valuations. In contrast, DA and adeonsine do not appear to affect decision making with cognitive effort costs, but instead cholinergic signaling has pronounced effects on effort allocation in this realm. In reference to brain areas mediating effort-based

decision making, the ACC appears to bias choice towards HR options in either the physical or cognitive space, while the prelimbic and infralimbic areas are preferentially involved in cognitive, but not physical, effort. And while the BLA has been implicated in both forms of decision making, in the cognitive effort literature these effects interact with individual willingness to "work" or "slack" at baseline.

However, a complete understanding of how physical and cognitive effort allocation compare is limited because only recently have efforts been made to model cognitive effort costs in animals. To address this, I will carry out a series of studies in which I use the rCET to probe how distinct brain regions and neurotransmitter systems regulate cognitive effort allocation. The neurotransmitters and regions up for investigation are those that have been previously assessed in the physical effort domain, but for which no data exists in regard to cognitive effort. The results of these experiments, paired with previous work on the rCET, will yield a point-to-point comparison of the major neurotransmitter and neural systems guiding effort-based choice. A summary of these experiments is described below.

**Experiment 1 (Chapter 3) will investigate cholinergic contributions to decision making on the rCET, using chemogenetics and a transgenic CHAT cre recombinase-driven rat line**. We have previously shown that systemic administration of cholinergic drugs affects rats' willingness to exert cognitive effort, and that these effects are dissociable from the role of acetylcholine in regulating attention (Hosking *et al*, 2014c). However, it is currently unknown

which cholinergic neuron population is responsible for regulating decision making with cognitive effort costs. To address this, I will selectively transfect cholinergic basal forebrain neurons with inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs). Prior to task onset, I will administer clozapine-n-oxide, which will selectively bind to these DREADDs and silence transfected neurons (Roth, 2016). I focus on cholinergic BF neurons in this experiment, given this is the main source of cortical cholinergic tone, and we have previously implicated medial prefrontal cortical areas in regulating the willingness to exert cognitive effort (Hosking *et al*, 2014a, 2015a).

**In experiment 2 (Chapter 4) I bilaterally inactivate dorsal and ventral parts of the striatum to assess their contributions to decision making with cognitive effort costs.** The ventral striatum plays a critical role in decision making with physical effort costs, but it is unknown how this area contributes to effort allocation in the cognitive domain (Ghods-Sharifi and Floresco, 2010; Hauber and Sommer, 2009). Imaging work suggests that NAc activity in anticipation of reward is degraded by a working memory challenge, but the causal role of this region to cognitive effort has not been identified (Botvinick *et al*, 2009). In contrast, regions dorsal to the ventral striatum have not received as much attention in the cost/benefit decision-making literature, although it generally appears that this region is not involved in physical effort allocation (Cousins *et al*, 1993; Farrar *et al*, 2008; Nunes *et al*, 2013a). Indeed, while ventral striatal activity has been shown to track expected reward, as well as predict behavioral

performance in tasks requiring both cognitive and physical effort, the ventral striatum is only co-activated with the dorsomedial striatum when cognitive, but not physical, task demands are high (Schmidt *et al*, 2012) Connectivity also suggests that the dorsal striatum may play a particularly important role in cognitive-effort based decision making, given widespread areas of the cortex project to this area, and aberrant signaling in this region can induce deficits in attention, working memory, and behavioural flexibility across species (Balleine *et al*, 2007; Bradfield *et al*, 2013; Cools *et al*, 2006; Ragozzino, 2007; Rogers *et al*, 2001). Neurons in dorsal striatal regions display task-related activity that is graded by reward magnitude, and neurons in this region appear to encode value signals necessary to guide choice during decision-making tasks (Cromwell, 2003; Hollerman *et al*, 1998; Kawagoe *et al*, 1998; Kim *et al*, 2013; Kimchi and Laubach, 2009; Lau and Glimcher, 2007, 2008). Thus I predict that temporarily silencing both striatal zones will decrease willingness to exert cognitive effort for preferred outcomes.

**The purpose of experiment 3 (Chapter 5) is to investigate orbitofrontal cortex contributions to decision making on the rCET via bilateral inactivation.** To date, dorsal and medial regions of the prefrontal cortex have been investigated on the rCET, while the role of orbitofrontal regions remain unknown. As discussed in section 1.10.1.1, the OFC generally mediates decisions in which costs are inherent to the outcome, such as those observed in delay or risk-based decision making (Rushworth *et al*, 2011b). This can be

dissociated from dorsal prefrontal regions, which are generally recruited when decision costs are incurred on the individual (physical, cognitive effort) (Rangel and Hare, 2010; Rudebeck *et al*, 2006). However, recent rodent work suggests that the LOFC may play a more nuanced role in decision making, and is particularly involved when task parameters make the objectively best option unclear, such that subjective evaluation of different outcomes is more pronounced (Winstanley and Floresco, 2016). This will be a particularly interesting area to probe, given lesions of the OFC do not affect attentional processes, and only mildly affect response control on the 5-CSRTT, unlike dorsal PFC regions (Chudasama *et al*, 2003b; Muir *et al*, 1996; Passetti, 2002). The OFC also sends projections to dorsal striatal areas implicated on the rCET (see Chapter 4), where signals from different frontal areas could be integrated via diffuse and focal projections (Mailly *et al*, 2013; Silveira MM, Tremblay M, 2016). We will focus on the ventral lateral orbital frontal cortex (VLO), given most of the decision-making literature has focused on this area, because neurons in this region encode expected outcomes, and because relative to the MO, the VLO sends more projections to the ACC – a cortical area known to be involved in rCET performance specifically and effortful decision making generally (Hoover and Vertes, 2011; Schoenbaum *et al*, 2003).

**Lastly, Experiment 4 (Chapter 6) uses a contralateral ACC-BLA disconnection procedure to determine how bidirectional amygdalocortical communication regulates decisions of cognitive effort allocation.** The BLA

appears to play a unique role on the rCET, whereby inactivations of this region decrease choice of subjects' generally preferred option (Hosking *et al*, 2014a). This finding corroborates recent imaging work in humans where the BLA encodes the subjective value of choices associated with cognitive, but not physical effort costs (Chong *et al*, 2017). Critically, the effect of BLA inactivations in models of cost/benefit decision making appears to depend on the nature of the costs and their severity specifically (Winstanley and Floresco, 2016). If costs are very aversive, such as signaled punishing timeouts or electric shock, the BLA appears to attenuate baseline choice of the more aversive option (Orsini *et al*, 2015a; Zeeb and Winstanley, 2011). In contrast, when rewards are especially salient relative to costs, such as omission of reward receipt, delays, or exerting physical effort, then the BLA drives choice towards more rewarding options (Ghods-Sharifi *et al*, 2009a). This may explain the divergent effects of BLA inactivation on choice in the rCET, in that workers decrease choice of the high effort, high reward (HR) option HR, whereas slackers increase HR choice (Hosking *et al*, 2014a). Indeed, this may suggest that the rewards associated with successful trial completion are more salient in workers, but that cognitive costs are especially aversive in slacker rats. BLA-ACC circuitry promotes effortful choice in a decision task involving physical effort costs, and we hypothesize the BLA similarly provides the ACC with value-related information needed to guide cognitive effort allocation on the rCET (Floresco and Ghods-Sharifi, 2007).

# Chapter 2: General Methods

## 2.1 Subjects

Unless otherwise specified (see Chapter 5 and Appendix 1), subjects were male Long–Evans rats (Charles River Laboratories, St. Constant, Quebec, Canada) weighing 275–300g at the start of testing. Two weeks following arrival, rats were food-restricted to 14 g of rat chow per day and maintained at 85% of their free-feeding weight. Water was available ad libitum. All subjects were pair or trio-housed in a climate-controlled colony room under 12 h reverse light–dark cycle (21◦C; lights off at 8 am). Behavioral testing took place 4-6 days per week. Housing and testing conditions were in accordance with the Canadian Council of Animal Care, and experimental protocols were approved by the UBC Animal Care Committee.

## 2.2 Behavioural Apparatus

Testing took place in 32 standard five-hole operant chambers (Figure 2.1), each of which was enclosed in a ventilated, sound-attenuating chamber (Med Associates Inc, Vermont). The dimensions of this chamber are 25 x 25 x 25cm (Figure 2.2). The front and back walls, and the roof of the chamber are made of clear polycarbonate. The front panel has a hinged door to allow the introduction of the animal. Both side walls are made of aluminum; one is curved and contains 5 square holes (2.5cm sides, 4cm deep) positioned 2cm above the metallic grid

floor. The floor is made of stainless steel rods. A stimulus light (28V, 100mA, 1W, 2.5cm diameter) is positioned at the back of each hole. The other side wall is not curved and contains a food well (5 x 5cm), which is 25cm away from each of the five holes. This side wall also contains a house light (28V, 100mA, 3W) located 1cm from the top, which is the source of illumination during the task, and a retractable lever on either side of the food well. Once the animal pokes its nose inside the food well, a sucrose pellet (45mg; Bioserv, New Jersey) is delivered from an automatic dispenser positioned outside the box. A horizontal infrared beam crosses the entrance of the holes and food well in order to record the animals' response. The chamber is enclosed in a ventilated sound-attenuating cubicle (inner dimensions 55.9 x 40.6 x 38.1cm). A micro-camera is mounted on the ceiling of this cubicle to monitor the rats' behaviour. The operant chambers were operated by software written in Med-PC by CAW, running on an IBM-compatible computer.

### 2.3 The Rat Cognitive Effort Task (rCET) Training and Testing

Habituation to the operant chambers took place over two daily sessions, during which the chambers were turned on and 5-10 sucrose pellets were placed in the response holes and food magazine.

Rats were then exposed to a variant of 5-choice serial reaction training, in which they had to detect a stimulus light appearing in one of the five nosepoke holes. Rats initiated a trial by poking their nose in the illuminated food well.

Following a 2 s inter-trial interval (ITI), a 30 s light stimulus illuminated one of the five holes. The rat was required to scan the five holes and respond by poking its nose in the lit hole within 30 s, at which point a food pellet was dispensed to reward this correct response. If the rat responded before the light stimulus turned on ("premature response"), responded by poking its nose in an un-lit hole ("incorrect response"), or failed to respond ("omission") within the limited hold (LH) period, this resulted in a 5 s time-out (TO) period where the house light was extinguished and no reward pellets were offered. Once the TO was complete, the food well illuminated to signal that the next trial could be initiated. These sessions lasted 30 minutes or 100 completed trials, whichever came first. Parameters were adjusted until rats could successfully complete at least 50 correct trials with a 5 s ITI, a stimulus duration of 10 s, and a 10 s limited hold period (equivalent to Stage 3 of standard 5-CSRTT training). This was achieved within 5-10 sessions.

In subsequent sessions, subjects were trained to respond on two retractable levers at a fixed ratio 1 schedule for reward. At the beginning of the session, the food tray light illuminated and one of the response levers extended from its slot (half of the animals were presented with the left lever, the other half with the right). When the animal depressed the lever, one sugar pellet was dispensed into the food tray. The session expired after 30 minutes. In the next session, the opposite response lever was presented and the animal was similarly rewarded for pressing the lever. Training progressed when *all* animals made 50 or more presses on each lever (approximately 2-3 sessions).

Rats then progressed to the forced choice version of the rCET. Prior to testing, the response levers were permanently designated as either “easy” or “difficult”. For half of the animals, the left response lever triggered a “low-effort/low reward” (LR) trial and the right response lever triggered a “high effort/high reward” (HR) trial; this arrangement was reversed in the other half of the animals. At the beginning of the session, the food tray light illuminated to signal that the first trial was available. The animal nose-poked the food tray to initiate the trial, and the food tray light extinguished. One of the two levers extended, chosen at pseudorandom; this indicated whether the trial was “easy” or “difficult”. The animal pressed the response lever and a 5 s inter-trial interval (ITI) followed. If the animal did not press the response lever within 10 s (choice omission), or if the animal nose-poked one of the five response holes during the ITI (premature response), the house light illuminated and the trial ended; after a 5 s “time out” punishment, the house light then extinguished, the food tray light illuminated, and the next trial could be initiated. Following the ITI, one of the five response hole lights illuminated, chosen at pseudorandom. The animal then had an opportunity to respond:

*Correct response*: the animal nose-poked the response hole that was illuminated

*Incorrect response*: the animal nose-poked in one of the four response holes that was not illuminated

*Response Omission*: the animal took no action

A correct response resulted in pellet delivery at the food tray, and the food tray light illuminated to signal that the next trial was available. Incorrect responses or omissions resulted in the illumination of the house light and a 5 s “time out” punishment, wherein the next trial was not available; following the punishment, the house light extinguished and the food tray light illuminated, signalling that the next trial could be initiated. During the initial forced choice sessions, the stimulus duration (SD) for the LR and HR levers were set at 10 s, and correct detection of these stimuli resulted in 1 sugar pellet reward. The levers were matched for SD and reward magnitude until rats were able to successfully detect a 1 s SD on the HR lever with 70% accuracy (along with <20% choice omissions, <20% hard omissions, and more than 25 correct responses made on the HR lever- see Table 2.1). Following this, the levers began to diverge in difficulty and reward magnitude. The SD and reward for correct responses remained the same for the LR lever (1 s and 1 sugar pellet, respectively), but correct responses on the HR lever now resulted in 2 sugar pellets, and SD for the HR lever incrementally decreased by .1 s until reaching a final duration of .2 s. If the animal surpassed the criteria for their respective stage of training (e.g. greater than 25 correct HR trials, greater than 70% accuracy and < 20% omissions on HR trials), they progressed to the next stage with correspondingly shorter stimulus durations. Training continued until all animals reached the stage corresponding to the free-choice stimulus durations (i.e. an LR stimulus duration of 1 s and a HR stimulus duration time of 0.2 s). Rats typically required 60-80 sessions of forced choice training before moving onto the free choice version of the task.

The free choice version of the rCET is nearly identical to the forced choice training, except that both of the levers extended after the animal initiated a trial. Thus, in this version rats can choose between easy or hard trials by selecting the corresponding lever (Figure 2.3). To recap, subjects began each trial by nose-poking in the illuminated food tray, thereby extending the levers. Pressing a lever would set the trial as LR or HR, at which point the levers would retract and a 5 s inter-trial interval (ITI) would be initiated. Following this ITI, one of the five stimulus lights would be briefly illuminated, with stimulus durations of 1.0 s for LR trials and 0.2 s for HR trials. Animals then had 5 s to nosepoke within the previously illuminated response hole (correct response) for reward. Subjects were rewarded with one sugar pellet for a correct LR trial and two sugar pellets for a correct HR trial, at which point the tray light would re-illuminate to signal the opportunity to start the next trial. Animals were tested 4-6 days per week in 30-minute sessions of no fixed trial limit.

Trials went unrewarded for a number of reasons: if animals failed to make a lever response within 10 s (a choice omission); if animals nosepoked during the ITI (a premature response, a long-used behavioural measure of motor impulsivity; Robbins, 2002); if animals nosepoked in any aperture other than the illuminated one (an incorrect response); and if animals failed to nosepoke any aperture within 5 s of stimulus light illumination (a response omission). All of these unrewarded trials were associated with a 5s time-out punishment period during which the houselight was illuminated and new trials could not be initiated

and thus reward could not be earned. Following the time-out, the tray light illuminated to signal that the rat could begin the next trial.

### 2.4 Behavioural Measures

Percent choice (rather than the absolute number of choices) was used to determine preference for lever/trial type, in order to minimize the influence of variation in the number of trials completed. Percent choice was calculated as follows: (number of choices of a particular lever / total number of choices) * 100. When baseline performance on the rCET was deemed statistically stable (no effect of session for choice, accuracy, and premature responding over the last three sessions when analyzed with a repeated-measures ANOVA; see "Data Analysis" below), animals were grouped as "workers" if they choice HR for >70% of trials and as "slackers" if they chose HR for $\leq$ 70% of trials. This subdivision was based on the mean split from the original rCET paper (Cocker *et al*, 2012b), where workers and slackers were categorized based on their preference for greater than or less than the average of 70% HR trials. To maintain consistency when discussing individual differences across studies, we held the worker/slacker distinction at 70% HR trials for all experiments.

The following variables were analyzed separately for LR and HR trials: percent accuracy ((number of correct responses/ number of correct and incorrect responses made) * 100); percent response omissions ((number of trials omitted / number of correct, incorrect, and omitted trials) * 100); percent premature responses ((number of premature responses / total number of trials initiated) *

100); latency to choose between LR and HR levers (lever choice latency); latency to correctly nosepoke in the illuminated aperture (correct latency); latency to collect reward (collection latency). Failures to choose a lever at the beginning of the trial (choice omissions), number of trials initiated, and total number of trials completed (correct + incorrect + response omissions) were also analyzed.

## 2.5 Data Analysis

Any analyses exclusive to specific experiments are described in subsequent chapters. All data were analyzed in SPSS (version 24.0; SPSS/IBM, Chicago, IL, USA). Variables expressed as a percentage were arcsine transformed to minimize the effects of an artificially imposed ceiling. Data were analyzed with a two-way repeated measures ANOVA with session (three levels: baseline sessions 1-3), inactivation (two levels: vehicle or inactivation), or dose (varying levels: vehicle plus different drug doses) as within-subjects factors, and group (two levels: worker or slacker) as a between-subjects factor for all analyses. A criterion was set wherein a subject had to select a given trial type (LR or HR) at least five times in order for behavioural measures associated with that lever to be analyzed (i.e. collect latencies, correct latencies, response omissions, choice latencies, premature responding, accuracy). Given that some rats selected the HR or LR option almost exclusively, behavioral measures for the non-sampled trial type were often unavailable. Thus including choice as a within-subjects factor would remove these rats from all analyses, so instead

behavioral measures for LR or HR trials were analyzed separately. Violations of sphericity were assessed using Mauchly's test, and when violated degrees of freedom were adjusted to more conservative values using the Greenhouse-Geisser correction. Corrected degrees of freedom are shown to the nearest integer. Any main effects or interactions of significance ($p < .05$) were further analyzed via *post hoc* one-way ANOVA or paired samples t-tests with a bonferonni correction for the number of comparisons made. Any *p*-values > .05 but < .10 were reported as a statistical trend.

## 2.6 Table

| Stage | Limited Hold | Size of Hard reward | LR Stimulus duration (s) | HR Stimulus Duration (s) | Criteria to Advance |
|---|---|---|---|---|---|
| 3 | 10 | 1 | 10 | 10 | < 20 choice omissions<br>≥ 25 LR correct<br>≥ 25 HR correct |
| 4 | 5 | 1 | 5 | 5 | < 20 choice omissions<br>≥ 25 LR correct<br>≥ 25 HR correct |
| 5 | 5 | 1 | 2.5 | 2.5 | < 20 choice omissions<br>≥ 25 LR correct<br>≥ 25 HR correct |
| 6 | 5 | 1 | 1.25 | 1.25 | < 20 choice omissions<br>≥ 25 LR correct<br>≥ 25 HR correct |
| 7 | 5 | 1 | 1 | 1 | **Criteria for remaining Stages**<br>< 20% choice omissions<br>≥ 25 HR correct<br>≥ 70% HR accuracy<br>≤ 20% HR omissions |
| 8 | 5 | 2 | 1 | .9 | |
| 9 | 5 | 2 | 1 | .8 | |
| 10 | 5 | 2 | 1 | .7 | |
| 11 | 5 | 2 | 1 | .6 | |
| 12 | 5 | 2 | 1 | .5 | |
| 13 | 5 | 2 | 1 | .4 | |
| 14 | 5 | 2 | 1 | .3 | |
| 15 | 5 | 2 | 1 | .2 | |

**Table 2.1. rCET training protocol**

The animals are placed on a forced choice variant of the rCET, at the equivalent of 5-CSRTT stage 3 and are trained to the task's final stimulus durations. Following this, animals are placed on the free-choice variant of the rCET until baseline behaviour is stable. LR: low-effort/low-reward; HR: high-effort/high-reward.

## 2.7 Figures

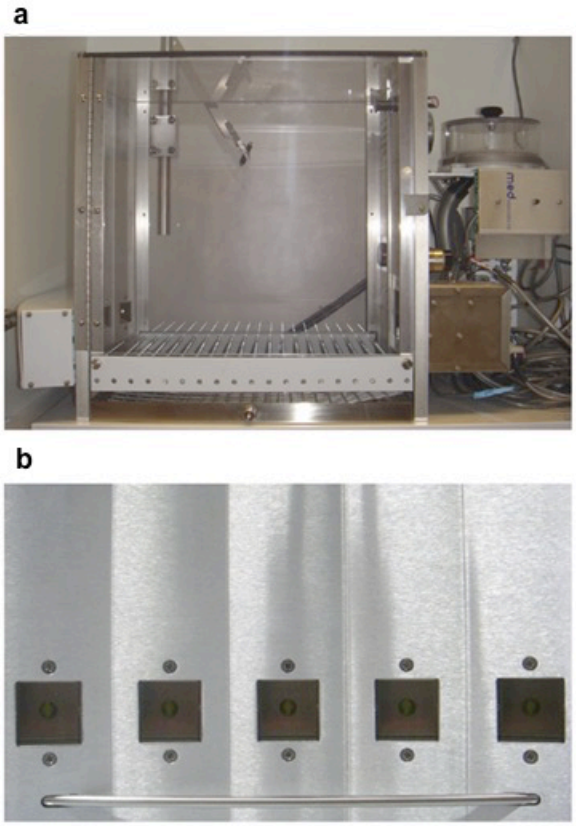


**Figure 2-1. The standard 5-hole operant chamber used for the rCET**

a) Front-facing view of operant chamber, with 5-hole stimulus array on the left and food tray on the right. b) The 5-hole stimulus array.

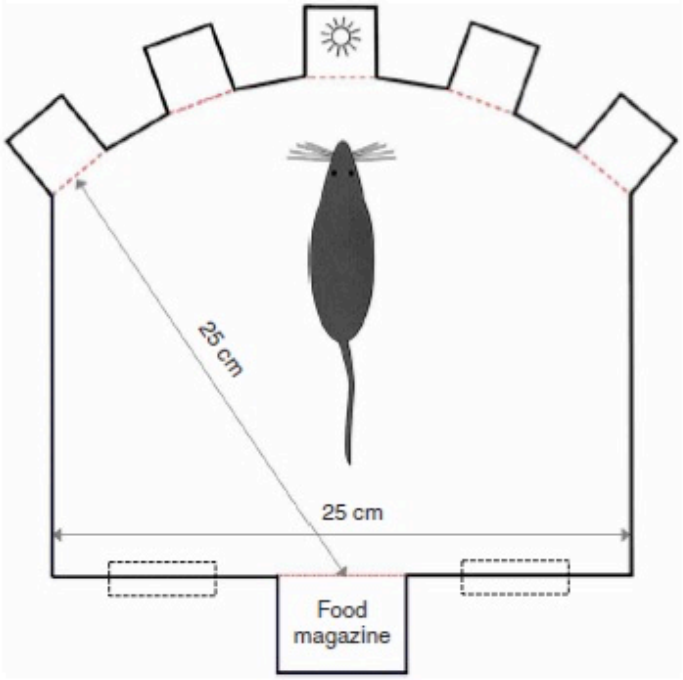


**Figure 2-2. The standard 5-hole operant chamber used for the rCET**

a) Aerial schematic of the 5-hole operant chamber, showing dimensions of the chamber, the 5-hole array, and the retractable levers on either side of the food well.

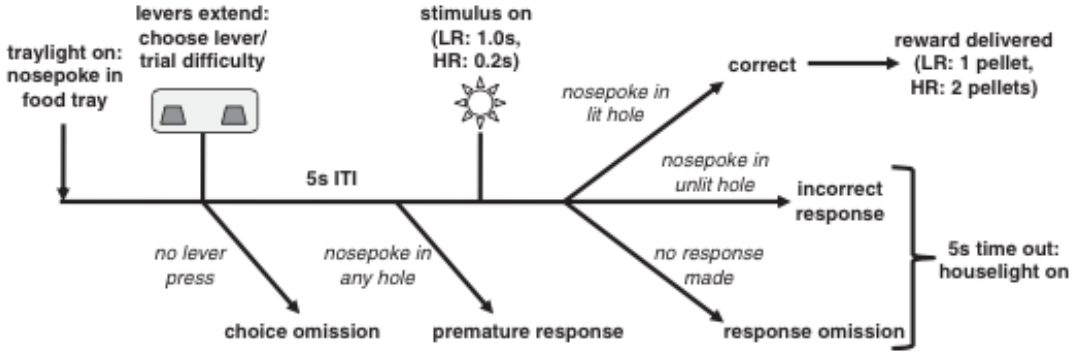


**Figure 2-3. Schematic diagram showing the trial structure of the rCET**

The task trial began with illumination of the tray light. A nose-poke response in the food tray extinguished the tray light, commencing a new trial and extending the levers. These levers were permanently designated to initiate either low-effort/LR or high-effort/HR trials. If one of the two levers was pressed, the levers retracted and a 5 s ITI would begin. Following the ITI, one of five stimulus lights would be briefly illuminated: 1.0 s for a LR trial and 0.2 s for a HR trial. A nose-poke response in the illuminated hole (i.e., correct response) led to a sugar reward-one pellet for a LR trial and two pellets for a HR trial-and the tray light would illuminate, indicating the opportunity to start the subsequent trial. A number of behaviors led to a 5 s time-out, signaled by house-light illumination: failure to make a lever response (choice omission); failure to withhold responding during the ITI (premature response); nose poke in an unlit hole following the stimulus (incorrect response); failure to make a nose-poke response following the stimulus (response omission.

# Chapter 3: Chemogenetic Silencing of Basal Forebrain Neurons Does Not Affect Cognitive Effort Allocation in a Rodent Model of Decision Making with Cognitive Effort Costs

## 3.1 Introduction

The cholinergic system is a neuromodulator that is implicated in a number of processes including arousal (Jones, 2004), learning and memory (Hasselmo, 2006), and attention (Klinkenberg *et al*, 2011). Disorders associated with aberrant cholinergic signaling, such as schizophrenia (Higley and Picciotto, 2014; Raedler *et al*, 2007) and Alzheimer's Disease (Court *et al*, 2001), are characterized by deficits in these cognitive domains, and for this reason many view the cholinergic system as a potential therapeutic target to ameliorate the cognitive impairments inherent to these disorders (Jones *et al*, 2011; Müller, 2007).

Emerging evidence suggests that the cholinergic system also contributes to the processes by which organisms evaluate choices in light of the costs and benefits associated with different candidate options. Indeed, impairments in cost/benefit decision making have been observed across Alzheimer's Disease and schizophrenia (Sevy *et al*, 2007; Sinz *et al*, 2008), but it is unclear whether cholinergic impairments contribute to these decision-making deficits. Animal models of cost/benefit decision making suggest that perturbations in acetylcholine transmission affect decision making involving delay (Mendez *et al*,

2012) and uncertainty (Mendez *et al*, 2012, 2013; Silveira *et al*, 2014) costs, and one investigation has demonstrated that muscarinic receptor agonism in the nucleus accumbens decreases rats' willingness to exert physical effort for lucrative rewards (Nunes *et al*, 2013a).

Recently, our lab has demonstrated that acetylcholine has dissociable roles in regulating attention versus the decision to engage in an attentional challenge, using the rodent Cognitive Effort Task (rCET) (Cocker *et al*, 2012b). In the rCET, rats decide whether to engage in easy or hard attentional trials, where correct detection of a 1s stimulus results in 1 sugar pellet reward, while correction detection of a difficult, 0.2s stimulus delivers 2 sugar pellets (Cocker *et al*, 2012b). The rCET is an adapted version of the 5-choice serial reaction time task (5-CSRTT), so in addition to measures of choice and accuracy, rates of premature responding are also assessed. Systemic administration of the muscarinic receptor antagonist scopolamine decreases choice of the high-effort/high-reward (HR) option across all rats without affecting attentional accuracy, while the muscarinic receptor agonist oxotremorine and the nicotine antagonist mecamylamine are without effect (Hosking *et al*, 2014c). In contrast, systemic administration of nicotine decreases choice of the HR option in a subset of rats who generally avoid the more demanding, albeit more lucrative attentional challenge. While nicotine decreases effortful choice in these "slacker" rats, it concomitantly increases their attentional accuracy on hard trials (Hosking *et al*, 2014c). Importantly, the improved accuracy in slackers following nicotine does not reflect amelioration of an attention deficit, as numerous studies have shown

that workers and slackers do not differ in attentional ability (Cocker *et al*, 2012b). Collectively, these results suggest that perturbing cholinergic signaling may impair or improve decision making with cognitive effort costs, the results of which depend on individual propensity to work or slack at baseline. Additionally, these effects on choice behaviour are separable from the established role of acetylcholine in regulating attentional processes (Himmelheber *et al*, 2000; Mcgaughy *et al*, 2002; Passetti *et al*, 2000).

Indeed, cholinergic regulation of attention versus decision making may map onto dissociable cholinergic neuron populations in the brain, such as the cortically projecting neurons of the basal forebrain (BF), versus the locally acting striatal interneurons (Bubser *et al*, 2012). Eight distinct cholinergic populations have been identified, but how any of these might regulate decision making is currently unknown (Bubser *et al*, 2012; Mesulam *et al*, 1983).

To begin to investigate this question, we took advantage of a transgenic rat line selectively expressing cre recombinase in ChAT neurons, and paired this with infusions of cre-driven Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) directed towards the BF (Witten *et al*, 2011). This procedure allows for cell-type specific insertion of DREADDs receptors into ChAT neurons of the BF, which are activated by the otherwise inert ligand clozapine N-oxide (Roth, 2016; Smith *et al*, 2016). The DREADD receptor utilized in this study is a mutated variant of the muscarinic $M_4$ receptor, which upon ligand binding leads to neuron downregulation given its linkage to inhibitory GPCR signaling pathways (Urban and Roth, 2015). Rats were injected with CNO prior to rCET

performance, to assess how downregulation of BF cholinergic neurons affects performance on-task. We also included a transgene negative group to control for potential nonspecific effects of CNO (Gomez *et al*, 2017). We also delivered a number of pharmacological challenges to assess whether transgenic rats expressing cre recombinase in ChAT neurons behave similarly to transgene negative controls.

## 3.2 Additional methods

### 3.2.1 Subjects and Baseline Choice

Subjects were 39 male Long-Evans rats bred in house. These rats were bred from breeding pairs obtained from Charles River Laboratories and the Rat Resource and Research Centre (RRRC, Columbia, MO) as part of a breeding program for transgenic rats that express cre recombinase (Cre) in neurons that contain choline acetyltranferase (ChAT; Long Evans –Tg (ChAT-Cre) 5.1 Deis, RRRC # 00658). Nineteen of these rats were positive for the transgene (TG+), while the remainder did not express the transgene (TG-). The transgenic status was included as a between-subjects variable for all analyses. One rat suffered health complications early on in training, leaving 38 males.

For this particular experiment, the mean choice of the HR option across rats was 76%. Animals were grouped as “workers” if they chose the HR option for >70% of trials (n= 29) and as “slackers” if they chose HR for ≤70% of trials (n= 9), as per previous work (Cocker et al., 2012), thereby enabling consistency

when discussing individual differences across studies.

### 3.2.2 Surgery

Animals were anesthetized with 2% isoflourane in $O_2$ and administered .1 ml of 5 mg.ml anafen and 10 ml lactated ringer subcutaneously. Rats were then secured in a stereotaxic frame (David Kopf) with the incisor bar set at -3.3. When a surgical plane of anesthesia was reached, rats were infused with AAV5-hsyn-DIO-$HM_4D_{Gi}$-mcherry (UNC Vector Core, 112 Durham, USA; titer 5.5 x $10^{12}$ gc/ml) bilaterally at two sites: Site 1: AP: -.8mm from bregma, ML: +/- 2.5 mm, DV: - 8 mm from dura; Site 2: AP: -1.5 mm, ML: + 2.5 mm, DV: -7 mm from dura. Using 32 G stainless steel injectors (Plastics One, Roanoke, VA), PE tubing (Instech) and 10 ul syringes (Hamilton), 1 ul of AAV was infused at each site a rate of 0.1 ul/minute, and injectors left in place for 10 minutes to ensure solution fully diffused from the injector tip. Animals were sutured and recovered for at least 5 days before resuming behavioural testing.

### 3.2.3 Drugs and Pharmacological Challenges

CNO was purchased from Toronto Research Chemical (North York, Ontario, Canada) and was dissolved in 5% DMSO in 0.9% saline. CNO was administered interperitoneally (I.P) 30 minutes before the start of the task. The muscarinic antagonist scopolamine hydrobromide was purchased from Tocris (Minneapolis, MN, USA), dissolved in 0.9% saline, and administered I.P right before task onset. The acetylcholinesterase inhibitor donepezil hydrochloride

monohydrate was purchased from Sigma (Oakville, Ontario, Canada), dissolved in 0.9% saline, and administered 30 minutes prior to task. All drugs were calculated as the salt and administered in a volume of 1ml/kg body weight.

### 3.2.4 Experimental Timeline

The transfection surgeries occurred while rats were being trained on the rCET. The first CNO (0, 0.3, 1.0, 3.0 mg/kg) Latin square occurred approximately 10 weeks after the final surgery, at which point their behaviour was stable. This was followed by a scopolamine Latin square (0, .03, 0.1, 0.3 mg/kg), and then by another CNO Latin square (0, 0.3, 1.0, 3.0 mg/kg) 15 weeks following the last transfection surgery. Rats then received crossover AB injections of vehicle or a high dose of CNO (10 mg/kg, approximately 20 weeks post-operation). The acetylcholinesterase inhibitor donepezil was then administered (0, 0.3, 1.0, 1.5 mg/kg). All latin squares were bookended by a one week washout period to prevent carryover effects, and stable baseline behaviour was established according to the procedures laid out in the general *Data Analysis* section in Chapter 2.

### 3.2.5 Immunohistochemistry

Rats were euthanized by transcardial perfusion of ice cold .9% saline in 1x PBS followed by 4% paraformaldehyde in 1x PBS. Rats were injected with 120 mg/kg ketamine and 15 mg/kg xylazine intraperitoneally, perfused, and brains were extracted and stored in 4% in 1x PBS paraformaldehyde for 48 hours

before being transferred to 30% sucrose solution in 1x PBS for at least 72 hours. The brains were then frozen and sliced at 35 μm on a cryostat. Sections encompassing the mPFC and BF were taken and either mounted onto gel-coated slides or stored as free-floating slices in 1x PBS.

Sections were processed for ChAT immunoreactivity. Sections were incubated in anti-ChAT primary antibody (Rabbit polyclonal, Millipore, cat # AB143, concentration used 1:200), and then secondary antibody (Goat anti Rabbit, Alexa Fluor 488conjugate, Invitrogen, cat # A11034, concentration used 1:500). Mounted slides were cover-slipped with VectaShield anti-fade Mounting Medium (Vector Laboratories, Burlingame, CA, USA). Expression of mCherry autoflourescence and ChAT was confirmed within the BF using an AxioZoom V16 microscope (Zeiss, Germany).

### 3.2.6 Data Analysis

For all analyses, transgene status was included as a between-subject factor. Any main effects of transgene status or interactions with dose and/or worker/slacker status were followed up post-hoc with a simple main effects analysis. Drug doses were compared to vehicle, and a bonferonni correction was applied to all *p* values to account for multiple comparisons. P values greater than .05 but less than .1 were reported as a statistical trend.

## 3.3 Results

### 3.3.1 Histology and Final Group Numbers

Verification of DREADDs transfection in ChAT neurons was confirmed by looking for co-expression of ChAT and mCherry flourescence. See Figure 3.1 for a representative image. hM4D(Gi) expression was primarily observed in the substantia innominata and globus pallidus, with less expression noted in the magnocellular preoptic nucleus. We did not observe expression in the diagonal band of Broca or the medial septum. We did not see expression of mCherry in 3 TG + samples, and so these rats were excluded. This left 16 TG + and 19 TG – rats for analysis. Including worker/slacker status, the final group numbers were as follows: TG + worker, n = 11; TG + slacker: n = 5; TG – worker: n = 16; TG – slackers: n = 3).

### 3.3.2 CNO Administration 10 Weeks Post Viral Transfection Surgeries

*Choice, Accuracy, Premature Responding*

Baseline behaviour has been described in detail in other chapters of this thesis, so will be briefly discussed here. As a group, rats chose the high-effort, high-reward (HR) option more than the low-effort, low-reward (LR) alternative (vehicle only- Choice: $F(1, 31) = 28.399$, $p < .001$). In keeping with their group categorization, workers chose the HR option more than slackers (vehicle only- Group: $F(1, 31) = 39.539$, $p < .001$). Rats who were transgene positive or negative did not differ in choice of HR (vehicle only- Transgene group: $F(1, 33) = 0.271$, NS). Transgene status also did not interact with lever choice or worker/slacker status (Choice x Transgene, Choice x Transgene x Group,

Transgene x Group: all *F*s < .419, NS), suggesting the respective worker/slacker groups were equivalent across transgene status. Administration of CNO had no selective effects on the HR choice of transgene positive rats (Dose x Transgene: $F(2, 56) = 1.432$, NS; Dose x Transgene x Group: $F(2, 93) = 0.51$, NS, Figure 3.2), or on HR choice in general (Dose, Dose x Group: all *F*s < 0.684, NS).

Animals were more accurate on LR versus HR trials (vehicle only- Choice: $F(1, 31) = 133.765$, $p < .001$). Workers and slackers did not differ in their performance across trials types (Choice x group: $F(1, 31) = 0.00$, NS; Group: $F(1, 31) = 0.002$, NS). Transgene positive and negative rats also did not differ in their attentional performance at baseline (Choice x Transgene, Choice x Transgene x Group, Transgene: all *F*s < 0.372, NS). CNO had no effect on accuracy across trial types (LR and HR trials- Dose, Dose x Transgene, Dose x Group, Dose x Transgene x Group: all *F*s < 2.315, NS, Figure 3.2).

There was a trending choice effect on premature responding, with rats making slightly more (3% versus 4%) premature responses on HR versus LR trials (vehicle only- Choice: $F(1,31) = 4.023$, $p = .054$). However, rates of premature responding did not differ between worker and slacker rats (Choice x Group, Group: all *F*s < .048, NS). Rats with different transgene statuses also did not differ in rates of premature responding (vehicle only- Transgene x Choice, Transgene x Choice x Group, Transgene: all *F*s < 2.135, NS). CNO had no effect on premature responding across trial types (LR and HR trials- Dose, Dose x Transgene, Dose x Group, Dose x Transgene x Group: all *F*s < 1.455, NS, Figure 3.2).

*Other Behavioural Measures*

Rats initiated approximately 130 trials in an rCET session, and choice omissions following lever presentation were generally low (~4). This did not differ between worker/slacker groups (vehicle only- Group: all *F*s < 1.764, NS), or transgene status (vehicle only- Transgene: all *F*s < 0.050, NS). Latencies to make a choice and to collect reward did not differ across trial types, worker/slacker status, or transgene status (Choice, Group, Transgene: all *F*s < 0.712, NS). Rats were faster to make a correct response on HR versus LR trials (vehicle only- Choice: $F(1, 31) = 4.973$, $p = .033$), and this did not differ between workers/slackers or transgene status (vehicle only- Transgene, Group: all *F*s < 0.460, NS). Omissions following stimulus presentation were low and did not differ between trial types (vehicle only- Choice: $F(1, 31) = 2.969$, NS). Response omissions did not differ between transgene positive or negative rats or between workers and slackers (Choice x Transgene, Choice x Group, Choice x Group x Transgene, Transgene, Group: all *F*s < 1.922, NS).

Administration of CNO increased trials initiated across all rats at the 1 mg.kg dose (Dose: $F(3, 93) = 2.816$, $p = .043$; sal versus 0.3 mg/kg: $F(1, 34) = 0.487$, NS; sal versus 1.0 mg/kg: $F(1, 34) = 6.914$, $p = .013$; sal versus 3.0 mg/kg: $F(1, 34) = 0.117$, $p = .734$), but this did not interact with worker/slacker or transgene status (Dose x Transgene, Dose x Group, Dose x Transgene x Group: all *F*s < 1.839, NS). CNO had no effect on any other measure (all *F*s < 1.358, NS) (Table 3.1).

### 3.3.3 CNO Administration 15 Weeks Post Viral Transfection Surgeries

CNO administration had no effect on choice, accuracy or premature responding across rats (Dose, Dose x Transgene, Dose x Group, Dose x Group x Transgene: all *F*s < 1.878, NS, Figure 3.3). CNO also did not affect any other rCET measure (Dose, Dose x Transgene, Dose x Group, Dose x Group x Transgene: all *F*s < 2.377, NS, Table 3.2).

### 3.3.4 High Dose (10 mg/kg) CNO Administration

We subsequently increased the dose of CNO to 10 mg/kg, as some researchers have reported only seeing behavioural effects at this higher dose (Smith *et al*, 2016). Again, CNO administration had no effect on choice, accuracy or premature responding across rats (Dose, Dose x Transgene, Dose x Group, Dose x Group x Transgene: all *F*s < 2.607, NS, Figure 3.4), although there was a trending increase in premature responding following CNO across all rats (Dose: $F(1, 31) = 4.171$, $p = .050$). The 10 mg/kg dose of CNO did not generally affect the other rCET measures (Dose, Dose x Transgene, Dose x Group, Dose x Group x Transgene: all *F*s < 2.706, NS, Table 3.3). However, there was a significant Dose x Transgene X Group ($F(1, 31) = 15.553$, $p < .001$) effect of CNO on HR collect latencies, but this was not significant following post hoc follow-up testing.

### 3.3.5 Reanalysis of CNO Data Excluding Worker/Slacker Status

There were a limited number of slacker rats in both the transgene positive and transgene negative conditions (n = 5 and n =3, respectively), thus limiting our ability to probe potential worker/slacker differences. Inclusion of worker/slacker status in the analyses reduces the degrees of freedom and thus the statistical power to detect any potential effects of CNO, and so we reanalyzed all the data, but did not include worker/slacker status as a between-subjects factor in the mixed model ANOVA. When reanalyzed in this way, CNO dose still did not interact with transgene status for any measure (Transgene x Dose: all *F*s < 2.830, NS).

### 3.3.6 Scopolamine Administration

*Choice, Accuracy, Premature Responding*

One rat did not start the task following injection of the middle scopolamine dose, and so was excluded from all analyses. In keeping with previous reports, the highest dose of scopolamine decreased choice of HR trials across all rats (Dose: $F(3, 90) = 4.464$, $p = .011$; sal versus 0.03 mg/kg: $F(1, 33) = 0.606$, NS; sal versus 0.1 mg/kg: $F(1, 33) = 4.908$, NS ; sal versus 1.0 mg/kg: $F(1, 33) = 16.594$, $p < .001$, Figure 3.5). This effect did not interact with worker/slacker designation or transgene status (Dose x Group, Dose x Transgene, Dose x Transgene x Group: all *F*s < 2.128, NS). Scopolamine administration had no effect on accuracy or premature responding (Dose, Dose x Group, Dose x Transgene, Dose x Transgene x Group: all *F*s < 1.99, NS, Figure 3.5).

*Other Behavioural Measures*

Scopolamine decreased the number of trials initiated across rats at the 0.1 and 0.3 mg/kg doses (Dose: $F(2, 66) = 19.725$, $p < .001$; sal versus 0.03 mg.kg: $F(1, 33) = 3.186$, NS; sal versus 0.1 mg/kg: $F(1, 33) = 18.888$, $p < .001$; sal versus 0.3 mg/kg: $F(1, 33) = 72.233$, $p < .001$; Dose x group, Dose x Transgene, Dose x Transgene x Group: all $F$s < 1.545, NS) and increased choice omissions at all doses (Dose: $F(3, 78) = 13.381$, $p < .001$; sal versus 0.03 mg.kg: $F(1, 33) = 9.425$, $p = .012$; sal versus 0.1 mg/kg: $F(1, 33) = 36.748$, $p < .001$; sal versus 0.3 mg/kg: $F(1, 33) = 38.069$, $p < .001$; Dose x group, Dose x Transgene, Dose x Transgene x Group: all $F$s < 0.920, NS, Table 3.4). Scopolamine sped up all rats' latencies to make a HR choice (HR trials- Dose: $F( 3, 90) = 3.5017$, $p = .018$; sal versus .3 mg/kg: $F(1, 33) = 15.50$, $p < .001$; Dose x group, Dose x Transgene, Dose x Transgene x Group: all $F$s < 1.981, NS, Table 3.4). Latencies to make a LR choice were unaffected (all $F$s < 1.96, NS). Scopolamine increased latencies to make a correct response on LR, but not HR trials, but none of the individual doses were significantly different from saline when bonferonni corrections were applied (LR trials – Dose: $F(2, 48) = 4.945$, $p = .012$; Dose x group, Dose x Transgene, Dose x Transgene x Group: all $F$s < 1.403, NS; HR trials – all $F$s < 0.62, NS). Latencies to collect reward were unaffected by scopolamine (all $F$s < 1.451, NS). At the highest dose, scopolamine increased response omissions on LR and HR trials (LR trials – Dose: $F(2, 74) = 3.983$, $p = .016$; sal versus 0.3 mg/kg: $F(1, 33) = 7.862$, $p = .024$; Dose x group, Dose x Transgene, Dose x Transgene x Group: all $F$s < 1.18, NS; HR trials- Dose: $F(2, 55) = 14.561$, $p <$

.001; sal versus 0.3 mg/kg: $F(1, 33) = 39.904$, $p < .001$; Dose x group, Dose x Transgene, Dose x Transgene x Group: all $F$s < 1.70, NS, Table 3.4).

### 3.3.7 Donepezil Administration

The acetylcholinesterase inhibitor donepezil did not affect choice or accuracy (Dose, Dose x Transgene, Dose x Group, Dose x Group x Transgene: all $F$s < 1.878, NS, Figure 3.6). There was a significant dose x group interaction for premature responding on HR trials, but this was not significant following post hoc testing (LR trials –all $F$s < 0.532, NS; HR trials – Dose x Group : $F(3, 93) = 2.838$, $p = .042$, Workers only and slackers only – all $F$s < 1.925, NS, Figure 3.6).

Donepezil increased choice omissions at the middle dose (Dose: $F(3, 93) = 4.276$, $p = .007$; sal versus 0.3 mg.kg: $F(1, 33) = 0.678$, NS; sal versus 1.0 mg/kg: $F(1, 33) = 7.986$, $p = .024$; sal versus 1.5 mg/kg: $F(1, 33) = 5.64$, NS; Dose x group, Dose x Transgene, Dose x Transgene x Group: all $F$s < 1.106, NS, Table 3.5). Latencies to make a choice, to make a correct response, and to collect reward were unaffected (Dose, Dose x group, Dose x Transgene, Dose x Transgene x Group: all $F$s < 2.561, NS, Table 3.5). Donepezil decreased trials initiated significantly in transgene negative worker rats at the highest dose (Dose x Transgene x Group: $F(2, 70) = 3.163$, $p = .043$; transgene negative only : Dose x Group: $F(2, 33) = 3.163$, $p = .056$; transgene negative workers only: $F(3, 45) = 3.513$, $p = .023$; sal versus 1.5 mg/kg: $F(1,15) = 10.388$, $p = .018$).

## 3.4 Discussion

The aim of the current investigation was to assess whether the basal forebrain contributes to the willingness to expend cognitive effort, above-and-beyond its role in regulating attentional and other cognitive processes. To achieve this, we used a recently developed line of transgenic rats expressing cre recombinase in ChAT neurons (Witten *et al*, 2011), and combined this with a cre-driven viral vector expressing a mutated muscarinic inhibitory GPCR (DREADD). This allowed us to selectively target ChAT neurons in the basal forebrain (Figure 3.1), and to downregulate these neurons via administration of clozapine-n-oxide (CNO) – an otherwise inert ligand that selectively binds to DREADDs. CNO administered at two timepoints (T= 10 weeks and T = 15 weeks) or at a very high dose (10 mg.kg) did not affect decision making or attentional ability in rats expressing the DREADDs receptor (transgene positive rats). As a group, the rats responded normally to a scopolamine challenge previously shown to selectively decrease effort invested towards larger, albeit more cognitively costly rewards (Hosking *et al*, 2014c), suggesting they were sensitive to manipulations of the cholinergic system. Although these results must be considered in light of the limitations of using DREADDs to selectively control neuronal populations, the current findings suggest that the basal forebrain does not contribute to the regulation of cognitive effort allocation.

Before discussing the implications of the null effects observed in the current study, we must consider the possibility that the technique we carried out was insufficient to target the cholinergic neurons of the basal forebrain.

According to stereological estimates, ChAT neurons account for ~5% of all BF neurons, a third synthesize GABA, and the vast majority synthesize glutamate (Gritti *et al*, 2006). We based our coordinates for viral transfection on studies investigating BF contributions to different aspects of visual attention (Botly and De Rosa, 2012; Chudasama *et al*, 2004), and which used the immunotoxin 192 IgG-saporin that targets cholinergic cells (Wiley *et al*, 1991). We delivered 2 ul of virus across two sites in each hemisphere, to ensure that we were able to transfect as many cholinergic BF neurons as possible. Indeed, we saw robust co-labelling of ChAT and mCherry, suggesting the inhibitory DREADD was localized to ChAT-expressing neurons. To our knowledge, no one has evaluated the transfection quality of $HM_4Di$ targeted towards cholinergic neurons of the NBM, but a previous study using a Cre-inducible channelrhodopsin (ChR2) viral construct found that 90% of neurons expressing ChAT also expressed EYFP tagged to ChR2 (Witten *et al*, 2011). Other studies using DREADDs generally show that approximately 2/3 of neurons express DREADDs using common AAV8-synapsin vectors in several structures (Gremel and Costa, 2013; Vazey and Aston-Jones, 2014). However, it is difficult to compare transfection rates across studies, as this will differ based on a number of factors including specific DREADD used, neuronal population targeted, serotype, and infusion protocol. Another notable limitation in the current study is that we did not carry out the in vivo or in vitro studies to demonstrate that CNO binding to the $HM4D_i$ receptor resulted in silencing of ChAT neurons. However, other studies have shown that systemic administration of CNO suppresses neuronal firing within 10

minutes, with response offset occurring at 70 minutes (Chang *et al*, 2015), and so it is likely the activity of transfected BF neurons would have been downregulated for the duration of an rCET session.

We have previously implicated the cholinergic system in the processes by which organisms allocate their cognitive resources for lucrative outcomes, by showing that systemic administration of cholinergic agents was capable of affecting decision making with cognitive effort costs (Hosking *et al*, 2014c). This prompted us to ask whether a specific cholinergic neuronal population in the brain mediated this process, and we considered the basal forebrain cholinergic neurons prime candidates. Acetylcholine synthesizing neurons are scattered throughout the BF nuclei of the medial septum-diagonal band of Broca (MS-DBB) and the nucleus basalis magnocellularis/substantia innominata (nBM/SI) (Mesulam *et al*, 1983). The cholinergic neurons of the MS-DBB and nBM/SI project predominantly to the hippocampus and neocortex, respectively, and we targeted the nBM given its ascribed role in regulating visual attention, and because we have previously implicated a number of medial prefrontal cortical regions in regulating decision making on the rCET (Hosking *et al*, 2014a, 2015a). Temporary silencing of these cholinergic neurons with CNO did not affect rats' choice of HR trials, nor did it affect attentional performance.

This latter finding is perhaps surprising considering a number of previous studies on the 5-CSRTT and related paradigms have implicated the nBM/SI in visual attention using the selective immunotoxin 192 IgG-saporin (Botly and De Rosa, 2012; Harati *et al*, 2008; Lehmann *et al*, 2003; Mcgaughy *et al*, 2002). In

one particular study, attentional impairments were most prominent in the high dose saporin group, which lesioned ChAT neurons in the nBM and the MS-DBB, while a lower dose selectively ablating ChAT neurons in the nBM produced subtler impairments in accuracy (Mcgaughy *et al*, 2002). A similar study also showed impairments in attentional accuracy at saporin doses that spared cholinergic neurons in the MS-DBB, suggesting that perturbation of cholinergic neurons in the nBM is sufficient to affect attentional processes (Harati *et al*, 2008). However, It is difficult to ascribe a purely attentional account to the deficits observed in some of these studies, as a number of cursory measures were also affected (including increased omissions, latencies, and premature responding), suggesting fundamental deficits in response control and motivation during the task (Lehmann *et al*, 2003; Mcgaughy *et al*, 2002). In contrast, other studies have failed to find attentional impairments in the 5-CSRTT or related tasks following nBM saporin lesions (Chudasama *et al*, 2004; Risbrough *et al*, 2002). Specifically, Risbrough et al., 2002 demonstrated a weak initial attentional deficit following NBM saporin lesions that quickly returned to pre-surgery levels. Similarly, Chudasama et al., (2004) found spared attentional processes in a variant of the 5-choice task, known as the Combined Attention and Memory Task, where rats have to detect a light stimulus and then recall its location after a delay. These particular studies reported increased omission rates following stimulus presentation -often considered a cursory measure of impaired attention- but primary indices of attentional processing were unaffected.

Indeed, a closer inspection of the available literature suggests that the

cholinergic neurons of the basal forebrain are not recruited in all situations taxing visual attention. Rather, these neurons appear to be play a prominent role in attention-recruiting situations that are especially difficult or novel, perhaps falling more in line with the role of the acetylcholine in the processes of cortical arousal (Teles-Grilo Ruivo *et al*, 2017). For example, in the study by Mcgaughy et al., (2002) reporting effects of NBM saporin lesions on attention, the original 5-CSRTT was modified so that incorrect trials and omissions automatically reinstated the ITI, meaning rats did not need to nosepoke the magazine tray to commence a new trial (Mcgaughy *et al*, 2002). This was done to increase the attentional demands of the task (Dalley *et al*, 2001), and is in contrast to the rCET where incorrect trials and omissions are followed by a 5s time-out and where a nosepoke is required to start the next trial. Attentional deficits following BF cholinergic neuron ablation also become pronounced in novel challenge sessions, where the parameters of the task, such ITI length or stimulus duration, change in a single session (Lehmann *et al*, 2003; Mcgaughy *et al*, 2002). In contrast, the two studies reporting null effects of saporin lesions on attention either used the standard five-choice, or used a variant of the task which reduced the number of stimulus presentations in a single session (Chudasama *et al*, 2004; Risbrough *et al*, 2002). If the BF cholinergic nuclei are particularly recruited in highly arousing situations, this may preclude their involvement in performance on the rCET. Indeed, while the rCET is an attentionally demanding task, rats receive extensive training, and the design of the task means that at trial onset rats have the opportunity to pace themselves before choosing the difficulty of the

upcoming trial. Had we instituted a rCET “challenge” session, or modified the pace of the rCET, it is possible we would have seen an effect of BF cholinergic downregulation on decision making and attention.

What also becomes clear after reviewing the literature is that a substantial number of cholinergic neurons must be depleted in order for effects on executive function to be observed. In the studies reporting no-to-minimal effects of saporin lesions, there was a 45-60 % reduction in ChAT BF neurons (Mcgaughy *et al*, 2002; Risbrough *et al*, 2002), while those reporting more substantial 5-CSRTT deficits saw almost complete ChAT BF loss, and in some cases these lesions also encroached on the MS-DBB cholinergic nuclei (Harati *et al*, 2008; Lehmann *et al*, 2003; Mcgaughy *et al*, 2002), Indeed, the study by McGaughy et al., (2002) reported a positive correlation between ChAT neurons in the nBM and accuracy on the 5-CSRTT. Collectively this work suggests that a majority of BF cholinergic nuclei must be silenced in order for behavioural effects to be observed. This may be another reason why the DREADDs technique we employed did not result in any noticeable behavioural changes. DREADDs are GPCRs that dampen, rather than eliminate, ongoing neural activity. Indeed, labs have reported that CNO suppresses neuronal activity to 60% of pre-CNO rates (Chang *et al*, 2015; Mahler *et al*, 2014). Thus it appears that DREADDs may not be the most appropriate method to investigate BF cholinergic neurons to attention and effort allocation. An alternative approach could be to make use of optogenetics, which utilizes light-gated ion channels, and which has been recently used to investigate the involvement of BF cholinergic nuclei to cue detection and vigilance in mice

(Gritton *et al*, 2016). However, a potential issue with this technique is the sparseness of BF cholinergic nuclei, making it unlikely that light would be able to penetrate brain tissue to silence enough cholinergic neurons in larger rodents.

While we chose to focus on BF cholinergic nuclei, there are a number of other cholinergic nuclei that might be responsible for regulating decision making on the rCET. Cholinergic nuclei are also found in the brainstem pedunculo-pontine and lateral dorsal tegmental nuclei, a subset of thalamic nuclei, and in the striatum, where they act as local interneurons (CINs) (Ballinger *et al*, 2016; Mesulam *et al*, 1983). Although CINs represent only a small fraction of the striatum total cell population (1–3% in rodents) they have dense and extensive axonal arborisation (Kawaguchi *et al*, 1995). These CINs would be another interesting target to pursue, given we have shown that the dorsomedial striatum regulates cognitive effort allocation (see Chapter 4). Cholinergic inputs to the dorsomedial striatum have also been detected originating from brainstem pedunculo-pontine nuclei (Dautan *et al*, 2014), thus providing another means by which cholinergic input may affect the striatal processes regulating decision making.

We subsequently carried out a number of pharmacological challenges to assess whether rats were sensitive to the effects of cholinergic drugs on the rCET. In keeping with a previous study, we demonstrated that scopolamine decreases HR choice, but does not affect ability to complete this more difficult challenge (Hosking *et al*, 2014c). We also administered the acetycholineserase inhibitor donepezil, but this did not affect decision making, attention, or

impulsivity. Notably, none of these drug effects interacted with transgene status, aside from donepezil significantly decreasing trials initiated in transgene negative workers. Overall, transgene positive rats demonstrated an identical behavioural phenotype to transgene negative rats, which is valuable to know given emerging evidence suggesting that method of transgenic development can lead to phenotypic variation. Indeed, a recent study has shown that ChAT– cre mouse lines created with the bacterial artificial chromosome (method) demonstrate significant deficits in intravenous nicotine self-administration, which are paralleled by an increase in vesicular acetylcholine transporter and choline acetyltransferase (ChAT) hippocampal expression (Chen *et al*, 2018). This was the same method used to generate the ChAT recombinase-driver rat lines (Witten *et al*, 2011), suggesting transgene status may pose as a confounding variable in some studies using these rat strains.

In conclusion, we were unable to identify a role for BF cholinergic nuclei in cognitive effort allocation and attention. We suggest that this reflects the selective recruitment of the BF cholinergic neurons in regulating arousal associated with demanding or novel conditions, which might explain the inconsistent literature implicating this neuronal population in visual attention as assessed by the 5-CSRTT and related paradigms. This work has important implications, and suggests that researchers must exercise caution when considering chemogenetic techniques to control cholinergic basal forebrain nuclei in relation to their role in mediating executive function. We also propose that cholinergic signalling in the striatum, mediated via CINs, may be another

potential candidate for regulating decision making with cognitive effort costs.

## 3.5 Tables

| | Vehicle | 0.3 mg/kg | 1.0 mg/kg | 3.0 mg/kg |
|---|---|---|---|---|
| **Choice omissions** | 4.40 ± 0.86 | 4.71 ± 0.76 | 4.11 ± 0.75 | 5.14 ± 1.01 |
| **LR choice latency** | 3.70 ± 0.25 | 3.80 ± 0.28 | 3.39 ± 0.21 | 3.63 ± 0.30 |
| **HR choice latency** | 3.67 ± 0.14 | 3.76 ± 0.14 | 3.64 ± 0.13 | 3.73 ± 0.15 |
| **LR correct latency** | 0.56 ± 0.03 | 0.58 ± 0.04 | 0.51 ± 0.02 | 0.51 ± 0.02 |
| **HR correct latency** | 0.47 ± 0.02 | 0.47 ± 0.02 | 0.47 ± 0.02 | 0.49 ± 0.02 |
| **LR collect latency** | 1.99 ± 0.11 | 1.77 ± 0.06 | 1.80 ± 0.10 | 1.91 ± 0.08 |
| **HR collect latency** | 1.84 ± 0.23 | 1.64 ± 0.06 | 1.57 ± 0.06 | 1.69 ± 0.10 |
| **LR response omissions (%)** | 7.03 ± 1.76 | 5.74 ± 1.64 | 5.00 ± 1.62 | 5.94 ± 1.34 |
| **HR response omissions (%)** | 5.98 ± 0.74 | 5.79 ± 0.66 | 4.37 ± 0.66 | 5.03 ± 0.74 |
| **Trials initiated** | 132.77 ± 3.15 | 134.17 ± 2.82 | 139.34 ± 2.58* | 133.40 ± 2.91 |

**Table 3.1. Other behavioural measures following CNO administered ten weeks post viral transfection surgery**

Data are collapsed across transgene status and worker/slacker designation.

Means are presented (+ SEM). $^{\#}p < .1$; * $p < .05$; ** $p < .001$

| | Vehicle | 0.3 mg/kg | 1.0 mg/kg | 3.0 mg/kg |
|---|---|---|---|---|
| **Choice omissions** | 3.62 ± 0.67 | 3.31 ± 0.90 | 3.77 ± 0.71 | 4.43 ± 1.02 |
| **LR choice latency** | 3.50 ± 0.24 | 3.38 ± 0.26 | 3.26 ± 0.23 | 3.62 ± 0.26 |
| **HR choice latency** | 3.60 ± 0.15 | 3.41 ± 0.12 | 3.59 ± 0.14 | 3.60 ± 0.13 |
| **LR correct latency** | 0.51 ± 0.02 | 0.50 ± 0.02 | 0.49 ± 0.02 | 0.50 ± 0.02 |
| **HR correct latency** | 0.46 ± 0.02 | 0.46 ± 0.02 | 0.46 ± 0.02 | 0.48 ± 0.02 |
| **LR collect latency** | 1.81 ± 0.08 | 1.91 ± 0.12 | 1.93 ± 0.18 | 2.00 ± 0.24 |
| **HR collect latency** | 1.55 ± 0.05 | 1.70 ± 0.15 | 1.55 ± 0.05 | 1.55 ± 0.05 |
| **LR response omissions (%)** | 5.44 ± 1.45 | 4.20 ± 1.40 | 4.89 ± 1.33 | 6.65 ± 3.03 |
| **HR response omissions (%)** | 4.83 ± 0.77 | 4.96 ± 0.74 | 4.75 ± 0.82 | 5.53 ± 0.80 |
| **Trials initiated** | 139.46 ± 3.37 | 141.57 ± 3.38 | 139.69 ± 3.32 | 138.39 ± 3.31 |

**Table 3.2. Other behavioural measures following CNO administered fifteen weeks post viral transfection surgery**

Data are collapsed across transgene status and worker/slacker designation.

Means are presented (+ SEM). $^{\#}p < .1$; * $p < .05$; ** $p < .001$

| | Vehicle | 10.0 mg/kg |
|---|---|---|
| **Choice omissions** | 5.23 ± 1.07 | 6.97 ± 1.22 |
| **LR choice latency** | 3.39 ± 0.26 | 3.52 ± 0.25 |
| **HR choice latency** | 3.55 ± 0.12 | 3.63 ± 0.14 |
| **LR correct latency** | 0.54 ± 0.03 | 0.55 ± 0.02 |
| **HR correct latency** | 0.47 ± 0.02 | 0.49 ± 0.02 |
| **LR collect latency** | 1.95 ± 0.12 | 2.00 ± 0.14 |
| **HR collect latency** | 1.64 ± 0.05 | 1.60 ± 0.05 |
| **LR response omissions (%)** | 13.42 ± 4.35 | 7.39 ± 1.37 |
| **HR response omissions (%)** | 6.16 ± 0.65 | 7.27 ± 1.14 |
| **Trials initiated** | 130.66 ± 3.02 | 127.63 ± 3.48 |

**Table 3.3. Other behavioural measures following 10 mg/kg CNO**

Data are collapsed across transgene status and worker/slacker designation.

Means are presented (+ SEM). $^{\#}p < .1$; * $p < .05$; ** $p < .001$

| | Vehicle | 0.03 mg/kg | 0.1 mg/kg | 0.3 mg/kg |
|---|---|---|---|---|
| **Choice omissions** | 3.68 ± 0.75 | 7.94 ± 1.69* | 13.65 ± 1.94** | 14.59 ± 1.82** |
| **LR choice latency** | 2.88 ± 0.25 | 3.30 ± 0.26 | 2.80 ± 0.19 | 2.57 ± 0.17 |
| **HR choice latency** | 3.61 ± 0.16 | 3.60 ± 0.15 | 3.26 ± 0.12 | 2.97 ± 0.13** |
| **LR correct latency** | 0.47 ± 0.02 | 0.48 ± 0.02 | 0.55 ± 0.04 | 0.63 ± 0.04 |
| **HR correct latency** | 0.47 ± 0.02 | 0.46 ± 0.02 | 0.46 ± 0.01 | 0.50 ± 0.03 |
| **LR collect latency** | 1.79 ± 0.07 | 1.81 ± 0.08 | 1.96 ± 0.13 | 2.54 ± 0.36 |
| **HR collect latency** | 1.54 ± 0.05 | 1.59 ± 0.06 | 1.63 ± 0.05 | 1.70 ± 0.09 |
| **Trials initiated** | 138.79 ± 3.47 | 132.56 ± 4.56 | 117.12 ± 5.10** | 89.88 ± 6.30** |

**Table 3.4. Other behavioural measures following systemic scopolamine administration**

Data are collapsed across transgene status and worker/slacker designation.

Means are presented (+ SEM). $^{\#}p < .1$; * $p < .05$; ** $p < .001$

| | Vehicle | 0.3 mg/kg | 1.0 mg/kg | 1.5 mg/kg |
|---|---|---|---|---|
| **Choice omissions** | 5.27 ± 0.89 | 5.80 ± 0.80 | 7.40 ± 1.16* | 7.30 ± 1.14 |
| **LR choice latency** | 3.51 ± 0.24 | 3.45 ± 0.33 | 3.84 ± 0.31 | 3.87 ± 0.34 |
| **HR choice latency** | 3.51 ± 0.13 | 3.47± 0.12 | 3.71 ± 0.14 | 3.72 ± 0.12 |
| **LR correct latency** | 0.50 ± 0.03 | 0.54 ± 0.03 | 0.50 ± 0.03 | 0.53 ± 0.02 |
| **HR correct latency** | 0.45 ± 0.02 | 0.47 ± 0.02 | 0.47 ± 0.02 | 0.47 ± 0.02 |
| **LR collect latency** | 3.14 ± 1.07 | 2.37 ± 0.30 | 1.88 ± 0.12 | 2.06 ± 0.19 |
| **HR collect latency** | 1.57 ± 0.06 | 1.67 ± 0.08 | 1.67 ± 0.07 | 1.76 ± 0.15 |
| **Trials initiated** | 132.76 ± 3.76 | 131.00 ± 3.78 | 121.89 ± 5.00 | 119.94 ± 5.00 |

**Table 3.5. Other behavioural measures following systemic donepezil administration**

Data are collapsed across transgene status and worker/slacker designation.

Means are presented (+ SEM). $^{\#}p < .1$; * $p < .05$; ** $p < .001$

## 3.6 Figures

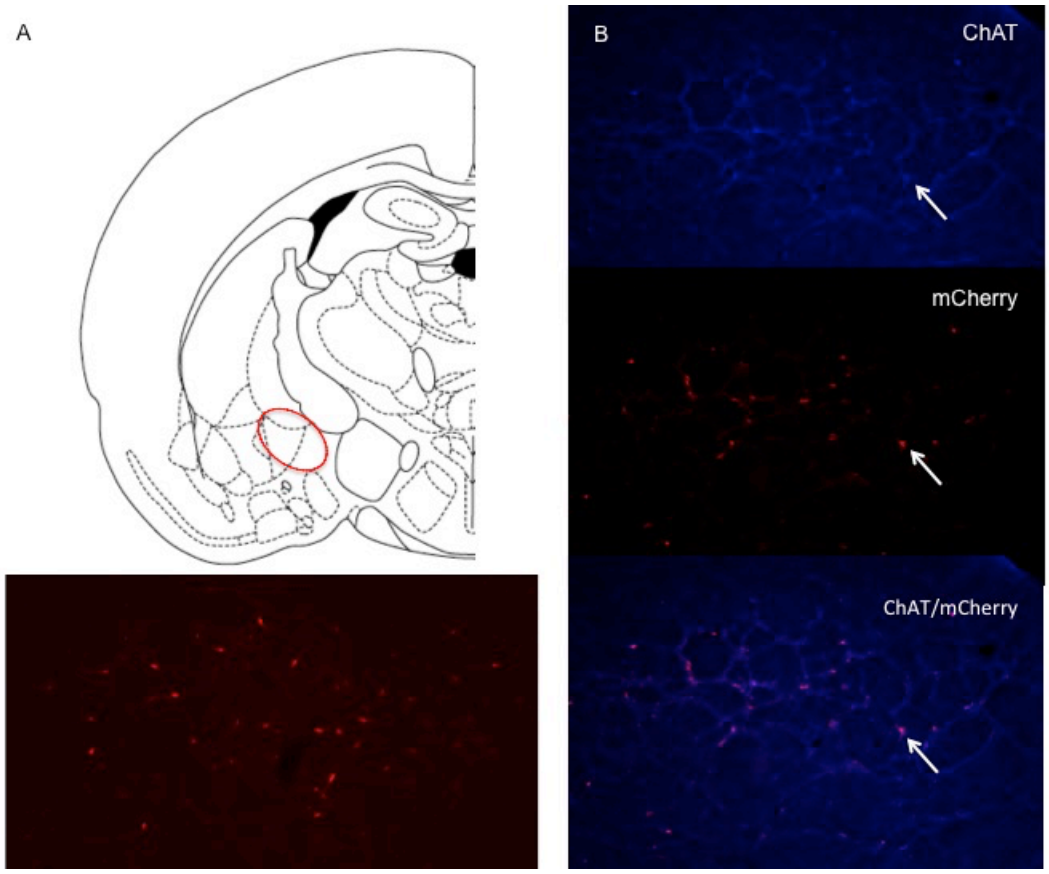


**Figure 3-1. Verification of HM4Di expression in cholinergic neurons of the basal forebrain**

A) Red circle corresponds to location of mCherry expression in image below brain atlas plate. hM4D(Gi) expression was primarily observed in the substantia innominata and globus palladus, with less expression noted in the magnocellular preoptic nucleus. We did not observe expression in the diagonal band of Broca or the medial septum. B) Cell bodies in the BF of a Tg + rat expressing ChAT (top), mCherry (middle), or co-expressing both mCherry and ChAT (bottom).

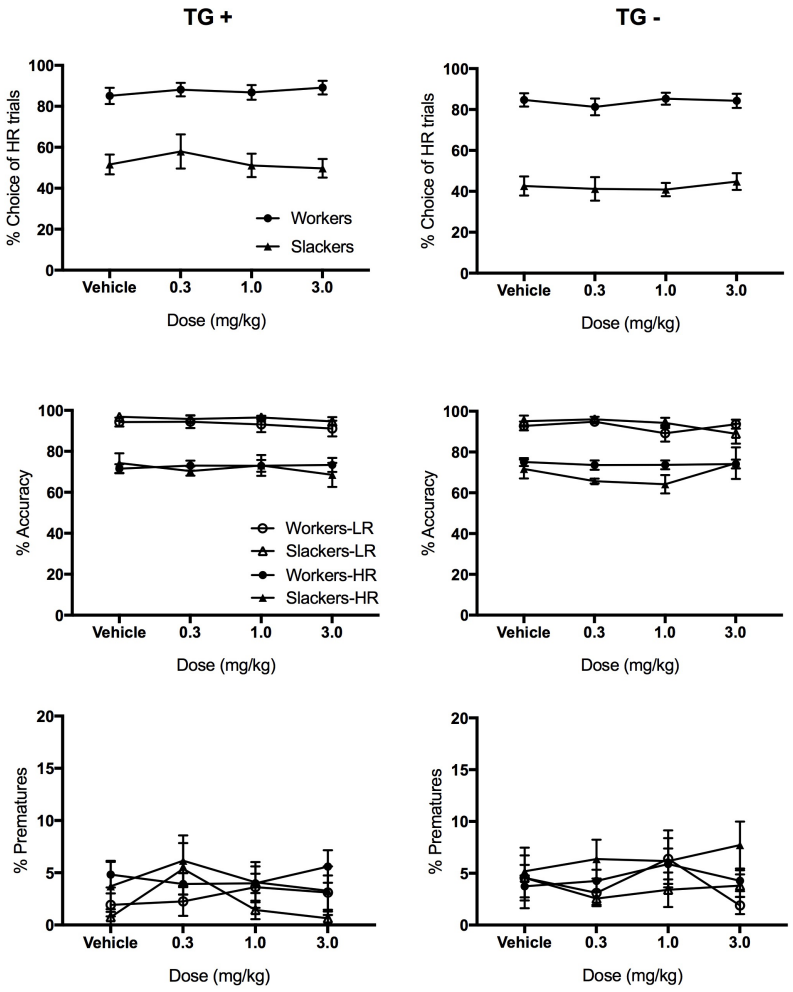


**Figure 3-2. Effects of CNO administration on choice of HR trials (%), accuracy (%), and premature responses (%) ten weeks post viral transfection surgeries**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. Data is shown separated by transgene status (transgene positive on left, transgene negative on right). CNO did not affect choice, accuracy, or premature responding.

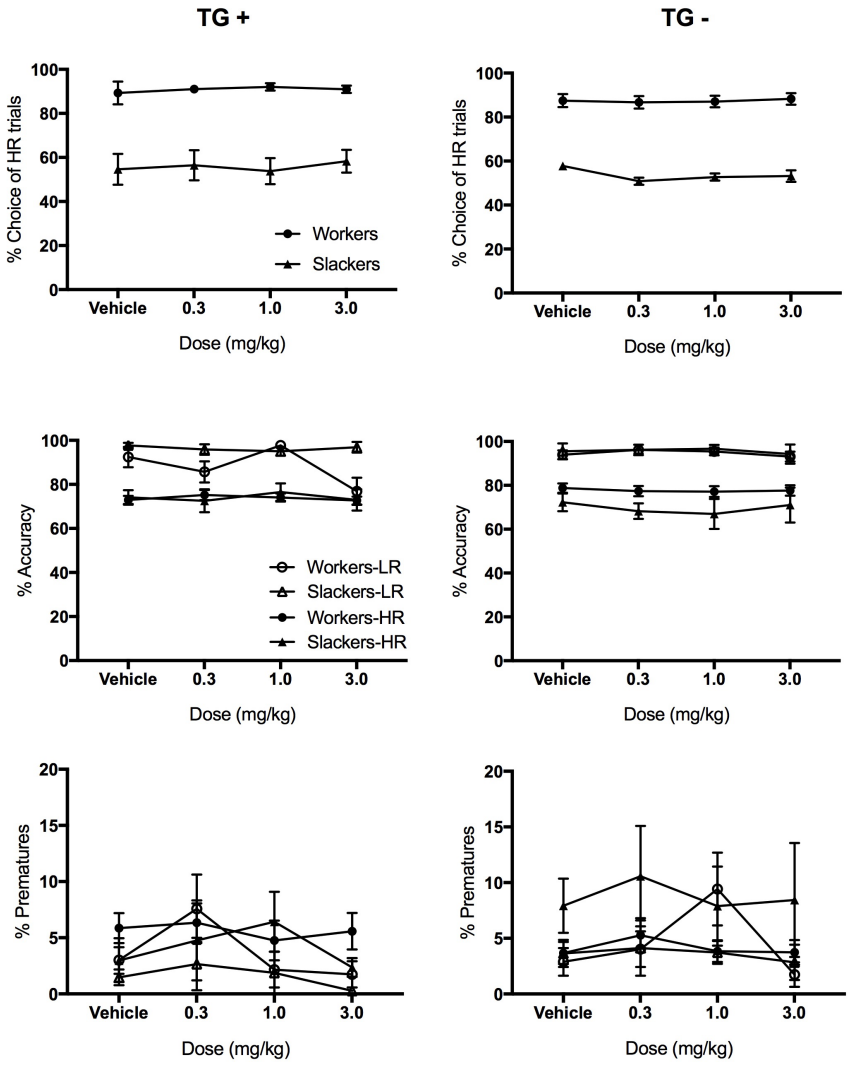


**Figure 3-3. Effects of CNO administration on choice of HR trials (%), accuracy (%), and premature responses (%) 15 weeks post viral transfection surgeries**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. Data is shown separated by transgene status (transgene postivie on left, transgene negative on right). CNO did not affect choice, accuracy, or premature responding.

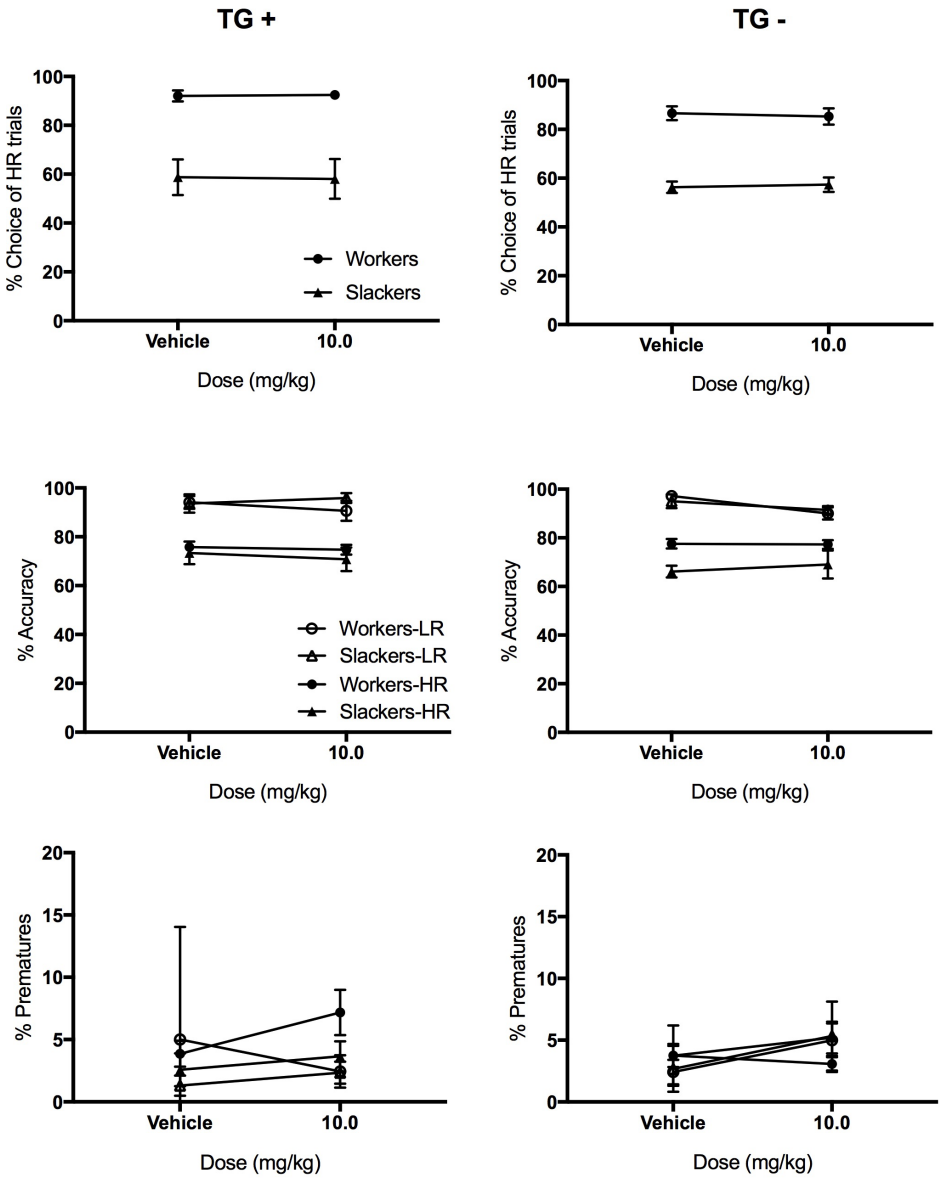


**Figure 3-4. Effects of high dose CNO administration on choice of HR trials (%), accuracy (%), and premature responses (%)**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. Data is shown separated by transgene status (transgene positive on left, transgene negative on right). CNO did not affect choice, accuracy, or premature responding.

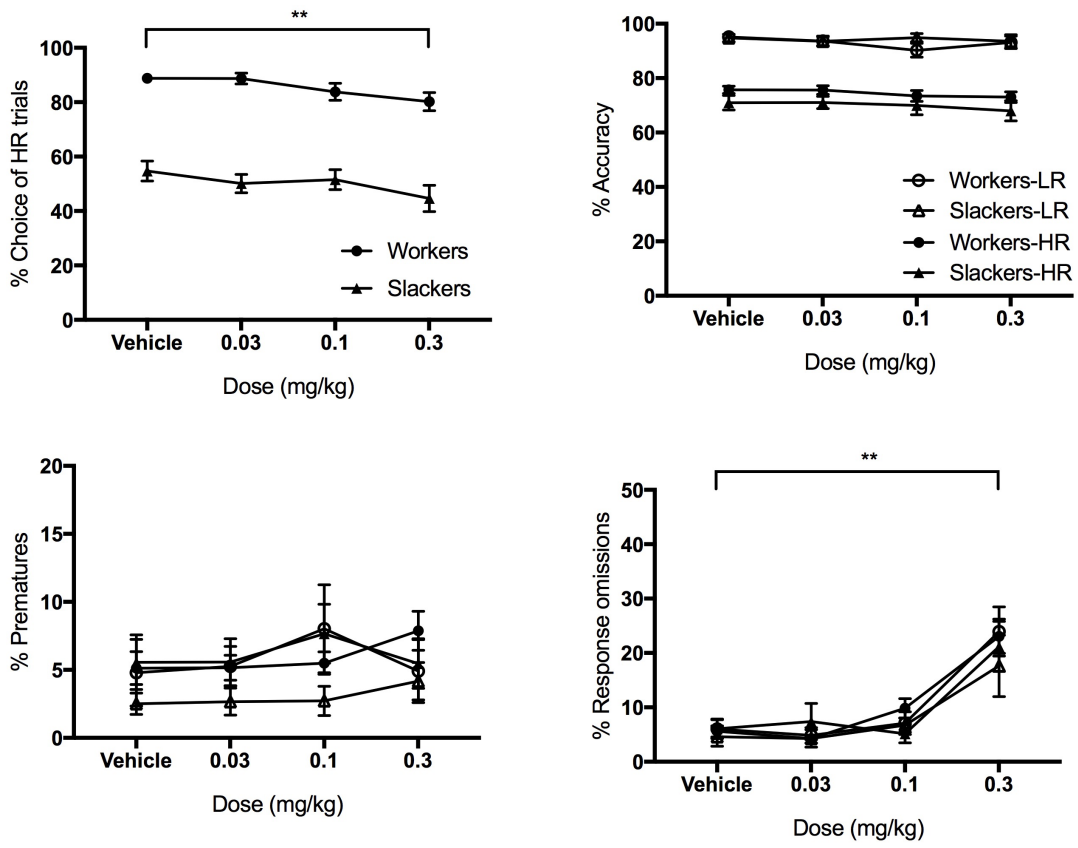


**Figure 3-5. Effects of scopolamine administration on (a) choice of HR trials (%), (b) accuracy (%), (c) premature responses (%), and (d) response omissions (%)**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. Transgene status did not interact with dose for any measure, so data is collapsed across transgene status. The highest dose of scopolamine decreased HR choice and increased response omissions across rats but did not affect accuracy or premature responding.

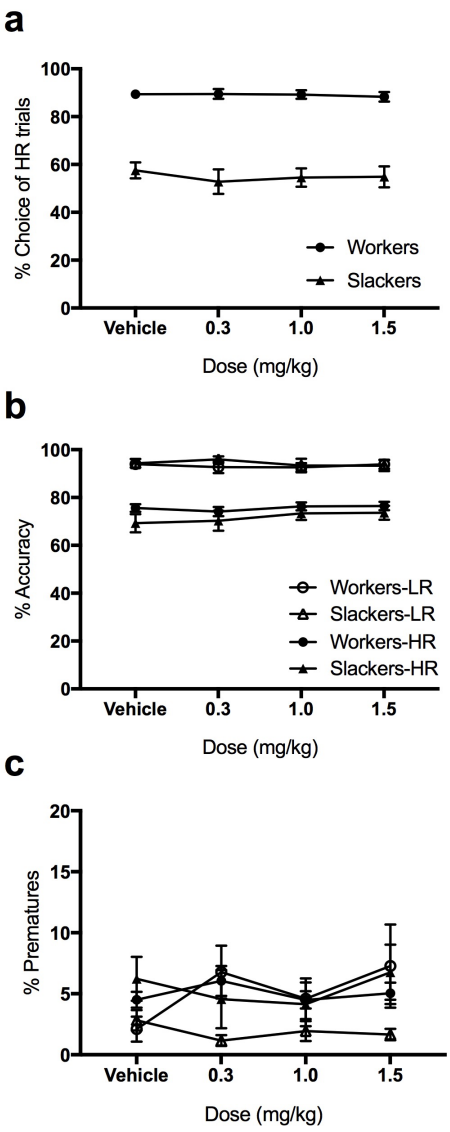


**Figure 3-6. Effects of donepezil administration on (a) choice of HR trials (%), (b) accuracy (%), and (c) premature responses (%)**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. Transgene status did not interact with dose for any measure, so data is collapsed across transgene status. Donepezil did not affect choice, accuracy, or premature responding.

# Chapter 4: Dissociable Contributions of Dorsal and Ventral Striatal Regions on a Rodent Cost/Benefit Decision-Making Task Requiring Cognitive Effort

## 4.1 Introduction

In order to successfully navigate their environment, an organism must choose between actions based on the subjective evaluation of the costs and benefits associated with the likely outcomes. The magnitude of reward, the motivational state of the animal, as well as the amount of effort required to obtain preferred goals are all variables used to guide optimal decision making. And while such processes may seem trivial when considering the routine decisions of day-to-day life, optimal cost/benefit decision making becomes crucial when choices carry with them the possibility of substantial gain or loss. Indeed, perturbations in decision making are observed in almost every mental illness (Goschke, 2014).

A substantial body of experimental data using both human and animal subjects has demonstrated that decisions involving physical effort costs are mediated by a cortico-limbic-striatal circuit generally including, but not limited to, the anterior cingulate cortex (ACC), basolateral amygdala (BLA), and ventral striatum (Winstanley and Floresco, 2016). Lesions or inactivations of these

regions in rodents decrease their willingness to traverse a barrier or press a lever repeatedly for larger reward (Floresco and Ghods-Sharifi, 2007; Ghods-Sharifi *et al*, 2009a; Ghods-Sharifi and Floresco, 2010; Hauber and Sommer, 2009; Rudebeck *et al*, 2006; Walton *et al*, 2003b), and imaging studies have found increased activity in these regions as humans or rodents engage in the evaluation of physically effortful options (Croxson *et al*, 2009; Endepols *et al*, 2010).

However, these manipulations fail to assess effort that is cognitive in nature, which is arguably more representative of the decisions humans face on a daily basis. Such cognitive effort is intrinsically costly; humans avoid cognitively demanding tasks, the subjective value of rewarding outcomes is discounted by difficult cognitive challenge, and cognitive allocation towards harder tasks can be biased by incentives (Kool *et al*, 2010a; Westbrook *et al*, 2013). While a single operational definition of cognitive effort remains elusive, recent proposals suggest cognitive effort, and its allocation, should be considered in an economic framework (Botvinick and Braver, 2015; Westbrook and Braver, 2015). Executive processes constitute a limited resource, and as such are valuable because they promote behaviour in pursuit of desired goals. Viewed in this way, effort investment towards any particular goal precludes allocation to other options, thus incurring opportunity costs. Under such a framework, studies in humans have employed standard choice experiments and demonstrated that as cognitive demands and incentives increase, the subjective value of a given option decreases and increases, respectively (Chong *et al*, 2017; Dixon and Christoff,

2012; Kool *et al*, 2010a; Westbrook *et al*, 2013). Notably, this has been observed in tasks probing working memory, cognitive flexibility, response inhibition, and attention (Kool et al, 2010; Westbrook et al, 2013, Dixon and Christoff, 2012; Reddy et al, 2015, Chong et al, 2017). Cognitive effort is thus an intrinsically valuable resource, appropriate deployment of which can maximize gains, but which is also subjectively and objectively costly. Whether cognitive effort, and its representation and regulation, should be conceptualized as unitary, pan-domain entities, or domain-specific processes, remains to be determined. Indeed, the mechanisms underlying the recruitment and allocation of cognitive effort in any aspect of cognition have yet to be firmly established.

To begin to address this discrepancy, we developed a rodent cognitive effort task (rCET), based on the 5-choice serial reaction time task, in which greater visuospatial attention is required to successfully obtain larger amounts of sucrose reward (Cocker *et al*, 2012b). As in human choice experiments manipulating cognitive effort costs, rats can decide at trial outset whether to detect a lengthy (1s) stimulus across five possible locations for one sugar pellet reward, or to earn two sugar pellets by detecting a brief (.2s) stimulus, which is much more difficult to identify (as reflected by lower task accuracy (ie, performance). Similar to animal physical-effort discounting tasks, decision making on the rCET is sensitive to inactivations of the ACC and BLA, but in the case of the BLA, is dependent on subjects' subjective preference for the harder option at baseline ("workers" vs "slackers"; (Hosking *et al*, 2014a)). Additionally, and unlike the physical effort literature, inactivations of the prelimbic and

infralimbic regions of medial prefrontal cortex (mPFC) decrease willingness to expend cognitive effort, suggesting the two forms of decision making share interrelated-yet-distinct neurobiological mechanisms (Hosking *et al*, 2015a; Walton *et al*, 2002, 2003b).

These discrepancies may likewise imply that the role played by striatal subregions may differ with respect to decisions involving the evaluation of cognitive versus physical effort. The ventral striatum, or nucleus accumbens (NAc), is histochemically and connectively segregated into a "core" and "shell" region, with previous work establishing a role for the core, but not shell, in decision making based on physical effort costs (Ghods-Sharifi and Floresco, 2010; Zahm, 2000). The NAc receives topographic excitatory inputs from the mPFC and BLA, and individual accumbal neurons receive converging inputs from various corticolimbic sites (Britt *et al*, 2012; Brog *et al*, 1993; Floresco *et al*, 2001; Mcdonald, 1991; Reynolds and Zahm, 2005). This organization allows the NAc to integrate signals from multiple regions, such as the effort and net value signals observed in the ACC, as well as valence signals observed from single unit recordings of the BLA (Belova *et al*, 2007; Cowen *et al*, 2012; Croxson *et al*, 2009; Jenison *et al*, 2011; Kennerley *et al*, 2009, 2011; Shidara and Richmond, 2002). For this reason the NAc has been deemed a "limbic-motor" interface, whereby these regions can influence motor output via accumbal projections to motor effector sites in the basal ganglia (Mogenson *et al*, 1980). Although imaging studies suggest ventral striatal activity is inversely related to cognitive demand (Botvinick *et al*, 2009), the exact contribution of the nucleus accumbens

to decisions based on cognitive effort costs remains unknown.

Moreover, effort tasks have mainly focused on the ventral, but not dorsal striatum, even though this latter region has been implicated in other forms of decision making and goal-directed behaviour (Balleine *et al*, 2007; Ferguson *et al*, 2013; Friedman *et al*, 2015). The dorsomedial portion of the striatum (DMS) receives efferent topographic projections from the medial prefrontal cortex, ACC and BLA (Mailly *et al*, 2013; Mcdonald, 1991; Sesack *et al*, 1989), and is thus ideally situated to integrate decision-related signals that can then bias action output. Indeed, the dorsal-ventral striatal boundary is an arbitrary one, with some proposing a ventromedial-to-dorsolateral striatal gradient based on functional organization (Voorn *et al*, 2004). It is possible that different corticostriatal "loops" processing facets of task performance (i.e., choice, attention, response inhibition) are integrated in the striatum, the resulting output of which can then bias motor output via palladal-thalamic-cortical pathways (Chudasama and Robbins, 2006; Haber and Behrens, 2014; Zahm and Brog, 1992). However, the contributions of striatal regions to cognitive effort allocation-versus-execution have not been investigated.

The goal of the current investigation was therefore to characterize the role of the DMS and NAc core on rCET performance, using pharmacological inactivation methods comparable to that used in investigations of the role played by these regions in evaluating physical effort costs (Ghods-Sharifi and Floresco, 2010).

## 4.2 Additional Methods

### 4.2.1 Subjects and Baseline Choice Profile

Subjects were 32 male Long Evans rats. When baseline performance on the rCET was deemed statistically stable, the mean choice of the HR option was 70%. Animals were grouped as “workers” if they chose the HR option for >70% of trials ($n$ = 15) and as “slackers” if they chose HR for $\leq$ 70% of trials ($n$ = 15), as per previous work (Cocker *et al*, 2012b), thereby enabling consistency when discussing individual differences across studies. Two rats failed to achieve criterion during rCET training and were thus excluded and not categorized.

### 4.2.2 Surgery to Implant Guide Cannulae

When baseline performance was deemed statistically stable, animals were implanted with 22-gauge stainless steel guide cannulae (Plastics One) bilaterally into the dorsomedial striatum (DMS) using standard stereotaxic techniques. Animals were anesthetized with 2% isoflurane in $O_2$ and cannulae implanted at the following coordinates: AP = +1.5 mm, ML = ±1.9 mm from bregma, DV = −3.8 mm from dura (Paxinos and Watson, 1998). Guide cannulae were fixed to the skull via 3-4 stainless steel screws and dental acrylic, and obdurators with dustcaps were inserted which ended flush with the end of the cannulae. Animals were given at least 1 week of recovery in their home cages before subsequent testing. Three animals were excluded due to poor recovery.

### 4.2.3 Microinfusion Procedure

Following recovery, animals performed 10 free-choice sessions, after which all individuals displayed stable behavior. Animals were then habituated to the microinfusion process with 2 mock infusions, during which 30-gauge injectors with tips extending 1 mm beyond the guide cannulae were inserted for 2 min but no infusion was performed, followed by a behavioural testing session. Infusions adhered to a 3-day cycle starting with a baseline session, followed by a drug or saline injection session, and then by a non-testing day. The DMS was inactivated by a mixture of the $GABA_B$ agonist baclofen and the $GABA_A$ agonist muscimol (Sigma–Aldrich), prepared separately at 0.5 µg/µL in saline, and mixed together in equal volumes to form a 0.25 µg/µL solution. 0.5 µL per hemisphere injections of saline or baclofen/muscimol (i.e. 0.125 µg of drug per hemisphere) were administered bilaterally at a rate of 0.4 µL/min, and injectors were left in place for an additional minute to allow diffusion. Pilot infusions indicated that this dosing regimen caused severe behavioural disruptions when infused into the nucleus accumbens core, and so the protocol was adjusted so that baclofen and muscimol were prepared separately at 0.25 µg/µL in saline, and mixed together in equal volumes to form a 0.125 µg/µL solution. 0.3 µL per hemisphere injections of saline or baclofen/muscimol (i.e. 0.0375 µg of drug per hemisphere) were administered into the nucleus accumbens core (NAcC) bilaterally at a rate of 0.4 µL/min, and injectors were left in place for an additional minute to allow diffusion. The NAcC was targeted using the same guide cannulae, but with injectors extending 3 mm beyond the cannuale tips.

Once the microinfusions were completed, injectors were removed, obdurators replaced, and animals were returned to their home cages for 10 min before being placed in the operant chambers and performing the rCET. Animals underwent 4 infusion sessions in total: On the first infusion day, half of the rats received saline infusions to the DMS (via injectors with +1 mm tips) whereas the other half received baclofen/ muscimol to the DMS; these administrations were reversed on the second infusion day, allowing for a within-subjects comparison. On the third infusion day, half of the rats received saline infusions to the NAcC (via injectors with +3 mm tips) whereas the other half received baclofen/muscimol; and again these administrations were reversed on the fourth infusion day. Animals were given a minimum of 1 week drug-free testing between DMS and NAcC inactivations, to minimize any carryover effects. DMS inactivation caused severe behavioral disruption in 1 animal and thus this rat was removed from analyses. Thus data from *n* = 15 workers and *n* = 11 slackers were included for behavioural analysis.

### 4.2.4 Histology

Following completion of all behavioral testing, animals were anesthetized with isoflurane and sacrificed by carbon dioxide exposure. Brains were removed and fixed in 4% formaldehyde for at least 24 h, transferred to a 30% sucrose solution, and then frozen and cut via cryostat into 40-μm coronal sections. These sections were stained with cresyl violet for visualization, and the projected locations of the injector tips protruding from the guide cannulae were mapped

onto standard sections from Paxinos and Watson (1998).

### 4.2.5 Data Analysis

Data were analyzed with a two-way repeated measures ANOVA with session (three levels: baseline sessions 1-3) or inactivation (two levels: vehicle or inactivation) as within-subjects factors, and group (two levels: worker or slacker) as a between-subjects factor for all analyses. Groups proved stable across the experiment: at rCET baseline and all saline infusions, workers chose a significantly greater percentage of HR trials than slackers (Group: all Fs > 22.553, $p < .001$). Given the behavioural disruptions observed following NAcC inactivation, choice data was only included from rats that made more than 10 choices.

## 4.3 Results

### 4.3.1 Cannula Placements

The locations of all acceptable placements are depicted in Figure 4.1. All DMS placements were acceptable, but one rat was excluded from the NAcC analysis due to inaccurate placement in one hemisphere, leaving a total of 26 ($n$ = 15 workers; $n$ = 11 slackers) and 25 ($n$ = 15 workers; $n$ = 10 slackers) rats for the DMS and NAcC analyses, respectively.

### 4.3.2 Dorsomedial Striatum Inactivation

*Choice, Accuracy, and Premature Responses.*

Baseline performance on the rCET has been discussed in detail elsewhere, and so will be only briefly discussed here. Animals chose the high-effort/high-reward (HR) option more than the low-effort/low-reward (LR) option when the dorsomedial striatum (DMS) was infused with saline (choice: $F(1, 24) = 22.967$, $p < .001$). In keeping with their group categorization, workers chose HR trials significantly more than slackers (saline only- group: $F(1, 24) = 24.910$, $p < .001$). Inactivation of the DMS decreased all animals' choice of HR trials (inactivation: $F(1, 24) = 9.538$, $p = .005$; inactivation x group: $F(1, 24) = 0.169$, NS; Figure 4.2).

As shown previously, animals were more accurate on LR versus HR trials (saline only- choice: $F(1, 21) = 135.561$, $p < .001$) and attentional performance did not differ between groups (saline only- choice x group: $F(1, 21) = 1.934$, NS; group: $F(1, 21) = .028$, NS), suggesting that subjects' choice of HR trials was not solely driven by their ability to complete these trials. Inactivation of the DMS decreased LR accuracy to a greater degree in worker rats, as revealed by a significant inactivation x group interaction (LR trials- inactivation: $F(1, 21) = 39.358$, $p < .001$; inactivation x group: $F(1, 21) = 4.549$, $p = .045$). However, subsequent post-hoc tests revealed that slackers also experienced attentional deficits on LR trials (Workers-LR: $F(1, 11) = 30.104$, $p < .001$; Slackers-LR: $F(1, 10) = 10.952$, $p = .008$). Inactivation of the DMS decreased HR accuracy to a similar extent across all rats (HR trials- inactivation: $F(1, 23) = 15.30$, $p = .001$; inactivation x group: $F(1, 23) = 2.402$, $p = .135$; Figure 4.2).

Premature responding was higher on HR relative to LR trials when the DMS was infused with saline (saline only- choice: $F(1, 21) = 10.063$, $p = .005$). As per previous cohorts, rates of premature responding did not differ between groups, again indicating that individuals' choice preferences were not driven by differences in motor impulsivity (saline only- choice x group/group: all $F$s < .544, NS). Inactivating the DMS significantly increased levels of premature responding on LR trials across subjects (LR trials- inactivation: $F(1, 22) = 65.915$, $p < .001$; inactivation x group: $F(1, 22) = .506$, NS). Although both groups exhibited increased impulsive responding on HR trials, this effect was again more pronounced in worker rats (HR trials- inactivation: $F(1, 23) = 52.265$, $p < .001$; inactivation x group: $F(1, 23) = 4.950$, $p = .036$; Workers-HR: $F(1, 14) = 46.918$, $p < .001$; Slackers-HR: $F(1, 9) = 14.837$, $p = .004$; Figure 4.2).

*Other behavioural measures.*

Rats initiated about 140 trials and made few (<5) choice omissions when the DMS was infused with saline, regardless of worker/slacker distinction (saline only- all $F$s < .147, NS; Table 4.1). Under normal conditions latencies to make a choice and collect reward were similar across levers and did not differ between groups (saline only- choice/ choice x group/ group: all $F$s < 2.983, NS). While workers and slackers did not differ in their latencies to make a correct response, rats generally were quicker to make responses for HR trials, perhaps unsurprisingly given the shorter stimulus duration (saline only- choice: $F(1, 20) = 13.282$, $p = .002$; choice x group / group: all $F$s < 2.204, NS). When infused with

saline, response omissions were equivalent for LR and HR trials across groups (choice/ choice x group / group: all *F*s < 2.789, NS). In line with the impairments in accuracy, inactivation of the DMS increased response omissions for both trial types, suggesting all rats were impaired in allocating attention to the five-hole array (LR trials- inactivation: $F(1, 22) = 9.99$, $p = .005$; inactivation x group: $F(1, 22) = 4.187$, $p = .053$; HR trials- inactivation: $F(1, 23) = 12.944$, $p = .002$; inactivation x group: $F(1, 23) = 0.686$, NS; Figure 4.2). This increase in response omissions does not seem indicative of general motor impairments, given choice omissions were unchanged, and the trending decline in trials initiated was modest following DMS inactivation (choice omissions- inactivation/ inactivation x group: all *F*s < 0.663, NS; trials initiated- inactivation: $F(1, 24) = 4.065$, $p = .055$; inactivation x group: $F(1, 24) = 2.466$, NS; Table 4.1). While trials completed did decline following DMS inactivation (inactivation: $F(1, 24) = 54.211$, $p < .001$), this is likely due to the pronounced increase in premature responding observed for LR and HR trials (see above). Additionally, choice latencies actually sped up following inactivation of the DMS (LR trials- inactivation: $F(1, 23) = 5.135$, $p = .033$; inactivation x group: $F(1, 23) = 1.139$ NS; HR trials- inactivation: $F(1, 22) = 9.564$, $p = .005$; inactivation x group: $F(1, 23) = 1.331$, NS). And while both groups took longer to make a correct response on LR and HR trials following DMS inactivation, latencies to collect reward for either trial type were unaffected, suggesting the inactivation did not affect motivation for sucrose reward (correct latency: LR trials- inactivation: $F(1, 21) = 6.164$, $p = .022$; inactivation x group: $F(1, 21) = 0.32$, NS; HR trials- inactivation: $F(1, 23) = 5.821$, $p = .024$; inactivation

x group: $F(1, 23) = 1.338$, NS; Collect latency: LR and HR trials – inactivation/ inactivation x group: all $F$s < 2.157, NS; Table 4.1).

### 4.3.3 Nucleus Accumbens Core Inactivation

In stark contrast to DMS inactivations, infusions of baclofen/muscimol into the nucleus accumbens rendered most rats incapable of performing the rCET. Both workers and slackers showed a drastic decline in trials initiated, only starting about 33% of trials relative to infusion of saline in the NAcC (Figure 4.3- inactivation: $F(1, 23) = 79.924$, $p < .001$; inactivation x group: $F(1, 23) = 0.827$, NS). Indeed, the high rate of response omissions for both trials types across groups (LR trials- inactivation: $F(1, 21) = 31.704$, $p < .001$; inactivation x group: $F(1, 21) = 1.049$, NS; HR trials- inactivation: $F(1, 19) = 19.043$, $p < .001$; inactivation x group: $F(1, 19) = 0.003$, NS) limits a meaningful analysis of choice patterns, attention, and impulsivity (Figure 4.3). Among rats that made at least ten choices ($n = 22$), inactivation of the NAcC produced a trending decline in choice of the HR option (inactivation: $F(1, 20) = 3.109$, $p = .093$; inactivation x group: $F(1, 20) = 0.088$, NS) (Figure 4.3). Visual inspection of the data also suggests this was accompanied by impairments in attentional accuracy, although these effects failed to reach significance (LR trials- inactivation: $F(1, 6) = 2.548$, NS; inactivation x group: $F(1, 6) = 0.001$, NS; HR trials- inactivation: $F(1, 12) = 0.949$, NS; inactivation x group: $F(1, 12) = 0.158$, NS), likely owing to the few number of cases analyzed for LR and HR trials (see table 4.2). Inactivation of the NAcC appeared to decrease premature responding, but only a trending decline

was observed for LR trials across groups (LR trials- inactivation: $F(1, 14) = 3.881$, $p = .069$; inactivation x group: $F(1, 14) = 0.041$, NS; HR trials- inactivation: $F(1, 15) = 0.778$, NS; inactivation x group: $F(1, 15) = 0.479$, NS) (Table 4.2). This decline in premature responding may reflect general motor impairments, but it is interesting to note that rats were quicker to initiate HR trials (LR trials- inactivation: $F(1, 14) = 0.008$, NS; inactivation x group: $F(1, 14) = 0.117$, NS; HR trials- inactivation: $F(1, 15) = 6.623$, $p = .021$; inactivation x group: $F(1, 15) = 0.005$, NS), and generally latencies to make a correct response and collect reward were unaffected following NAcC inactivation (all $F$s < 3.284, NS) (Table 4.2). Lastly, inactivation of the NAcC did not affect choice omissions (inactivation: $F(1, 23) = 2.320$, NS; inactivation x group: $F(1, 23) = 0.166$, NS).

## 4.4 Discussion

Here we show that temporarily silencing dorsal and ventral regions of the striatum produce dissociable patterns of effects on a complex decision-making task requiring the evaluation and application of cognitive effort. In line with recent theories of cognitive effort, this study viewed its allocation as an economic choice, in which larger rewards were discounted by the cognitive effort costs associated with increased attentional demand (Westbrook and Braver, 2015). Inactivation of the dorsomedial striatum (DMS) decreased all animals' choice of the high effort/high reward (HR) option, while concomitantly impairing accuracy, increasing premature responding, and increasing omission rates. This

behavioural profile mimics lesions of the dorsomedial striatum on the standard 5-CSRTT (Rogers *et al*, 2001), and as suggested below, these deficits may arise not only from attentional impairments per se, but more generally from an inability to organize both attentional and response control processes necessary for optimal task performance. With these processes impaired, rats appeared to shift their choice towards a low cost strategy. In contrast, inactivations of the nucleus accumbens core (NAcC) region of the ventral striatum resulted in profound behavioural impairments that rendered rats incapable of successfully engaging in the task. The current investigation helps to delineate the cortico-striatal-limbic circuits guiding decision making based on cognitive effort costs, albeit restricted to the domain of attentional demands. As noted earlier, cognitive effort discounting has also been reported in the domains of working memory, response inhibition, and cognitive flexibility (Dixon and Christoff, 2012; Kool *et al*, 2010; Reddy *et al*, 2015; Westbrook *et al*, 2013). Future studies can now address whether similar circuits are involved in the allocation of cognitive effort across these executive functions.

It is possible that DMS inactivation impaired subjects' ability to discriminate LR from HR trials, such that the increased choice of LR trials reflects an indiscriminate sampling of the two levers, rather than a deliberate selection of the easier option. If so, then selection of the HR option should approximate 50% for both workers and slackers after DMS inactivation, yet this is clearly not the case. Additionally, temporary inhibition of direct pathway striatal neurons does not impair performance in a simple magnitude discrimination task, and likewise

inactivation of the ventral striatum does not impair selection of lever options associated with larger sucrose reward (Ferguson *et al*, 2013; Ghods-Sharifi and Floresco, 2010). Thus, there is little evidence to suggest that subjects were unaware of the specific outcomes associated with the different levers following DMS inactivation. Although this manipulation impaired accuracy on HR trials, choice of the LR option cannot be considered “advantageous”, as accuracy was also impaired on these easier trials.

Alternatively, increased preference for the LR option, along with impaired accuracy, could reflect decreased motivation for sucrose reward. Indeed, prefeeding produces a similar behavioural profile as that observed following DMS inactivation, including a shift towards LR, impaired accuracy, and decreased completed trials (Cocker *et al*, 2012b). Again this explanation seems unlikely, as the decline in trials initiated was modest following inactivation, and latencies to collect reward - considered indirect measures of motivation for sucrose reward in the standard 5-CSRTT (Robbins, 2002) - were otherwise unaffected. Lesions to the DMS also do not affect progressive ratio performance for sucrose, and do not affect the amount of sucrose eaten during a consumption test (Eagle *et al*, 1999; Rogers *et al*, 2001). Although the striatum plays an integral role in motor control, the task deficits observed here are unlikely to reflect general impairments in motor ability given the robust number of trials completed, faster response latencies, and increased premature responding seen after DMS inactivations.

### 4.4.1 Dorsomedial Striatum Contributions to Executive Control and

**Decision Making**

Following inactivation of the DMS, rats displayed impaired accuracy and an increase in premature responding (Table 4.3), which together suggests that the striatum is necessary for the integration of cognitive and motor signals underlying the executive control required for optimal task performance. Subjects were unable to adequately time and organize their behaviour during a trial, such that they were quicker to make a choice, but were unable to wait for the light stimulus, or nosepoke at the appropriate (illuminated) aperture (Table 4.3). This account of the DMS deficit is in accordance with various theories of basal ganglia function, which suggest that the direct and indirect pathways are involved in context-appropriate action selection, sequence programming, as well as the initiation and termination of motor output (Macpherson *et al*, 2014; Schroll and Hamker, 2013).

Our findings replicate previous work with the standard 5-CSRTT, in which lesions of the medial striatum produced long-lasting attentional impairments as well as impairments in response inhibition (Rogers *et al*, 2001), and accord with more recent pharmacological evidence suggesting that glutamatergic, dopaminergic and serotonergic signaling in this region regulates attention and response control (Agnoli *et al*, 2012; Agnoli and Carli, 2011, 2012). As noted above, this contrasts with lesions of the lateral striatum, which render rats incapable of engaging with the task (Rogers *et al*, 2001). Similar deficits are also observed following disconnection of the medial prefrontal cortex-dorsomedial striatal pathway, suggesting that a mPFC-DMS projection regulates aspects of

visual attention and response control (Christakou *et al*, 2001). Indeed deficits in attention and impulsivity induced by altered cortical signaling (via intra-mPFC NMDA antagonism) can be remedied by infusions of dopaminergic or serotonergic antagonists into the DMS (Agnoli *et al*, 2012; Agnoli and Carli, 2012), further emphasizing that, at baseline, cortical and striatal networks are fine tuned to optimize executive function and behaviour. Notably, bilateral dorsal mPFC lesions impair accuracy and response control, akin to lesions of the dorsal striatum (Chudasama *et al*, 2003b; Muir *et al*, 1996; Passetti, 2002; Rogers *et al*, 2001). Disrupting dorsal striatal activity may also affect its interactions with other cortical inputs converging on this region, such as the posterior anterior cingulate cortices, which regulate response inhibition but not attention (Muir *et al*, 1996). Thus lesions or inactivations of DMS may result in more widespread deficits relative to manipulations that target specific cortical regions. Indeed, inactivations of the anterior cingulate, prelimbic, and infralimbic cortices lead to distinct attentional or response control impairments on the rCET (Hosking *et al*, 2014a, 2015a), but the DMS inactivation here mirrored a global cortical dysfunction.

Unlike previous work with the 5-CSRTT, we show here that the attentional and response control deficits observed following DMS silencing are also accompanied by alterations in decision making (Table 4.3). DMS inactivation decreased choice of the hard option across both worker and slacker rats, raising an interesting question as to whether the shift in choice is driven by, or independent of, the impaired ability to complete the more cognitively difficult HR trials. Indeed, we have shown previously that decision-making impairments

following cortical inactivation are accompanied by performance deficits on the rCET, but that silencing of the BLA can affect decision making devoid of effects on attention and impulsivity (Hosking *et al*, 2014a, 2015a). We have also shown that these behavioural variables- choice vs attentional performance vs impulsivity- can be independently affected by pharmacological challenges (Cocker *et al*, 2012b; Hosking *et al*, 2014c; Silveira *et al*, 2016). As described above, the shift to LR trials does not reflect an advantageous decision in this instance, as rats were similarly incapable of completing LR trials. The current behavioural data may suggest then that rats are making a shift to LR choice based on their learned experience with the easier option, but are not updating this representation with their current ability to complete these trials.

While possible, another and perhaps more parsimonious explanation is that the dorsal striatum, like the ventral striatum, may play a role in enabling animals to overcome cognitive effort-related costs associated with larger rewards (Floresco *et al*, 2008a). To our knowledge, no previous studies have temporarily inactivated or lesioned the dorsal striatum to assess its contributions to effort-based choice. However, ventral striatal manipulations that affect valuations of physical effort allocation (e.g. intra-accumbens adenosine, acetylcholine, or dopamine neurotransmission manipulation) are ineffective when targeted toward a dorsal striatal site, suggesting that the contributions of this striatal zone to decision making with physical effort costs is minimal (Cousins *et al*, 1993; Farrar *et al*, 2010; Font *et al*, 2008; Nunes *et al*, 2013a). Indeed, human imaging data suggests that while the ventral striatum may be a common motivational node,

cognitive and physical effort may preferentially recruit the dorsomedial and dorsolateral striatum, respectively (Schmidt *et al*, 2012). The dorsal striatum may play a particularly important role in decisions based on cognitive effort costs, given widespread areas of the cortex project to this “associative” area, and aberrant signaling in this region can induce deficits in a number of cognitive processes, such as attention, working memory, and behavioural flexibility across species (Bradfield *et al*, 2013; Cools *et al*, 2006; Ragozzino, 2007; Rogers *et al*, 2001).

In line with this, a medial-prefrontal-dorsal striatal circuit has recently been shown necessary for cost/benefit valuations under approach-avoidance conditions. During a T-maze task, in which rats chose between rewards discounted by an aversive overhead light, optogenetic inhibition of a medial prefrontal-DMS pathway increased rats’ choice of the high-cost/high-reward arm (Friedman *et al*, 2015). Subsequent experiments revealed that activation of this pathway during the approach-avoidance task dampens striatal neuron activity via an inhibitory interneuron microcircuit, suggesting that under basal conditions DMS activation may bias choice towards more rewarding, yet costlier “avoidable” options. This is in line with more general evidence that activity in dorsal striatal neurons display task-related activity that is graded by reward magnitude (Cromwell, 2003; Hollerman *et al*, 1998; Kawagoe *et al*, 1998), and that phasic neuronal activity in this region covaries with action value and chosen value, thus providing a means by which striatal activity may guide choice and evaluate consequent outcomes (Kimchi and Laubach, 2009; Lau and Glimcher, 2007,

2008; Tai *et al*, 2012). Evidence that signals for previous goal choice also persist in the DMS provide further evidence that this region may be actively involved in the cost-benefit computations necessary for rCET decision making (Kim *et al*, 2013).

The effect of DMS inactivation on attentional, response control, and decision-making processes are also in accordance with evidence that corticostriatal "loops" may regulate distinct facets of task performance (i.e., choice, attention, response inhibition), the resulting output of which can then bias motor output via palladal-thalamic-cortical pathways (Chudasama and Robbins, 2006; Haber and Behrens, 2014). For example, while the mPFC-DMS circuit appears necessary for 5-CSRTT performance, the ACC-NAc pathway is not, even though lesions to each area increase premature responding on task (Christakou *et al*, 2001, 2004; Muir *et al*, 1996). In contrast, this ACC-NAc projection is necessary for cost/benefit valuations in which rats must decide to overcome physical barriers for larger reward (Hauber and Sommer, 2009). And while we focus our discussion here on corticostriatal pathways, we cannot preclude the contribution of other basal ganglia structures and striatal circuits to task performance. Indeed, the STN, as well as the projection between the mPFC and STN, regulate attention and impulsivity on the standard 5-CSRTT (Baunez, 1997; Chudasama *et al*, 2003a). This structure is also implicated in some forms of decision making (Adams *et al*, 2017b). Additionally, amygdala projections to the striatum are involved in reward processing, and it is possible such amygdalo-striatal inputs may guide decision making on the rCET (Namburi *et al*, 2015;

Stuber *et al*, 2011).

### 4.4.2 Nucleus Accumbens Inactivations Preclude Responding on Complex Cognitive Tasks

In stark contrast to inactivations of the DMS, inactivation of the nucleus accumbens core region rendered rats incapable of performing the rCET (Table 4.2). For this reason meaningful interpretation of choice, accuracy, and premature responding data is limited. Visual inspection of the data suggests that rats decreased choice of the HR option following inactivation, but this was only a trend. Decreased choice of the HR option is in line with the general role of the ventral striatum in biasing animals towards high reward options, even when discounted by physical effort, delay, or uncertainty costs (Cardinal *et al*, 2001; Cardinal and Howes, 2005; Ghods-Sharifi and Floresco, 2010; Stopper and Floresco, 2011), and supports human neuroimaging data suggesting that activity in the ventral striatum varies as a function of the reward and costs associated with different options (Botvinick *et al*, 2009; Croxson *et al*, 2009; Levy *et al*, 2010).

While rats are still capable of performing the 5-CRSTT following lesions of the nucleus accumbens core region, emerging evidence suggests that temporarily inactivating the region prior to task onset may preclude task engagement (Feja *et al*, 2014). The discrepancy observed following lesions and inactivations of the nucleus accumbens highlights the compensatory mechanisms associated with lesion studies, which might explain why the two

techniques can ascribe different roles to the ventral striatum and the corticostriatal pathways targeting it (Christakou *et al*, 2004; Feja and Koch, 2015). It is unlikely that the cessation of behaviour reflects a decreased motivation for sucrose reward, given NAcC inactivations do not affect responding for sucrose on a FR1 schedule, and do not affect preference for larger sucrose reward in a reward magnitude discrimination test (Ghods-Sharifi and Floresco, 2010; Moscarello *et al*, 2010). Rather, the effects of NAcC inactivation on-task might reflect general impairments in motor function, such that subjects were not able to keep pace with the rapid nature of an rCET trial. Indeed, inactivations of the core, but not shell region, decrease locomotor activity and increase response latencies on a probability discounting task (Pothuizen *et al*, 2005; Stopper and Floresco, 2011).

Another possibility is that the cessation of rCET engagement following NAcC inactivation might reflect a profound inability for rewarding outcomes to invigorate approach or effortful behavior (Floresco, 2015). Lesions of the nucleus accumbens decrease lever pressing for increasing PR ratios, and, inactivations or lesions of the NAcC decrease choice of high effort/high reward options in T-maze or lever-based versions of physical effort-based discounting tasks (Ghods-Sharifi and Floresco, 2010; Hauber and Sommer, 2009; Moscarello *et al*, 2010). Importantly, rats are still capable of engaging in these tasks, presumably because of the low number of trials completed in the former, or the relatively minimal movement required in the latter. In contrast, the rCET requires animals to execute a complex series of carefully timed motor movements involving both

response selection and inhibition, and this may be impossible with the NAcC completely off-line.

### 4.4.3 Conclusion

When considered with previous studies completed using the rCET, the current investigation contributes to our understanding of the cortico-limbic-striatal circuitry mediating the allocation of cognitive effort, at least in the attentional domain. Indeed, the current findings may relate to the apathy observed in basal ganglia disorders such as Huntington's disease, as well as the anergia and avolition observed in schizophrenia, whose etiology is based in part on deregulated corticostriatal signaling (Culbreth *et al*, 2016b; Duijn *et al*, 2014; Shepherd, 2013). Work with the rCET may identify the aberrant mechanisms giving rise to the motivational deficits characteristically observed in such patient populations, and may one day reveal ways in which these can be treated.

## 4.5 Tables

| | LR - Saline | LR - Bac/Mus | HR - Saline | HR - Bac/Mus |
|---|---|---|---|---|
| Choice latency | 2.94 ± 0.24 | 2.39 ± 0.22 | 2.83 ± 0.15 | 2.22 ± 0.15 |
| Correct latency | 0.61 ± 0.02 | 0.69 ± 0.04 | 0.52 ± 0.02 | 0.68 ± 0.08 |
| Collect latency | 1.74 ± 0.07 | 2.22 ± 0.31 | 1.63 ± 0.09 | 1.81 ± 0.14 |
| Choice omissions | (For Session) | | 3.54 ± 0.97 | 4.57 ± 1.03 |
| Trials Initiated | (For Session) | | 139. 23 ± 4.49 | 124.50 ± 7.26 |
| Trials Completed | (For Session | | 121.46 ± 5.42 | 77.85 ± 6.04 |

**Table 4.1. Other rCET behavioural measures following DMS inactivation**

Means are presented (± SEM). Values presented for choice omissions, trials initiated, and trials completed reflect data for saline and inactivation session.

$^{\#}p < .1$; * $p < .05$; ** $p < .001$.

| | LR - Saline | LR - Bac/Mus | HR - Saline | HR - Bac/Mus |
|---|---|---|---|---|
| Accuracy (%) | 88.46 ± 2.64 | 65.47 ± 12.33 | 66.02 ± 3.77 | 42.62 ± 11.68 |
| Premature (%) | 15.61 ± 4.09 | 6.37 ± 2.80 | 15.53 ± 3.49 | 13.98 ± 4.29 |
| Choice latency | 2.46 ± 0.23 | 2.48 ± 0.35 | 3.20 ± 0.31 | 2.25 ± 0.25 |
| Correct latency | 0.61 ± 0.05 | 1.02 ± 0.26 | 0.55 ± 0.03 | 0.61 ± 0.06 |
| Collect latency | 1.82 ± 0.18 | 2.64 ± 0.37 | 1.86 ± 0.24 | 2.67 ± 0.91 |
| Choice omissions | (For Session) | | 5.16 ± 1.37 | 2.92 ± 0.92 |

**Table 4.2. Other rCET behavioural measures following nucleus accumbens core inactivation**

Means are presented (± SEM). Values presented for choice omissions reflect data for saline and inactivation session.

$^{\#}p < .1$; * $p < .05$; ** $p < .001$.

| | Dorsomedial striatum | Nucleus accumbens core |
|---|---|---|
| Choice of HR | ↓ | ↓# |
| Accuracy (LR/HR) | ↓/ ↓ | - / - |
| Premature responding (LR/HR) | ↑/↑ | ↓#/ - |
| Omissions at choice onset | - | - |
| Response omissions following stimulus presentation (LR/HR) | ↑/↑ | ↑/↑ |
| Latencies to make choice (LR/HR) | ↓/ ↓ | - / ↓ |
| Latencies to make a correct response (LR/HR) | ↑/↑ | - / - |
| Latencies to collect reward (LR/HR) | - / - | - / - |
| Trials initiated | - | ↓ |

**Table 4.3. Summary of behavioural effects observed following dorsomedial striatum and nucleus accumbens core inactivation**

HR = high effort/high reward; LR = low effort/low reward. Note that the severe behavioural disruptions observed following NAcC inactivation limit a meaningful analysis of choice patterns, attention, and impulsivity. # denotes a trending effect.

## 4.6 Figures

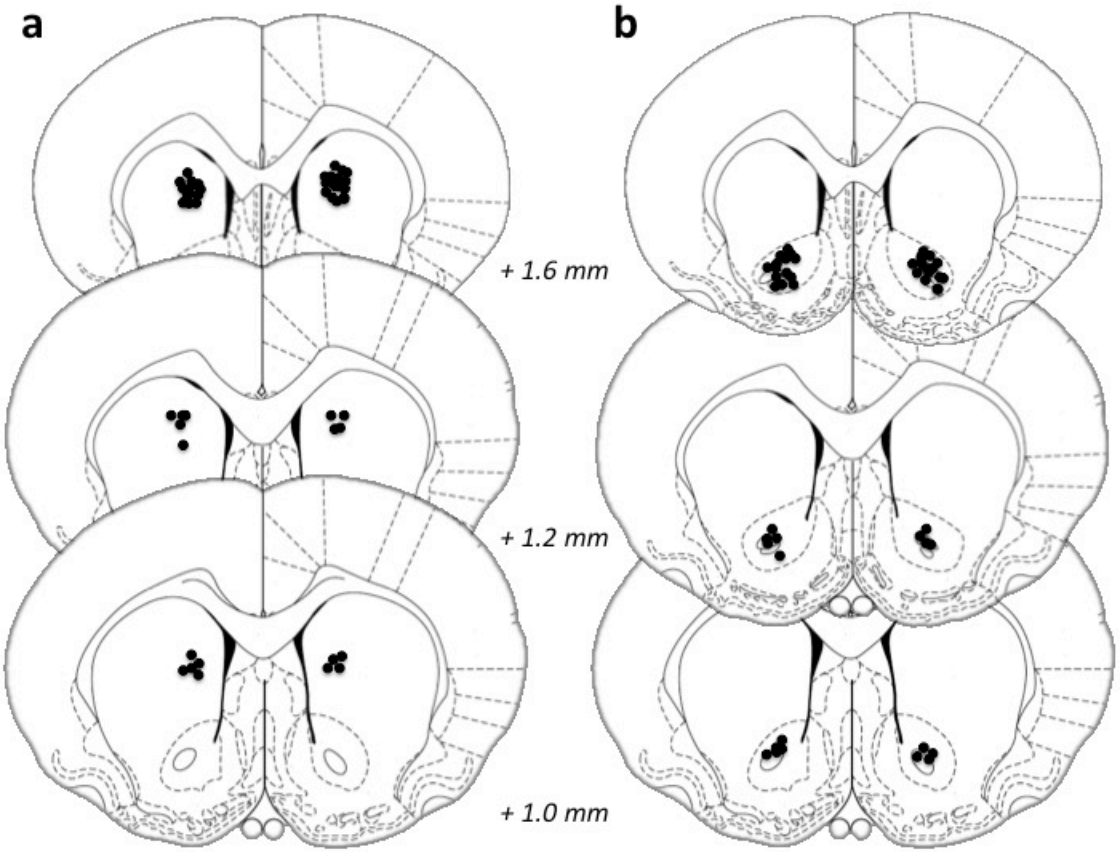


**Figure 4-1. Histological analysis of cannulae implantation**

Location of all acceptable dorsal striatal (a) and nucleus accumbens core (b) infusions. Coordinates are relative to bregma. Plates modified from Paxinos and Watson (1998).

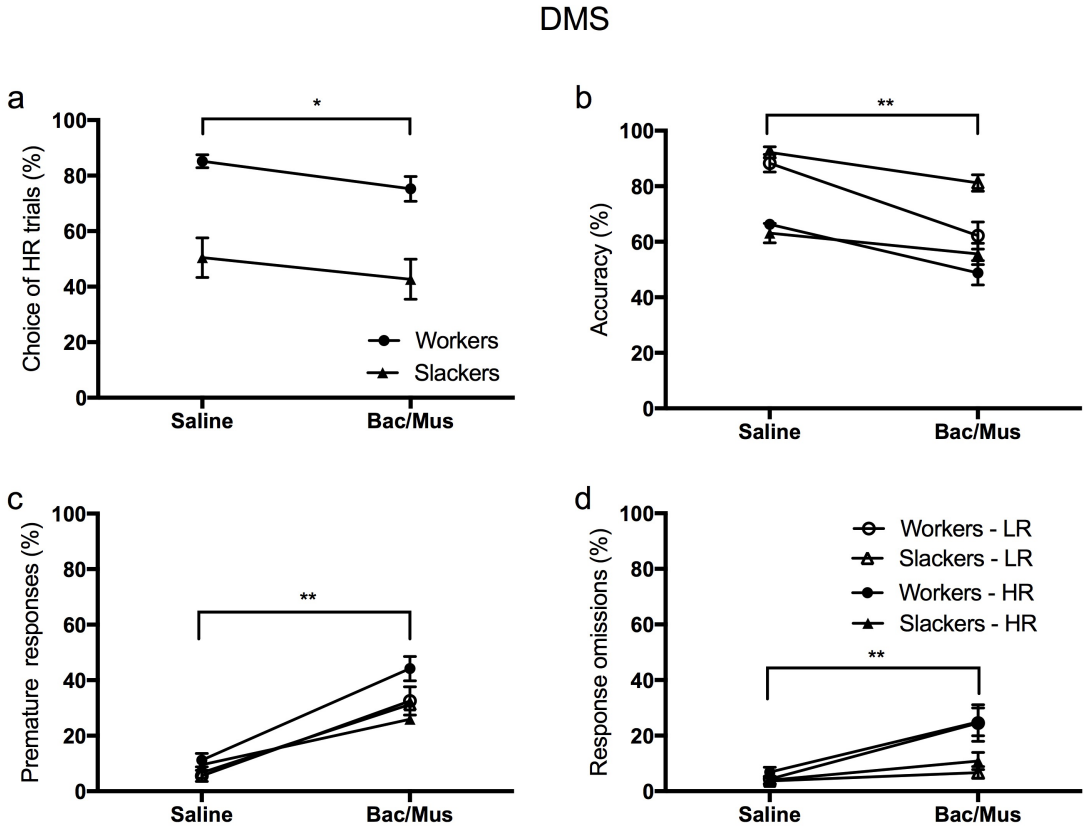


**Figure 4-2. Effects of DMS Inactivations on (a) choice of HR trials (%), (b) accuracy, (c) premature responses (%), and response omissions (%)**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. DMS inactivation decreased choice of the HR option across both groups, and impaired accuracy for both trial types. DMS Inactivation also increased premature responding and response omissions for LR and HR trials. # $p < .1$; * $p < .05$; ** $p < .001$

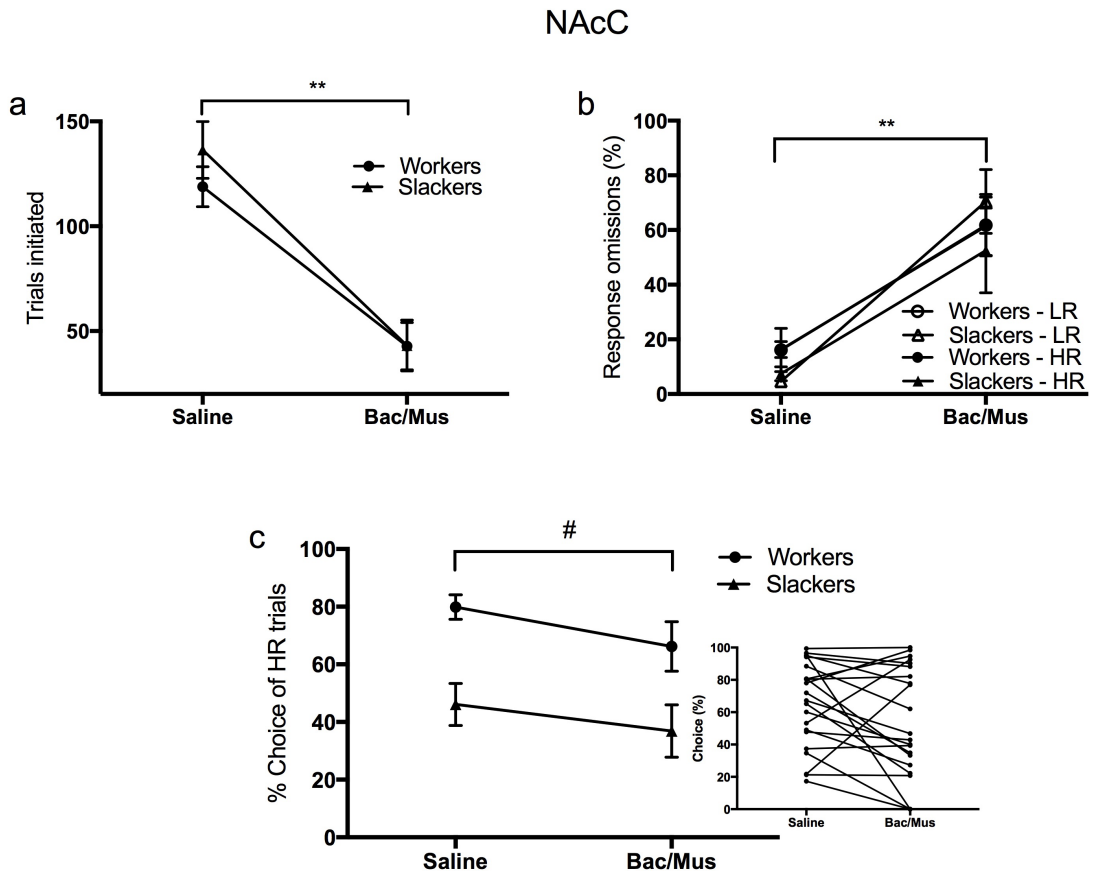


**Figure 4-3. Effect of nucleus accumbens core (NAcC) inactivations on (a) trials initiated, (b) response omissions (%), and (c) choice of HR trials (%).**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. NAcC inactivation decreased trials initiated and increased response omissions across both groups. There was a trending decline in HR choice across rats (individual choice data inlaid). # $p < .1$; * $p < .05$; ** $p < .001$

# Chapter 5: Investigating Orbitofrontal Cortex Contributions to Decision Making Involving Cognitive Effort Costs

## 5.1 Introduction

The student choosing to study for an exam, the worker spending late nights at the office, and the scientist who devotes years of work towards a single research question are all similar in that they have decided to allocate their cognitive resources towards a particular task, in the hopes that it will lead to lucrative future outcomes (e.g. degrees, promotions, scientific discovery). In all cases described above, executive processes constitute a limited resource, and so engagement in a particular cognitive episode precludes effort allocation towards alternative options that might yield beneficial, albeit less rewarding, outcomes. The decision to expend cognitive effort can thus be parlayed as an economic decision, whereby cognitive resources will only be allocated to a particular task if the expected value of the goal is high, thereby offsetting the opportunity costs incurred from persevering with a cognitively effortful option (Botvinick and Braver, 2015; Westbrook and Braver, 2015)

Accordingly, a number of regions have been implicated in the processes by which the brain mediates such valuations of mental effort allocation, but none have received as much attention as the dorsal anterior cingulate cortex (dACC)

(Shenhav *et al*, 2013). Previous research has shown that dACC blood-oxygen-level dependent (BOLD) signals reflect the subjective value of a chosen cognitively effortful reward, and that activity in this region is higher during cognitive effort versus delay discounting tasks (Chong *et al*, 2017; Massar *et al*, 2015). More generally, objective measures of task difficultly correlate with activity in the ACC, and stimulation of this region can elicit a feeling of "gearing up" for an effortful challenge, whereas lesions to this region can abolish conscious feelings of mental effort (McGuire and Botvinick, 2010b; Mulert *et al*, 2005; Naccache *et al*, 2005; Parvizi *et al*, 2013).

Recently, our group has also demonstrated a causal role for the ACC in valuations of cognitive effort, using a rodent model of cost-benefit decision making known as the rodent Cognitive Task (rCET). In this task, rats choose between two levers associated with easy versus hard attentional trials, whose successful completion is associated with one or two sugar pellet rewards, respectively (Cocker *et al*, 2012b). In agreement with the human cognitive effort literature, inactivations of the rodent dorsal mPFC (including prelimbic and pregenual Cg1 regions, corresponding to areas of the primate dACC (Heilbronner *et al*, 2016)) uniformly decrease choice of the more effortful option on the rCET, regardless of individuals' preference for hard or easy trials at baseline (Hosking *et al*, 2014a, 2015a).

Thus, while dorsal prefrontal cortical areas have been implicated in cognitive effort allocation across species, few, if any, studies have implicated the orbital frontal cortices in such processes. Indeed, the animal cost/benefit

decision-making literature generally supports dissociable roles for the ventral and dorsal cortical areas in decision making, in which lesions or inactivations of the anterior cingulate cortex affect physical or cognitive effort-based valuations (Croxson *et al*, 2009; Rudebeck *et al*, 2006; Walton *et al*, 2003b), while similar manipulations to the orbital frontal regions selectively affect decision making involving delay, risk, and probability costs (Barrus *et al*, 2017; Orsini *et al*, 2015a; Stopper *et al*, 2014; Winstanley, 2004; Winstanley and Floresco, 2016). Similar dissociable roles for dorsal and ventral prefrontal areas in goal-directed choice are also observed in the human literature (Mobini *et al*, 2002; Prevost *et al*, 2010; Rangel and Hare, 2010; Rushworth *et al*, 2011a).

However, the role of the orbital frontal regions in cognitive effort allocation has never been causally investigated, and the tendency to consider effort as a unitary construct may mask distinct neural substrates regulating cognitive versus physical effort. Indeed, there is mounting evidence across species that these forms of decision making are mediated by unique mechanisms (Chong *et al*, 2017; Hosking *et al*, 2015b; Schmidt *et al*, 2012), and our own with work with the rCET suggests the orbital areas may play a selective role in cognitive effort-based decision making.

For one, rats display a diverse propensity to “work” or “slack” at baseline, a behavioural distribution which is not generally observed in the physical effort literature (although see Randall *et al*, 2012), given most subjects in these paradigms willingly scale a barrier or lever press at high ratios for larger reward (Cocker *et al*, 2012b). A review of the animal model decision-making literature

has led to a recent proposal that the OFC is recruited in situations where task parameters make the objectively best option unclear, such that subjective evaluation of different outcomes is most pronounced (Winstanley and Floresco, 2016). Given baseline choice patterns on the rCET are not driven by differences in cognitive ability (i.e. attention and response control), the OFC may be a critical region for establishing subjective preference for cognitive episodes associated with lucrative outcomes.

Secondly, the OFC sends dense projections to the dorsomedial striatum, and inactivation of this region decreases choice of the hard lever on the rCET, suggesting that at baseline this striatal zone may bias choice towards high effort, high rewards options (Mailly *et al*, 2013; Silveira MM, Tremblay M, 2016). Thus, signals from the OFC, along with input from other prefrontal cortical, thalamic, and limbic areas may interact to bias activity in the dorsomedial striatum to optimize decisions with cognitive effort costs.

In this study, we use standard pharmacological inactivation techniques to test whether the orbitofrontal cortices play a causal role in deciding how to allocate cognitive resources for beneficial outcomes. Although there is increasing appreciation of the functional heterogeneity within the rat PFC as it relates to reward learning and decision making (Izquierdo, 2017), we focus on the ventral lateral orbital frontal cortex (VLO). This area is the most often targeted in other rodent decision-making paradigms, thus facilitating direct comparisons of how this region contributes to cost/benefit decision making with distinct costs.

## 5.2 Additional Methods

### 5.2.1 Subjects and Baseline Choice

Subjects were 24 female Long-Evans rats bred in house. These rats were bred from breeding pairs obtained from Charles River Laboratories and the Rat Resource and Research Centre (RRRC, Columbia, MO) as part of a breeding program for transgenic rats that express cre recombinase (Cre) in neurons that contain choline acetyltranferase (ChAT; Long Evans –Tg (ChAT-Cre) 5.1 Deis, RRRC # 00658). Twelve of these rats were positive for the transgene (TG+), while the remainder did not express the transgene (TG-). The transgenic status of the rats was not utilized for the current study, but instead the subjects were considered naïve given they exhibit a similar behavioural profile to Long Evans rats purchased from a commercial supplier. To account for potential differences, transgene status (TG + or TG -) was included as a between-subjects factor for all analyses. These results will not be reported, unless a significant a main effect of TG status or interaction with TG status is observed for any of the rCET behavioural measures. Animals weighed at least 200 g at the start of the experiment and were food restricted to 85% of their free-feeding weight (maintained on 10 g rat chow daily). Water was available ad libitum. Rats were housed in groups of three or four in a climate controlled colony room maintained at 21° C on a reverse 12-hr light-dark schedule (lights off at 8 am).

For this particular experiment, the mean choice of the HR option across rats was 66%. Animals were grouped as “workers” if they chose the HR option

for >70% of trials (n=12) and as “slackers” if they chose HR for ≤70% of trials (n=12), as per previous work (Cocker et al., 2012), thereby enabling consistency when discussing individual differences across studies.

### 5.2.2 Surgery to Implant Guide Cannulae

Following stable baseline performance, animals were implanted with 22-gauge stainless steel guide cannulae (Plastics One) bilaterally into the lateral orbitofrontal cortices using standard stereotaxic techniques. Animals were anesthetized with 2% isoflurane in $O_2$ and cannulae implanted at the following coordinates: AP = +3.8 mm, ML = ±2.6 mm from bregma, DV = −2.6 mm from dura (Paxinos and Watson, 1998). The cannulae were fixed to the skull by 3-4 stainless steel screws and dental acrylic. Obdurators flush with the cannulae tip were inserted to prevent clogging prior to testing. Animals recovered for at least 5 days before resuming behavioural testing. Two rats died during surgery, and so 22 rats moved on to microinfusions.

### 5.2.3 Microinfusion Procedure

Once recovered, rats performed 10 free-choice sessions, at which point their behaviour was deemed statistically stable (see data analysis). To habituate rats to the microinfusion process, two mock infusion sessions were carried out prior to the critical testing sessions. During these mock infusions, obdurators were removed and 28-gauge injectors extending 1 mm beyond the guide cannulae were inserted for two minutes, but no infusion occurred. Ten minutes

following this, rats were tested on the rCET. Infusions adhered to a 3-day cycle, starting with a baseline session, followed by a drug or saline injection, and ending with a non-testing washout day. The LOFC was inactivated by a mixture of the $GABA_B$ agonist baclofen and the $GABA_A$ agonist muscimol (Sigma–Aldrich), prepared separately at 0.5 μg/μL in saline, and mixed together in equal volumes to form a 0.25 μg/μL solution. 0.5 μL per hemisphere injections of saline or baclofen/muscimol (i.e. 0.125 μg of drug per hemisphere) were administered bilaterally at a rate of 0.4 μL/min, and injectors (injectors extending +1 mm from cannula tips) were left in place for an additional minute to allow diffusion. Once the microinfusions were completed, injectors were removed, obdurators replaced, and animals were returned to their home cages for 10 min before being placed in the operant chambers and performing the rCET. Animals underwent two infusions in total: on the first infusion day, half of the rats received saline and the other half received baclofen/muscimol These infusions were reversed on the second infusion day, thus permitting a within-subjects comparison of how silencing the LOFC affects rCET performance. Three rats did not have data from at least one microinfusion day due to health complications, and so they were excluded from the study. Thus complete data was obtained from 19 subjects.

### 5.2.4 Histology

When behavioural testing terminated, animals were anesthetized with 2% isoflurane in $O_2$ and then euthanized via $C0_2$. Brains were extracted and submerged in 4% paraformaldehyde for 24h, before being transferred to a 30%

sucrose solution where they remained until sectioning. Brains were frozen and cut into 40-µm coronal sections using a cryostat. The sections were placed on gelatin-coated slides and stained with cresyl violet for visualization. The projected locations of the injector tips protruding from the guide cannulae were mapped onto the coronal section images from Paxinos and Watson (1998).

### 5.2.5 Data Analysis

Data were analyzed with a repeated measures ANOVA with session (three levels: baseline sessions 1-3) or inactivation (two levels: vehicle or inactivation) as within-subjects factors, and group (two levels: worker or slacker) as a between-subjects factor for all analyses. TG status (two levels: TG+ or TG-) was also included as a between-subjects factor all analyses. These results will not be reported unless there is a significant main effect of TG status, or interaction with TG status for any of the variables analyzed.

## 5.3 Results

### 5.3.1 Cannula Placements

The locations of all acceptable placements are depicted in Figure 5.1. Two rats were excluded because the infusions were ventral to the OFC, leaving a total of 17 rats (*n* = 7 workers and *n* = 10 slackers) for analyses.

### 5.3.2 Inactivation Analyses

*Choice, accuracy, and premature responses.*

Baseline performance on the rCET has been discussed in detail previously (Cocker *et al*, 2012b), and so will be briefly discussed here. As a group, rats chose the high-effort/high-reward (HR) option more than the low-effort/low-reward (LR) option when the orbitofrontal cortex (OFC) was infused with saline (saline only- choice: $F(1, 13) = 43.473$, $p < .001$). In line with their categorization, workers ($M = 86.70\%$) chose the HR option significantly more than slackers ($M = 51.53\%$) (saline only- group: $F(1, 13) = 39.252$, $p < .001$). Temporary inactivation of the OFC did not affect choice of the HR option across both groups of rats (inactivation: $F(1, 13) = .963$, NS; inactivation x group: $F(1, 13) = 0.151$, NS; Figure 5.2a).

Accuracy on HR trials was lower than accuracy on LR trials, suggesting that HR trials were indeed more difficult for subjects to attend to (saline only-choice: $F(1, 12) = 56.701$, $p < .001$). In line with previous work on the task,

workers and slackers did not differ in attentional performance (saline only- choice x group: $F(1, 12) = 3.402$, NS; group: $F(1, 12) = .411$, NS). This suggests that slackers' reduced preference for HR was not due to a profound inability to successfully attend to the .2s stimulus. Inactivation of the OFC did not affect accuracy on LR (LR trials- inactivation: $F(1, 12) = 0.155$, NS; inactivation x group: $F(1, 12) = 1.75$, NS) or HR trials (HR trials- inactivation: $F(1, 11) = 2.293$, NS; inactivation x group: $F(1, 11) = 0.087$, NS) for either group (Figure 5.2b).

Premature responding did not differ between LR or HR trial types (saline only- choice: $F(1, 12) = 0.185$, NS), but groups did differ at baseline on rates of premature responding (saline only- choice x group: $F(1, 12) = 7.036$, $p = .021$). While workers and slackers did not differ in rates of premature responding on HR trials (HR trials-saline only: $F(1, 13) = 0.81$, NS), there was a trend for workers to be more impulsive on easy, LR trials (LR trials-saline only: $F(1, 12) = 3.494$, $p = .086$), This trend was driven by one worker rat who infrequently made LR choices, but who acted impulsively on most of these trials. In any case, slackers' reduced choice of HR cannot be explained by an inability to wait for the .2s stimulus on HR trials. Across all animals, inactivation of the OFC robustly increased premature responding for both trials types (LR trials- inactivation: $F(1, 12) = 17.286$, $p < .001$; inactivation x group: $F(1, 12) = .372$, NS; HR trials-inactivation: $F(1, 11) = 5.187$, $p = .044$; inactivation x group: $F(1, 11) = .434$, NS, Figure 5.2c).

*Other behavioural measures.*

When infused with saline, rats initiated 130 trials on average, and this did not differ between worker and slacker rats (saline only-group: $F(1, 13) = 0.946$, NS). OFC inactivation decreased trials initiated by both groups, but rats still initiated over 100 trials during the experimental test session (inactivation: $F(1, 13) = 13.128$, $p = .003$; inactivation x group: $F(1, 13) = 0.896$, NS). Following trial initiation and extension of both HR and LR levers, rats typically made few choice omissions under baseline conditions and this did not differ between groups (saline only-group: $F(1, 13) = 0.73$, NS). However, when the OFC was infused with baclofen/muscimol, choice omissions almost tripled (inactivation: $F(1, 13) = 30.788$, $p < .001$; inactivation x group: $F(1, 13) = 0.187$, NS, Figure 5.2d). Latencies to make a trial type choice, to correctly detect the visual stimulus, and to collect sucrose from the magazine tray following successful trial completion did not differ between LR or HR trial types, nor did they differ between workers and slackers when the OFC was infused with saline (saline only- choice/ choice x group/ group: all Fs < 4.571, NS). Following OFC inactivation, there was a trend for rats to be slower to initiate LR trials (choice latency: LR trials- inactivation: $F(1, 12) = 4.430$, $p = .057$; inactivation x group: $F(1, 12) = 3.602$, $p = .082$), but otherwise latencies were unaffected (HR choice latency, LR and HR correct latency, LR and HR collect latency- inactivation/ inactivation x group: all Fs < 2.608, NS). Previous work has shown that OFC inactivation increases general latencies to make a choice in a probability discounting task (St Onge and Floresco, 2010), and so we collapsed latencies across trial types and analyzed this general choice latency measure. However, OFC inactivation did not affect

overall choice latencies (inactivation, inactivation x group: all *F*s < 1.902, NS). In line with the increased attentional demands of HR trials, response omissions following stimulus presentation were slightly higher for this trial type, but did not differ between worker and slacker rats (saline only- choice: $F(1, 12) = 12.476$, $p = .004$; choice x group/ group: all Fs < 2.441, NS). Unlike the pronounced increase in choice omissions, response omissions for either trial type were otherwise unaffected (LR and HR trials- inactivation / inactivation x group: all Fs < 3.378, NS). Infusions of the OFC significantly decreased the number of trials completed (inactivation: $F(1, 13) = 47.957$, $p < .001$; inactivation x group: $F(1, 13) = 1.194$, NS, Figure 5.2e), reflecting the rise in incomplete trials due to increased choice omissions and premature responding.

## 5.4 Discussion

The cost/benefit decision-making literature generally supports dissociable roles for dorsal and ventral cortical regions in effort allocation. Across species, the anterior cingulate cortices are implicated in the processes by which organisms decide to allocate effort towards lucrative outcomes, while no work has shown a causal role for the orbitofrontal cortices in these cost/benefit computations (Winstanley and Floresco, 2016). However, this literature has almost entirely focused on the physical effort costs that discount preferred rewards, and has neglected the cognitive effort costs that are arguably more representative of the decisions individuals face in modern societies. Like physical effort, cognitive effort is an inherently costly resource whose deployment can be biased by incentives, suggesting it is amenable to the cost/benefit framework that has been used to study physical effort allocation (Westbrook and Braver, 2015). Here we directly investigated the role of the OFC in mediating valuations of cognitive effort allocation while rats performed the rodent cognitive effort task (rCET). Inactivations of the OFC did not affect rats' willingness to exert cognitive effort for larger rewards, nor did they affect the attentional ability required to complete difficult, high reward trials. However, inactivations selectively and dramatically increased choice omissions, and in keeping with previous literature, increased measures of impulsive action (Chudasama *et al*, 2003b). The effects are in striking contrast to previous work on the rCET, in which inactivation of medial and dorsal cortical areas simultaneously affect willingness to exert

cognitive effort as well as executive performance (Hosking *et al*, 2014a, 2015a). Indeed, the current work suggests that cognitive performance deficits induced by cortical perturbation are not necessarily accompanied by shifts in effort allocation. More generally, this work provides further support for a dorsal/ventral divide in effort-based decision making, with dorsal, but not ventral, prefrontal regions recruited when decision costs require effort expenditure in the physical or cognitive domain.

Before discussing the behavioural effects observed following OFC inactivation, it is worth speculating whether our infusion protocol was sufficient to produce changes in choice behaviour. The pharmalogical inactivations we carried out encompass a relatively small region (~1mm, see Floresco, 2006) relative to the lesions previously used to investigate the role of the OFC in physical effort-based decision making (Ostrander *et al*, 2011; Rudebeck *et al*, 2006). However, the infusion protocol is identical to those previously used to investigate prelimbic, infralimbic, and ACC contributions to rCET performance (Hosking *et al*, 2014a, 2015a). As will be discussed below, inactivations of these regions alter effort allocation while concomitantly affecting other measures of performance. While OFC inactivation did not affect decision making, it had pronounced effects on premature responding and choice omission rates, suggesting the infusions were selective and behaviourally effective.

Our infusions targeted the lateral orbitofrontal cortices, but there is growing recognition that the orbitofrontal cortex is a functionally heterogenous structure, and that behavioural neuroscientists may oversample some areas

while undersampling from others (Izquierdo, 2017). We focused on the lOFC because most of the previous cost/benefit decision making literature has targeted this region, thus allowing us to easily compare and contrast our observed effects with those seen in tasks probing decision making with risk (Orsini *et al*, 2015a), uncertainty (Barrus *et al*, 2017; St Onge and Floresco, 2010; Zeeb and Winstanley, 2011), delay (Mar *et al*, 2011; Winstanley, 2004), and most importantly effort (Ostrander *et al*, 2011; Rudebeck *et al*, 2006) costs. Additionally, this area was previously lesioned on the 5-choice serial reaction time task, the predecessor of the rCET, thus allowing us to directly relate our behavioural effects on attention and impulsivity with those seen previously (Chudasama *et al*, 2003b). Some researchers have investigated medial orbital (MO) contributions to decision making under conditions of uncertainty (Stopper *et al*, 2014) and delay (Mar *et al*, 2011) and reported effects on choice. While lesions targeting the infralimbic/prelimbic areas and encompassing MO do not affect physical effort allocation (Walton *et al*, 2003b), this may be an interesting target for future studies on the rCET.

We opted to use female rats in the current study, given that they display similar patterns of behaviour on the rCET to the more commonly used males. Indeed, choice of the HR lever in this study was 66 %, while HR choice has ranged from 56% (Hosking *et al*, 2014a) to 73% (Hosking *et al*, 2014b) across male cohorts (HR choice is typically ~70%). Accuracy and premature responding rates are also similar to those typically observed in male cohorts. While we did not track females' estrous cycle, previous research using a risk discounting task

and lever-based physical effort discounting task have shown that this does not affect baseline choice patterns (Orsini *et al*, 2015b; Uban *et al*, 2012). It could be argued that a different pattern of behavioural results might have been observed if males were used. This is possible, in light of research showing c-fos expression in the lateral OFC is correlated with choice in a rodent analog of the Iowa Gambling Task in females, but not males (van Hasselt *et al*, 2012), as well as some human imaging studies showing differential OFC activity between sexes in risk-based decision making tasks (Bolla *et al*, 2004; Lee *et al*, 2009). However, we are not aware of any preclinical or human literature suggesting that the OFC is differentially recruited between males and females in regard to effort allocation, and cannot find any example of a brain lesion or inactivation effect fundamentally affecting one sex selectively. Given we also replicated an impulse control deficit previously documented on the 5-CSRTT in males (Chudasama *et al*, 2003b), it is likely the current study can be directly compared to previous investigations on the rCET using male rats.

### 5.4.1 The OFC is Not Involved in the Cost/Benefit Computations Regulating Cognitive Effort Allocation

Following OFC inactivation, worker and slacker rats did not alter their baseline preference for the HR option. This is in agreement with work in the domain of physical effort allocation, in which OFC lesions similarly do not affect rats' willingness to scale a barrier for larger rewards (Ostrander *et al*, 2011; Rudebeck *et al*, 2006). Rather, dorsal prefrontal regions, encompassing the

anterior cingulate cortex (ACC), play a central role in processes guiding effort-based decision making. In the physical effort domain, imaging studies have shown increased activity in this region in anticipation of an effortful physical challenge (Croxson *et al*, 2009) as well as correlates of subjective value as subjects choose between options differing in their effort/reward contingencies (Chong *et al*, 2017; Prevost *et al*, 2010). Lesions of the ACC decrease rats' willingness to scale a barrier (Rudebeck *et al*, 2006; Schweimer, 2005; Walton *et al*, 2002, 2003a), or to repeatedly press a lever for larger reward (Hart *et al*, 2017; Walton *et al*, 2009) -processes regulated by dopaminergic (Schweimer *et al*, 2005; Schweimer and Hauber, 2006) and cannabinoid signaling (Khani *et al*, 2015) in this region. In contrast, intra-OFC $CB_1$ receptor signaling does not regulate valuations of physical effort, providing further evidence that this form of decision making is localized to the ACC (Khani *et al*, 2015). Likewise, cognitive effort allocation appears to be mediated primarily by dorsal prefrontal regions, as temporary inactivation of the region decreases effortful choice on the rCET (Hosking *et al*, 2014a). This is in accordance with imaging studies showing coordinated activity in the ACC in anticipation of a mental challenge (Botvinick *et al*, 2009), and aligns with recent work showing that the value of a cognitively effortful option is encoded in the ACC (Chong *et al*, 2017; Massar *et al*, 2015). Indeed, according to recent theories of effort allocation, the ACC plays an executive role in calculating the "expected value" of cognitive control, and uses this information to select among competing cognitive tasks in ways that are most worthwhile (Shenhav *et al*, 2013).

In contrast, the OFC appears to play a central role in other forms of decision making, including those involving delays (Kable and Glimcher, 2007; Mar *et al*, 2011; Prevost *et al*, 2010; Winstanley, 2004), uncertainty (Barrus *et al*, 2017; Mobini *et al*, 2002; St Onge and Floresco, 2010; Stopper *et al*, 2014), and risk of punishment (Orsini *et al*, 2015a). Inherent to all these costs is the fact that none of them have a perceptible effect on the decider at the time of choice, but manifest themselves only when the outcomes are revealed. Said another way, the OFC may preferentially mediate decisions in which costs are inherent to the outcome, while dorsal prefrontal regions are recruited when decision costs are incurred on the individual, akin to physical or cognitive effort (Rangel and Hare, 2010; Rushworth *et al*, 2011b). These dissociable roles in decision making may stem from the roles of the OFC and ACC in stimulus learning and action learning, respectively, as lesions to either area disrupt subjects' ability to use feedback about visual stimuli or actions to learn about outcomes (Camille *et al*, 2011; Rudebeck *et al*, 2008).

While OFC inactivation did not affect choice profiles, it increased choice omissions three-fold. There was also a trending increase in latency to choose the LR option following inactivation, but unlike previous work we did not observe a general rise in latencies to make a choice following this manipulation (St Onge and Floresco, 2010). This striking rise in choice omissions is unlikely to reflect a motivational deficit, as rats initiated a high number of trials, response omissions following stimulus presentation were unaffected, and latencies throughout the trial did not change. There is a rich literature implicating the OFC in the

processes by which stimuli or cues can be used to predict expected (Baxter *et al*, 2000; Schoenbaum *et al*, 1998), as well as inferred future outcomes (Takahashi *et al*, 2013). Previous work in the realm of delay-based decision making has shown that cues can exert powerful effects on baseline choice profiles, effects which are mediated by the OFC (Mobini *et al*, 2002; Zeeb *et al*, 2010; Zeeb and Winstanley, 2011). Indeed, while OFC lesions do not affect rats' willingness to scale a barrier for larger reward under normal conditions, one study has demonstrated that pre-training OFC lesions can affect physical effort allocation if cues predict reward availability in the high effort arm (Ostrander *et al*, 2011). In the rCET, the HR and LR lever are identical but vary in their spatial location, their cognitive demand, as well as their reward outcomes both within a trial (1 vs 2 sugar pellets) and across a session. To the extent that the OFC may be involved in associating outcomes to each lever, or in establishing a cognitive map of "task space" as has been recently proposed (Wilson *et al*, 2014), the increase in choice omissions following OFC inactivation might reflect a disruption in representing this information when both levers are extended. Aberrations in these processes might then affect the cost/benefit computations occurring in other prefrontal regions, such as the medial prefrontal and anterior cingulate cortices, which are specifically involved in allocating cognitive resources in worthwhile ways (discussed below). Indeed, some computational models have proposed a goal-processing "pyramid" in the brain, in which the OFC may relay stimulus identity information to the ACC, which then uses this information to guide motor control during goal pursuit (O'Reilly *et al*, 2014). Relative to medial

areas of the OFC, the LOFC we targeted sends more projections to the medial wall of the frontal cortex, and so connectivity supports this theoretical account of OFC-ACC communication during decision making (Hoover and Vertes, 2011). In further support of this cortical hierarchy, OFC inactivations appear to preferentially play a role in decision making during initial acquisition when task contingencies (e.g stimulus-outcomes) are first being learned, but play less of a role once acquired (Zeeb and Winstanley, 2011), and a PET study in rats showed increased metabolic activity in the OFC while rats performed a physical effort allocation task, even though this region does not affect this type of decision making when lesioned (Endepols *et al*, 2010). Given the extensive training on-task, disrupting OFC signaling may affect the component processes required to make an effort-based decision, but ultimately other prefrontal areas can compensate when this information is otherwise offline.

### 5.4.2 Cortical Contributions to Decision Making on the rCET

Previous work on the rCET has demonstrated that the medial wall of the rodent prefrontal cortex plays an important role in allocating attentional resources for lucrative outcomes. Specifically, selective inactivations of the prelimibic, inflralimbic, and anterior cingulate cortex all decrease choice of the HR option in both worker and slacker rats (Hosking *et al*, 2014a, 2015a). However, these shifts in decision making are also associated with deficits in the executive processes necessary to successfully complete trials and gain reward: inactivations of the prelimbic cortex selectively affect the ability to attend to LR

and HR stimuli (Hosking *et al*, 2015a), while inactivations of the infralimbic and anterior cingulate cortices increase premature responding (Hosking *et al*, 2014a, 2015a), thus precluding stimulus presentation. While attentional difficulty is explicitly manipulated between easy and hard trials, rats ultimately must exhibit a certain degree of restraint in order for the stimulus to appear. Thus, deficits in both impulse control and/or attentional ability might affect the processes by which rats choose between easy and hard response alternatives during the rCET. Notably, any attentional or impulse control deficits that have been observed following inactivation of these cortical regions are observed on both easy and hard trials, suggesting that the shift in choice toward the cognitively easier option is not the adoption of an "advantageous" choice strategy. Instead, we speculate rats are defaulting to the easier trials when executive processes are impaired, based on their previous experience with these trial types.

Unlike the simultaneous effects on cognitive allocation and ability following inactivation of the infralimbic, prelimbic, and anterior cingulate cortices, the current OFC findings suggest that at the level of the prefrontal cortex, cognitive allocation and ability can in part be dissociated. OFC inactivation did not affect effort allocation, but robustly increased impulsive action, replicating previous lesion work on the 5-CSRTT (Chudasama *et al*, 2003b). Thus, rats were impaired in their ability to complete successfully complete trials, but this did not affect their decision-making profiles. This is in contrast to the role of the basolateral amygdala on the rCET, which does not affect measures of performance, but appears to regulate rats' baseline willingness to "work" or "slack" at baseline

(Hosking *et al*, 2014a). The observed effects are also distinct from inactivations of the dorsomedial striatum, which simultaneously decrease HR choice, impair accuracy, and increase impulsivity (Chapter 4), in line with this being a downstream cortical target that may integrate the various cortical signals used to rCET performance. Considered together, this work suggests that while many cortical areas regulate optimal performance on the rCET, the medial wall plays a selective role in allocating cognitive effort in pursuit of desired outcomes, above and beyond its role in attention and impulse control.

### 5.4.3 Conclusion

To summarize, the present findings suggest that valuations of cognitive effort allocation are not dependent on OFC signaling. Rather, the current work supports evidence for a dorsal-ventral divide in decision-making processes, whereby cognitive effort costs are processed by medial prefrontal structures encompassing the prelimbic, infralimbic, and anterior cingulate cortices in rodents. This is in agreement with current conceptualizations of cognitive effort allocation in humans, which attribute a prominent role to the anterior cingulate cortex in adjudicating cognitive resources for maximal gain (Shenhav *et al*, 2013). How cognitive effort allocation is specifically mediated by interactions with the ACC and other cortical and subcortical regions is still unknown, but future work on the rCET holds great promise towards delineating the cortical-striatial-limbic interactions guiding effort allocation in the cognitive domain.

## 5.5 Table

| | LR - Saline | LR - Bac/Mus | HR - Saline | HR - Bac/Mus |
|---|---|---|---|---|
| Choice latency | 3.28 ± 0.26 | 3.69 ± 0.22[#] | 3.28 ± 0.22 | 3.40 ± 0.22 |
| Correct latency | 0.53 ± 0.02 | 0.77 ± 0.11 | 0.52 ± 0.03 | 0.68 ± 0.09 |
| Collect latency | 5.04 ± 3.12 | 1.82 ± 0.09 | 1.45 ± 0.07 | 1.52 ± 0.12 |
| Trials Initiated | (For Session) | | 130.06 ± 5.47 | 108.06 ± 7.93 |
| Trials Completed | (For Session) | | 103.65 ± 6.46 | 52.65 ± 6.73 |

**Table 5.1. Other rCET behavioural measures following OFC inactivation**

Means are presented (± SEM). Values presented for trials initiated and trials completed reflect data for saline and inactivation session. [#] $p < .1$; * $p < .05$; ** $p < .001$.

## 5.6 Figures

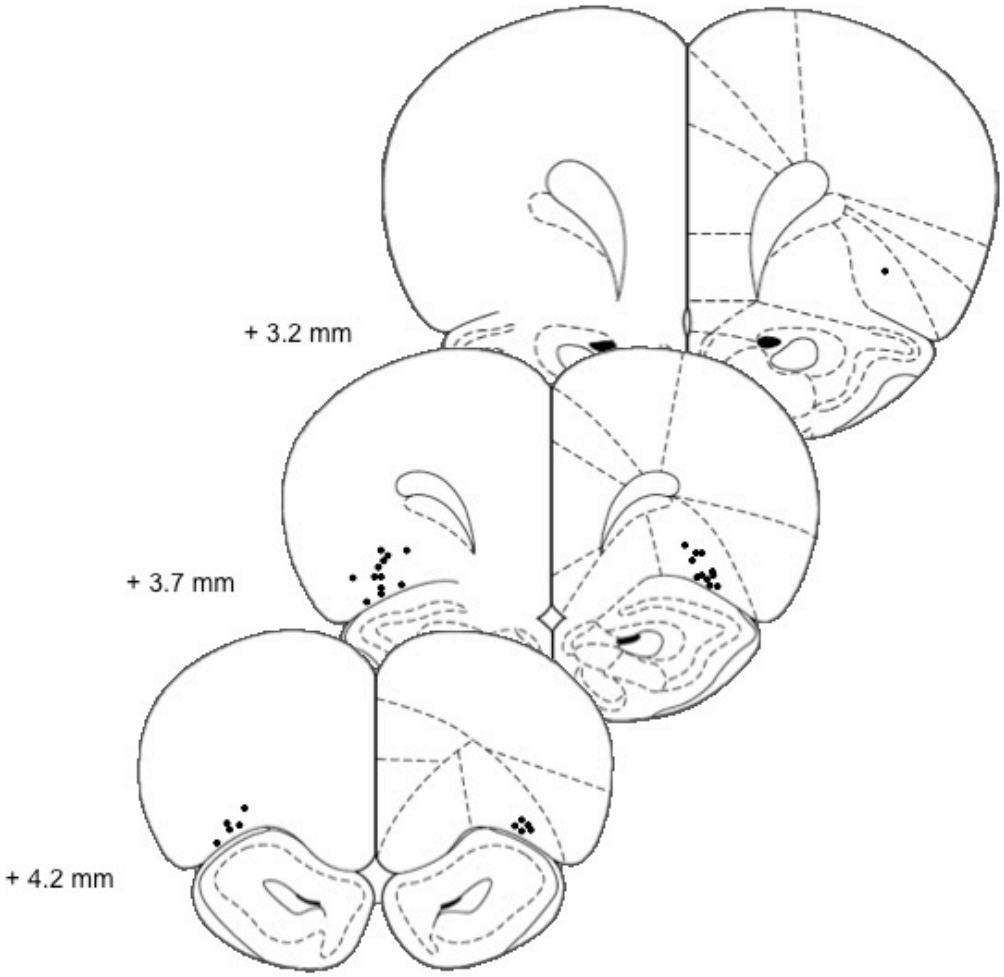


**Figure 5-1. Histological analysis of cannulae implantation**

Location of all acceptable orbitofrontal cortex infusions. Coordinates are relative to bregma. Plates modified from Paxinos and Watson (1998).

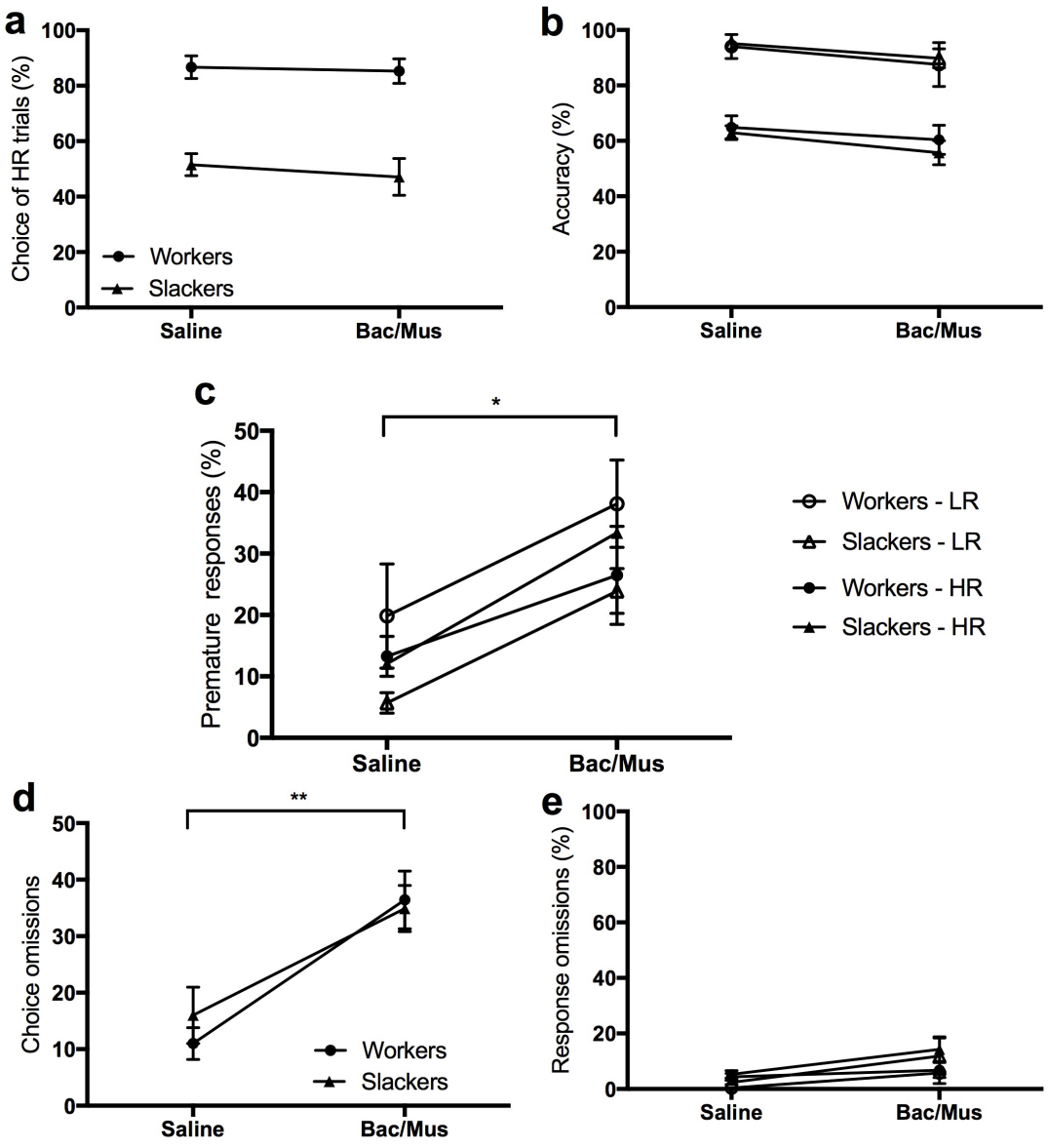


**Figure 5-2. Effects of OFC inactivations on (a) choice of HR trials (%), (b) accuracy (%), (c) premature responses (%), (d) choice omissions, and (e) response omissions (%)**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. OFC inactivation increased premature

responding and choice omissions but did not affect choice, accuracy, or response omissions.

# Chapter 6: Investigating Amygdala-Prefrontal Cortical Circuit Involvement in the Allocation of Cognitive Effort

## 6.1 Introduction

Humans must frequently evaluate the amount of effort that must be invested for potential rewards. Research in this area of cost/benefit decision making has focused on the physical effort costs that discount lucrative outcomes, but recent work has emphasized the cognitive effort costs that are arguably more representative of the decisions individuals face in modern societies. Current theories conceptualize cognitive effort allocation as a neuroeconomic choice, whereby mental processes will only be allocated to a particular task if the expected value of the goal is high, thus offsetting the opportunity costs incurred from persevering with an effortful option in lieu of simpler, less rewarded alternatives (Shenhav *et al*, 2013; Westbrook and Braver, 2015). In this framework, cognitive effort costs are those that are non-physical in nature and tax limited executive resources, as observed through psychological constructs such as working memory, attention and response inhibition (Schmidt *et al*, 2012). While such allocation processes may seem trivial when considering the routine decisions of day-to-day life, the consequences of not applying cognitive effort can be quite severe, as observed by the angeria, avolition, and impairments in effort

allocation inherent to conditions such as depression and schizophrenia (Culbreth *et al*, 2016a; Duijn *et al*, 2014; Gold *et al*, 2013; Treadway *et al*, 2009).

Two areas known to be critical for cost/benefit decision making are the anterior cingulate cortex (ACC) and basolateral amygdala (BLA). Patients with lesions to either area display deficits in tests of real-world decision making, in which the magnitude of rewards and costs are varied across options (Bechara *et al*, 1999; Brand *et al*, 2007; Manes *et al*, 2002; Naccache *et al*, 2005). Single unit recordings similarly suggest that BLA and ACC neurons may register reward, cost, and value information which could be used to guide choice behavior (Belova *et al*, 2008; Jenison *et al*, 2011; Shidara and Richmond, 2002). Regarding effort-based decision making, stimulation of the ACC can elicit a feeling of "gearing up" for an effortful challenge, whereas lesions to this region can abolish conscious feelings of mental effort (Mulert *et al*, 2005; Naccache *et al*, 2005; Parvizi *et al*, 2013). Functional imaging studies also demonstrate coordinated activation in the ACC in anticipation of effort exertion (Botvinick *et al*, 2009; Kurniawan *et al*, 2013; Vassena *et al*, 2014) and signals encoding the subjective value of a physically or cognitively effortful option have been observed in both the ACC and BLA (Chong *et al*, 2017; Massar *et al*, 2015; Prevost *et al*, 2010).

Recently, we have demonstrated a causal role for the ACC and BLA in the processes by which rodents must decide how to allocate attentional effort for lucrative outcomes, by using a rodent model of cost/benefit decision making

known as the rat Cognitive Effort Task (rCET) (Hosking *et al*, 2014a). The task is a modified version of the standard 5-choice serial reaction time task (Bari *et al*, 2008), in which rats can decide at trial outset whether to respond for a visuospatial stimulus that is either easy (1s) or hard (0.2s) to detect across five possible locations, and where successful completion of easy or hard trials results in one or two sugar pellets, respectively (Cocker *et al*, 2012b). Bilateral inactivations of the ACC uniformly decrease choice of the high-effort/high reward option (.2 s stimulus/ 2 pellet reward; HR), thus mimicking the effect ACC perturbations have on rodent tasks of physical effort which require rats to scale a barrier (Rudebeck *et al*, 2006; Schweimer, 2005; Walton *et al*, 2003b) or repeatedly press a lever (Hart *et al*, 2017; Walton *et al*, 2009) to access larger reward. In contrast, BLA inactivations move subjects away from their propensity to “work” or “slack” at baseline on the rCET: rats initially preferring the hard option at baseline decrease selection of this option, whereas rats opting for the easier of the two options at baseline now increase hard lever choice following BLA inactivation (Hosking *et al*, 2014a). These findings are in contrast to the observation that BLA inactivation decreases choice of physically effortful options across rats (Floresco and Ghods-Sharifi, 2007; Ghods-Sharifi *et al*, 2009b; Hart and Izquierdo, 2017). These nuances in BLA contributions to choice involving the evaluation of different forms of effort are particularly interesting given recent imaging work in humans suggesting that activity in the right amygdala appears to process subjective value uniquely for rewards associated with cognitive, but not physical effort costs (Chong *et al*, 2017).

However, interpreting the contributions of these regions to effort-based decision making in isolation lacks external validity (Floresco *et al*, 2008a). Rather, it is important to assess how different brain areas, such as the ACC and BLA, communicate to bias choice towards options differing in cognitive effort. Imaging work has provided some insight into the pathways regulating effort allocation, but these findings are correlational and have exclusively focused on corticostriatal pathways (Botvinick *et al*, 2009; Croxson *et al*, 2009; Schmidt *et al*, 2012). A previous study has demonstrated that contralateral disconnection of the BLA-ACC pathway decreases rats' willingness to scale a barrier for larger reward, suggesting that under normal conditions this circuit biases subjects towards lucrative, physically effortful options (Floresco and Ghods-Sharifi, 2007). However, given differences in the role of the BLA in physical versus cognitive effort, and growing appreciation that these manifestations of effort are regulated by dissociable neural mechanisms, it is important to address the role of the BLA-ACC signaling to decisions with cognitive effort costs (Hosking *et al*, 2014b).

The current study utilizes a disconnection procedure to assess how ACC-BLA communication regulates aspects of cognitive effort allocation. Specifically, unilateral inactivation of the ACC is paired with unilateral inactivation of the contralateral BLA, thus disrupting serial communication between these structures in each hemisphere. Variations of this technique have been employed to successfully disrupt serial transfer of information between brain regions in both rodents (Chudasama *et al*, 2003a; St. Onge *et al*, 2012; Zeeb and Winstanley, 2013) and nonhuman primates (Baxter *et al*, 2000; Eldridge *et al*, 2015). We

hypothesized that BLA-ACC disconnection would mimic the effects of bilateral BLA inactivation on choice, suggesting that the BLA may send information about rats' subjective preference for easy or difficult mental challenges to the ACC, which then uses this information to compute whether a lucrative outcome is worth the effort (Shenhav *et al*, 2013).

To preview, we failed to confirm this hypothesis. In the course of carrying out the inactivations, we discovered that a single unilateral inactivation of the BLA was sufficient to affect cognitive effort allocation in a subset of rats. Although this finding precludes a discussion of how BLA-ACC circuitry regulates cognitive effort allocation using the current design, it does suggest that the BLA plays a critical role in this type of decision making.

## 6.2 Additional Methods

### 6.2.1 Baseline Choice

Thirty-seven Long-Evans rats were used in the currently study. For this particular experiment, the mean choice of the HR option across rats was 69% following post-operative baseline. Animals were grouped as "workers" if they chose the HR option for >70% of trials (n= 20) and as "slackers" if they chose HR for ≤70% of trials (n= 17), as per previous work (Cocker et al., 2012), thereby enabling consistency when discussing individual differences across studies.

### 6.2.2 Guide Cannulae Implantation

Thirty-seven rats were run on the free choice version of the rCET daily, at which point their behaviour was stable for at least three sessions (session main effect and interactions $p > .05$ when a repeated measures measures ANOVA was carried out on the choice, accuracy, and premature responding variables). Animals were anesthetized with 2% isoflourane in $O_2$ and administered .1 ml of 5 mg.ml anafen and 10 ml lactated ringer subcutaneously. Rats were then secured in a stereotaxic frame (David Kopf) with the incisor bar set at -3.3. When a surgical plane of anesthesia was reached, rats were implanted with guide cannulae targeting the ACC or BLA. Twenty-two gauge bilateral cannulae were used to target the ACC in both hemispheres, whereas a single 22-gauge cannula was aimed at either the left or right BLA. The hemisphere chosen for BLA cannulation was counterbalanced across rats, accounting for worker/slacker and hard lever (left or right) designations to ensure a similar number of left or right BLA were targeted across these between-subjects factors. The coordinates (Paxinos and Watson, 1998) for ACC and BLA cannulation were as follows: ACC: anterioposterior (AP) = + 2.0 mm, mediolateral (ML) = $\pm$ .75 mm from bregma, dorsoventral (DV) = - .8 mm from dura; BLA: AP = -3.1 mm, ML = $\pm$ 5.2 from bregma, DV: - 6.5 mm from dura. These coordinates are similar to the ones used previously to independently target these structures on the rCET (Hosking *et al*, 2014a). Cannulae were fixed to the skull by 3-4 stainless steel screws and dental acrylic. Obdurators flush with the cannulae tip were inserted to prevent clogging prior to testing. Animals recovered for at least 5 days before resuming behavioural testing.

### *6.2.3* Disconnection Design

Disconnections are a sophisticated method to assess the functional interaction between two brain regions. They rely on the assumption that information flows serially from one area to an efferent region in both hemispheres in parallel. In this procedure, a structure in one hemisphere is lesioned or inactivated, and the corresponding brain region in the functional circuit is similarly lesioned or inactivated in the alternate hemisphere. For example, if decision making on the rCET is dependent on the serial flow of information from the BLA to the ACC, then inactivating the BLA in one hemisphere will prevent any signals from being generated and sent to the ipsilateral ACC. In the alternate hemisphere, the BLA is able to generate and send information to the ipsilateral ACC, but the ACC is offline and thus unable to act on those incoming signals. Thus, while there is an intact BLA and ACC in each hemisphere to carry out the functions dependent on those structures, the functional interaction between them is compromised.

Also implicit to a disconnection design is the assumption that ipsilateral inactivation of a structure alone will not have an effect on behaviour. To address this possibility, a condition is included where both ipsilateral structures are inactivated, because the intact ipsilateral circuit should be able to compensate for the unilateral disruption of function. Finally, in order for a disconnection to be successful, contralateral connections between structures should be minimal. In regards to the pathway of interest, the ACC sends both ipsi-and contralateral

projections to the rostral portions of the BLA, whereas efferents from the BLA to the ACC are primarily ipsilateral. Thus, any disruption observed following this disconnection is likely mediated by the serial transfer of information from the BLA to the ACC, given the procedure will spare projections from one ACC to the contralateral BLA. The positioning of the BLA inactivation towards its caudal pole would also block signal flow from the BLA, while leaving ACC inputs at the rostral pole intact. However, while the available evidence suggests that any behavioural effects observed following the contralateral disconnection are likely due to impaired information flow from the BLA-ACC, we cannot preclude the involvement of top-down ACC –> BLA signaling with the current design.

In accordance with the disconnection procedure described above, each rat will receive three sets of infusions: 1) unilateral saline infusion into the ACC and into the contralateral BLA (control); 2) unilateral inactivation of the ACC and BLA ipsilaterally (ipsilateral inactivation control; and 3) unilateral inactivation of the ACC and the contralateral BLA (disconnection).

### 6.2.4 Microinfusion

Following recovery from surgical implantation of cannula, rats performed an additional 10 baseline rCET sessions, at which point their behaviour was deemed stable (see *Data Analysis*). Rats were habituated to the microinfusion process via two mock infusions. During these mocks, dust caps and obdurators were removed, 28-gauge injectors extending 1 mm below the cannula tip were inserted, but no infusion was performed. The injectors were left in place for two

minutes before being removed and replaced with obdurators. Ten minutes following the mock infusion, rats began an rCET session. One rat got sick following their second mock infusion, leaving n= 36 (n = 19 workers, n = 17 slackers) rats who moved on to the infusion stage.

Inactivations adhered to a three-day cycle, consisting of a baseline session, a test session where rats received intracerebral infusion or drug, followed by a washout day in which rats were not run. Inactivations were achieved using a mixture of the $GABA_B$ agonist baclofen and the $GABA_A$ agonist muscimol (Sigma–Aldrich), prepared separately at 0.5 μg/μL in saline, and mixed together in equal volumes to form a 0.25 μg/μL solution. 0.5 μL per hemisphere injections of saline or baclofen/muscimol (i.e. 0.125 μg of drug per hemisphere) were administered at a rate of 0.4 μL/min, and injectors (extending +1mm from cannula tips) were left in place for an additional minute to allow diffusion. Once the microinfusions were completed, injectors were removed, obdurators replaced, and animals were returned to their home cages for 10 min before being placed in the operant chambers and performing the rCET.

In keeping with the disconnection protocol described above, each rat received three sets of infusions: 1) unilateral saline infusion into the ACC and into the contralateral BLA (control); 2) unilateral inactivation of the ACC and BLA ipsilaterally (ipsilateral inactivation control; and 3) unilateral inactivation of the ACC and the contralateral BLA (disconnection). The order of these infusions was counterbalanced across rats. Immediately after these infusions, rats received an additional three sets of infusions, the order of which was counterbalanced across

rats. These included a 1) unilateral BLA inactivation, a 2) unilateral left ACC inactivation, and a 3) unilateral right ACC inactivation. The three-day cycle (baseline, infusion, washout) was similarly carried out with these infusions.

### 6.2.5 Histology

When behavioural testing terminated, animals were anesthetized with 2% isoflurane in $O_2$ and then euthanized via $C0_2$. Brains were extracted and submerged in 4% paraformaldehyde for 24h, before being transferred to a 30% sucrose solution where they remained until sectioning. Brains were frozen and cut into 40-μm coronal sections encompassing the ACC and BLA were obtained using a cryostat. The sections were placed on gelatin-coated slides and stained with cresyl violet for visualization. The projected locations of the injectors tips protruding from the guide cannulae were mapped onto the coronal section images from Paxinos and Watson (1998)

### 6.2.6 Data Analysis

Data analysis was carried out as described in Chapter 2. However, ipsilateral and contralateral ACC-BLA inactivation caused some rats to complete less than ten trials. Specifically, two rats in the ipsilateral condition and 9 rats in the contralateral condition completed less than ten trials, and so were excluded from the data analysis. Conducting a one-way repeated measures ANOVA across both conditions would exclude many rats from the entire analysis, so the contralateral and ipsilateral conditions were individually compared to the saline

condition.

## 6.3 Results

### 6.3.1 Cannula Placements

The locations of all acceptable ACC and BLA placements are shown in Figure 6.1. Given we used bilateral mounted cannulae to target the ACC, six placements were too lateral in one hemisphere and too medial in another. Four BLA placements were located dorsal to the BLA. When considered together, this meant 8 rats were excluded from the ipsilateral and contralateral analyses, leaving $n$ = 14 workers and $n$ = 14 slackers. In subsequent experiments where we analyzed the effects of unilateral ACC or BLA inactivation, rats were only excluded if they had missed placements in the area of interest.

### 6.3.2 Baseline rCET Behavior

#### 6.3.2.1 Choice, Accuracy, and Premature Responses

When infused with saline, rats as an entire group showed a preference for the high-effort/high-reward (HR) option compared to the low-effort/low-reward option (saline only- choice: $F(1, 27) = 12.074$, $p = .002$). In keeping with their baseline categorization, workers ($M$ = 85.74%) chose the HR option significantly more than slackers ($M$ = 44.96%) (saline only- group: $F(1, 26) = 83.730$, $p < .001$).

Accuracy for LR trials was significantly higher than accuracy for HR trials, suggesting that the HR trials were indeed more demanding (saline only- choice: $F(1, 26) = 131.055$, $p < .001$). While a repeated measures ANOVA did not suggest a difference in HR trial accuracy between workers and slackers (saline only- choice x group: $F(1, 26) = 0.223$, NS; group: $F(1, 26) = 2.822$, NS), there was a 10% difference in accuracy between groups (workers: $M = 63.62$, slackers: $M = 53.79$) in the saline condition. A follow-up one-way ANOVA comparing HR accuracy between groups suggests that this difference is significant (saline only-group: $F(1, 26) = 7.852$, $p = .009$). This finding is in contrast to all other investigations in the rCET, in which workers and slackers do not differ in their attentional ability. Thus, in the current study we cannot rule out the possibility that slackers' preference for LR trials is due to their impaired ability to successfully complete HR trials.

Premature responding did not differ between LR or HR trial types (saline only- choice: $F(1, 26) = 2.350$, NS) and groups did not differ at baseline on rates of premature responding (saline only- choice x group: $F(1, 26) = 1.362$, NS; group: $F(1, 26) = 0.533$, NS). This suggests that slackers' reduced choice of HR cannot be explained by an inability to wait for the .2s stimulus on HR trials.

#### 6.3.2.2 Other Behavioural Measures

When infused with saline, rats initiated about 130 trials on average, and this did not differ between worker and slacker rats (saline only-group: $F(1, 26) = 4.011$, NS). Following trial initiation and extension of both LR and HR levers, rats

typically made few choice omissions (~6 on average), with slackers omitting more at this phase compared to workers (saline only- group: $F(1, 26) = 4.791$, $p = .038$). Latencies to make a choice and to collect reward did not differ between trial types or groups (saline only- choice/ choice x group/ group: all Fs < 2.151, NS). Rats were generally quicker to make responses for HR trials, perhaps unsurprisingly given their shorter stimulus duration (saline only- choice: $F(1, 26) = 7.717$, $p = .010$). Response omissions following stimulus presentation were low, and this did not differ between groups (saline only- choice/ choice x group/ group: all Fs < 0.446, NS).

### 6.3.3 Contralateral and Ipsilateral Inactivation Analyses

#### 6.3.3.1 Trials Completed

We noticed that upon inactivating the ACC and BLA either ipsilaterally or contralaterally, some rats completed less than 10 total trials, and this appeared to be disproportionally higher following contralateral inactivation. Specifically, two slackers (/14) failed to complete at least ten trials in the ipsilateral condition, whereas in the contralateral inactivation condition this included four workers (/14) and five slackers (/14). Conducting a one-way repeated measures ANOVA across both conditions would thus exclude many rats from the entire analysis, so the contralateral and ipsilateral conditions were individually compared to the saline condition.

#### 6.3.3.2 Contralateral Inactivation Analyses: Choice, Accuracy, and Premature Responding

The following analyses are based on the 10 workers and 9 slackers who completed at least ten trials following contralateral ACC-BLA inactivation. When considered as a homogenous group, inactivation of the ACC and contralateral BLA did not affect choice of the HR option (inactivation: $F(1, 17) = .497$, NS). Rather, the effects of this inactivation condition depended on rats' worker/slacker status (inactivation x group: $F(1, 17) = 6.051$, $p = .025$). A simple main effects analysis revealed that contralateral ACC-BLA inactivation specifically decreased HR choice in workers, but not slacker rats (workers only- inactivation: $F(1, 9) = 4.840$, $p = .055$) (Figure 6.2). While a visual inspection of the data suggests that slackers' choice of HR increased following inactivation, this effect was not significant (slackers only- $F(1, 8) = 1.632$, $p = .237$). Contralateral ACC-BLA inactivation did not affect accuracy on LR or HR trials (inactivation/ inactivation x group: all Fs < 3.060, NS), nor did it affect measures of premature responding for either option (inactivation/ inactivation x group: all Fs < 1.779, NS) (Figure 6.2).

#### 6.3.3.3 Contralateral Inactivation Analyses: Other Behavioural Measures

Contralateral inactivation of the ACC-BLA significantly increased latencies to make a LR and HR choice across both groups (LR choice- inactivation: $F(1, 17) = 10.554$, $p = .005$, inactivation x group: $F(1, 17) = 0.009$, NS; HR choice- inactivation: $F(1, 17) = 4.658$, $p = .046$, inactivation x group: $F(1, 17) = 1.22$, NS), and increased choice omissions when both levers extended at trial onset

(inactivation: $F(1, 17) = 23.451$, $p < .001$; inactivation x group: $F(1,17) = 0.046$, NS) (Table 6.1). Latencies to make a correct HR response likewise increased across groups, but LR correct response latencies increased to a greater extent in worker rats (HR correct latency- inactivation: $F(1, 17) = 4.382$, $p = .052$, inactivation x group: $F(1,17) = 0.942$, NS; LR correct latency- inactivation: $F(1,17) = 18.405$, $p < .001$, inactivation x group: $F(1,17) = 4.369$, $p = .052$; workers only- inactivation: $F(1, 9) = 22.842$, $p < .001$; slackers only- inactivation: $F(1, 8) = 2.155$, NS). In contrast, latencies to collect reward on either trial type were unaffected (inactivation/inactivation x group: all $F$s < 1.905, NS). Following stimulus presentation, response omissions on LR trials were unaffected by ACC-BLA inactivation, but this manipulation did increase response omissions on HR trials (LR response omissions- inactivation/inactivation x group: all $F$s < 3.361, NS; HR response omissions- inactivation: $F(1, 17) = 22.409$, $p < 001$; inactivation x group: $F(1,17) = 0.391$, NS). Trials initiated and trials completed significantly decreased following contralateral ACC-BLA inactivation (trials initiated- inactivation: $F(1, 17) = 27.363$, $p < .001$, inactivation x group: $F(1, 17) = 0.000$, NS; trials completed- inactivation: $F(1, 17) = 44.412$, $p < .001$, inactivation x group: $F(1, 17) = 0.591$, NS) (Table 6.1).

#### 6.3.3.4 Ipsilateral Inactivation Analyses: Choice, Accuracy, and Premature Responding

The following analyses are based on the 14 workers and 12 slackers who completed at least ten trials following ipsilateral ACC-BLA inactivation. Similar to

the effects on HR choice in the contralateral condition, ipsilateral inactivation did not have any discernible effect on the entire group (inactivation: $F(1, 24) = 0.130$, NS). However, there was a trending inactivation x group interaction (inactivation x group: $F(1, 24) = 3.544$, $p = .072$), but subsequent simple main effects analyses were not significant, even though a visual inspection of the data suggests this condition had a similar effect on HR choice compared to the contralateral condition (workers only- inactivation: $F(1, 13) = 2.37$, $p = .147$; slackers only- inactivation: $F(1, 11) = 1.303$, $p = .278$) (Figure 6.3). In contrast to contralateral ACC-BLA inactivation, ipsilateral ACC-BLA inactivation decreased accuracy on both LR and HR trials across groups (LR trials- inactivation: $F(1, 19) = 7.759$, $p = .012$, inactivation x group: $F(1,19) = 0.016$, NS; HR trials- inactivation: $F(1, 22) = 6.649$, $p = .017$, inactivation x group: $F(1, 22) = 0.416$, NS), while rates of premature responding were unaffected (inactivation/ inactivation x group: all $F$s < 1.090, NS) (Figure 6.3).

#### 6.3.3.5 Ipsilateral Inactivation Analyses: Other Behavioural Measures

Latencies to make a choice increased for HR, but not LR, trials following ipsilateral ACC-BLA inactivation (HR trials- inactivation: $F(1, 23) = 7.845$, $p = .010$, inactivation x group: $F(1, 23) = 1.588$, NS; LR trials- inactivation/ inactivation x group: all $F$s < 1.343, NS) and so did the number of choice omissions following lever presentation (inactivation: $F(1, 24) = 25.998$, $p < .001$; inactivation x group: $F(1, 24) = 1.469$, NS) (Table 6.2). Latencies to make a correct response and to collect reward were not affected for LR trials

(inactivation/ inactivation x group: all *F*s < 3.091, NS), but these latencies increased on HR trials following ipsilateral ACC-BLA inactivation (correct latency- inactivation: $F(1, 21) = 5.415$, $p = .03$, inactivation by group: $F(1, 21) = 0.112$, NS; collect latency- inactivation: $F(1, 21) = 19.046$, $p < .001$; inactivation x group: $F(1, 21) = 1.228$, NS). Response omissions also increased for both trial types following stimulus presentation (LR trials- inactivation: $F(1, 20) = 8.102$, $p = .01$, inactivation x group: $F(1, 20) = 0.071$, NS; HR trials- inactivation: $F(1, 21) = 9.334$, $p = .006$, inactivation x group: $F(1, 21) = 0.721$, NS). Lastly, both trials initiated and trials completed significantly decreased following ipsilateral ACC-BLA inactivation (trials initiated- inactivation: $F(1, 24) = 33.065$, $p < .001$; inactivation x group: $F(1, 24) = 1.318$, NS; trials completed- inactivation: $F(1, 24) = 54.966$, $p < .001$; inactivation x group: $F(1, 24) = 0.464$, NS) (Table 6.2).

### 6.3.4 Follow-up Unilateral Analyses

A core assumption of a disconnection design is that ipsilateral inactivation should have minimal effects on behavior, given communication between two areas is still intact. Additionally, unilateral inactivation of a brain region should not affect behaviour, because the intact structure in the opposite hemisphere is still on-line to mediate the processes normally carried out by the structures bilaterally. Our observation that ipsilateral inactivation caused a trending choice impairment that mirrored the effects of the contralateral condition prompted us to ask whether these effects are primarily due to a unilateral inactivation of the ACC or BLA. We subsequently infused the BLA, left ACC, and right ACC unilaterally, and

tested rats on the rCET. We recoded the left and right ACC data to correspond to whether it was the ACC targeted in the original ipsilateral or contralateral conditions. Two rats suffered health complications from prior infusions, and so could not contribute data to any of these subsequent conditions. Additionally one rat completed less than 10 total trials in each of the unilateral BLA, ipsilateral ACC and contralateral ACC conditions, while an additional rat in each of the ipsilateral and contralateral unilateral ACC conditions experienced cannula blockages and thus data could not be obtained. For this reason each dose was individually compared to the original saline condition, in which the BLA and contralateral ACC were infused with saline. The sample size for each analysis is as follows: unilateral BLA inactivation (n = 14 workers, 15 slackers), unilateral ipsi-ACC inactivation (n = 15 workers, n = 12 slackers), and unilateral contra-ACC inactivation (n = 15 workers, n = 12 slackers).

#### 6.3.4.1 Effects of Unilateral Inactivations on Choice

Like the choice profiles observed in the ipsilateral and contralateral inactivation conditions, unilateral BLA inactivation did not affect choice as a homogenous group, but differentially affected rats based on their worker-slacker status (inactivation: $F(1, 27) = 2.07$, NS; inactivation x group: $F(1, 27) = 4.716$, $p = .039$. Specifically, workers' choice of the HR option decreased, while slackers' choice of HR was unaffected (workers only- inactivation: $F(1, 13) = 7.485$, $p = .017$; slackers only- inactivation: $F(1, 14) = .243$, NS). This unilateral effect persisted when looking specifically at rats included in the contralateral ACC-BLA

inactivation condition (inactivation: $F(1, 15) = 0.338$, NS; inactivation x condition: $F(1, 15) = 5.039$, $p = .040$; workers only- $F(1, 8) = 4.567$, $p = .065$, slackers only – $F(1, 7) = 1.209$, NS), as well as rats included in the ipsilateral ACC-BLA inactivation condition (inactivation: $F(1, 22 = 0.366$, NS; inactivation x condition: $F(1, 22) = 8.197$, $p = .009$; workers only- $F(1, 12) = 6.630$, $p = .024$, slackers only – $F(1, 10) = 2.324$, NS). In contrast, unilateral inactivation of the ACC did not affect choice, regardless of it whether it was the ACC targeted ipsilateral or contralateral to the BLA (inactivation/ inactivation x group: all $F$s < .400 NS). Unilateral ACC inactivation similarly did not affect choice when we re-ran the analyses according to whether the left or right ACC was targeted (inactivation/ inactivation x group: all $F$s < .751, NS). A comparison of the three choice profiles - unilateral BLA, unilateral ipsi-ACC, and unilateral contra-ACC -is provided in Figure 6.4.

#### 6.3.4.2 Effects of Unilateral BLA Inactivation on Other Measures

We focus on the general effects of unilateral BLA inactivation given this is the only unilateral inactivation that affected HR choice. BLA inactivation impaired accuracy on LR, but not HR trials (LR trials inactivation: $F(1, 26) = 11.511$, $p = .002$, inactivation x group: $F(1, 26) = 0.64$, NS; HR trials- inactivation, inactivation x group: all $F$s < 1.00, NS), and did not affect measures of premature responding (inactivation/ inactivation x group: all $F$s < 2.232, NS). Latencies to make a HR choice increased, but LR choice latencies were unaffected (HR trials – inactivation: $F(1, 26) = 5.749$, $p = .024$; inactivation x group: $F(1, 26) = .803$, NS;

LR trials- inactivation/ inactivation x group: all *F*s < 1.906, NS), and otherwise latencies to make a correct response and collect reward were not changed following unilateral BLA inactivation (all *F*s < 3.00, NS). Response omissions did not change, but choice omissions increased significantly (choice omissions: inactivation: $F(1, 26) = 9.935$, $p = .004$, inactivation x group: $F(1, 26) = 2.207$, NS; LR and HR trial response omissions: all *F*< 1,273, NS). Lastly, unilateral BLA inactivation decreased the number of trials initiated, but did not affect trials completed (trials initiated- inactivation: $F(1, 27) = 4.413$, $p = .045$, inactivation x group: $F(1,27) = 0.267$, NS; trials completed- inactivation/ inactivation x group: all *F*s < 2.56, NS). A summary of these behavioural results is provided in Table 6.3.

## 6.4 Discussion

The purpose of this study was to investigate whether BLA-ACC signaling contributes to decision making where cognitive effort must be exerted in pursuit of lucrative rewards. This was based in part on previous work with the rCET, in which bilateral inactivations of the basolateral amygdala (BLA) and anterior cingulate cortex (ACC) affect this form of decision making independently (Hosking *et al*, 2014a), and also by related work showing that BLA-ACC signaling regulates effort allocation in the physical domain (Floresco and Ghods-Sharifi, 2007). Following unilateral inactivation of the ACC and contralateral BLA, intended to produce a functional disconnection between these structures, we observed a decline in choice of the high-effort/high-reward option selectively in

"worker" rats inherently displaying a preference for this option at baseline. However, when we carried out ipsilateral inactivations of the ACC and BLA, a condition intended to act as a control, we observed an identical shift in HR choice in worker rats. Subsequent unilateral inactivations of the ACC and BLA revealed this effect on choice could be entirely attributed to a unilateral inactivation of the BLA. Although this finding precludes a discussion of how BLA-ACC circuitry regulates decision making with cognitive effort costs, it reinforces the critical role of BLA signaling in valuations of cognitive effort. Indeed, as will be discussed below, this adds to a growing literature suggesting that the BLA may play a unique role in decision making with cognitive effort costs, over and above its role in decision making in the physical effort domain.

Before discussing the observed effects on choice as an aberration in decision-making processes, it is important to consider alternative explanations. For one, the inactivations we carried out in this study, and in particular the unilateral BLA inactivation, could have produced indifference between options, as if rats were unable to recall the contingencies associated with each lever. This seems unlikely, given workers' choice of the HR option was still well above 50% following inactivation. It is also possible that the inactivations reduced motivation for sucrose reward. Pre-feeding before the task decreases trials completed, decreases HR choice, increases collect latencies, and impairs accuracy (Cocker *et al*, 2012b). However, we did not see a similar profile following any of our inactivations, and collect latencies – considered indirect measures of motivation (Robbins, 2002) - were generally unaffected. This also seems unlikely

considering other studies showing that inactivation or lesion of the amygdala and ACC do not affect preference for larger sucrose rewards in similar tasks (Ghods-Sharifi *et al*, 2009b; Rudebeck *et al*, 2006).

One notable limitation in the current study is that slackers' accuracy on HR trials was significantly lower than their worker counterparts. Indeed, this is the first rCET study where accuracy on the HR lever fell below 60%. Thus, it is possible rats' reduced choice is not just based on their aversion to cognitive effort costs as previously established (Cocker *et al*, 2012b), but also by their fundamental inability to successfully perform the HR trials. This is important to consider when discussing the role of the BLA in cognitive effort allocation, because we have previously shown that this region may regulate subjective preference to "work" or "slack" at baseline (Hosking *et al*, 2014a). Indeed, a visual inspection of the contralateral and ipsilateral inactivation data, as well as the unilateral BLA inactivation data, appears to replicate the effects of bilateral BLA inactivation in slackers, but this was not significant. Given the LR response option might now be the objectively better option for some slackers, this might have precluded BLA involvement in this form of decision making. In the ensuing discussion, it is important to consider whether a unilateral BLA inactivation would have had an effect on slacker rats had their accuracy been similar to previous cohorts.

Inherent to a disconnection design is the assumption that unilateral inactivation of a structure will have minimal to no effect on behaviour, given there is still an intact structure in the alternate hemisphere capable of carrying out its

normal functions. Along these lines, ipsilateral inactivation of two connected brain regions in one hemisphere should have minimal impact, given serial transfer information is unobstructed in the other hemisphere. Following unilateral inactivation of the ACC and contralateral BLA, intended to produce a functional disconnection of BLA-ACC signaling, choice of the HR option decreased in worker rats, while slackers' choice of HR appeared to increase, though this was not significant. However, a similar pattern of effects was observed following ipsilateral inactivation of the BLA and ACC. While there is some evidence that interhemispheric inactivations of the BLA and striatal or cortical regions can have effects on behaviour (Fuchs *et al*, 2007; Lasseter *et al*, 2014; St. Onge *et al*, 2012), this is generally rare. Rather, the similar effect on HR choice across ipsi- and contralateral inactivation groups could also reflect a mass action effect (Lashley, 1931), where the magnitude of the behavioral impairment is related simply to the volume of tissue damage, regardless of its location. However, this account is unlikely, as a single unilateral BLA inactivation was sufficient to replicate the effects observed in the contralateral and ipsilateral inactivation conditions.

Notably, the magnitude of the unilateral BLA effect (~12.5% decline in choice HR) was almost identical to that observed following contra- or ipsilateral ACC-BLA inactivation (~13%). This was specific to unilateral BLA inactivation, as unilateral inactivations of the ACC were without effect. This is a rare example of unilateral BLA disruption of function, and to our knowledge is the first demonstration of a decision-making deficit induced by unilateral BLA

perturbation. Previous work has established a number of functions that are impaired when a single BLA is inactivated or lesioned, but most of this work has been in the domain of Pavlovian conditioning or simple instrumental responding. For example, unilateral pre-training BLA lesions partially attenuate fear conditioning to cues (LaBar and LeDoux, 1996), while post-training lesions abolish contextual conditioned fear in rats (Flavell and Lee, 2012). Deficits in PPI are also observed following unilateral BLA inactivation (Forcelli *et al*, 2012), and this manipulation attenuates context-induced renewal of Pavlovian-conditioned alcohol seeking (Chaudhri *et al*, 2013). Unilateral BLA perturbation also disrupts numerous aspects of reward processing, including reinforcer devaluation in monkeys (Izquierdo, 2004), as well instrumental learning and reward memory in rodents (Baldwin *et al*, 2000; Coleman-Mesches *et al*, 1996).

The effects here are in stark contrast to the effects of unilateral BLA inactivation in the related domain of physical effort allocation, in which BLA-ACC signaling biases rats towards larger reward obstructed by a scalable barrier (Floresco and Ghods-Sharifi, 2007). In this study unilateral inactivation of the BLA with lidocaine caused a slight, nonsignificant decrease in choice of the HR option, but the magnitude of this effect was much smaller than the choice shift observed following functional BLA-ACC disconnection. This is interesting considering the differential roles the BLA appears to play in either form of decision making. While bilateral BLA inactivations uniformly decrease willingness to exert physical effort across rats, regardless of whether the effort is defined by barrier climbing or lever pressing (Floresco and Ghods-Sharifi, 2007; Ghods-

Sharifi *et al*, 2009a), the effects of BLA inactivation on cognitive effort allocation appear to depend on rats' baseline preference to "work" or "slack". Specifically, workers decrease their choice of the high-effort/high (HR) reward option, while slackers increase HR choice following inactivation (Hosking *et al*, 2014a). A visual inspection of the current data suggests unilateral inactivation of the BLA reproduced the effects of bilateral BLA inactivation on rCET choice, but the choice shift was not significant in the slacker group (possible reasons for this were described above). It is possible the unilateral BLA inactivation was effective in producing a similar choice profile via directly or indirectly downregulating the contralateral, intact BLA. While diffuse interamygdaloid connectivity has been observed in rats via the anterior commissure, a previous study showed that unilateral lesion of the BLA does not affect measures of fos activity in the contralateral BLA (Flavell and Lee, 2012). Alternatively, the effects might reflect a functional breakdown in the intra- and interhemispheric projections originating from the BLA. Regardless of the mechanism, BLA signaling appears to play a more prominent role in valuations of effort in the cognitive domain, as both nuclei are required for optimal effort allocation to occur.

This regulation of cognitive effort is also interesting considering recent imaging data showing that while a network of structures appear to process the subjective value of effortful options (including the dACC, dmPFC, dlPFC, intraparietal sulcus, and anterior insula), the amygdala appears to uniquely encode the subjective value of rewards discounted by cognitive, but not physical effort (Chong *et al*, 2017). The same study also found distinct computational

functions encode the subjective value of rewards discounted by physical or cognitive effort costs (Chong *et al*, 2017). Together, the current and previous work on the rCET support the notion that the BLA might specifically encode the subjective value of lucrative outcomes discounted by mental effort. In contrast, rather than supporting subjective value computations in the domain of physical effort, BLA involvement in rodent physical effort tasks may preferentially encode expected reward, thus biasing rats towards the high effort, high reward response option. This appears to be true given the effects of BLA inactivations on rodent decision-making tasks appear to depend on the nature and severity of the costs (Winstanley and Floresco, 2016). If costs are very aversive, such as signaled punishing timeouts or electric shock, the BLA appears to attenuate baseline choice of the more aversive option (Orsini *et al*, 2015a; Zeeb and Winstanley, 2011). In contrast, when rewards are especially salient relative to costs, such as omission of reward receipt, delays, or exerting physical effort, then the BLA appears to drive choice towards more rewarding options (Ghods-Sharifi *et al*, 2009a; Winstanley, 2004). Comparatively, the physical effort costs employed in rodent tasks may not be considered severe, perhaps because rats are always guaranteed reward following effort expenditure. In contrast, this may explain the divergent effects of BLA inactivation on choice in the rCET, whereby the rewards associated with successful trial completion are more salient in workers, but cognitive costs are especially aversive in slacker rats. This hypothetical role for the BLA in cost/benefit decision making may be mediated by distinct neuron populations shown to encode positive and negative value, as well as by distinct

circuits processing positive (BLA-NAc) or negative (BLA-CEA) associations (Belova *et al*, 2007, 2008; Namburi *et al*, 2015; Paton *et al*, 2006).

While we are the first group to show a unilateral BLA contribution to value-based decision making, we are not the first to show a lateralized cognitive deficit resulting from unilateral silencing of a structure in the domain of effort allocation. Croxson and colleagues demonstrated that a unilateral mPFC lesion, encompassing the prelimbic (PL), infralimbic (IL), and anterior cingulate cortices (ACC), was sufficient to decrease HR arm choice in the effort T-maze paradigm (Croxson *et al*, 2014). This effect is likely driven by an interaction of the PL/IL and ACC lesions, as selective lesions of the PL/IL do not affect decision making in this paradigm (Walton *et al*, 2003a), but other investigators have unilaterally lesioned or inactivated the ACC without effect (Floresco and Ghods-Sharifi, 2007; Hauber and Sommer, 2009). Notably, unilateral mPFC lesions were particularly effective at decreasing HR choice when the HR arm was contralateral to the lesion, suggesting that the mPFC may play an important role in evaluating the costs and benefits of actions directed to specific spatial locations (Croxson *et al*, 2014). We carried out a similar analysis, coding whether the BLA inactivation was ipsi- or contralateral to the HR option, but found no evidence that the inactivation interacted with the relative location of the HR option (data not shown).

While we cannot speculate on the role of ACC-BLA signaling in cognitive effort allocation given the nature of the unilateral BLA effect, it is worth noting that many rats in the contralateral inactivation condition did not engage with the task.

Specifically, nine rats completed less than 10 total trials following contralateral ACC-BLA inactivation, compared to two rats in the ipsilateral condition. It is difficult to say what is driving this profound inability to engage, but it is unlikely a motor or motivational impairment, considering effects on general latencies and omissions rates were more pronounced in the ipsilateral inactivation condition (see tables 6.1 and 6.2). It is also not due to an impaired ability to perform the attentional challenge, as accuracy and rates of premature responding were unaffected. Rather, this might reflect a fundamental deficit in willingness to apply effort generally, as has been previously described following functional disconnection of the ACC and BLA (Floresco and Ghods-Sharifi, 2007). It is worth noting that the design of the rCET necessarily makes it both a cognitively and physically demanding task; in a typical session, rats initiate approximately 130 trials in which they hit a lever, orient towards the stimulus array to detect the stimulus, then turn around to either collect reward or commence the next trial. If the BLA and ACC work in tandem to energize organisms towards lucrative outcomes discounted by an effortful challenge, then downregulating this circuit might preclude task rCET engagement.

In conclusion, we were unable to implicate ACC-BLA circuitry in the processes guiding decision making with cognitive effort costs. However, we discovered the BLA plays a powerful, albeit nuanced, role in guiding cognitive effort allocation.

## 6.5 Tables

| | LR - saline | LR - inactivation | HR - saline | HR - inactivation |
|---|---|---|---|---|
| **Choice omissions** | (For Session) | | 5.00 ± 1.32 (6.50 ± 1.40) | 17.00 ± 2.27* (13.46 ± 1.87) |
| **Correct latency (s)** | 0.58 ± 0.03 (0.57 ± 0.03) | 0.75 ± 0.04 (0.73 ± 0.04) | 0.47 ± 0.02 (0.47 ± 0.02) | 0.75 ± 0.13* (0.87 ± 0.17) |
| **Collect latency (s)** | 3.21 ± 1.23 (3.12 ± 1.17) | 2.20 ± 0.19 (2.19 ± 0.10) | 1.83 ± 0.29 (1.79 ± 0.27) | 2.60 ± 0.50 (2.65 ± 0.48) |
| **Response Omissions (%)** | 7.01 ± 2.65 (6.67 ± 2.54) | 11.67 ± 2.64 (11.09 ± 2.57) | 3.68 ± 1.06 (3.82 ± 1.04) | 18.90 ± 3.47* (24.25 ± 5.13) |
| **Trials Initiated** | (For Session) | | 129.42 ± 5.57 (127.71 ± 4.44) | 91.47 ± 7.63* (65.21 ± 8.99) |
| **Trials Completed** | (For Session) | | 102.18 ± 7.32 (98.41 ± 5.62) | 58.21 ± 6.85* (43.73 ± 6.89) |

**Table 6.1. Other rCET behavioural measures following contralateral ACC-BLA inactivation**

Means are presented (± SEM). Data in brackets shows means and SEM for all rats, including those excluded from analyses because they completed less than 10 trials. Values presented for choice omissions, trials initiated, and trials completed reflect data for saline and inactivation session.

$^{\#}p < .1$; * $p < .05$; ** $p < .001$

| | LR - saline | LR - inactivation | HR - saline | HR - inactivation |
|---|---|---|---|---|
| **Choice omissions** | (For Session) | | 5.65 ± 1.31 | 14.58 ± 1.88* |
| **Correct latency (s)** | 0.57 ± 0.02 (0.55 ± 0.02) | 0.84 ± 0.15 (0.84 ± 0.14) | 0.47 ± 0.02 (0.46 ± 0.02) | 0.75 ± 0.11* (0.75 ± 0.11) |
| **Collect latency (s)** | 3.11 ± 1.12 (3.09 ± 0.98) | 2.57 ± 0.35 (2.51 ± 0.33) | 1.51 ± 0.07 (1.49 ± 0.07) | 1.83 ± 0.06* (1.84 ± .08) |
| **Response Omissions (%)** | 7.20 ± 2.42 (6.60 ± 2.25) | 20.72 ± 3.87* (22.57 ± 4.57) | 5.41 ± 1.33 (5.41 ± 1.33) | 29.34 ± 4.09* (29.34 ± 4.09) |
| **Trials Initiated** | (For Session) | | 129.42 ± 4.55 (127.71 ± 4.44) | 83.12 ± 6.64* (78.29 ± 7.02) |
| **Trials Completed** | (For Session) | | 101.52 ± 5.55 (98.41 ± 5.62) | 56.42 ± 6.23* (52.75 ± 6.31) |

**Table 6.2. Other rCET behavioural measures following ipsilateral ACC-BLA inactivation**

Means are presented (± SEM). Data in brackets shows means and SEM for all rats, including those excluded from analyses because they completed less than 10 trials. Values presented for choice omissions, trials initiated, and trials completed reflect data for saline and inactivation session.

$^{\#}p < .1$; * $p < .05$; ** $p < .001$

| | LR - saline | LR - inactivation | HR - saline | HR - inactivation |
|---|---|---|---|---|
| **Choice latency (s)** | 3.44 ± 0.26 | 3.70 ± 0.26 | 3.13 ± 0.17 | 3.55 ± 0.17 |
| **Choice omissions** | (For Session) | | 6.48 ± 1.36 (7.03 ± 1.42) | 12.82 ± 1.61* (12.93 ± 1.56) |
| **Premature responses (%)** | 16.93 ± 3.48 | 11.18 ± 2.14 | 21.41 ± 3.29 | 15.86 ± 1.97 |
| **Accuracy (%)** | 88.49 ± 1.92 | 80.48 ± 2.34 | 59.79 ± 2.11 | 53.41 ± 2.82 |
| **Correct latency (s)** | 0.55 ± 0.02 | 0.59 ± 0.03 | 0.48 ± 0.02 | 0.53 ± 0.02 |
| **Collect latency (s)** | 2.87± 0.85 | 2.57 ± 0.65 | 1.66 ± 0.21 | 1.66 ± 0.11 |
| **Response Omissions (%)** | 7.00 ± 2.03 | 5.60 ± 1.39 | 5.43 ± 1.23 | 7.04 ± 1.99 |
| **Trials Initiated** | (For Session) | | 127.74 ± 4.24 (126.93 ± 4.17) | 113.97 ± 5.37* (110.87 ± 6.04) |
| **Trials Completed** | (For Session) | | 98.95 ± 5.43 (98.45 ± 5.27) | 87.69 ± 5.27 (84.90 ± 5.81) |

**Table 6.3. Other rCET behavioural measures following unilateral BLA inactivation**

Means are presented (± SEM). Data in brackets shows means and SEM for all rats, including the one rat that completed less than ten trials following unilateral BLA inactivation. This rat did not contribute individual lever data because they initiated less than five trials for each lever type. Values presented for choice omissions, trials initiated, and trials completed reflect data for saline and inactivation session. $^{\#}$ $p < .1$; * $p < .05$; ** $p < .001$

## 6.6 Figures

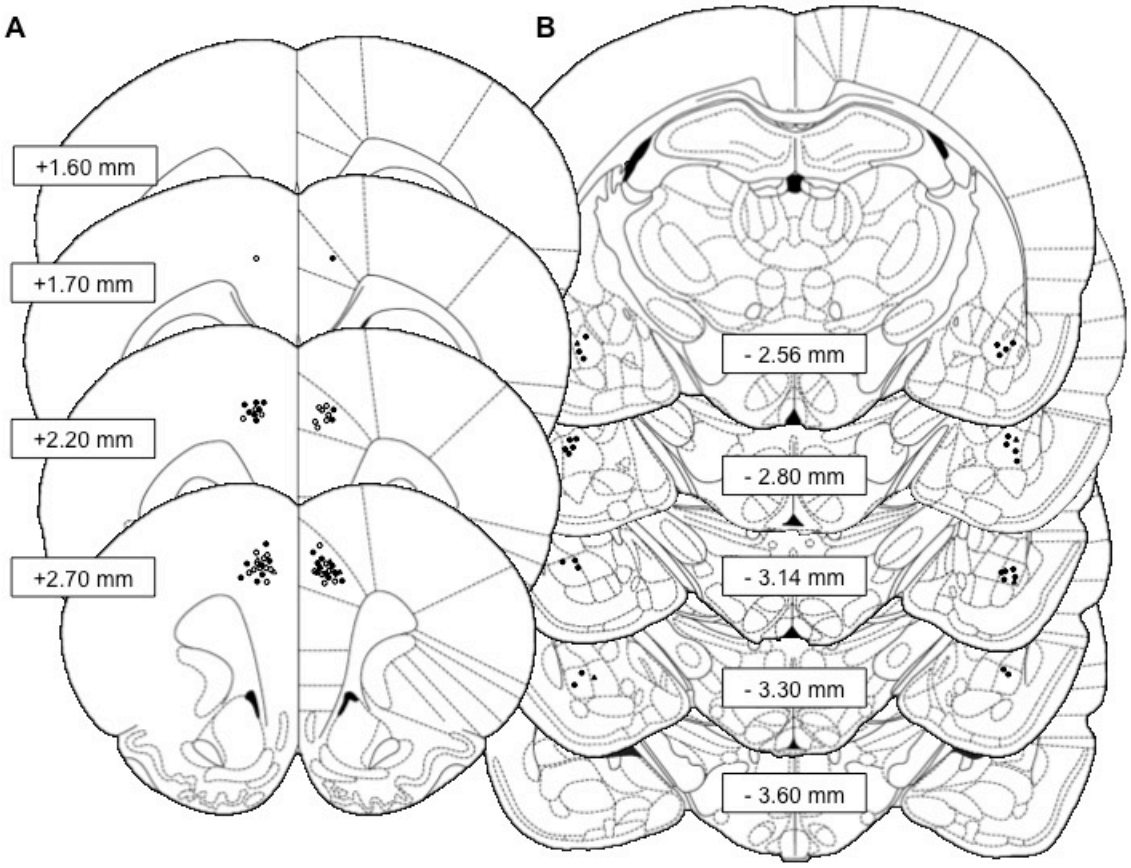


**Figure 6-1. Histological analysis of cannulae implantation**

Location of all acceptable a) anterior cingulate cortex (ACC) and b) basolateral amygdala infusions. On the ACC plates, white circles reflect ipsilateral infusions, while black circles are contralateral infusions. Black triangles are placements that were not acceptable for the ipsilateral/contralateral analyses, but were included in the unilateral analyses. Coordinates are relative to bregma. Plates modified from Paxinos and Watson (1998)

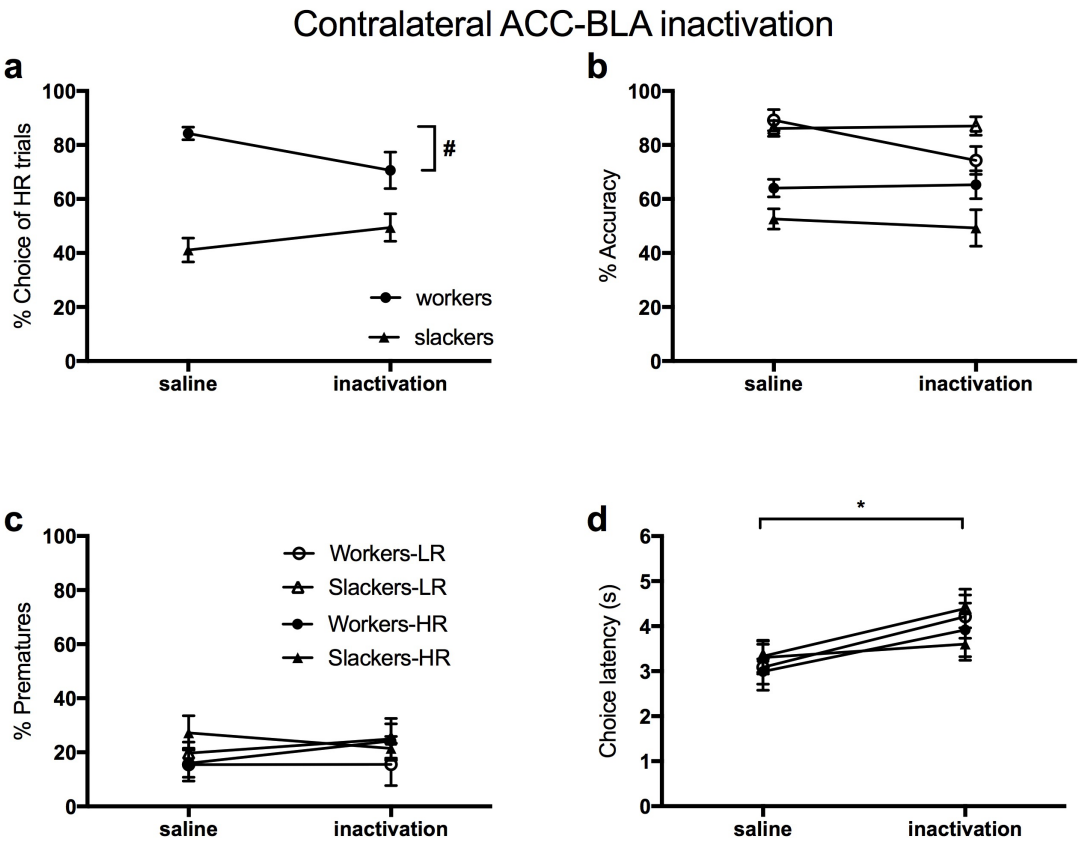


**Figure 6-2. Effects of contralateral ACC-BLA inactivation on (a) choice of HR trials, (b) accuracy (%), (c) premature responses, and (d) choice latencies**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. Contralateral ACC-BLA inactivation decreased HR choice in workers and increased choice latencies across groups.

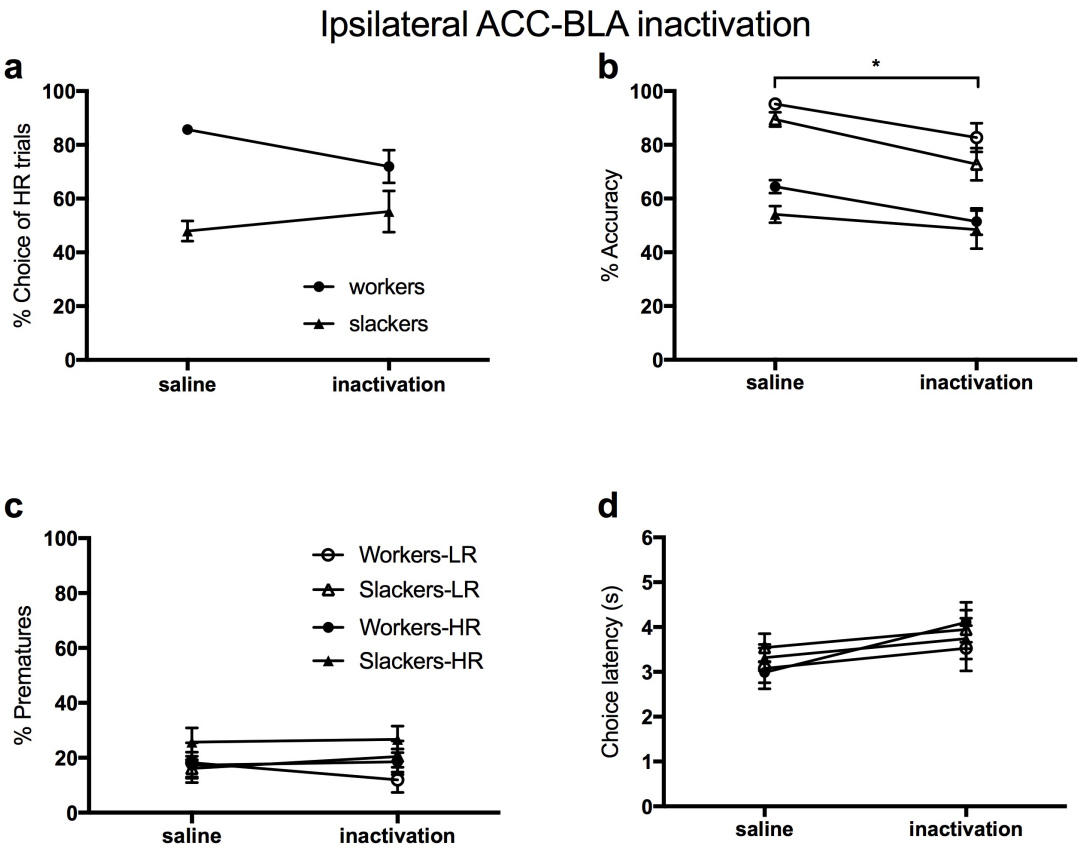


**Figure 6-3. Effects of ipsilateral ACC-BLA inactivation on (a) choice of HR trials (%), (b) accuracy (%), (c) premature responses (%), and (d) choice latencies**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. Ipsilateral ACC-BLA inactivation appeared to decrease HR choice in workers and increase HR choice in slackers, but this was not significant when the inactivation x group interaction was followed up with a simple main effects analysis. This condition also impaired accuracy for both trial types and increased HR choice latencies across groups.

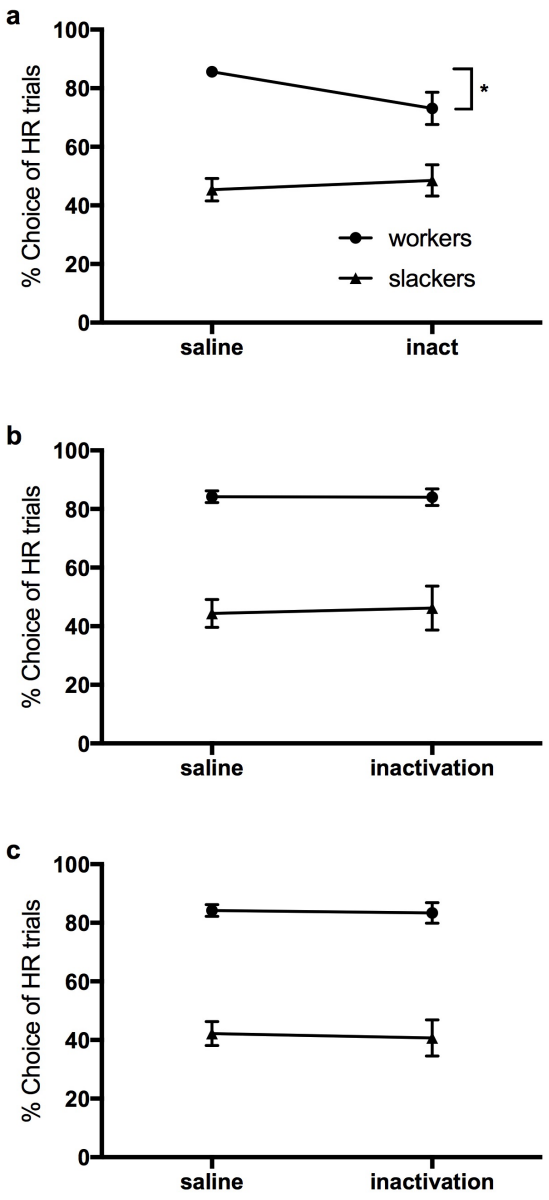


**Figure 6-4. Effects of (a) unilateral BLA, (b) unilateral IPSI-ACC, and (c) unilateral contra-ACC inactivation on choice of HR trials (%)**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial.

## Chapter 7: General Discussion

The purpose of the experiments described in the preceding chapters was to improve our understanding of the neural processes guiding the allocation of cognitive effort. This was primarily focused on specific brains regions, circuits, and neurotransmitter systems that have been studied in the related field of physical effort allocation, but which had not been studied in the cognitive domain. This limited a full comparison of the processes underlying both forms of effort allocation, and has often led to generalizations regarding the substrates guiding cognitive effort. To this end, I identified brain regions (i.e., OFC, NAc) that appear to play similar roles across effort types, others that play a more nuanced role in valuations of cognitive effort (i.e., BLA), and some which exclusively mediate decision making with cognitive effort costs (i.e., DMS). Together, these studies provide some of the first causal evidence of the neural processes regulating decision making with cognitive effort costs. My hope is this work will inform the corresponding human literature, and that it might motivate behavioural neuroscientists to develop preclinical models investigating effort allocation in other executive domains.

In experiment 1, I used chemogenetic techniques to temporarily donwregulate basal forebrain cholinergic neurons as rats performed the rCET. This manipulation did not affect performance on the rCET, even though multiple doses and timepoints were assessed. However, I was able to replicate a

previously observed effect of scopolamine of rCET choice, in which muscarinic receptor antagonism decreased high-effort/high-reward choice while leaving attentional processes intact (Hosking *et al*, 2014c) . Thus, cholinergic signaling appears to play an important role in rCET decision making, but the current experiments suggest that other cholinergic neuron populations are mediating these effects. I suggest that striatal cholinergic interneurons are a worthy target for future work on the rCET, given the role of the striatum in cognitive effort allocation, as identified in experiment 2.

In experiment 2, I showed that the striatum plays an integral role in performance of the rCET. Temporary inactivation of the dorsomedial striatum decreased choice of the HR option, whilst impairing accuracy and increasing impulsivity. In contrast, nucleus accumbens core inactivation precluded task engagement, which I suggest might reflect a profound unwillingness to engage with the rCET. Indeed, while this work complements human imaging work associating striatal areas with cognitive effort discounting (Botvinick *et al*, 2009; Schmidt *et al*, 2012), this is the first experiment to causally implicate the striatal regions in cognitive effort allocation.

The purpose of experiment 3 was to assess whether orbital frontal cortices contribute to the processes guiding rCET decision making. Temporary bilateral inactivation of this region did not affect decision making or attention on the rCET, but led to a pronounced increase in motor impulsivity and latency to make a decision. These results have important implications. For one, they establish specificity in the prefrontal regions regulating cognitive effort allocation; while

orbital areas do not appear play a role, the medial wall of the prefrontal cortex has been previously implicated in decision making on the rCET (Hosking *et al*, 2014a, 2015a). Additionally, this work shows that deficits in ability to perform the task – in this case a lack response control- are not always accompanied by shifts in decision making. This is in stark contrast to the effects observed following inactivation other prefrontal regions, which affect decision making while simultaneously impairing attention (i.e prelimbic cortex) or response control (i.e infralimbic, anterior cingulate cortex). Indeed, the results of experiment 3 confirm that, at the level of the prefrontal cortex, the processes guiding cognitive willingness and cognitive ability are dissociable.

Lastly, in experiment 4 I attempted to examine how cortical and limbic regions interact to guide decision making with cognitive effort costs, by functionally disconnecting the BLA and ACC while rats performed the rCET. However, proper investigation of BLA-ACC signaling was precluded by the observation that unilateral inactivation of the BLA was capable of affecting decision making in isolation. This is a surprising result, given few studies have reported disruption of behavioural function following unilateral lesion or inactivation of a brain structure. However, this is not the first study to report effects on decision making following unilateral inactivation (Croxson *et al*, 2014), and the results are in keeping with recent imaging work showing that the right BLA appears to encode the subjective value of options characterized by cognitive, but not physical, effort costs (Chong *et al*, 2017). Indeed, the results of the experiment 4 suggest that encoding in the BLA bilaterally strongly regulates

innate willingness to exert cognitive effort for lucrative outcomes.

**Comparing Effort Allocation Across Cognitive and Physical Domains**

The goal of the experiments in this thesis was to further characterize the neurobiology regulating decision making with cognitive effort costs, so that this type of effort allocation could be compared and contrasted with existing work in the physical effort literature. Indeed, the current experiments add support to the notion that cognitive and physical effort allocation are subserved by overlapping, albeit distinct, neural mechanisms. At the level of the prefrontal cortex, both forms of effort allocation appear to depend on neural activity in dorsal regions encompassing the anterior cingulate cortex (Hosking *et al*, 2014a; Rudebeck *et al*, 2006), while orbital regions appear to play a nominal role in either form of decision making (Rudebeck *et al*, 2006, Chapter 4). This finding lends further support for "dorsal-ventral" streams in decision making, by showing that costs intrinsic to the individual (i.e. physical or cognitive effort) are preferentially mediated by dorsal prefrontal cortical regions (Klein-Flugge *et al*, 2016; Rangel and Hare, 2010). In contrast, the prelimbic and infralimbic regions of the rodent prefrontal cortex appear to play a selective role in cognitive effort allocation (Hosking *et al*, 2015a), which is likely owing to the nature of the cognitive challenge, given these areas regulate the processes needed to successfully complete rCET trials (Chudasama *et al*, 2003b; Passetti, 2002). To the extent that the PL/IL regions are functionally analogous to the primate dorsolateral prefrontal cortex (reviewed in section 1.10.1), these findings fit with current

theories of cognitive effort allocation, which suggest that signalling between the ACC and dorsolateral prefrontal areas of the human PFC direct and intensify cognitive resources to maximize lucrative outcomes (Shenhav *et al*, 2013).

The striatum is another area this thesis has implicated in cognitive effort allocation, and is one area that appears to differentially regulate effort allocation depending on the nature of the effortful challenge. Decision making with physical effort costs appears to be exclusively mediated by the ventral striatum (and specifically the nucleus accumbens core region) (Ghods-Sharifi and Floresco, 2010). In contrast, I identified a possible role for the nucleus accumbens in deciding between options that differ in cognitive load, but also a prominent role for the dorsal striatum in mediating this process (Chapter 4). The preferential involvement of this region in cognitive effort allocation is likely related to the number of cortical inputs to this region from areas previously implicated in rCET decision making (i.e. prelimbic, infralimbic, and anterior cingulate cortices) (Hosking *et al*, 2014a, 2015a; Mailly *et al*, 2013).

Previously our lab has shown that the BLA plays a nuanced role in decision making with cognitive effort costs, such that inactivation of the region interacts with subjects' baseline preference to work or slack at baseline (Hosking *et al*, 2014a). This is in contrast to effort allocation in the physical domain, where inactivation of BLA decreases effortful choice across subjects (Ghods-Sharifi *et al*, 2009b). BLA-ACC signaling regulates rats' willingness to scale a larger barrier for larger reward, and I wanted to assess whether this circuit plays a similar role in biasing rats towards hard cognitive challenges. However, a proper assessment

of this pathway was precluded by the fact that a unilateral inactivation of the BLA was capable of affecting choice in a subset of rats preferring the high-effort/high-reward option at baseline. This partially replicates the effects of bilateral BLA inactivation, where choice of HR decreases in worker rats but increases in slackers, as if rats were moving away from their generally preferred option. The findings of experiment 4(Chapter 6) are in contrast to work with physical effort costs, where unilateral inactivation of the BLA Is without effect. Given the major difference between the two types of decision making is the type of cost, it appears then that intact bilateral BLA signaling is necessary for valuations of cognitive effort, which may reflect a specialized role for the BLA in subjective valuations of cognitive effort. Indeed, recent human imagining works suggests that activity in the right BLA encodes the subjective value of a choice with cognitive, but not physical, effort costs (Chong *et al*, 2017).

In Chapter 1 I carried out an exhaustive review of the neurotransmitter systems involved in either form of effort-based decision making. Some neurotransmitters, like the cannabinoid system, play similar roles regardless of whether the challenge is physical or cognitive (section 1.8.4), while others, such as noradrenaline and serotonin, do not appear to contribute to the cost/benefit computations regulating either form of effort allocation (Section 1.8.2, 1.8.6, and appendix 1). In contrast, some neurotransmitters appear to be preferentially involved in physical effort allocation, such as dopamine (section 1.8.1) and adenosine (1.8.5), whereas the cholinergic system appears to play a more prominent role in valuations of cognitive effort (sections 1.8.3). We attempted to

further characterize this role of acetylcholine in this form of decision making, by selectively downregulating cholinergic neurons of the basal forebrain using chemogenetics. This manipulation did not affect choice on the rCET, which I take as evidence that this neuron population is not critical for mediating decision making with cognitive effort costs (although in Chapter 3 I discuss potential limitations of using chemogenetics to target basal forebrain cholinergic neurons). However, I replicated the effect of scopolamine on rCET decision making, suggesting that these rats were sensitive to challenges of the cholinergic system. While I was unable to identify which cholinergic neuron population might regulate cognitive effort allocation, experiment 1 does help narrow down the targets for future studies (such as striatal cholinergic interneurons, as discussed in Chapter 3).

**Implications, Outstanding Questions, and Future Directions**

Aside from permitting a thorough comparison of the processes guiding cognitive and physical effort allocation, the current thesis experiments add to a relatively new literature studying valuations of cognitive effort (Westbrook and Braver, 2015). However, interest in decision making with cognitive effort costs is growing, as evidenced by the number of human behavioural paradigms that have recently been developed (section 1.1.2). Indeed, the number of cognitive allocation tasks now trumps the number of available physical effort discounting paradigms (reviewed in Section 1.1.1). However, only one animal model of decision making with cognitive effort costs currently exists, thus limiting causal

investigations of the neural mechanisms guiding this decision process. In the sections that follow I propose an expansion of the tasks developed to probe valuations of cognitive effort in rodents, and suggest avenues for future research that I believe will make important contributions to this area of decision making literature.

**Are the Processes Guiding Effort allocation Domain-Specific or Generalized?**

A core feature of the rCET is that it manipulates cognitive effort via titrating the attention required to detect a visuospatial stimulus. The task is a variant of the 5-choice serial reaction time task, and so there is a rich literature we can directly draw from when assessing how different manipulations affect decision making, attention, and response control (Chudasama and Robbins, 2006; Robbins, 2002). Another clear advantage of the rCET design is that there are no delay confounds between trial types, as latencies to reward delivery are similar. However, the rCET models only one type of effort, and it is interesting to consider whether different neurotransmitters and circuits would be recruited if another executive function were being taxed. Indeed, the human literature has models probing visuospatial attention, in addition to tasks manipulating working memory, cognitive flexibility, and response inhibition (Dixon and Christoff, 2012; Kool *et al*, 2010b; Massar *et al*, 2015; Westbrook *et al*, 2013). Generally speaking, the imaging literature suggests that valuations of cognitive effort are subserved by a similar network, including the ACC and dorsolateral prefrontal cortex (Chong *et*

*al*, 2017; Massar *et al*, 2015). However, some regions appear to be domain specific, such as the role of the BLA in encoding the subjective value of a difficult attentional challenge (Chong *et al*, 2017). This might be related to the role of the BLA in motivationally guided attention, via its connections to the basal forebrain as previously described (Peck and Salzman, 2014). Indeed, the BLA appears to plays a critical role in the evaluative processes guiding choice on the rCET, but this might be driven by the attentional nature of the cognitive challenge (Hosking *et al*, 2014a). Would the BLA still be implicated in cognitive effort allocation if a working memory challenge were used instead? This is an open question, but a review of the animal decision-making literature suggests it would play some role (Winstanley and Floresco, 2016).

To this end, I think it is imperative that other models be developed which assess cognitive effort allocation in other domains. For example, an operant task could be developed in which rats choose between easy or hard options that differ in the working memory load they require. In this task rats would nosepoke one of the five lit stimulus holes, orient towards the magazine and make a sustained nosepoke response, wait a specified delay period, and then try to make a nosepoke in the original lit aperture. On low-effort/ low reward trials, the lit hole could remain constant across trials, and so working memory requirements on a given trial would be minimal, while on high-effort/ high-reward trials rats would need to hold online the location of the correct stimulus hole as it varied between trials. This design is loosely based on the combined attention-memory (CAM) task developed by Chudasama and colleagues (Chudasama *et al*, 2004). This

procedure would also allow a researcher to increase the delay period across blocks of trials, thus producing a discounting curve showing how reward is devalued by increasing working memory load. Regardless of the specific nature of the task, other animal models would help us determine whether decision making with cognitive costs is mediated by a common system, or by distinct processes. This is an exciting avenue for future work.

### The Role of Other Brain Regions and Site-Specific Neurotransmitter Regulation in Cognitive Effort Allocation

As described in Chapter 1, a number of prefrontal cortical, striatal, and limbic regions have been implicated in the processes guiding effort allocation. Indeed, in the realm of cost/benefit decision making, the literature has focused almost exclusively on the OFC, ACC, and medial prefrontal areas, the nucleus accumbens, and the basolateral amygdala (Winstanley and Floresco, 2016). In this thesis I identified a role for the dorsomedial striatum in cognitive effort allocation, which is notable considering this striatal zone has not been studied in many other domains. However, it is unlikely these regions are the sole regulators of decision making, and there are a number of regions I think would be useful to investigate on the rCET. I am primarily interested in the role of the insula in valuations of cognitive effort, as imagining evidence suggests that this area is recruited during valuations of physical effort (Arulpragasam *et al*, 2018; Prevost *et al*, 2010), and activity in this region correlates with the subjective value of choices that are either physically or cognitively effortful (Chong *et al*, 2017).

Another notable area to assess would be the lateral habenula, as work from Floresco and colleagues has shown that the region strongly regulates subjective preference in decision making with probability and delay costs (Stopper and Floresco, 2014)

Now that we have identified the general regions and neurotransmitter systems guiding decision making with cognitive effort costs, future work should specifically investigate where certain neurotransmitters are mediating their effects on decision making. For example, cholinergic and cannabinoid signaling regulate rCET decision making, but it is currently unknown where this regulation is taking place. We attempted to address this by studying the contribution of basal forebrain cholinergic neurons to the rCET, but we suggest that the chemogenetic technique might have been inadequate to fully test this hypothesis. Instead, lesions of BF cholinergic or striatal interneurons could be carried out using the immunotoxin 192- IgG-saproin as previously described (Chudasama *et al*, 2004; Mcgaughy *et al*, 2002), and likewise intra-PFC or intra-striatal infusions of scopolamine or similar cholinergic antagonists could also be used. In relation to cannabinoid signaling, $CB_1$ receptor activation in the ACC and NAcc biases rats towards low effort, low reward options, and work with the rCET suggests a similar role for the $CB_1$ receptor in the mPFC (Fatahi and Haghparast, 2018; Khani *et al*, 2015; Silveira *et al*, 2016).

**Trial-by-Trial Analyses**

The baseline propensity to “work” or “slack” at baseline interacts with a

number of pharmacological challenges and appears to be uniquely mediated by some brain regions, suggesting that different neural substrates may regulate baseline willingness to exert cognitive effort. Our understanding of exactly why workers and slackers differ in their baseline choice is limited, but it appears that slackers view cognitive costs as especially aversive, rather than their decreased HR choice being reflective of an insensitivity to reward (Cocker *et al*, 2012b). It would be interesting to conduct a trial-by-trial analysis of rCET performance, to see if workers and slackers fundamentally differ in the pattern by which they allocate their cognitive resources in a session. For example, there is evidence that individuals with Parkinson's Disease are less willing to exert effort because they need to recuperate after a difficult challenge (Müller and Apps, 2018, personal communication). It would be interesting to assess whether something similar is happening in slackers, whereby following an HR trial they select the LR lever for a few trials before resuming selection of HR. This and other decision-making patterns can be evaluated, thus identifying key ways that workers and slackers differ in valuations of a cognitive challenge.

**Physiological Factors Affecting Cognitive Effort Allocation and Modeling Disease States**

The utility of the rCET is that it is designed to model the decision making humans face on a day-to-day basis. Indeed, compared to the costs modeled in other decision-making paradigms, humans consistently find themselves in settings where cognitive effort expenditure is necessary to achieve desired or

lucrative outcomes. Notably, physiological states such stress, fatigue, and sickness are associated with impaired academic and workplace performance (Kronholm *et al*, 2015; LeBlanc, 2009; Odeen *et al*, 2013), which are prime situations where individuals must decide how to allocate cognitive resources. Indeed, these impairments can result in significant personal and economic consequences, and subdomains of economics and occupational psychology study how these adverse consequences can be ameliorated by environmental or behavioural modifications. It is possible relationships between adverse physiological states and impaired functional outcomes are mediated by a reduced willingness to exert cognitive effort, and the rCET provides an ideal model to test these hypotheses directly. For example the effects of acute, chronic, or unpredictable stress could be manipulated as rats perform the rCET (Bryce and Floresco, 2016; Shafiei *et al*, 2012), or fatigue could be induced by having rats treadmill run for an extended period of time or sleep depriving them prior to task performance (Iodice *et al*, 2017). Similarly, sickness could be induced by the administration of inflammatory cytokines or lipopolysaccharide as has been recently investigated in the domain of physical effort allocation (Nunes *et al*, 2014). If stress, fatigue, and sickness affect willingness to engage in a cognitive challenge, then the rCET provides a useful model to identify pharmacological or behavioural therapies that can reverse these impairments.

During the writing of this thesis, I became familiar with the work of Salamone and colleagues, and specifically their attempt to model the motivational symptoms inherent to depression (Nunes *et al*, 2013b). This work

was borne out of the fact that typical antidepressant SSRIs are ineffective in treating the motivational deficits of apathy and anergia in depression (Rothschild *et al*, 2014). Administration of the VMAT inhibitor tetrabenazine decreases willingness to exert physical effort for larger rewards across physical effort allocation paradigms, and these effects are reversed by a number dopaminergic and adrenergic drugs, but not by typical SSRIs (Yohn *et al*, 2016b). It would be interesting to test whether the effects of DA depletion via tetrabenazine similarly affect decision making in the cognitive domain. Although previous work on the rCET has shown that DA plays no role in guiding decision making with cognitive effort costs, selective D1 and D2 antagonists were administered that do not mirror the mechanism of action of tetrabenazine (Hosking *et al*, 2014b). Additionally, motivational deficits are not just limited to depression, but are also observed in Parkinson's Disease and schizophrenia (Chong *et al*, 2015; Reddy *et al*, 2015), and at least one study has shown that individuals with schizophrenia display deficits in cognitive effort allocation (Culbreth *et al*, 2016b; Gold *et al*, 2014). To this end, it would be interesting to test animal models of Parkinson's Disease (i.e. 6-OHDA lesions) and schizophrenia (I.e. isolation rearing, neonatal ventral hippocampal lesion, gestational methylazoxymethanol (Jones *et al*, 2011)) to assess whether they emulate the decision-making deficits inherent to the disorders they model.

**Limitations and Critical Considerations**

The rCET is has proven itself a valuable tool in probing the brain

mechanisms guiding effort allocation in the cognitive domain. As with any study, however, the findings must be considered in light of certain limitations.

Firstly, two of our experiments (Chapter 5 and Appendix 1) were notable in that they used female subjects. Indeed, the animal cost/benefit decision-making literature has almost exclusively focused on male subjects, with few exceptions (Orsini *et al*, 2015b; Uban *et al*, 2012). Female performance on the rCET is similar to males, but we did not assess how this behaviour may fluctuate with estrous cycle. Previous work in the physical effort allocation literature suggests that this phenomenon does not map onto changes in estrous cycling (Uban *et al*, 2012), but this is something that might be worth investigating in future rCET studies.

The rCET requires extensive training, and this might preclude involvement of some brain regions and neurotransmitters that might otherwise be involved in effort allocation in novel situations. In the human literature, participants experience a task once or twice, but in the rCET rats are trained for months on a task where contingencies do not change. Areas such as the OFC appear to be preferentially recruited early on during task acquisition, and neurotransmitters like dopamine play an important role in learning stimulus-outcome associations- processes that are probably not occurring following extended training (Schulz *et al*, 2013; Zeeb and Winstanley, 2011). Indeed, this may be one reason why DA does not appear to play a role in decision making on the rCET, but the physical effort literature and computational models of cognitive effort implicate DA in effort allocation (Salamone and Correa, 2012; Westbrook and Braver, 2016). One

possible way to get around this might be to administer rCET “challenge” sessions in which parameters such as stimulus duration or inter-trial-interval change. These challenges were used to great effect in the original 5-CSRTT literature, and were used to probe the extent of effects of different lesions and pharmacological manipulations. The extensive training on the rCET also precludes any investigation of decision making at earlier developmental time points, such as adolescence. This latter point is more a comment on the animal decision-making literature in general, as the training required extends beyond the 15-20 day window considered adolescence in rodents.

Another limitation requires a theoretical consideration of why a given cognitive challenge is difficult in the first place. A cognitive task may be aversive because it is inherently costly, but might also be aversive because it is less like to be successfully completed (Westbrook and Braver, 2016). In the rCET, difficulty between challenges is evidenced by the lower accuracy on hard trials, suggesting that one of the reasons the HR option is aversive is because it is less likely to result in reward. Previous work has shown that the effort discounting observed on the rCET is not simply related to the probability of receiving reward for LR and HR trials types (Cocker *et al*, 2012b). Rather, the imposition of cognitive load biases rats’ away from the HR option, above and beyond its probability of reinforcement. In any case, many human behavioural paradigms are designed so that performance on easy and hard trials is equivalent or similar, so that any effort discounting is directly due to the inherent cost of cognitive control. It might be interesting for future studies to play with the parameters of the

rCET, to see if there is a set of stimulus durations that result in similar accuracy rates, but which are valued differently on the basis of preference experiments where reward magnitudes are matched.

A final limitation that is not specific to the current thesis, but is pervasive across the animal cost/benefit decision-making literature is that choices are made between concurrently available options. Indeed, most paradigms present animals with a low cost, low reward response option, or a high-cost, high-reward alternative, while other models present subjects with multiple, albeit concurrently available choices (Cousins and Salamone, 1994; de Visser *et al*, 2011; Winstanley and Floresco, 2016). These paradigms were designed to model the decisions humans face on a day-to-day basis, but it is not always the case that humans choose between concurrently available options. Rather, the choice is deciding whether to stick with a current course of action, or to move on in hopes that a more fruitful course of action will be available. This “explore” versus “exploit” form of decision making, also known as foraging choice, recruits distinct neural circuits relative to concurrent choice paradigms, and may be related to the accept/reject format used by some newer human effort-based decision-making models (Chong *et al*, 2015; Daw *et al*, 2006; Kolling *et al*, 2012). Currently no rodent model has been developed to assess this type of decision making, but it is interesting to consider whether similar brain regions regulate cognitive effort when the decision is whether to continue engaging in a cognitive episode, or to disengage and instead seek out other cognitively challenging opportunities that might be more lucrative.

## Conclusion

The experiments carried out in this thesis contribute to a growing literature investigating decision making with cognitive costs. I identified striatal contributions to this process, and confirmed that the BLA plays a critical, albeit nuanced role in the processes guiding cognitive effort allocation. I also demonstrate that akin to decision making with physical effort costs, the orbitofrontal cortices play a minimal role in valuations of cognitive effort. Additionally, I further characterized the previously ascribed role of the cholinergic system in rCET performance. The current findings complement existing work in the domain of physical effort, and support the notion that these two forms of effort-based decision making are mediated by distinct, albeit overlapping circuitries. Now that the main brain regions and neurotransmitters systems have been investigated on the rCET, future work can address specific circuits and neurotransmitter interactions guiding this allocation process. And while this work fundamentally contributes to our understanding of how organisms navigate their environment, it may also have practical utility. Indeed, the rCET may help to identify behavioural or pharmacological therapies that can boost cognitive willingness, and conversely has the potential to better understand the decision-making deficits observed in disorders such as Parkinson's Disease, schizophrenia, and depression. The motivational dysfunction observed in some of these disorders is predictive of poor functional outcomes (Green *et al*, 2015), and an inherent unwillingness to exert cognitive effort may preclude attempts to

seek out or adhere to often challenging treatment regimens. In this regard, identifying the mechanisms giving rise to these cognitive deficiencies may be a therapeutically lucrative target warranting further investigation.

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# Appendix 1: Investigating Serotonergic Contributions to Cognitive Effort Allocation Using a Rodent Model of Cost/Benefit Decision Making

## Introduction

Serotonin plays a limited role in physical effort allocation (Denk *et al*, 2005), but it is still unknown what, if any, contribution this neurotransmitter makes to decision making with cognitive effort costs. To address this gap in the literature, I will investigate how serotonergic agents working at different receptors affect decision making on the rCET. While the serotonin system is notoriously complex, comprised of over 14 distinct receptors subtypes (Barnes and Sharp, 1999), I focus here on the 5-$HT_{2A}$ and 5-$HT_{2c}$ receptor subtypes. Specifically, I will administer the 5-$HT_{2c}$ agonist Ro-60-0175, the 5-$HT_{2c}$ antagonist SB 242,084, as well as the 5-$HT_{2A}$ antagonist M100907. I chose to study these subtypes because 5-$HT_{2A}$ and 5-$HT_{2c}$ receptors have received particular attention for their roles in regulating motor impulsivity, as assessed by the original 5-CSRTT. For example, the 5-$HT_{2c}$ agonist Ro-60-0175 and 5-$HT_{2A}$ antagonist M100907 both decrease motor impulsivity (Fletcher *et al*, 2011; Winstanley *et al*, 2003b, 2004b), while the 5-$HT_{2c}$ antagonist SB 242,084 has the opposite effect (Fletcher *et al*, 2007; Winstanley *et al*, 2004b). Thus, I will be able to assess whether doses normally affecting impulse control likewise affect allocation of cognitive resources.

## Additional Methods

### Subjects and Baseline Choice

Subjects were 24 female Long-Evans rats bred in house. These rats were bred from breeding pairs obtained from Charles River Laboratories and the Rat Resource and Research Centre (RRRC, Columbia, MO) as part of a breeding program for transgenic rats that express cre recombinase (Cre) in neurons that contain choline acetyltranferase (ChAT; Long Evans –Tg (ChAT-Cre) 5.1 Deis, RRRC # 00658). Twelve of these rats were positive for the transgene (TG+), while the remainder did not express the transgene (TG-). The transgenic status of the rats was not utilized for the current study, but instead the subjects were considered naïve given they exhibit a similar behavioural profile to Long Evans rats purchased from a commercial supplier. To account for potential differences, transgene status (TG + or TG -) was included as a between-subjects factor for all analyses. These results will not be reported, unless a significant a main effect of TG status or TG x dose interaction is observed for any of the rCET behavioural measures. Animals weighed at least 200 g at the start of the experiment and were food restricted to 85% of their free-feeding weight (maintained on 10 g rat chow daily). Water was available ad libitum. Rats were housed in groups of three or four in a climate controlled colony room maintained at 21° C on a reverse 12-hr light-dark schedule (lights off at 8 am).

For this particular experiment, the mean choice of the HR option across

rats was 66%. Animals were grouped as “workers” if they chose the HR option for >70% of trials (n=12) and as “slackers” if they chose HR for ≤70% of trials (n=12), as per previous work (Cocker et al., 2012), thereby enabling consistency when discussing individual differences across studies. We experienced a box issue during the first Latin square (Ro-60-0175), and so one worker was excluded from this analysis, but was included in subsequent Latin squares.

**Effects of Systemic R0-60-0175, M100907, and SB 242,084 on rCET Performance**

Animals received Ro-60-0175 (0, .1, .3, .6 mg/kg), then M100907 (0, .01, .03, 0.1 mg/kg) and lastly SB 242,084 (0, 0.1, 0.25, 0.5 mg/kg) in a series of counterbalanced Latin squares (four doses: ABCD, BDAC, CDAB, DCBA). Ro-60-0175 was administered subcutaneously, whereas M100907 and SB 242,084 were injected intraperitoneally. All drugs were administered 15 minutes prior to starting the behavioural task. Drug injections were given on a three-day cycle, starting with a baseline session. On the second day, rats received a drug or vehicle injection prior to testing, and on the third day they were not tested. To prevent carry-over effects, animals were tested drug-free for a minimum of one week before starting the next Latin square.

**Drugs**

All drugs were purchased from Tocris. A stock solution of the highest concentration was prepared, and four aliquots frozen at -20°C. One aliquot was

thawed on each drug day and diluted as necessary to produce the required doses. Ro-60-0175 was dissolved in 0.9% saline. M100907 was dissolved in 0.9% saline and pH adjusted to 6.25 using 0.1 M NaOH and 0.1 M HCl. SB 242,084 was dissolved in 25 mM citric acid in 8% cyclodextrine in 0.9% saline. Drug doses were calculated as the salt. Systemic injections of drugs were given in a volume of 1 ml/kg body weight. Doses were selected on the basis of a previously published study from out lab (Adams *et al*, 2017a).

## Data Analyses

Data were analyzed with a repeated measures ANOVA with session (three levels: baseline sessions 1-3) or dose (four levels: vehicle plus three doses) as within-subjects factors, and group (two levels: worker or slacker) and TG (+ or -) status as between-subjects factors for all analyses. If analyses produced significant main effects of dose or dose x group interaction, doses were compared post hoc to vehicle with paired samples t-tests. A bonferonni correction was applied to these subsequent analyses. Significance was set at $p < .05$ and $p$ values < .1 reported as statistical trend.

## Results

### Ro-60-0175 Administration

#### Choice, Accuracy, and Premature Responding

Baseline behaviour on the rCET has been discussed in detail previously, and so will be briefly discussed here. As an group, rats selected HR trials more often than LR trials (saline only- choice: $F(1, 19) = 26.838$, $p < .001$). In keeping with their group categorization, workers ($M = 80.83\%$) chose HR significantly more than slackers ($M = 52.76\%$) (saline only- group: $F(1, 21) = 22.739$, $p < .001$). Systemic administration of Ro-60-0175 did not affect choice of HR trials (Dose: $F(3, 57) = .257$, NS; dose x group: $F(3, 57) = 2.192$, NS; Figure 7.1a).

Accuracy on HR trials was lower than accuracy on LR trials, in keeping with this option being more attentionally demanding (saline only- choice: $F(1, 19) = 102.914$, $p < .001$). In line with previous studies, workers and slackers did not differ in attentional performance (saline only- choice x group: $F(1, 19) = 0.00$, NS; group: $F(1, 19) = .067$, NS), suggesting slackers' reduced preference for the HR option is not driven by a deficit in ability. Across rats Ro-60-0175 affected LR and HR accuracy (LR trials- Dose: $F(3, 51) = 3.045$, $p = .037$; Dose x group: $F(3, 51) = 1.705$, NS; HR trials- Dose: $F(2, 37) = 4.535$, $p = .007$; Dose x group: $F(2, 37) = 0.582$, NS). Follow-up tests revealed that LR accuracy was not significantly affected by Ro-60-0175, but HR accuracy significantly increased at the 0.1, 0.3, and 0.6 mg/kg doses (LR trials- sal vs. 0.1 mg/kg: $F(1, 20) = 3.522$, $p = .225$, sal vs. 0.3 mg/kg: $F(1, 20) = 1.125$, $p = .903$, sal vs. 0.6 mg/kg: $F(1, 20) = 0.600$, $p = 1.00$; HR trials- sal vs. 0.1 mg/kg: $F(1, 19) = 12.924$, $p = .006$, sal vs. 0.3 mg/kg = $F(1, 19) = 6.776$, $p = .051$, sal vs. 0.6 mg/kg = 13.935, $p = .003$, Figure 7.1b).

Rates of premature responding did not differ between LR and HR trials (saline only- choice: $F(1, 19) = .046$, NS), and did not differ between groups

(saline only- choice x group: $F(1, 19) = 2.913$, NS; group: $F(1, 19) = .355$, NS). Ro-60-0175 produced a trending decrease in premature responding, but none of the individual doses were significant following post hoc tests (Dose: $F(3, 51) = 2.284$, $p = .09$; Dose x group: $F(1 , 51) = 1.901$, NS; all post hoc tests NS). Ro-60-0175 did not affect HR premature responding (Dose: $F(3, 54) = 1.164$, NS, Dose x group: $F(3, 54) = 0.263$, NS, Figure 7.1c).

**Other Behavioural Measures**

When injected with saline, rats initiated ~130 trials on average and made few choice omissions when the levers were extended. This did not differ between groups (all $F$s < 0.436, NS). Latencies to make a lever choice, to correctly detect the light stimulus, and to collect reward did not differ between trial types (Saline only- Choice: all $F$s < 4.00, NS). Workers and slackers generally did not differ In latencies to make a choice and to make a correct response (Saline only- Choice x group, Group: all $F$s < .718, NS), but slackers were generally slower to collect reward across trials (Saline only- Group: $F(1, 19)$: 7.350, $p = .014$). Response omissions following stimulus presentation were higher for HR trials, in line with this being the more attentionally demanding trial type (Saline only- Choice: $F(1, 19) = 11.630$, $p = .003$). Omissions rates did not differ between groups (Saline only- Choice x group, Group: all $F$s < 1.059, NS).

Following Ro-60-0175 administration, trials initiated and trials completed significantly decreased at all doses across groups (Trials initiated- Dose: $F(3, 57) = 30.133$, $p < .001$, Dose x group: $F(3, 57) = 0.05$, NS; sal vs. 0.1 mg/kg: $F(1, 22)$

= 8.90, $p$ = .007, sal vs. 0.3 mg/kg: $F(1, 22) = 33.33$, $p < .001$, sal vs. 0.6 mg/kg: $F(1, 22) = 75.78$, $p < .001$; Trials completed- Dose: $F(3, 57) = 33.595$, $p < .001$, Dose x group: $F(3, 57) = 0.028$, NS; sal vs. 0.1 mg/kg: $F(1, 22) = 10.634$, $p$ = .012, sal vs. 0.3 mg/kg: $F(1, 22) = 30.758$, $p < .001$, sal vs. 0.6 mg/kg: $F(1, 22) = 77.86$, $p < .001$). There was a trending effect on choice omissions, but none of the post hoc comparisons were significant (Dose: $F(2, 45) = 2.895$, $p$ = .063, all posthoc tests $F$s < 5.457, NS). None of the latency measures were affected by Ro-60-0175 (Choice latency, Correct Latency, Collect latency – Dose, Dose x group: all $F$s < 1.726, NS). However, the drug had biphasic effects on response omissions, decreasing response omissions on HR trials at the lowest dose, and increasing response omissions for both trial types at the middle dose (LR response omissions- Dose: $F(3, 51) = 2.852$, $p$ = .048, Dose x group: $F(3, 51)$ = 0.045, NS; sal vs. 0.1 mg/kg: $F(1, 20) = 0.822$, $p$ = 1.00, sal vs. 0.3 mg/kg: $F(1, 20) = 12.447$, $p$ = .006, sal vs. 0.6 mg/kg: $F(1, 20) = 2.637$, $p$ = .360; HR response omissions- Dose: $F(3, 54) = 3.214$, $p$ = .030, Dose x group: $F(3, 54)$ = 1.477, NS; sal vs. 0.1 mg/kg: $F(1, 21) = 8.976$, $p$ = .007, sal vs. 0.3 mg/kg: $F(1, 21) = 9.078$, $p$ = .007, sal vs. 0.6 mg/kg: $F(1, 21) = 3.276$, $p$ = .085, Figure 7.1d).

**M100 907 Administration**

**Choice, Accuracy, and Premature Responding**

M100 907 did not affect choice (Dose: $F(3, 60) = 1.95$, NS; Dose x group: $F(3, 60) = 1.31$, NS, Figure 7.2a), and did not affect accuracy for LR or HR trials (LR trials- Dose: $F(3, 57) = 1.051$, NS, Dose x group: $F(3, 57) = 2.577$, $p$ = .063;

HR trials – Dose: $F(3, 57) = 0.321$, NS, Dose x group: $F(3, 57) = 1.716$, NS, Figure 7.2b). Systemic administration of M100 907 did not affect premature responding for LR trials (LR trials- Dose: $F(3, 57) = 1.367$, NS, Dose x group: $F(3, 57) = 0.322$, NS) but decreased motor impulsivity at all doses for HR trials (HR trials- Dose: $F(3, 57) = 6.115$, $p < .001$, Dose x group: $F(3, 57) = 1.095$, NS; veh versus 0.01 mg/kg: $F(1, 22) = 16.17$, $p = .003$; veh versus 0.03 mg/kg: $F(1, 22) = 7.96$, $p = .03$; veh versus 0.1 mg.kg: $F(1, 22) = 11.73$, $p = .006$, Figure 7.2c).

**Other Behavioural Measures**

M100 907 increased choice omissions at the middle and high dose when both levers were extended at trial onset (Dose: $F(3, 60) = 7.309$, $p < .001$, Dose x group: $F(3, 60) = 0.057$, NS; veh versus 0.01 mg/kg: $F(1, 23) = 0.03$, NS; veh versus 0.03 mg/kg: $F(1, 23) = 8.11$, $p = .03$; veh versus 0.1 mg.kg: $F(1, 23) = 11.12$, $p = .009$), and increased the latencies to make an LR or HR choice (LR trials- Dose: $F(2, 45) = 4.910$, $p = .009$, Dose x group: $F(2, 45) = 2.508$, $p = .084$; veh versus 0.01 mg/kg: $F(1, 22) = 2.17$, NS; veh versus 0.03 mg/kg: $F(1, 22) = 6.02$, NS; veh versus 0.1 mg.kg: $F(1, 22) = 13.14$, $p = .003$; HR trials- Dose: $F(2, 40) = 8.509$, $p = .001$, Dose x group: $F(2, 40) = 1.380$, NS; veh versus 0.01 mg/kg: $F(1, 22) = 4.101$, NS; veh versus 0.03 mg/kg: $F(1, 22) = 18.122$, $p < .001$; veh versus 0.1 mg.kg: $F(1, 22) = 10.24$, $p = .012$). Latencies to make a correct response were affected for HR, but not LR trials at all doses (HR trials- Dose: $F(3, 57) = 8.138$, $p < .001$, Dose x group: $F(3, 57) = 0.587$, NS; veh versus 0.01

mg/kg: $F(1, 22) = 13.513$, $p = .003$; veh versus 0.03 mg/kg: $F(1, 22) = 19.246$, $p < .001$; veh versus 0.1 mg.kg: $F(1, 22) = 17.825$, $p < .001$; LR trials- Dose: $F(2, 38) = 2.756$, $p = .076$, Dose x group: $F(2, 38) = 0.671$, NS). However, latencies to collect reward were unaffected (LR and HR trials- Dose, Dose x group: all $F$s < 2.716, NS). Response omissions significantly increased for HR but not LR trials (HR trials- Dose: $F(2, 41) = 5.789$, $p = .005$, Dose x group: $F(2, 41) = 1.478$, NS; veh versus 0.01 mg/kg: $F(1, 22) = 12.738$, $p = .006$; veh versus 0.03 mg/kg: $F(1, 22) = 12.917$, $p = .006$; veh versus 0.1 mg.kg: $F(1, 22) = 11.216$, $p = .009$; LR trials- Dose: $F(3, 57) = 0.856$, NS, Dose x group: $F(3, 57) = 0.479$, NS, Figure 7.2d). Lastly, M100 907 decreased the number of trials completed at all doses (Dose- $F(3, 60) = 10.668$, $p < .001$, Dose x group: $F(3, 60) = 0.305$, NS; veh versus 0.01 mg/kg: $F(1, 23) = 8.346$, $p = .024$; veh versus 0.03 mg/kg: $F(1, 23) = 15.713$, $p = .003$; veh versus 0.1 mg.kg: $F(1, 23) = 21.856$, $p < .001$).

**SB 242 Administration**

**Choice, Accuracy, and Premature Responding**

SB 242, 084 did not affect choice (Dose: $F(3, 60) = 2.139$, NS; Dose x group: $F(3, 60) = 0.446$, NS, Figure 7.3a), and did not affect accuracy for LR or HR trials (LR trials- Dose: $F(2, 33) = 1.106$, NS, Dose x group: $F(2, 33) = 1.830$, NS; HR trials – Dose: $F(3, 60) = 1.875$, NS, Dose x group: $F(3, 60) = 0.097$, NS, Figure 7.3b). However, SB 242, 084 increased premature responding across trial types at all doses tested (LR trials- Dose: $F(3, 57) = 8.503$, $p < .001$, Dose x group: $F(3, 60) = 0.643$, NS; veh versus 0.1 mg/kg: $F(1, 22) = 8.83$, $p = .021$; veh

versus 0.25 mg/kg: $F(1, 22) = 14.74$, $p = .003$; veh versus 0.5 mg.kg: $F(1, 22) = 45.62$, $p < .001$; HR trials- Dose: $F(3, 60) = 7.221$, $p < .001$, Dose x group: $F(3, 60) = 2.426$, NS; veh versus 0.1 mg/kg: $F(1, 23) = 12.84$, $p = .006$; veh versus 0.25 mg/kg: $F(1, 23) = 14.34$, $p = .003$; veh versus 0.5 mg.kg: $F(1, 23) = 11.34$, $p = .009$ Figure 7.3c).

**Other Behavioural Measures**

SB 242, 084 appeared to invigorate responding on the rCET, increasing trials completed at all doses tested (Dose- $F(3, 60) = 14.332$, $p < .001$, Dose x group: $F(3, 60) = 2.727$, NS; veh versus 0.1 mg/kg: $F(1, 23) = 22.629$, $p < .001$; veh versus 0.25 mg/kg: $F(1, 23) = 19.892$, $p < .001$; veh versus 0.5 mg.kg: $F(1, 23) = 19.874$, $p < .001$). This was accompanied by faster latencies to make a choice or a correct response on either trial type (choice latency: LR trials- Dose: $F(3, 57) = 14.438$, $p < .001$, Dose x group: $F(3, 57) = 0.326$, NS; veh versus 0.1 mg/kg: $F(1, 22) = 20.693$, $p < .001$; veh versus 0.25 mg/kg: $F(1, 22) = 22.137$, $p < .001$; veh versus 0.5 mg.kg: $F(1, 22) = 23.814$, $p < .001$; HR trials- Dose: $F(3, 60) = 36.418$, $p < .001$, Dose x group: $F(3, 60) = 0.766$, NS; veh versus 0.1 mg/kg: $F(1, 23) = 40.679$, $p < .001$; veh versus 0.25 mg/kg: $F(1, 23) = 71.605$, $p < .001$; veh versus 0.5 mg.kg: $F(1, 23) = 61.12$, $p < .001$; Correct latency: LR trials- Dose: $F(3, 57) = 9.147$, $p < .001$, Dose x group: $F(3, 57) = 0.581$, NS; veh versus 0.1 mg/kg: $F(1, 22) = 13.928$, $p = .003$; veh versus 0.25 mg/kg: $F(1, 22) = 20.512$, $p < .001$; veh versus 0.5 mg.kg: $F(1, 22) = 5.712$, $p = .078$; HR trials- Dose: $F(3, 60) = 3.943$, $p = .012$, Dose x group: $F(3, 60) = 1,152$, NS; veh versus 0.1 mg/kg:

*F*(1, 23) = 11.243, *p* = .009; veh versus 0.25 mg/kg: *F*(1, 23) = 2.424, NS; veh versus 0.5 mg.kg: *F*(1, 23) = 10.831, *p* = .009). In contrast, SB 242, 084 did not affect latencies to collect larger reward following successful stimulus detection, but decreased latencies to collect reward selectively in slacker rats on LR trials at the highest dose (HR trials-Dose: *F*(2, 32) = 1.753, NS; Dose x group: *F*(2, 32) = 1.357, NS; LR trials-Dose: *F*(2, 33) = 5.722, *p* = .009; Dose x group: *F*(2, 33) = 4.900, *p* = .017, Workers only – all Fs < 1.172, NS, Slackers only – Dose: *F*(2, 15) = 4.226, *p* = .048, veh versus 0.1 mg/kg: *F*(1, 11) = 3.042, NS; veh versus 0.25 mg/kg: *F*(1, 11) = 4.834, NS; veh versus 0.5 mg.kg: *F*(1, 11) = 11.492, *p* = .018). Lastly, SB 242, 084 administration did not affect choice or response omissions (Dose, Dose x group: all Fs < 2.379, NS).

**Discussion and Conclusion**

The aim of this experiment was to assess serotonergic contributions to decision making on the rCET. I show here that serotonergic signaling at the 5-$HT_{2A}$ and 5-$HT_{2C}$ receptors has dissociable roles in regulating attention, impulsivity, and decision making as probed by the rCET. None of the drugs affected cognitive effort allocation, but all had subtle effects on either attention or impulsivity. The 5-$HT_{2C}$ agonist Ro-60-175 improved HR trial accuracy, while the 5-$HT_{2C}$ antagonist SB 242, 084 and the 5-$HT_{2A}$ antagonist M100 907 increased and decreased impulsivity, respectively. These effects are in keeping with previous work on the standard 5-CSRTT (Fletcher *et al*, 2007, 2011, Winstanley *et al*, 2003b, 2004b), as well as behavioural paradigms such as the rodent Gambling Task which provide cursory measures of impulsive action (Adams *et al*, 2017a). While the focus of these experiments was limited to the 5-$HT_2$ receptor subtype, the results clearly demonstrate that doses affecting different dimensions of cognitive function were unable to shift rats' baseline willingness to allocate cognitive resources for reward. When considered with the negligible role of serotonin signaling in decision making with physical effort costs (Denk *et al*, 2005), the current works suggests that this neurotransmitter system play a minor role in decision making with effort costs.

**Tables**

| | Vehicle | 0.1 mg/kg | 0.3 mg/kg | 0.6 mg/kg |
|---|---|---|---|---|
| Choice omissions | 7.04 ± 1.33 | 8.70 ± 1.59 | 8.00 ± 1.21 | 11.43 ± 1.55 |
| LR choice latency | 3.51 ± 0.22 | 3.74 ± 0.28 | 3.68 ± 0.25 | 3.82 ± 0.26 |
| HR choice latency | 3.07 ± 0.22 | 3.21 ± 0.19 | 3.47 ± 0.24 | 3.38 ± 0.21 |
| LR correct latency | 0.55 ± 0.02 | 0.57 ± 0.02 | 0.58 ± 0.02 | 0.59 ± 0.02 |
| HR correct latency | 0.51 ± 0.03 | 0.52 ± 0.03 | 0.50 ± 0.02 | 0.52 ± 0.03 |
| LR collect latency | 2.04 ± 0.22 | 2.03 ± 0.13 | 2.22 ± 0.25 | 3.61 ± 1.50 |
| HR collect latency | 1.54 ± 0.18 | 1.61 ± 0.23 | 2.02 ± 0.55 | 1.50 ± 0.07 |
| Trials completed | 114.30 ± 5.52 | 101.17± 6.63* | 90.09 ± 6.60** | 69.35 ± 5.81** |

**Table 7.1. Other behavioural measures following systemic Ro-60-0175 administration**

Means are presented (+ SEM). $^{\#}p < .1$; * $p < .05$; ** $p < .001$

| | Vehicle | 0.01 mg/kg | 0.03 mg/kg | 0.1 mg/kg |
|---|---|---|---|---|
| **Choice omissions** | 5.63 ± 1.45 | 5.50 ± 1.16 | 8.04± 1.44* | 9.42 ± 1.67* |
| **LR choice latency** | 3.22 ± 0.22 | 3.38 ± 0.22 | 3.70 ± 0.24 | 3.82 ± 0.24* |
| **HR choice latency** | 3.13 ± 0.21 | 3.34 ± 0.21 | 3.69 ± 0.20** | 3.69 ± 0.21* |
| **LR correct latency** | 0.53 ± 0.02 | 0.58 ± 0.03 | 0.59 ± 0.02 | 0.60 ± 0.03 |
| **HR correct latency** | 0.46 ± 0.02 | 0.54 ± 0.03* | 0.56 ± 0.03** | 0.58 ± 0.03** |
| **LR collect latency** | 2.10 ± 0.50 | 2.75 ± 0.64 | 2.36 ± 0.22 | 2.14 ± 0.15 |
| **HR collect latency** | 1.34 ± 0.05 | 1.47 ± 0.06 | 1.60 ± 0.12 | 1.50 ± 0.05 |
| **Trials completed** | 125.25 ± 4.99 | 117.50± 6.08* | 108.50 ± 5.51* | 107.58 ± 5.76** |

**Table 7.2. Other behavioural measures following systemic M100 907 administration**

Means are presented (+ SEM). $^{\#}p < .1$; * $p < .05$; ** $p < .001$

| | Vehicle | 0.1 mg/kg | 0.25 mg/kg | 0.5 mg/kg |
|---|---|---|---|---|
| **Choice omissions** | 4.75 ± 1.23 | 2.58 ± 1.12 | 3.58 ± 1.20 | 3.46 ± 0.99 |
| **LR choice latency** | 3.25 ± 0.22 | 2.47 ± 0.18** | 2.60 ± 0.18** | 2.33 ± 0.15** |
| **HR choice latency** | 3.31 ± 0.17 | 2.50 ± 0.16** | 2.49 ± 0.13** | 2.30 ± 0.12** |
| **LR correct latency** | 0.55 ± 0.02 | 0.49 ± 0.02* | 0.46 ± 0.01** | 0.50 ± 0.02 |
| **HR correct latency** | 0.49 ± 0.02 | 0.43 ± 0.02* | 0.45 ± 0.02 | 0.43 ± 0.02* |
| **LR collect latency** | 1.97 ± 0.21 | 1.62 ± 0.11 | 1.51 ± 0.12 | 1.48 ± 0.08 |
| **HR collect latency** | 1.71 ± 0.29 | 1.26 ± 0.05 | 1.33 ± 0.08 | 1.46 ± 0.17 |
| **Trials completed** | 121.21 ± 5.04 | 136.71± 4.81** | 134.92 ± 5.66** | 137.08 ± 4.27** |

**Table 7.3. Other behavioural measures following systemic SB 242, 084 administration**

Means are presented (+ SEM). $^{\#}p < .1$; * $p < .05$; ** $p < .001$

## Figures

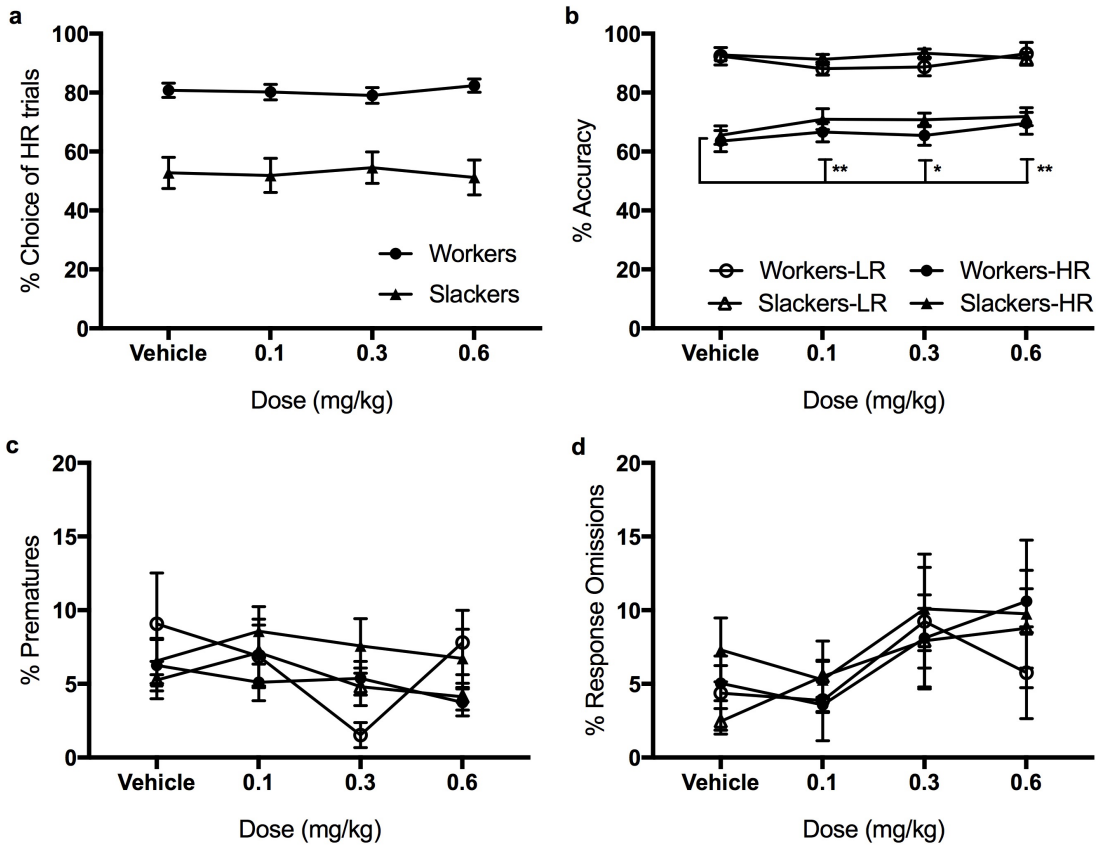


**Figure 7-1. Effects of the 5-HT$_{2C}$ receptor agonist Ro-60-0175 on (a) choice of HR trials (%), (b) accuracy, (c) premature responses (%), and (d) response omissions (%)**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. Ro-60-0175 did not affect choice at any dose tested, and did not affect rates of premature responding. However, all doses tested increased HR trial accuracy, and the low (0.1 mg/kg) and middle (0.3 mg/kg) doses increased HR response omissions.

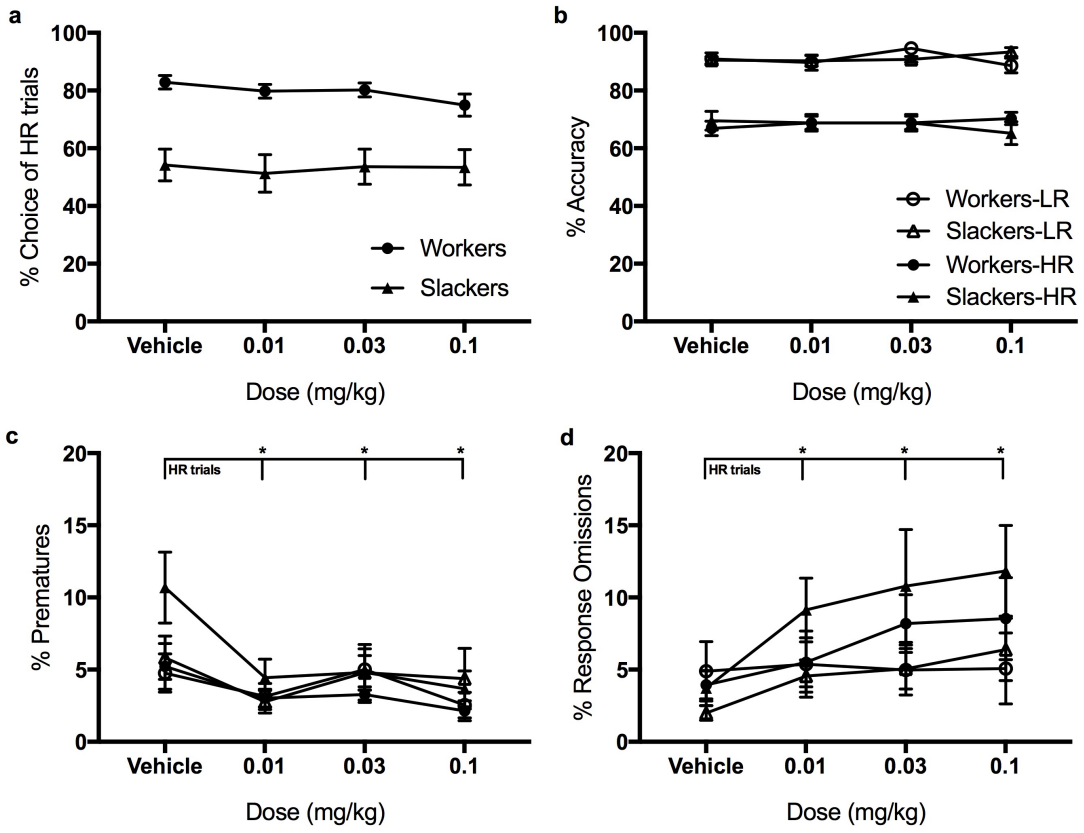


**Figure 7-2. Effects of the 5-HT$_{2A}$ receptor antagonist M100 907 on (a) choice of HR trials (%), (b) accuracy (%), (c) premature responses (%), and (d) response omissions (%)**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. M100 907 did not affect choice at any dose tested, and did not affect accuracy. However, all doses tested decreased premature responding and increased response omissions on HR trials.

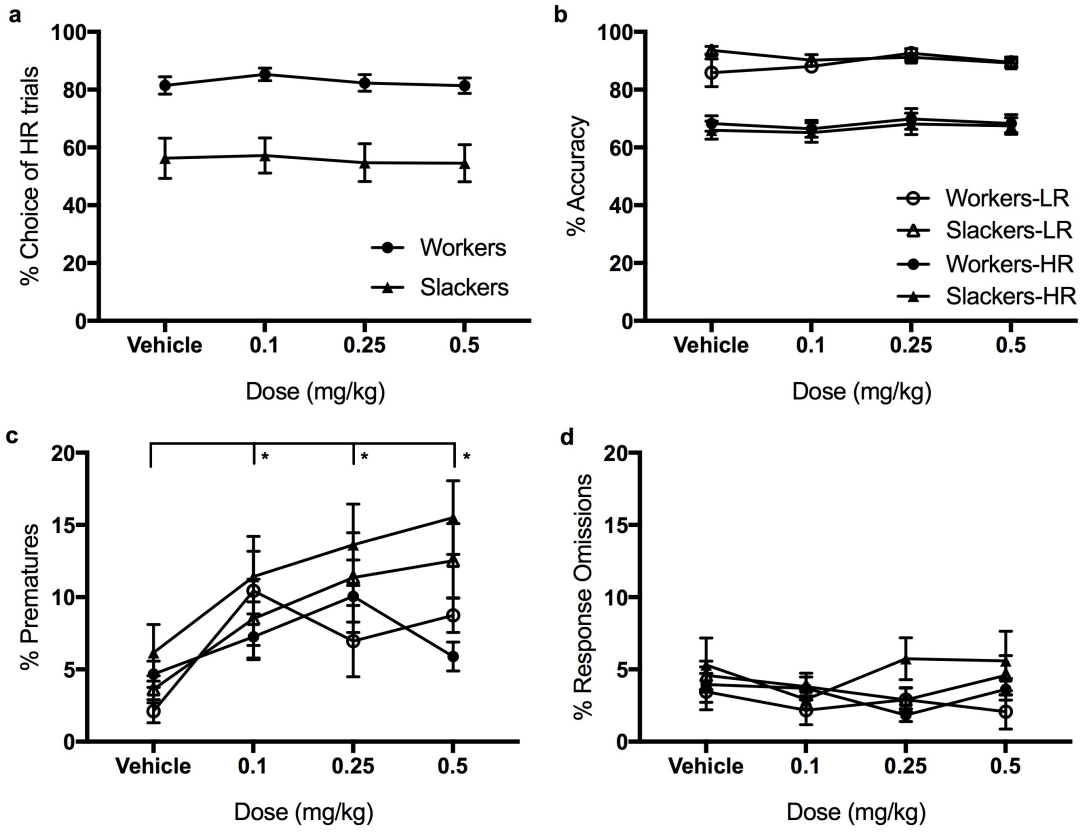


**Figure 7-3. Effects of the 5-HT$_{2C}$ receptor antagonist SB 242, 084 on (a) choice of HR trials (%), (b) accuracy, (c) premature responses (%), and (d) response omissions (%)**

Data shown are the mean for each variable (±SEM). LR: Low effort/low reward trial; HR: High effort/high reward trial. SB 242, 084 did not affect choice at any dose tested, and did not affect accuracy or response omissions. However, all doses tested increased premature responding across trial types.