Novel approaches towards pharmacological enhancement of motivation

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The work described in this thesis, can be divided into two sections. Initially, a series of experiments were conducted that aimed to increase the likelihood of cross-species translation of motivation research. This was achieved by firstly optimising and validating a battery of tasks to assess effort-related behaviour, a preclinical measure of motivation, in rats for use within an operant touchscreen platform. This will allow tasks to be performed with high face validity, across species. Secondly, we applied a highly translatable functional imaging measure, *in vivo* oxygen amperometry, to explore whether a neural correlate of motivated behaviour could be detected in rats.

The second section describes the identification and exploration of a novel pharmacological target for treating apathy. By using a progressive ratio (PR) schedule of reinforcement, muscarinic acetylcholine receptor antagonists were found to facilitate motivated behaviour in intact mice. Furthermore, through the application of several compounds, these actions appeared to be driven by the M_1 receptor subtype. Subsequently, nonpathological aging was examined as a potential model of impaired motivation, based upon previous reports. However, the deficit was found not to be reliable. Therefore, the effects of the muscarinic acetylcholine receptor antagonist biperiden was tested following administration of the dopamine receptor antagonist haloperidol, a well-validated model of impaired motivation. Biperiden was able to successfully, reverse the effects of haloperidol on effort-based behaviour. This suggests that the drug biperiden may be a therapeutic option in the treatment of apathy.

Declaration

The following work was conducted within the Department of Psychology, University of Cambridge between 2014-2018, including an industrial placement at Eli Lilly and Co. during 2015-2016, under the supervision of Professors Timothy J. Bussey and Lisa M. Saksida. This dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text.

This thesis is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other university or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my dissertation has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text

This thesis does not exceed 60,000 words

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Summary

This work explores novel approaches towards pharmacological enhancement of motivated behaviour. A loss of motivation remains a severe unmet clinical need in a number of neuropsychiatric and neurodegenerative disorders.

The work described in this thesis, can be divided into two sections. Initially, a series of experiments were conducted that aimed to increase the likelihood of cross-species translation of motivation research. This was achieved by firstly optimising and validating a battery of tasks to assess effort-related behaviour, a preclinical measure of motivation, in rats for use within an operant touchscreen platform. This will allow tasks to be performed with high face validity, across species. Secondly, we applied a highly translatable functional imaging measure, *in vivo* oxygen amperometry, to explore whether a neural correlate of motivated behaviour could be detected in rats.

The second section describes the identification and exploration of a novel pharmacological target for treating apathy. By using a progressive ratio (PR) schedule of reinforcement, muscarinic acetylcholine receptor antagonists were found to facilitate motivated behaviour in intact mice. Furthermore, through the application of several compounds, these actions appeared to be driven by the M_1 receptor subtype. Subsequently, nonpathological aging was examined as a potential model of impaired motivation, based upon previous reports. However, the deficit was found not to be reliable. Therefore, the effects of the muscarinic acetylcholine receptor antagonist biperiden was tested following administration of the dopamine receptor antagonist haloperidol, a well-validated model of impaired motivation. Biperiden was able to successfully, reverse the effects of haloperidol on effort-based behaviour. This suggests that the drug biperiden may be a therapeutic option in the treatment of apathy.

Publications

The work described in this dissertation contributed to the following manuscripts:

Hailwood JM., Gilmour GG., Robbins TW., Saksida LM., Bussey TJ., Marston HM., Gastambide F. (2018) Oxygen responses within the nucleus accumbens are associated with individual differences in effort exertion in rats. *European Journal of Neuroscience.*

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Hailwood JM., Heath CJ., Phillips BU., Robbins TW., Saksida LM., Bussey TJ. (2018) Blockade of muscarinic acetylcholine receptors with biperiden facilitates motivated behaviour and rescues a model of antipsychotic-induced amotivation *Neuropsychopharmacology.*

The following unrelated manuscripts were also completed during this thesis:

Lopez-Cruz, L., Phillips., BU., Hailwood JM., et al., (2018) Translational approaches to evaluating motivation in laboratory rodents: conventional and touchscreen-based procedures *Current Opinion in Behavioural Sciences,* 22, 21-27.

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Abbreviations

ACC	Anterior Cingulate Cortex
ACh	Acetylcholine
AChEI	Acetylcholinesterase Inhibitor
AD	Alzheimer's Disease
ALS	Amyotrophic Lateral Sclerosis
ANOVA	Analysis of Variance
AUC	Areas Under the Curve
BLA	Basolateral Amygdala
BOLD	Blood-Oxygenation Level Dependent
BP	Breakpoint
CANTAB	Cambridge Neuropsychological Test Automated Battery
CIN	Cholinergic Interneuron
CNS	Central Nervous System
CPA	Constant Potential Amperometry
CPE	Carbon Paste Electrode
CPT	Continuous Performance Task
cTUNL	Continuous Trial Unique Matching to Location
DA	Dopamine
DAT	Dopamine Transporter
EEfRT	Effort Expenditure for Rewards Task
EFD	Effort Discounting
EPS	extrapyramidal symptoms
ERC	Effort-related choice
fMRI	Functional Magnetic Resonance Imaging
FR	Fixed ratio
FSCV	Fast scan cyclic voltammetry
FTD	Fronto temporal dementia
GABA	Gamma-Aminobutyric acid
GPCR	G-protein coupled receptor
HD	Huntington’s disease
IRI	Inter-response interval
ITI	Inter trial interval

KO	Knock-out
mAChR	Muscarinic acetylcholine receptors
MAO	Monoamine-oxidase
MRI	Magnetic resonance imaging
MSN	Medium spiny neuron
NAc	Nucleus Accumbens
nAChR	Nicotinic acetylcholine receptor
NAM	Negative allosteric modulator
NPI	Neuropsychiatric inventory
OFC	Orbitofrontal cortex
PAL	Paired associates learning
PAM	Positive allosteric modulator
PANS-N	Negative subscale of the Positive and Negative Syndrome Scale
PD	Parkinson's Disease
PFC	Prefrontal Cortex
PIT	Pavlovian -instrumental Transfer
PPI	Paired pulse inhibition
PR	Progressive Ratio
PRP	Post Reinforcement Pause
RCT	Randomised control trial
RED	Rearing effort discounting
RDoC	Research Domain Criteria
SANS	Scale for Assessment of Negative Symptoms
SSRI	Selective serotonin reuptake inhibitor
TUNL	Trial Unique Matching to Location
VMAT	Vesicular monoamine transporter
VTA	Ventral Tegmental Area

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Male Wistar rats (Charles River, UK) were the subjects used in this study. A total of twenty-one rats underwent behavioural testing. Two were subsequently excluded due to poor signal quality resulting in all trials containing multiple artefacts (see *4.2.4* below). Four rats were excluded due to all electrodes being either mis-located NAc electrodes or having unidentifiable co-ordinates (see *4.3.1*). Resulting in fifteen rats being included in the main (NAc) analysis. A further five rats were excluded from the OFC analysis due to incorrectly located or unidentifiable OFC electrodes. All animals were group housed (3-4 per cage) throughout the study, in a temperature (20-22°c) and light controlled (lights on 0700-1900) environment. All testing took place during the light phase. Animals were given at least seven days of acclimation in the facility prior to surgical implantation of oxygen recording electrodes. Following a two-week surgical recovery period, animals were placed on a schedule of controlled feeding and maintained at no less than 85% of their free feeding body weight measured post-surgery. No correction was applied to this 85% control weight to match the animals expected growth curve (weight at training: 585g ± 10.38; weight at testing: 612g ± 16.45). Water was freely available throughout the study. All experimental procedures were

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Chapter 1. General Introduction

1.1 What is apathy

1.1.1 Defining apathy

The ability to initiate and maintain goal-directed behaviour is crucial to everyday functioning. This ability, loosely referred to as motivation, encompasses a range of cognitive processes that govern how we interact with our environment to obtain key goals. The loss of motivation, commonly known as apathy or amotivation, is a severe unmet clinical need in a number of disorders. Apathy, as a clinical term, has received several definitions. Marin (1990) defined apathy as a disturbance of motivation that cannot be attributed to cognitive impairment or emotional distress. This definition was subsequently operationalised to suggest apathy was the loss of voluntary goal-directed behaviour (Levy & Dubois 2006). A further definition suggested that apathy may arise from deficits in three distinct processes, in which apathy could consist of auto-activation, cognitive and emotional-affective types (Levy & Dubois 2006). The auto-activation, or behavioural domain refers to a loss of self-initiated behaviour or a loss of response to externally driven behaviour. The cognitive domain refers to a loss of curiosity, routine or ideas both self-initiated and externally stimulated. Finally, the emotional domain refers to the loss of spontaneous emotions or emotional reactivity (Robert et al., 2009).

One important distinction is the difference between apathy and depression. Depression and apathy share a high degree of co-morbidity as apathy itself is a prevalent symptom in major depressive disorder (Treadway & Zald, 2011). Apathy and depressive symptoms also frequently co-occur in a number of neurodegenerative disorders (Rovner et al. 1989; Cummings 1992; Paulsen et al. 2005; Kirsch-Darrow et al. 2011). Furthermore, apathy and depression share a number of clinical features such as a loss of interest and psychomotor retardation (Landes et al. 2001). Given this close association, it is important to distinguish between these two syndromes as conventional treatments for major depression have little effect on apathy (Nutt et al. 2007). However, it is possible to distinguish apathy and depression in clinical samples. For example, some characteristics of depression, such as dysphoria are not observed in apathy (Levy et al. 1998; Lanctôt et al. 2017). Furthermore, apathy and depression have been associated with differential clinical outcomes in neurodegenerative diseases (Naarding et al. 2009). Apathy and depression show dissociable neuroimaging correlates (Starkstein et al. 2009; Dan et al. 2017) and across neurodegenerative disorders, high rates of

apathy are observed in non-depressed patients (Levy et al. 1998; Paulsen et al. 2005; Starkstein et al. 2001). Perhaps the key distinction between the two constructs is that apathy is an emotionally neutral state, whereas depression occurs in a severely negative emotional state (Radakovic et al. 2015).

A separate distinction is the relationship between apathy and anhedonia. Anhedonia, an inability to experience pleasure, is also commonly observed in a number of disorders associated with apathy (Der-Avakian & Markou 2012). Distinguishing the two constructs in clinical samples can be difficult as a loss of an ability to experience pleasure is a diagnostic symptom of emotional apathy (Landes et al. 2001; Robert et al. 2009). This means that apathy and anhedonia will likely share a high degree of overlap, as anhedonia will likely also contribute to a loss of goal directed behaviours. Consequently, in both the general population and neurodegenerative disease samples, apathy and anhedonia are highly correlated (Pluck & Brown 2002; Kaji & Hirata 2011; Ang et al. 2017). However, the lack of a complete overlap suggests some features may be separate. Apathy can be observed in the presence of otherwise intact reward processing in Schizophrenia and Parkinson's disease (Lee et al. 2015; Leentjens et al. 2008). Furthermore, in a study of healthy subjects, apathy was shown to be dissociable from both anhedonia and depression (Bonnelle et al. 2015). Rodent studies have also demonstrated that apathy and anhedonia have at least partially distinct neural substrates (Salamone et al. 1994; Der-Avakian & Markou 2012). Together, these studies suggest in spite of the overlap in clinical samples, apathy and anhedonia represent two distinct constructs, that may respond differentially to pharmacological interventions. An example of the overlap of neuropsychiatric symptoms observed in AD can be seen in figure 1.1.

1.1.2 Apathy prevalence and severity

The presence of symptoms of apathy is observed in neurodegenerative diseases, such as Alzheimer's disease (AD, Starkstein et al. 2001), Parkinson's disease (PD, Pedersen et al. 2009), Huntington's disease (HD, Naarding et al. 2009), frontotemporal dementia (FTD, Chow et al. 2009), vascular dementia (Staekenborg et al. 2010) and amyotrophic lateral sclerosis (Radakovic et al. 2016); as well as following both traumatic (Kant et al. 1998) and nontraumatic brain injury (Brodaty et al. 2005). Additionally, apathy is a common symptom in neuropsychiatric disorders such as Schizophrenia (Foussias et al. 2014) and major depressive disorder (Treadway & Zald 2011). The prevalence of motivational impairments in these diverse clinical groups is consistently high. Studies have reporting rates of apathy of up to 72% in AD (Mega et al. 1996); 38%-42% in PD (Starkstein et al. 1992; Pedersen et al. 2009), 28% in HD

(van Duijn et al. 2014) and 50% in cases of Schizophrenia (Bortolon et al. 2017). Across disorders, the presence of apathy is associated with significantly lower quality of life and increased caregiver burden (Ho et al. 1998; Boyle et al. 2003; Starkstein et al. 2006; Aarsland et al. 2007; Barone et al. 2009; Strauss et al. 2013; Spalletta et al. 2015; Dauphinot et al. 2015; Hongisto et al. 2018). AD patients with apathy show accelerated rates of cognitive decline and increased mortality rates (Starkstein et al. 2006; Spalletta et al. 2015). Together, these studies highlight the prevalence and severity of a loss of motivation.

Several studies have also suggested that apathy may emerge early in neurodegenerative diseases. For example, the presence of apathy is often noted several years prior to formal diagnosis of HD (Tabrizi et al. 2013; Epping et al. 2016). The presence and severity of apathy is also associated with a greater risk of developing AD in those with mild cognitive impairment (Robert et al. 2006). Finally, individuals with rapid eye-movement sleep behaviour disorder, who are likely to develop a neurodegenerative disorder such as PD or dementia with Lewy bodies (Iranzo et al. 2013) show significantly higher rates of apathy than control subjects (Barber et al. 2017; Barber et al. 2018). Together, these studies suggest that the presence of apathy can precede full disease diagnosis, highlighting the importance of understanding apathy in the context of diseases.

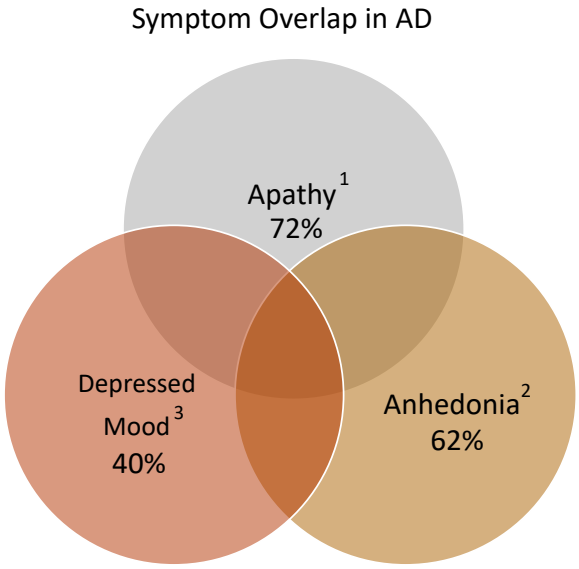


Figure 1.1: Example of the overlap and prevalence of symptoms in Alzheimer's disease (AD). [1]Mega et al., 1996. [2]Husain & Roiser 2018. [3] (Reichman & Coyne 1995)

1.1.3 Methods of assessment of apathy in humans

The majority of clinical studies assess apathy using questionnaire-based measures. These can consist of specific assessments such as the apathy evaluation scale (Marin et al. 1991), or

consist of subsets of more general scales such as the Neuropsychiatric Inventory (Cummings et al. 1994). They are also scales that have been validated for specific clinical populations such as the Lille apathy rating scale for PD (Sockeel et al. 2006). Although, such specific scales do not exist for a number of neurodegenerative disorders (Radakovic et al. 2015).

There are a number of considerations in the use of questionnaire-based apathy scales. Firstly, such measures may overlook any multidimensional aspects of apathy that can exit within a clinical population. Although certain scales that probe apathy subtypes do exist, (Radakovic & Abrahams 2014), they have not yet been widely used in large scale clinical populations. Secondly, scales may be confounded by a lack of self-awareness caused by the cognitive impairments that co-occur with apathy in many disorders (Lanctôt et al. 2017). A number of scales also rely on responses from caregivers; however, the agreement between self-report and caregiver ratings can be low (Zanetti et al. 1999; Chatterjee et al. 2005; McKinlay et al. 2008). For example, caregiver ratings on the severity of apathy appear to be heavily influenced by the burden placed on the caregiver (Mangone et al. 1993; Zanetti et al. 1999). It is possible that the responses given may be biased to reflect caregiver burden rather than apathy per se. The large variation in the quality of each scale may also confound comparison between studies that employ different questionnaires (Radakovic et al. 2015). Finally, it is not clear how well questionnaire based measures compare to the behavioural tasks used preclinically (see below, Luther et al. 2018).

1.1.4 Neuroimaging correlates of apathy

A number of studies have used structural and functional neuroimaging methods to determine the neural correlates of apathy. Recent in-depth reviews (Kos et al. 2016; Moretti & Signori 2016) highlight, that disruptions to fronto-striatal circuitry have been consistently associated with the presence of apathy, across disorders. Magnetic resonance imaging (MRI) studies of stroke patients with apathy have identified higher rates of frontal and prefrontal cortex damage compared to non-apathetic stroke patients (Kang & Kim 2008; Brodaty et al. 2005; Hama et al. 2007). Studies of post-stroke apathy have also implicated lesions within the basal ganglia as being predictive of the presence of apathy (Onoda et al. 2011; Hama et al. 2007). Similar findings have been reported in studies of neurodegenerative diseases. Studies using both structural and functional MRI techniques have associated abnormalities within the frontal lobe with apathy in PD (Reijnders et al. 2010; Skidmore et al. 2013), as well as reduced fronto-striatal resting state connectivity (Baggio et al. 2015). Apathy in PD has also been associated with reduced ventral striatum volume (Carriere et al. 2014). The association between structural

MRI-measured brain changes in AD and apathy is less clear. Some studies have reported an association between medial frontal lobe volume (Bruen et al. 2008; Tunnard et al. 2011) whereas others have not (Starkstein et al. 2009). Neuroimaging studies of frontotemporal dementia (FTD) have linked atrophy within the frontal lobe to motivational impairments (Rosen et al. 2005; Bertoux et al. 2012; Zamboni et al. 2008). Ventral striatal volume within FTD patients has also been linked to the prevalence of apathy (Rosen et al. 2005). However, no association between apathy and structural MRI measures was found in patients with HD (Scahill et al. 2013).

The neural correlates of motivational impairments have been widely studied in Schizophrenia. Like neurodegenerative samples, apathy in schizophrenia has been associated with reduced frontal volumes (Roth et al. 2004; Mørch-Johnsen et al. 2015) and hypoactivation of the ventral striatum during anticipation of rewards (Kirschner et al. 2016; Simon et al. 2010; Waltz et al. 2009; Wolf et al. 2014). Furthermore, the degree of this hypoactivation has been correlated with the severity of motivational impairments (Kirschner et al. 2016; Wolf et al. 2014)

To summarise, a loss of apathy is clearly present in a number of disorders including AD, PD and schizophrenia. Aberrant fronto-striatal function and structure have been consistently linked with the presence of apathy in these disorders. This suggests an approach, in line with RDoC, in which we bypass traditional diagnostic categories and instead focus on understanding the neurobiology of specific symptoms that exist across disorders (Insel et al. 2010; Cuthbert & Insel 2013). By understanding the biological basis of apathy, we may be able to treat it across a range of disorders.

1.2 Assessment of motivation in rodents

1.2.1 Parcellation of apathy

A great deal of work has focused on defining and operationalising motivation for preclinical assessment. Motivation can be divided into appetitive/anticipatory and consummatory processes (Craig 1918; Ikemoto & Panksepp 1996). The appetite-anticipatory phase refers to behaviours involved in approaching a goal; whereas the consummatory components are involved in the terminal phase of behaviour, governing the direct interaction with the goal (Ikemoto and Panksepp 1996; Salamone and Correa, 2012).

Another partition of motivation divides motivated behaviours into directional and activational processes (e.g. Duffy 1941; 1957; Bindra 1968). Directional components of motivation, which contain some aspects of reward seeking or approach behaviour, allow behaviour to be directed towards and away from appropriate goals. Activational aspects of motivation provide an organism with the vigour or energy required to overcome any costs or obstacles to obtain key stimuli. This governs the ability to initiate and maintain goal-directed behaviour (Salamone & Correa 2012). In humans, the presence of apathy appears to reflect changes in activational behaviours, rather than a deficit in directional or consummatory processes (Treadway et al. 2012; Wolf et al. 2014; Barch et al. 2014; Chong et al. 2015). Although much of the research has focused on effort exertion for appetitive rewards, a number of preclinical studies have suggested that activational behaviours are also necessary to avoid negative outcomes, such as footshocks (McCullough et al. 1993; Sokolowski et al. 1994). These studies suggest that it is possible to disrupt the ability of rodents to overcome the costs to needed to avoid an aversive event but leave the basic reactivity to the outcome intact (Salamone 1994). Deficits in exerting effort to avoid negative outcomes have also been reported in apathetic PD patients (Porat et al. 2014).

1.2.2 Preclinical assays to measure effort

In laboratory rodents, activational aspects of motivation can be probed through examining the expenditure of effort for food rewards (Salamone & Correa 2012; Salamone 1988). A number of behavioural assays have been developed to study effort, a number of which are outlined in table 1.1. One of the most commonly used assays of effort expenditure involves studying behaviour under a progressive ratio (PR) schedule of reinforcement (Hodos 1961). PR schedules probe the ability to maintain instrumental responding (such as lever-pressing) for rewards under escalating work demands. As the work requirement for each reward grows, an animal will eventually cease responding – which is known as the breakpoint. Breakpoints, therefore, can provide a measure of how much effort an animal is willing to exert for a given reward (Stewart 1975). This has been widely interpreted as giving a reflection of the motivational state of the organism. A drug-induced increase or decrease in breakpoint suggests that the compound is able to facilitate or disrupt motivated behaviour respectively. PR schedules were originally developed for use in the rat (Hodos 1961), however they have since been utilised across an number of nonhuman species including mice, pigeons and nonhuman primates (Dardano & Sauerbrunn 1964; Randt & Quartermain 1972; Griffiths et al. 1975).

Preclinical Assay	Advantages	Disadvantages
Progressive ratio (PR)	Highly sensitive to bi-directional modulations in performance Human equivalent	Could be confounded by changes in hedonic (appetitive) state
Effort-related Choice (ERC) tasks	Can dissociate changes in motivational and hedonic processes Human equivalent	Subject to ceiling effects. Typically requires the use of a deficit-model
Progressive Choice (PR Choice) tasks	Can dissociate changes in motivational and hedonic processes Does not require the use of a motivational-deficit model	Less widely used

Table 1.1: Overview of some of the assays used to probe effort-based behaviour in rodents

One disadvantage with the use of PR schedules is that a number of psychological processes other than a disruption in motivation could affect performance. For example, any manipulation that causes a disruption to motoric function or appetite could suppress breakpoint. Alternatively, any animal that was hyperactive, or compulsive may show elevated breakpoints. However, the risk of these potential confounds can be mitigated through the use of a battery of control tasks that test for potential mediating behaviours, such as change in hedonic state (Bailey et al., 2015)

An alternative or complementary approach is to employ effort-based decision-making tasks to probe activational processes of motivation (Salamone, Yohn, et al. 2016). The major advantage is these tasks can dissociate changes in motivational (i.e. effort exertion) and hedonic state (Salamone et al. 1997). The first effort related choice (ERC) task was developed for operant chambers (Salamone et al. 1991). During this task, rats can choose between performing an action (pressing a lever a given number of times) for a highly palatable reward (sucrose pellet) or consuming a freely available, but less preferred reward (such as standard lab chow). During these conditions, rats would readily respond for the highly preferred reward and consume little lab chow (Salamone et al., 1991). Crucially, perturbations that are believed to suppress effort-exertion result in a behavioural shift away from lever pressing to the low-effort, low-reward option (Salamone et al. 1991; Cousins et al. 1994).This is in contrast to the effects of appetite suppressant drugs, which result in a suppression of both lever pressing and chow consumption (Sink et al. 2008). ERC tasks have also been developed for use in T-mazes, where

animals are given a choice between entering one maze arm for a small reward, or scaling a barrier in the other arm to receive a larger reward (Salamone et al. 1994).

A potential limitation with the use of standard ERC tasks to test for enhancements in motivation is that ceiling effects may obscure a facilitation in performance following drug administration. This is because, at baseline, healthy animals choose the higher effort, high reward option, in the majority of trials. Therefore, many studies have tested the efficacy of pharmacological interventions in rescuing a deficit model. These deficit models include pre-treatment with the dopamine receptor antagonist haloperidol, or tetrabenazine, a vesicular monoamine transport inhibitor, both of which shift behaviour to the low-effort option without causing any significant change in appetite or motor function (Salamone et al. 1991; Nunes, Randall, Hart, et al. 2013). An alternative approach has been to combine the PR and ERC tasks into PR choice tasks (Schweimer & Hauber 2005; Randall et al. 2012). In these tasks, the work requirement for the valued reward increases throughout the experimental session. The escalating work requirement reduces ceiling effects but still allows for the dissection of hedonic and activational processes.

1.2.3 *Performance based tasks in humans*

In recent years some of the behavioural tasks used in rodents have been translated for use in humans. Several human versions of PR tasks exist that require participants to complete an easy but mundane task to emulate operant responding (e.g. Roane et al. 2001; Wolf et al. 2014; Strauss et al. 2016; Bland et al. 2016). Like the rodent PR the number of times this task is required for trial completion escalates throughout the session. This allows for the calculation of breakpoint in human participants. However, the same caveats apply for the use of PR with clinical samples, as with rodent versions. For example, patients with frontal lobe damage are known to show high rates of apathy (Eslinger & Damasio 1985) as well as high rates of perseverative responding on computerised tests (Owen et al. 1993). This perseverative responding would be expected to produce high breakpoints in this clinical population, in spite of the presence of apathy.

Like PR, versions of effort-based decision-making tasks have been translated for used for use in humans. One widely used assay is the Effort Expenditure for Rewards Task (EEfRT, Treadway et al. 2009). The task involves rapid button pressing for monetary rewards, with participants able to select from hard or easy versions of the task on each trial that yield larger or smaller rewards respectively. Other behavioural tasks manipulate effort through exerting hand grip force (Chong et al. 2015). Although such performance based tasks are promising,

and have been used to probe motivational deficits in clinical populations (Treadway et al. 2012; e.g. Barch et al. 2014; Treadway et al. 2015; Hershenberg et al. 2016; Wolf et al. 2014), there have been no large-scale studies to date using such measures to test pharmacological interventions in humans. Therefore, the findings reviewed below rely on traditional, questionnaire-based assessments of motivation. In spite of this, there appears to be good agreement between these measures and the behavioural tests used in rodents to probe motivated behaviours.

1.3 Apathy as an unmet clinical need: Pharmacological approaches to enhancement of motivated behaviour

1.3.1 Neurocircuitry of effort-based behaviour

The neurocircuitry of effort-related behaviour has been widely characterised. Regions including the anterior cingulate cortex (Walton et al. 2003); medial orbitofrontal cortex (Münster & Hauber 2017); basolateral amygdala (Floresco & Ghods-Sharifi 2007) and hippocampus (Schmelzeis & Mittleman 1996) have all been shown to be involved in effort-expenditure for appetitive rewards. One system that has been extensively relative to effort-based behaviour studied is the mesolimbic dopamine (DA) pathway. The mesolimbic pathway, one of the major DAergic pathways of the brain, consists of DA neurons projecting from the ventral tegmental area to the striatum. Dopaminergic depletions or local receptor blockade within the nucleus accumbens (NAc) region of the ventral striatum impairs PR performance (Aberman et al. 1998; Hamill et al. 1999; Bari & Pierce 2005). In contrast, excitotoxic lesions to the NAc increase PR breakpoints (Bowman & Brown 1998); whereas lesions to dorsal regions of the striatum do not affect breakpoint (Eagle et al. 1999).

Similar (but not identical) findings have been observed using effort-based decision-making tasks (reviewed in Bailey, Simpson, et al. 2016). Dopamine depletions of the NAc or local DA receptor blockade shift performance from the higher-effort high-reward options, to the low-cost less preferred choices in ERC tasks (Salamone et al. 1991; Cousins & Salamone 1994; Nowend et al. 2001). The NAc can be further subdivided into the core and shell sub-regions (Záborszky et al. 1985). The effects of DAergic manipulations on effort-related behaviour appear to driven by the core, not the shell, sub-region of the NAc (Sokolowski & Salamone 1998; Bari & Pierce 2005).

A number of brain regions beyond the nucleus accumbens have also been implicated in supporting effort exertion. As reviewed above, human imaging studies have implicated altered activity within the prefrontal cortex (PFC) in patients displaying apathy. In rodents, an appreciation of the neural circuitry supporting behaviour can be gained through disconnection lesion-based approaches, where asymmetric unilateral lesions or infusions of GABA agonists are administered. In mice, lesions to the prelimbic region of the PFC significantly reduces PR breakpoint (Gourley et al. 2010). Numerous studies have also implicated the anterior cingulate cortex (ACC) in effort-based behaviour. Excitotoxic lesions to the ACC shift behaviour from preforming a high-effort high-reward behaviour to a lower effort smaller reward option, in both maze- and lever-based ERC tasks (Walton et al. 2003; Schweimer & Hauber 2005; Walton et al. 2009). The ACC has strong projections to the NAc core (Brog et al. 1993). Disconnecting the ACC and the NAc Core significantly impaired ERC performance, demonstrating a necessary role of the ACC-NAc circuitry in effort-based behaviour (Hauber & Sommer 2009). Like the ACC, lesions to the basolateral nucleus of the amygdala (BLA), disrupts effort-based decision making (Floresco & Ghods-Sharifi 2007; Ghods-Sharifi et al. 2009). The BLA also projects to the NAc (McDonald 1991) and receives projections from the ACC (Mcdonald et al. 1996). Disconnecting this BLA and ACC circuit through asymmetric lesions impairs performance (Floresco & Ghods-Sharifi 2007). Effort-based behaviour can also be disrupted through interfering with the output projections from the NAc. Disconnecting the NAc from the ventral pallidum also shifts behaviour to the low effort, low reward action (Mingote et al. 2008), mirroring the effects of DAergic lesions to the NAc. Taken together, these studies demonstrate an extended network of brain regions involved in supporting effort-based behaviour.

1.3.2 Dopaminergic based approaches

As disruptions to DA function impair effort-based behaviour, manipulations that enhance NAc DA function may facilitate motivated behaviour. Neurotransmission of DA can be enhanced with psychostimulants. For example the indirect catecholamine agonist d-amphetamine, which enhances DA release within the striatum (Westerink et al. 1987), facilitates PR performance in rodents. Infusions of amphetamine into the nucleus accumbens (NAc) region of the striatum enhances PR breakpoints (Zhang et al. 2003; Wirtshafter & Stratford 2010). Furthermore, systemic administration of amphetamine facilitates PR performance in rats and mice (Poncelet et al. 1983; Bensadoun et al. 2004; Heath et al. 2015). Amphetamine can also facilitate selection of high-effort high-reward options in some effort-related decision-making tasks (Floresco et al. 2008; Bardgett et al. 2009), but not others (Cousins et al. 1994).

In healthy human subjects, amphetamine facilitates high-effort selection in the EEfRT task (Wardle et al. 2011), particularly in trials where the likelihood of reward was low. There have also been a number of amphetamine studies investigating the effects in clinical samples using observational and questionnaire-based measures. Amphetamine has been reported to be effective in reducing apathy in small group of patients with FTD (Huey et al. 2008). Furthermore, in elderly subjects residing in care homes, amphetamine was effective at significantly reducing rates of apathy in the majority of subjects (Clark & Mankikar 1979).

Other psychostimulant compounds have also demonstrated efficacy in facilitating motivated behaviours in rodents. The dopamine reuptake inhibitor methylphenidate, (Ferris & Tang 1979) is typically prescribed as a treatment in attention deficit hyperactivity disorder as well as narcolepsy (Wenthur 2016), Like amphetamine, one of the major physiological actions of methylphenidate is to increase the levels of dopamine within the NAc (Kuczenski & Segal 1997). Systemic administration of methylphenidate to rats increases PR breakpoints (Poncelet et al. 1983; Mayorga et al. 2000). Furthermore, methylphenidate is able to rescue an induced deficit in ERC performance following pre-treatment with the pro-inflammatory cytokine, interleukin-6 (Yohn, Arif, et al. 2016). The ability to facilitate effort exertion in intact rodents, and rescue a motivational deficit provides strong preclinical evidence for pro-motivational efficacy.

A number of clinical studies have reported that methylphenidate has been successful in reducing symptoms of apathy, across a number of disorders. Single patient or small scale case reports have noted that motivational deficits were improved following methylphenidate treatment, patients with subcortical infarcts (Watanabe et al. 1995), PD (Chatterjee & Fahn 2002), hydrocephalus (Keenan et al. 2005), major depression (Padala et al. 2005), and acquired brain injuries (Spiegel et al. 2009). The effects of methylphenidate on apathy have also been studied in larger randomised control trials (RCTs). An initial small study of patients with AD reported a significant reduction in the severity of apathy (Galynker et al. 1997). Subsequent larger RCTs, investigating the effect of methylphenidate treatment on apathy in AD patients have replicated this finding (Herrmann et al. 2008; Rosenberg et al. 2013; Padala et al. 2017). Together, these studies present strong support for the use of methylphenidate in AD to treat apathy; however, further research is needed to explore the potential therapeutic benefits in other disorders.

Bupropion also acts as a dopamine reuptake inhibitor (Dwoskin et al. 2006), and has been used as a treatment for depression as well as a smoking cessation aid (Stahl et al. 2004). Bupropion has been shown to produce a dose-dependent increase in PR breakpoints (Bruijnzeel & Markou 2003). Furthermore, systemic administration of bupropion increases selection of the high-effort, high reward option in a PR-choice task in rats (Randall, Lee, Podurgiel, et al. 2014). Bupropion is also effective at rescuing motivational impairments in rodents. Administration of bupropion can rescue deficits in effort-related decision-making induced by pre-treatment with tetrabenazine (Randall, Lee, Nunes, et al. 2014; Nunes, Randall, Hart, et al. 2013) and the pro-inflammatory cytokine interleukin-6 (Yohn, Arif, et al. 2016).

Bupropion has been reported to improve symptoms of apathy in cases of acquired brain injury, major depression (Corcoran et al. 2004) and frontotemporal dementia (Lin et al. 2016). However, several larger placebo-controlled studies suggest only limited effects of bupropion. In a study of 40 patients with schizophrenia, bupropion was found to have no significant effect on apathy or negative symptoms as a whole (Yassini et al. 2014). Furthermore, in a recent RCT of bupropion in HD, apathy was not significantly affected by the drug (Gelderblom et al. 2017). It is not clear whether bupropion lacks clinical efficacy, or whether bupropion as a whole is not affective at treating motivational impairments, or simply not effective in the clinical populations tested

Novel dopamine reuptake inhibitors have also been demonstrated to facilitate motivated behaviour indexed by PR and PR Choice tasks in healthy rodents (Sommer et al. 2014) and are able to rescue a tetrabenazine deficit model (Yohn, Gogoj, et al. 2016). Another compound which may act, as a weak dopamine reuptake inhibitor is the wakefulness promoting drug modafinil (Minzenberg & Carter 2008). In rodents, modafinil has been shown to increase extracellular DA within the NAc (Murillo-Rodríguez et al. 2007). However, the evidence for motivational enhancing effects of modafinil in rodents is mixed. One study suggested modafinil produced a small but significant increase in PR breakpoints in mice (Young & Geyer 2010). Whereas, another study has reported no effects on either PR or PR choice performance in rats (Sommer et al. 2014). However, modafinil has been reported to partially reverse a ERC deficit (Yohn, Gogoj, et al. 2016). Together, this suggests that modafinil may be more effective at reversing a deficit rather than facilitating effort-exertion in healthy rodents.

Like the mixed preclinical results, it is not clear if modafinil is a useful therapeutic option for apathy in clinical cases. There are several single case reports suggesting that modafinil can improve symptoms of apathy in elderly subjects (Padala et al. 2007; Camargos & Quintas 2011). A systematic review of five RCTs in schizophrenia concluded that modafinil or armodafinil (the R-enantiomer of modafinil) may be effective at reducing negative symptoms (Andrade et al. 2015). However, it is not clear whether these effects are linked to changes in motivated behaviour specifically. The RCTs in question all employed composite measures of negative symptoms as endpoints – the subscale of the Positive and Negative Syndrome Scale (PANSS-N) or the Scale for Assessment of Negative Symptoms (SANS), rather than a specific measure of apathy. Although apathy is a subdomain of both of these rating scales (Kay et al. 1987; Andreasen 1989), both scales consist of a number of other domains independent from apathy, that may underlie the global change in negative symptoms score. In particular, several domains are linked to inattentiveness across both scales (Kay et al. 1987; Andreasen 1989). Given the stronger association between modafinil and attention in schizophrenia (Morein-Zamir et al. 2007). It is possible that the effects reported here are primarily driven by a domain other than apathy. Furthermore, a small RCT of the efficacy of modafinil at treating apathy in AD, found no significant difference from placebo on symptoms of apathy (Frakey et al. 2012). Overall, the mixed evidence for the efficacy of modafinil may be due to its weaker effect on DA function compared to other psychostimulants such as methylphenidate.

Several studies have investigated the effects of monoamine-oxidase (MAO) inhibitors on effort expenditure in rodents. The MAO-B inhibitor deprenyl (also known as selegiline) is used as a treatment in Parkinson's disease and depression (Miklya 2016) Although it is not clear whether administration of deprenyl is sufficient for an increase extracellular DA levels (Kato et al. 1986; Butcher et al. 1990; Yohn et al. 2018), the compound does potentiate stimulant induced DA release (Schiffer et al. 2003). Acute administration of deprenyl has been shown to partially rescue a tetrabenazine-induced deficit on both ERC and PR choice tasks (Randall, Lee, Nunes, et al. 2014; Contreras-Mora et al. 2018). Deprenyl is also able to facilitate high effort selection on a PR choice task in intact rats (Yohn et al. 2018). Together, these studies suggest that DAT inhibition as well as MAO-B inhibitors can increase effort expenditure in intact rodents as well as reverse pharmacological-induced motivational deficits.

The MAO-B inhibitor deprenyl has been linked to positive changes in motivation in clinical samples. Case reports suggest deprenyl may be effective at treating apathy in cases of traumatic brain injury (Newburn & Newburn 2005; Moutaouakil et al. 2009). An initial open-label study

suggested deprenyl may also be effective at reducing negative symptoms in schizophrenia (Bodkin et al. 1996), which has since been replicated in a placebo controlled RCT (Amiri et al. 2008). Furthermore, deprenyl is able to significantly reduce symptoms of apathy in patients with schizophrenia relative to placebo (Bodkin et al. 2005). However, there is no clear evidence of any benefit of deprenyl administration to those with AD (Birks & Flicker 2003, for a review).

Many of the effective compounds described above (e.g. amphetamine, methylphenidate, bupropion) act on the noradrenaline system (Kuczenski & Segal 1997). However, tests of selective noradrenaline reuptake inhibitors atomoxetine and desipramine suggest that the effects of psychostimulants on motivated drugs are not driven by affecting noradrenaline function. In rats, atomoxetine does not affect effort-related decision making (Hosking et al. 2015) nor affect responding in a PR choice task (Yohn, Errante, et al. 2016). Furthermore, desipramine does not rescue a tetrabenazine-induced deficit in ERC (Yohn, Collins, et al. 2016). In line with preclinical evidence, atomoxetine does not reduce symptoms of apathy in PD (Weintraub et al. 2010).

Taken together there is clear evidence that enhancing dopamine neurotransmission can facilitate effort-related behaviour in rodents and improve symptoms of apathy on clinical samples. However, it is also important to note that there may be several problems with the use of dopaminergic therapy, particularly in clinical groups. These include exacerbation of pre-existing symptoms, particularly in the case of schizophrenia (Janowsky & Davis 1976; Janowsky et al. 1973; Lindenmayer et al. 2013); abuse liability (Morton & Stockton 2000); and increasing maladaptive behaviours such as impulsivity (Cools et al. 2003).

1.3.3 Effects of serotoninergic compounds

Research has also investigated the therapeutic potential of non-dopaminergic targets in motivated behaviours. Compounds that affect the activity of another monoamine, serotonin (5-HT) can also affect effort exertion in rodents. Serotonin reuptake inhibitors (SSRIs) are amongst the most common forms of antidepressant medication (Jakobsen et al. 2017). However, in laboratory animals, administration of SSRIs has been shown to either reduce PR breakpoints or have no effect (Cilia et al. 2001; Sanders et al. 2007; Mathes et al. 2013). Additionally, SSRIs are not effective at reversing pharmacological-induced deficits on effort-related decision-making (Yohn, Collins, et al. 2016; Yohn, Lopez-Cruz, et al. 2016).

Different 5-HT receptor subtypes appear to have differential roles in motivated behaviour. For example, 5-HT_6 stimulation can facilitate PR breakpoint, whereas 5-$HT_{1/7}$ receptor stimulation reduces breakpoints (Pratt et al. 2012). A number of studies have focused on the 5-HT_{2c} subtype, due to a relationship with dopamine transmission (Di Giovanni et al. 1999; Alex et al. 2005). Micro-dialysis studies have allowed for the investigation of the effects of 5-HT_{2c} ligands on dopamine release. In particular several studies have investigated the effects of such drugs on NAc dopamine efflux. Systemic and local administration of 5-HT_{2c} agonists results in a decrease in NAc levels (Alex et al. 2005). Conversely, 5-HT_{2c} receptor agonists can result in an increase in dopamine influx. Studies with genetic KO mice have confirmed this relationship (Huang et al. 2011). Similar 5-HT_{2c} – DA associations have been observed within the dorsal striatum and prefrontal cortex (Bailey et al. 2018; Di Giovanni et al. 2000).. Due to this relationship it would be anticipated that 5-HT_{2c} agonists and antagonists would decrease and increase effort expenditure respectively. In line with this, activation of 5-HT_{2c} receptors reduces PR breakpoints (Fletcher et al. 2010; Bezzina et al. 2015; Wolff & Leander 2000). Conversely, PR performance can be facilitated by the 5-HT_{2c} receptor antagonist SB242084 (Simpson et al. 2011; Bailey, Williamson, et al. 2016). Furthermore, SB242084 also facilitates high effort choices on a modified ERC task (Bailey, Williamson, et al. 2016).

Much of the research in humans has investigated the efficacy of SSRIs on apathy. In line with the preclinical evidence, these studies suggest SSRIs have little effect on the symptoms of apathy and may even exacerbate motivational impairments. SSRIs typically do not affect rates of apathy present in major depression (Nutt et al. 2007). In elderly patients, SSRI use is associated with higher levels of apathy (Wongpakaran et al. 2007) (Padala et al. 2012; Barnhart et al. 2004). Similar findings have been reported in other clinical populations. A number of RCTs have reported that SSRIs do not reduce apathy in AD patients (Pollock et al. 2002; Siddique et al. 2009; Porsteinsson et al. 2014). The use of SSRIs as an add-on to antipsychotic medication appear to have no effect on symptoms of apathy in schizophrenia (Hayashi et al. 1997), or HD (Beglinger et al. 2014). Whereas, SSRI use is associated with greater levels of apathy in PD (Zahodne et al. 2012). Together, these studies suggest that SSRIs have little positive impact on apathy in clinical populations. There is however, evidence is evidence to suggest that 5-HT_{2c} antagonists may be able to treat apathy, in line with the preclinical findings. Agomelatine is a nonselective 5-HT_{2c} antagonist (Millan et al. 2003), used as an antidepressant treatment. In a case report, agomelatine was reported to reduce SSRI-induced apathy (Callegari et al. 2016). Furthermore agomelatine was found to reduce apathy in a group of non-depressed patients with FTD (Callegari et al. 2016). This early result suggests that 5-HT_{2c} receptors may

be a viable target for treating motivational impairments; however, this finding should be fully explored with the use of larger -scale RCTs.

One caveat with the therapeutic use of 5-HT_{2c} agonists are the effects of this class of drugs on appetite. In rodents, activation of 5-HT_{2c} receptors facilitates the onset of satiety and reduces free food consumption (Hewitt et al. 2002; Clifton et al. 2000). Conversely genetic deletion of 5-HT_{2c} increases feeding behaviour in mice (Heisler & Tecott 1999). This may potentially confound the rodent studies of effort, reviewed above, which used food-reinforced assays to measure motivation. Although some studies have utilised sucrose consumption tests as a control (Bailey, Williamson, et al. 2016), the results on breakpoint should be interpreted with caution due to a potentially mediating effect of appetite. In humans, long-term use pharmacotherapies with of 5-HT_{2c} antagonist properties are associated with alterations in appetite regulation (Reynolds & Kirk 2010). This is believed to underlie the significant weight-gain associated with atypical antipsychotic use (Lord et al. 2017); a hypothesis that is supported by preclinical studies (Kirk et al. 2009). Together, this may limit the potential therapeutic benefit of targeting the 5-HT_{2c} receptor for the treatment of apathy.

1.3.4 Effects of adenosinergic compounds

Another pharmacological target that has been linked with effort-based behaviour is the adenosine system. The widely used psychostimulant caffeine acts as a nonselective adenosine receptor antagonist (El Yacoubi et al. 2000). In rodents, caffeine increases effort expenditure for food reward. When administered prior to PR testing, acute and chronic administration of caffeine increases breakpoint (Poncelet et al. 1983; Sheppard et al. 2012; Retzbach et al. 2014). In intact rats, a moderate dose of caffeine can produce a small but significant facilitation of PR-choice performance (SanMiguel et al. 2018). Furthermore, caffeine can partially rescue the effort-related decision-making deficit induced by the D_2 receptor antagonist haloperidol (Salamone, Farrar, et al. 2009).

Studies investigating the effects of caffeine on effortful behaviour in humans date back over a century (Rivers & Webber 1907). A small observational study of elderly patients with dementia reported a significant negative correlation between the quantity caffeine consumption and severity of apathy (Kromhout et al. 2014). This finding suggests that caffeine may reduce the severity of apathetic symptoms. Although a recent case study suggests that caffeine may not work as a direct intervention for apathy (Kromhout et al. 2017), a larger study of elderly

subjects aims to investigate the therapeutic potential of caffeine (the BeCAF study), which remains an ongoing trial, with no results yet published (Kromhout et al. 2018).

Many of the behavioural actions of caffeine may be driven primarily through actions at the adenosine A_{2A} receptor subtype (El Yacoubi et al. 2000). Within the striatum, adenosine A_{2A} receptors are colocalised with DA receptors on D_2-like MSNs (striatopallidal neurons) where they exert opposing effects on the adenylyl cyclase signalling pathway (Ferré et al. 2008). Antagonism of adenosine A_{2A} receptors with systemic administration of the compound MSX-3, can reverse a motivational deficit defect on ERC tasks induced by systemic haloperidol (Farrar et al. 2007; Mott et al. 2009) and tetrabenazine (Nunes, Randall, Hart, et al. 2013). Systemic administration of MSX-3 also facilitates high effort choices in a PR choice performance in intact rats (Randall et al. 2012). Other A_{2A} receptor antagonists are also able to reverse the motivational deficits induced by dopamine D2 receptor blockade (Nunes et al. 2010; Collins et al. 2012). Together, these results suggest a possible therapeutic benefit of A_{2A} receptor blockade in the treatment of motivational impairments.

Several studies have investigated adenosine antagonists as pharmacological treatments for Parkinson's disease. A recent RCT investigated the effect of caffeine in Parkinson's patients (Postuma et al. 2017). Although apathy was not specifically examined, the authors reported that caffeine did not produce any benefit on quality of life, cognitive function or mood (Postuma et al. 2017). Selective A_{2A} receptor antagonists appear effective at treating motoric symptoms of Parkinson's Disease (Sako et al. 2017). Several case reports suggest that A_{2A} receptor antagonists may benefit mood and fatigue in Parkinson's disease (Nomoto et al. 2014). However, there is currently no studies specifically examining the effects of adenosine A_{2A} receptor antagonists on apathy in Parkinson's disease. Further research is needed to investigate the potential therapeutic benefits of A_{2A} receptors as targets for motivational dysfunction.

Pharmacological Target / Mechanism	Preclinical findings	Clinical findings
Dopamine reuptake transporter / inhibition	Increases PR breakpoint in healthy rodents Rescue a tetrabenazine-induced ERC deficit Facilitates high effort selections on a PR-choice task in healthy rodents	Can reduce apathy in Alzheimer's disease May be effective at treating apathy in PD, depression and following brain injuries

		No evidence of efficacy in Huntington's disease or schizophrenia
Monoamine oxidase B / inhibition	Rescue tetrabenazine-induced ERC and PR-choice deficits Facilitates high effort selections on a PR-choice task in healthy rodents	May be effective at reducing motivational impairments in schizophrenia
Serotonin 2c receptor / antagonism	Increases PR breakpoint in healthy rodents Facilitates high effort selections on a modified ERC task	May be effective at reducing apathy in cases of frontotemporal dementia
Adenosine A2A receptor / antagonism	Rescue a tetrabenazine-induced ERC deficit Facilitates high effort selections on a PR-choice task	Further research needed

Table 1.2: Overview of the effects of the effects of pharmacological interventions for apathy.

1.3.5 Resistance to current treatments

The typical front-line treatments for many neuropsychological and neurodegenerative disorders have little impact on apathy. In schizophrenia, neither typical nor atypical antipsychotics are effective in treating motivational impairments (Fusar-Poli et al. 2015; Fervaha et al. 2015). Furthermore, there is some evidence to suggest antipsychotic treatment may in fact exacerbate symptoms of apathy in patients (Artaloytia et al. 2006) and induce impairments in healthy subjects (Mas et al. 2013). Symptoms of apathy arising during cases of major depression are also resistant to traditional pharmacological treatments (Nutt et al. 2007), and may be exacerbated by serotoninergic-based antidepressant medication (Barnhart et al. 2004). In AD, there is no clear evidence whether the acetylcholinesterase inhibitors (AChEIs) used to treat cognitive impairments can produce clinically meaningful effects on rates of apathy (Ruthirakuhan et al. 2018). Apathy also appears resistant to the non-AChEI treatment, memantine (Kishi et al. 2017). Likewise, the only approved pharmacological treatment for HD, tetrabenazine, does not help reduce the severity of apathy (Mestre et al. 2009). These studies highlight how conventional treatment approaches However, one possible exception arises in PD, where treatment where dopaminergic-based treatments, such as levodopa, may reduce the symptoms of apathy associated with the disease (Sousa et al. 2018; Chong et al. 2015).

As the foregoing review indicates, apathy remains an unmet clinical need. However, there is clear therapeutic promise with a number of pharmacological approaches, in particular methylphenidate. Further work is needed to fully explore the potential benefits of 5-HT_{2c} and adenosine A_{2A} receptor antagonists in treating motivational impairments. In spite of differences in the way motivation is assessed in laboratory animals and clinical groups, the similarity in the efficacy in a number of compounds suggests that examining effort exertion can model the motivational processes that occur in humans. This strengthens the case for clinical studies to build on future preclinical work discovering new targets.

1.4 The Muscarinic system

1.4.1 Muscarinic receptors location and pharmacology

As reviewed above, there is a clear association between DA function and motivated behaviour. Furthermore, both 5-HT_{2c} and adenosine A_{2A} receptor antagonists are able to modulate DA function ((Salamone, Farrar, et al. 2009; Valencia-Torres et al. 2017). A separate neurotransmitter that can act as a neuromodulator is acetylcholine (ACh) (Picciotto et al. 2012). In particular, ACh plays a key role in modulating striatal circuitry and function (for reviews see: Cragg 2006; Exley & Cragg 2008; Picciotto et al. 2012). ACh receptors consist of muscarinic (mAChR) and nicotinic (nAChR) types. Of the muscarinic type, there are five receptor subtypes, M_1-M_5(Dörje et al. 1991; Bonner et al. 1987). Based on morphology and function these have been divided into M_1-type (M_1, M_3, M_5) and M_2- type (M_2, M_4) mAChRs (Caulfield & Birdsall 1998). mAChRs are expressed in peripheral and central locations (Hulme et al. 1990). In peripheral tissue, mAChRs are expressed within the gut, heart and bladder (Caulfield 1993). Centrally, some degree of regional localisation of receptor subtypes has been found within the rodent brain (table 1.3). The M_1 receptor is the most common mAChR in the CNS (Langmead et al. 2008). M_1 receptors are the highest expressed subtype in the cortex and hippocampus, (Levey et al. 1991; Levey et al. 1995). M_1 receptors are also widely expressed in both dorsal and ventral regions of the striatum (Weiner et al. 1990) and to a lesser extent the thalamus and hindbrain (Levey 1993). M_2 receptors are also located within the striatum, along with high expression within thalamus and cortex (Levey et al. 1991; Levey 1993). Unlike the other subtypes, M_3 receptors show far lower levels of CNS expression; however, M_3 receptors are expressed within the cortex and hippocampus (Wall et al. 1991). M_4 receptors, like the M_1 subtype are expressed throughout the brain, particularly within the striatum where they are the most widely expressed mAChR (Bernard et al. 1992). Finally, M_5 receptors are the most

discretely localised receptor, with expression largely confined to the substantia nigra and ventral tegmental area (Vilaró et al. 1990). M_1 receptors show similar levels of expression in both the dorsal and ventral striatum, whereas M_2 and M_4 receptors are expressed in higher levels in the ventral striatum (Tayebi et al., 2004, table 1.3).

Receptor Subtype	CNS Location	Striatal Location	Signalling pathway
M_1	Hippocampus Striatum Thalamus Cortex	Similar ventral and dorsal expression Post-synaptic: D1-type and D2-type MSNs	$G_{q/11}$ diacylglycerol and inositol 1,4,5-triphosphate
M_2	Thalamus Striatum Cortex	Ventral > dorsal expression Presynaptic: CINs, glutamatergic neurons	$G_{i/o}$ Inhibits adenylyl cyclase
M_3	Thalamus Cortex	Minimal	$G_{q/11}$ Activates diacylglycerol and inositol 1,4,5-triphosphate
M_4	Thalamus Striatum Cortex	Ventral > dorsal expression Presynaptic: CINs, DA neurons Post-synaptic: D1-type MSNs	$G_{i/o}$ Inhibits adenylyl cyclase
M_5	Substantia Nigra pars compacta Ventral Tegmental Area	Minimal	$G_{q/11}$ Activates diacylglycerol and inositol 1,4,5-triphosphate

Table 1.3: Outline of the localisation and signalling pathways of centrally-located muscarinic acetylcholine receptors. CIN: cholinergic interneurons; CNS: Central Nervous System; DA: dopamine; MSN: Medium Spiny Neurons.

Muscarinic receptors also differ according to their pharmacological profile. Muscarinic receptors are G-protein coupled receptors (GPCRs) M_1-type (M_1, M_3, M_5) receptors couple to the $G_{q/11}$ protein, and M_2- type (M_2, M_4) receptors couple to the $G_{i/o}$ protein. Figure 1.2 shows how the stimulatory M_1 class of receptor activates phospholipase c, which causes the generation of the second messengers diacylglycerol and inositol 1,4,5-triphosphate (Felder 1995). Inositol 1,4,5-triphosphate subsequently mobilises intracellular calcium and diacylglycerol activates protein kinase C (Felder 1995). Activation of M_2- type receptors inhibits adenylyl cyclase,

which reduces the availability of the second messenger cyclic adenosine monophosphate (cAMP, table 1.3, figure 1.2).

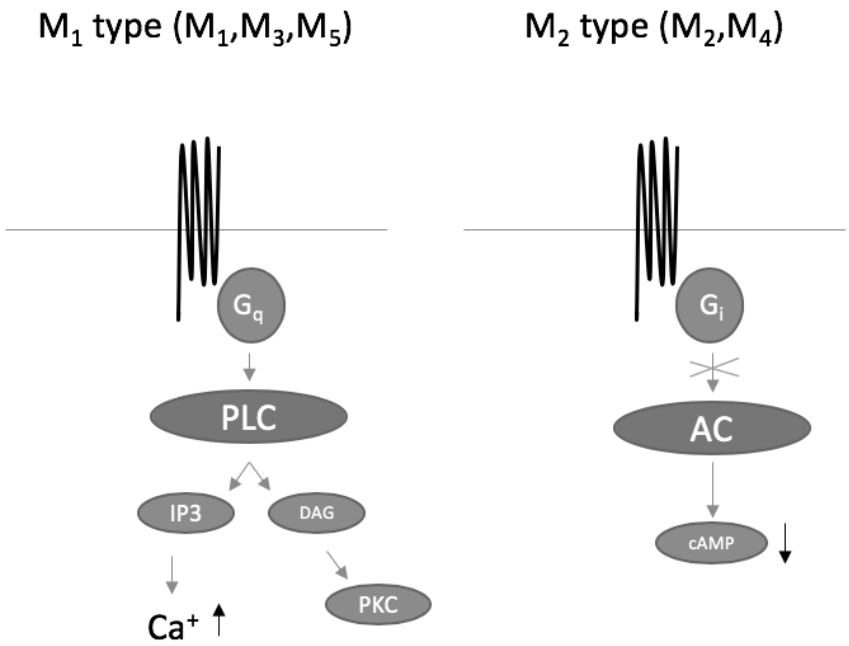


Figure 1.2 Visualisation of the signalling pathways of M_1 and M_2 type muscarinic receptors. M_1 type receptors activate phospholipase C .M_2 receptors inhibit adenyl cyclase. PLC: phospholipase C; IP3: inositol 1,4,5-triphosphate; DAG: diacylglycerol activates; PKC: protein kinase C; AC: adenylyl cyclase; cAMP: cyclic adenosine monophosphate

Striatal levels of ACh, as well as acetylcholinesterase expression are amongst the highest of anywhere in the brain (Macintosh 1941; Weiner et al. 1990). Throughout the striatum, cholinergic tone is governed by tonically active cholinergic interneurons (CINs, Zhou et al. 2002). CINs only comprise of 1-2% of the striatal neuron population (Phelps et al. 1985; Bolam et al. 1984); however, they have been heavily implicated in modulating striatal function (Goldberg et al. 2012; Gonzales & Smith 2015). CINs express presynaptic M_2 and M_4 receptors (Bernard et al. 1992), where they function as autoreceptors. Activation of these receptors has been shown to supress ACh release from CINs (Zhang et al. 2002). The majority of the remaining striatal neurons (90-95%) are GABAergic medium spiny neurons (MSNs, Goldberg et al. 2012). These MSNs are the primary output neurons of the striatum and have been divided into two pathways. The dopamine D_1 receptor expressing striatonigral MSNs (also known as the direct pathway) and the dopamine D_2 receptor expressing striatopallidal MSNs (also known as the indirect pathway, Gerfen et al. 1990). M_4 receptors appear largely co-expressed with D_1 receptors on striatonigral MSNs, where they exert opposing effects to D_1 receptors which activate adenylyl cyclase, and subsequent cAMP signalling (Girasole & Nelson 2015).

In the striatum, D_1 receptor dependent cAMP signalling is mediated by the $G_{\alpha olf}$ protein, unlike in cortical areas where D_1 receptors are believed coupled to $G_{\alpha s}$ proteins, due to the clear regional distribution of the expression of these G-protein subunits (Hervé et al. 1993; Hervé 2011; Yano et al. 2018).

M_1 receptors, in contrast to M_4 receptors , are expressed on both MSN types (Goldberg et al. 2012). M_1 receptors increase MSN excitability through promoting the closure of potassium channels (Shen et al. 2005). Interestingly, M_1 receptor modulation of dendritic excitability by Kir2 potassium channels is found almost exclusively in striatopallidal MSNs, due to elevated expression of the Kir2.3 subunits in these neurons (Shen et al. 2007). This raises the possibility that activation of M_1 receptors preferentially modulates excitability in the D_2-containing striatopallidal MSNs (Shen et al. 2007; Oldenburg & Ding 2011).

It is worth noting that muscarinic receptors express highly similar acetylcholine binding pockets. In particular there is a high degree of similarity in the structure of $M_1$and M_4 receptors (Thal et al. 2016) This has made development of highly selective compounds that act on the orthosteric binding sites difficult (Bradley et al. 2017). As a consequence, there is a lack of highly selective ligands that can act exclusively on one mAChR subtype. The lack of selective ligands may have led to some inconsistency in the literature, as compounds have at best, preference for one mAChR subtype. Therefore, a cautious approach should be adopted in interpreting pharmacological effects of one receptor subtype over another. mAChR allosteric binding sites are topographically separate from the site at which endogenous ligands bind on, are less evolutionarily conserved than the orthosteric binding sites (Bridges et al. 2010). Therefore, it may be possible to produce allosteric compounds with far greater selectivity for mAChR subtypes (Conn et al. 2009). Additionally, combining the use of genetic mAChR knock-out mice along with mice have helped researchers to understand the function of mAChRs (Wess et al. 2007).

1.4.2 mAChRs and Dopamine function

Converging evidence suggests that mAChRs can modulate dopamine (DA) release and function within the striatum. However, reports of the effects of mAChR ligands on DA release have been somewhat conflicting. For example, mAChR agonists have been reported to both potentiate and supress striatal DA release (de Belleroche & Gardiner 1982; Kudernatsch & Sutor 1994; Chapman et al. 1997). It is likely that differing receptor subtypes have opposing

roles and DA release (Threlfell et al. 2010). The conflicting results may be due to the poor selectivity of many classical mAChR orthosteric compounds (Conn et al. 2009). However, a combined approach of using more selective ligands as well as muscarinic receptor knock out (KO) mice, has provided evidence of the modulatory role mAChRs have on DA release. Pharmacological activation of M_2 and M_4 receptors inhibits striatal DA release, through an autoreceptor-mediated reduction in cholinergic tone (Threlfell et al. 2010; Shin et al. 2015). The reduction in local cholinergic tone, reduces the activity of nAChRs expressed on DAergic axons (Threlfell et al. 2010), reducing DA release. Conversely, M_5 receptor activation potentiates striatal DA transmission (Shin et al. 2015), likely through actions on VTA DA neurons. Alongside mAChR subtype differences, there are also regional influences on the muscarinic-dopamine relationship. For example, M_2 and M_4 are necessary for mAChR mediated control of DA release within the dorsal striatum (Threlfell et al. 2010). In contrast only M_4, but not M_2 receptors, are necessary for mAChR regulation of NAc DA (Threlfell et al. 2010). In a separate study, M_4 KO mice were shown to display enhanced amphetamine–induced NAc DA release relative to wild-type mice, whereas M_2 KO mice did not (Tzavara et al. 2004); however, no behavioural data was reported in this study. It is also worth noting, that the ability of mAChRs to modulate DA function is at least partially dependent on the activity of the DA system (Threlfell et al. 2010). This suggests, that different results may be observed depending on, for example, whether an animal is behaving or idle. Therefore, there is a well-established pathway for presynaptic M_4 receptors modulating DAergic function, via a decrease in ACh tone. M_4 receptors are also able to modulate DA function independently of this established nAChR-mediated pathway. Pre-treatment with a nAChR antagonist does not entirely abolish the mAChR-mediated inhibition of DA release (Foster et al. 2016). It is likely, therefore, that postsynaptic M_4 receptors are also involved in the modulating striatal DA function. This suggests that M_4 receptor induced inhibition of NAc DA release, appears largely mediated by presynaptic autoreceptors; however, as the effect was not entirely abolished, an independent pathway may underlie part of the M_4-DA relationship. The use of regionally specific KO mice has allowed this claim to be better investigated. Mice lacking M_4 receptors solely on D1-type MSNs, show enhanced DA-release in following amphetamine challenge relative to WT mice (Jeon et al., 2010). This indicates a key role for these post-synaptic receptors in mediating the effects of ACh on DA function

M_1 receptors also appear to have a similar inhibitory role. M_1 KO mice display enhanced DA release following amphetamine administration (Gerber et al. 2001). Antagonists with some preference for the M_1 receptor, potentiate cocaine-induced DA release within the NAc (Tanda

et al. 2007). Given the similarity in the location of postsynaptic M_1 and M_4 receptors within the striatum (table 1.3), it is possible that there is a similar mechanism underlying the effects of M_1 and M_4 receptor ligands on DA release. However, to date, there have been no studies using region specific M1-KO mice needed to fully investigate this hypothesis.

Alongside the physiological evidence, a number of studies have also implicated mAChRs in modulating DA-dependant behaviours. In particular, the antipsychotic-like effects of muscarinic receptor agonists have been investigated. In humans, the M_1/M_4 receptor agonist xanomeline, is reported to produce antipsychotic effects (Shekhar et al. 2008). One commonly used preclinical rodent model of psychotic-like behaviour is amphetamine-induced locomotion (Arnt 1995; Stanhope et al. 2001). Amphetamine challenge is used as a model of psychotic like behaviour, due to its validity in predicting the efficacy of a number of antipsychotic treatments (Moore & Kenyon 1994), as well as being potentiated in several rodent models of schizophrenia (Forrest et al. 2014; Jones et al. 2011). Xanomeline treatment attenuates amphetamine-induced locomotor activity in rats (Stanhope et al. 2001). Furthermore, this attenuating effect of xanomeline is entirely abolished in M_4 KO mice and significantly reduced in M_1 KO mice (Woolley et al. 2009). Xanomeline has also been shown to attenuate amphetamine-induced arousal and stereotypies in nonhuman primates (Andersen et al. 2003), as well as reducing nonmotoric behavioural effects of amphetamine such as latent inhibition and pre-pulse inhibition (Barak & Weiner 2011). A preferential M_1 receptor allosteric agonist has also been reported to reduce amphetamine induced locomotion (Jones et al. 2008). Although M_1-specific positive allosteric modulators (PAMs) can produce antipsychotic-like effects in other rodent models, such as MK-801–induced pre-pulse inhibition and spatial memory deficits(Digby et al. 2012; Melancon et al. 2013), a number of M_1 receptor PAMs have been reported to be ineffective at attenuating the behavioural effects of amphetamine (Choy et al. 2016). The antipsychotic effects of xanomeline may, therefore, be primarily mediated through actions on the M_4 subtype. In line with this, M_4 PAMs attenuate the behavioural effects of methamphetamine and amphetamine (Byun et al. 2014; Wood et al. 2017; Le et al. 2013; Chan et al. 2008; Brady et al. 2008).

Conversely, mAChR antagonists have been shown to potentiate amphetamine and cocaine-induced locomotor activity (Carlton 1961; Barrett et al. 1973; Thomsen 2014). Non-selective mAChR antagonism can also enhance amphetamine-induced stereotypies (Klawans et al. 1972) and amphetamine toxicity (Mennear 1965). Preferential M_1 receptor antagonists can also potentiate cocaine-induced locomotor activity (Tanda et al. 2007). Together, these studies

suggest mAChR agonists attenuate and antagonists enhance NAc DA function, these effects also appear to be largely mediated through M_1 and M_4 receptors. There is also limited evidence from human studies for mAChR modulation of DA function. mAChR antagonists can be used in the treatment of the hypodopaminergic disorder, PD (Brocks 1999) and the extrapyramidal symptoms (EPS), that follow D_2 receptor antagonist treatment (Pisani et al. 2007).

In rodents, mAChRs have also been implicated in a number of reward-related behaviours. Local administration of scopolamine into the NAc disrupts lever pressing for food reward, as well as free-feeding sucrose consumption (Pratt & Kelley 2004; Pratt & Kelley 2005). mAChRs also affect Pavlovian-instrumental transfer (PIT). PIT refers to the process of reward paired-cues producing excitatory (Pavlovian) effects on instrumental behaviour. Like effort-based behaviour, PIT is highly dependent on intact NAc DA signalling (Dickinson et al. 2000; Wyvell & Berridge 2000; Wassum et al. 2013). Systemic administration of scopolamine reduces the excitatory influences of Pavlovian cues on behaviour (Ostlund et al. 2014). Furthermore, infusion of scopolamine into the NAc was found to supress the normal cue-evoked NAc DA release observed during PIT, as well as its behavioural expression (Collins et al. 2016). These behavioural and neurochemical effects of scopolamine are in contrast to the studies that suggest mAChR antagonists facilitate NAc DAergic function. One possibility, is that these results are linked to the previously mentioned activity-dependent relationship between mAChRs and DA (Threlfell et al. 2010), although this has not been investigated.

1.4.3 Muscarinic receptors as potential targets for effort related behaviour

As previously mentioned, M_1 and M_4 receptor subtypes are expressed upon the striatal output MSNs. Both dorsal and ventral striatal MSNs express complimentary populations of excitatory and inhibitory G-protein coupled receptors (Girasole & Nelson 2015). Striatopallidal MSNs preferentially express the $G_{i/o}$-coupled dopamine D_2 receptors and the G_s -coupled adenosine A_{2A} receptors. Striatonigral MSNs express the inhibitory $G_{i/o}$–coupled muscarinic M_4 receptors and the $G_{\alpha olf}$-coupled dopamine D_1 receptors.

Generally, compounds that act on these receptors have been shown to affect effort-based behaviour in rodents (with the current exception of M_4 receptors, the effects of which are unknown). More specifically, compounds that would be expected to facilitate activation the striatopallidal pathway (such as D_2 receptor antagonists and A_{2A} receptor agonists, as reviewed above), have been shown to supress effort-based behaviour measured with assays such as PR and ERC (Cheeta et al. 1995; Mingote et al. 2008). Conversely, compounds that would be

expected to inhibit striatopallidal MSNs (such as adenosine A_{2A} receptor antagonists), enhance effort-based behaviour in rodents (Randall et al. 2012). In support of this, chemogenetic suppression of striatopallidal neurons enhances PR performance (Carvalho Poyraz et al. 2016). Together this is in line with the supposed function of this "no-go" pathway (Albin et al. 1989), as decreasing activity within these neurons should facilitate motor output. Additionally, it would be expected that suppression of the striatonigral pathway would impair effort-based behaviour. In line with this, D_1 receptor antagonists impair PR performance (Bari & Pierce 2005). It may also be expected that facilitation of this pathway could enhance effort expenditure. However, no study has shown an enhancement of effort expenditure with compounds that facilitate this pathway (e.g. D_1 receptor agonists, M_4 receptor antagonists). However, as reviewed above, despite being expressed on both MSN types, M_1 receptors appear to preferentially excite striatopallidal neurons (Shen et al. 2007). M_1 (as well as M_4) receptor antagonism might therefore be predicted to facilitate effort-related behaviour, by reducing the normal actions of endogenous ACh on these neurons. In line with this prediction, genetic deletion of M_1 receptors in mice results in enhanced levels of locomotor activity in mice (Gerber et al. 2001) and increased levels of behavioural output in touchscreen systems (Bartko et al. 2011).

1.5 Translational Approaches to measuring motivation

1.5.1 RDoC Approach

It is clear that motivational disruptions are associated with a number of heterogeneous disorders. In recent years, the research domain criteria (RDoC) initiative has emphasised understanding the neurobiology of specific symptoms that exist across disorders (Insel et al. 2010; Cuthbert & Insel 2013). It is also necessary to understand the physiological basis of behaviours in healthy individuals. One element of the RDoC matrix is approach motivation, which includes the subconstruct effort/valuation willingness to work. Understanding the pharmacological substrates of such constructs, across species, may facilitate successful clinical outcomes.

1.5.2 Touchscreen operant systems

A recent refinement in preclinical testing has been the use of touchscreen technology to test cognition in rodents (Horner et al. 2013; Mar et al. 2013; Oomen et al. 2013). Automated touchscreen batteries have been used for a number of years to assess cognition in humans (Sahakian & Owen 1992), and nonhuman primates (Weed et al. 1999). Touchscreen operant

system allow for a battery testing approach where a broad range of cognitive domains can be tested, often within the same animals. The advantages of touchscreen battery testing include the ability to probe domains such as attention and working memory in the same environment using the same reinforcers. This is advantageous as many traditional assays vary considerably in terms of environment. For example, many behavioural assays are aversively motivated (Vorhees & Williams 2006), whereas others employ appetitive food rewards as motivation (Carli et al. 1983; Dunnett 1985). This can make comparison problematic, as any difference in performance between assays, may result from an interaction between the experimental manipulation and task environment. The versatility of a touchscreen operant system also allows for assessments of complex cognitive domains that are difficult to assess within traditional operant systems, such as working/long-term memory, as shown in table 1.4 (Talpos et al. 2009; Talpos et al. 2010).

Another advantage of touchscreen-based operant systems is the translational nature of the tasks that are possible using this method. One of the criticisms of preclinical behavioural testing is that many results fail to translate to humans (Garner 2014). This is especially problematic in the field of drug development, where large numbers of compounds that are efficacious in rodents fail during clinical trials (Zahs & Ashe 2010; Paul et al. 2010). One of the possible causes of this 'translational gap' is the difference in the assays used to probe the same cognitive process, across species (Garner 2014). Traditionally, the behavioural assays used in rodents vary considerably from the ones used in humans, which are often questionnaire-based. Due to this, there has been an effort to increase to similarity (or face validity) between human and rodent assays. One approach has been to back-translate some of the assays used in humans, such as the Cambridge Neuropsychological Test Automated Battery (CANTAB). This has allowed for near identical cognitive tasks to be administered in rodents and humans (Nithianantharajah et al. 2015; Bussey et al. 2012). Like other cognitive domains, effort-exertion can also be probed using touchscreen systems. Touchscreen based PR was first developed as part of a nonhuman primate battery (Weed et al. 1999), and have recently been developed for mice (Heath et al. 2015) and humans as part of the EMOTICOM battery (Bland et al. 2016).

It should be noted that face validity does not equate to predictive or construct validity. Attempts to establish validity of touchscreen tasks have been restricted to studies demonstrating similar pharmacological (MacQueen et al. 2018) and genetic (Nithianantharajah et al. 2015) effects across species. Whereas this may demonstrate some degree of predictive

validity, such evidence does not exist for the majority of touchscreen tasks. Therefore, although face validity is an attractive feature of cognitive assays and may increase the likelihood of results translating across species (Bussey et al. 2012), this does not necessarily indicate that assays are tapping the same cognitive processes in rodents and humans. There are a number of differences between touchscreen assays in humans and those administered to rodents. Although performance on touchscreen tasks can be compared across species, rodents are required to learn a touchscreen task through appetitive reinforcement over the course of a number of weeks. Whereas, in human versions of the tasks, no learning of the task is required, and performance can be measured within a session. This, coupled with the way performance is reinforced between the human and rodent versions, may result in different behavioural processes being adopted to perform the task. Indeed, the approaches used by rodents to perform many traditional touchscreen assays are often ambiguous (Piantadosi et al. 2019; Phillips et al. 2018). As a consequence, face validity should not be assumed to equate to construct validity.

Cognitive domain	**Rodent Touchscreen Assay**		**Human equivalent**	
Attention	5-CSRTT CPT	(Bartko et al. 2011; Kim et al. 2015)	No Yes*	(Beck et al. 1956)
Memory	PAL	(Talpos et al. 2009)	Yes	(Sahakian et al. 1988)
Working Memory	TUNL/cTUNL	(Talpos et al. 2010; Oomen et al. 2015)	No	
Attentional Set Shifting	ID/ED set shifting	(Brigman et al. 2005)	Yes	(Downes et al. 1989)
Cognitive flexibility	Reversal Learning	(Bussey et al. 2008)	Yes	(Sahakian et al. 1988)
Motivation	PR ERC	(Heath et al. 2015)	Yes Yes*	(Treadway et al. 2009; Bland et al. 2016)

Table 1.4: Overview of some of the range of tasks available within rodent touchscreen system and human equivalents. 5-CSRTT: Five Choice Serial reaction time task; CPT – continuous performance task; PAL: Paired Associates Learning; c/TUNL: Continuous/Trial unique nonmatching to location; ID/ED: Intra dimensional/extradimensional set shifting task; PR; Progressive Ratio; ERC: Effort-Related Choice. *Human task is non-touchscreen based.

1.5.3 In Vivo oxygen amperometry

A complementary approach to facilitating cross-species translation of behavioural results can be achieved through the use of functional imaging. Neuroimaging techniques can be used to

demonstrate equivalent neural correlates of cognitive tasks across species (Keeler & Robbins, 2011). In other words, that a given assay is engaging homologous neural circuitry in humans and rodents. Additionally, functional imaging could be used to demonstrate that drugs exert similar actions on neural measures across species. The most widely used imaging measure in humans is the Blood Oxygenation Level Dependent (BOLD) contrast measured with functional magnetic resonance imaging (fMRI, Ogawa, 1990). BOLD-fMRI relies on a concomitant increase in the level of oxygenated blood in a given brain region following neural activity (Logothetis 2008). In spite of only being a surrogate measure of neural activity, BOLD-fMRI has been widely used to probe the neural correlates of cognitive processes in humans as well as additional measures of the efficacy of pharmacological treatments (Wise & Tracey 2006; Poldrack 2012).

A number of imaging techniques can be performed in laboratory animals that directly probe the neurophysiological and neurochemical correlates of behaviour. These include techniques such as electrophysiological recording that allow for a direct measure of neuronal activity with a far higher temporal resolution than fMRI (Seamans et al. 2008). Other techniques include fast scan cyclic voltammetry (FSCV), which can be used to measure sub-second changes in the neurotransmitter dopamine (Robinson et al. 2003). Additional techniques are available that allow for assessment of other neurotransmitters such as acetylcholine with similar spatial and temporal precision (Teles-Grilo Ruivo et al. 2017). However, given the invasive nature of these procedures, they cannot be widely performed in humans (with some exceptions such as intracranial electrophysiological recordings prior to neurosurgery, Hill et al. 2012). Furthermore, none of the aforementioned rodent imaging techniques can provide an adequate proxy measure of BOLD-fMRI. fMRI can be performed in rodents; however, it requires animals to be restrained or anesthetised. This severely limits the ability of fMRI to probe the neural correlates of cognitive processes in rodents. An alternate approach would be to use amperometric recording to measure changes in brain tissue oxygen (O_2). Crucially, this technique can be performed in awake animals performing complex behavioural tasks. Evoked and resting-state amperometric measures in rodents, closely resembles BOLD-fMRI in humans (Lowry et al. 2010; Francois et al. 2012). This makes it ideally suited to produce a translatable measure of the neural correlates of cognitive tasks or pharmacological effects. For example, amperometry and BOLD-FMRI produce equivalent reward prediction error signals in rats and humans (Francois et al. 2012; Lowry et al. 2010) as well as equivalent effects of ketamine on reward processing (Francois et al. 2016), . Together, these studies suggest that O_2 amperometry can be a viable and valid proxy measure of BOLD-fMRI, and a powerful translational tool.

1.6 Conclusions

Apathy, or a lack of motivation represents a widespread and severe unmet clinical need across a number of patient groups. In spite of differences in how motivation is assessed in humans and preclinical settings, there is general agreement in the actions of a number of compounds in facilitating effort-based behaviour in rodents and reducing symptoms of apathy in clinical populations (table 1.2). However, through the use of touchscreen testing of effort-based behaviour and O_2 amperometry, it may be possible to further increase the likelihood of cross-species translation of pharmacological effects. There is also evidence that effort-based behaviour may be facilitated through compounds that act on muscarinic acetylcholine receptors. Furthermore, this facilitation may be driven through the M_1 and M_4 receptor subtypes. Therefore, these receptors should be investigated as novel targets for pharmacological enhancements of motivation. This is the primary aim of this thesis.

Part 1: Facilitating cross species research into motivation through the use of translational assays and functional imaging

Chapter 2. *Validation and optimisation of a progressive ratio test of motivation for the rat touchscreen*

2.1 Introduction

Contemporary clinical trials for novel CNS medicines have been plagued by failure (Kola & Landis 2004; Cummings et al. 2014). The high rate of attrition in clinical trials is contrasted by the large number of studies demonstrating compound efficacy in rodents (Zahs & Ashe 2010; Geerts 2009; Garner 2014). Whereas this is almost certainly due to a combination of factors, one problem that has been highlighted is the markedly different ways that the cognitive endpoints of interest (e.g. long-term memory, psychosis) are measured across species. Therefore, an attempt at increasing the similarity of assays across species may help reduce this gap and facilitate cross-species translation. Recently, in an attempt to refine preclinical animal testing, rodent operant testing using touchscreen-based systems have been developed. One recent refinement in preclinical animal testing has been the development of touchscreen operant systems (Bussey et al. 2012; Hvoslef-Eide et al. 2015). These systems allow the assessment of a number of cognitive domains including attentional processes and long-term and working memory (Oomen et al. 2013; Horner et al. 2013; Mar et al. 2013) within a single environment. These systems also allow for the use of assays that share a high degree of face validity with the automated computerised testing batteries increasingly used in clinical populations (Sahakian & Owen 1992; Barnett et al. 2010; Bland et al. 2016) and nonhuman primates (Weed et al. 1999). Although face validity does not guarantee construct validity, it may help facilitate cross-species translation of results.

As discussed previously, it is clear that impaired motivated behaviour represents an unmet clinical need in a number of neuropsychiatric and neurodegenerative disorders. Therefore, the ability to measure motivated behaviours in rodents is of crucial importance. Motivated behaviour can be divided into activational and directional components (Robbins & Everitt 1982; Salamone 1988). Directional processes allow behaviour to be directed towards appetitive and away from aversive stimuli. Activational aspects of motivation allow organisms to overcome costs or obstacles that are associated with obtaining goals (Salamone 1988). In a number of disorders associated with motivational impairments, activational processes appear

disrupted (Barch et al. 2014; Chong et al. 2015; Salamone, Yohn, et al. 2016). Activational components of motivated behaviour can be probed in the laboratory through studying the exertion of effort. One widely used assay involves studying behaviour under a progressive ratio (PR) schedule of reinforcement (Hodos 1961). This task probes the ability of an organism to maintain instrumental responding (such as lever pressing or nose-poking) under increasing work demands. As the response requirement increases, an animal will eventually cease responding. The amount of effort an animal is willing to expend in pursuit of appetitive reinforcement, expressed as the maximum number of responses to obtain a single reward, is referred to as the breakpoint (BP, Stewart 1975). PR schedules have been used to study effort exertion across a number of species including rats (Hodos 1961); mice (Randt & Quartermain 1972); pigeons (Dardano & Sauerbrunn 1964); nonhuman primates (Griffiths et al. 1975) and humans (Roane et al. 2001).

Previous research has shown that, similar to lever and nose-poke manipulanda, rodent touchscreens can support the sustained repetitive response behaviour required in ratio schedules such as PR, at least in mice (Heath et al., 2015). The majority of the previous research into pharmacological enhancement of motivation in rodents has used the rat as the model species (reviewed in chapter 1). The development of a validated rat touchscreen PR test would allow the assessment of motivation in the rat using the same reinforcers, responses and test setting as those used in the assessment of other complex behavioural constructs in the same apparatus. This would allow motivated behaviour to be assessed alongside and in a comparable way to other cognitive processes as part of a battery approach in situations where the rat is the favoured species. Recently, a touchscreen based PR task was developed for use in humans (Bland et al. 2016). The validation of a rat touchscreen PR task would allow for measurement of activational processes of motivation with high face validity with humans, as well as mice and nonhuman primates (Weed et al. 1999; Heath et al. 2015; Kangas et al. 2016). The present study aimed to validate a PR task by testing the sensitivity of touchscreen PR in rats to a number of manipulations known to affect performance in traditional lever-based versions of PR.

In spite of being widely used as assays of motivation, there are no standard set of parameters used in PR. Specifically, PR tasks can vary in the nature of the schedule of reinforcement used. Some PR schedules increase in a linear fashion (e.g. Skjoldager et al. 1993; Aberman et al. 1998; Bensadoun et al. 2004; Heath et al. 2015), whereas others employ exponentially increasing ratios (e.g. Poncelet et al. 1983; Mobini et al. 2000; Rickard et al. 2009). It is not known whether manipulations that affect PR performance differentially affect behaviour

reinforced under these different schedule types. Therefore, performance was assessed on two separate reinforcement schedules: the linear PR4 and the exponential PREXP schedule.

Studies using PR schedules to assay motivation often use breakpoint as the sole outcome variable. The use of analysis of additional measures of performance may be useful in establishing the psychological mechanisms underlying a change in PR performance. Breakpoint has been criticised as a blunt measure of motivation, ignoring within-session changes in responding (Olarte-Sánchez et al. 2015). A complimentary approach is to examine with-session changes in response rates. Through analysis of the decline in response rates throughout a session it is possible to extract measures of the peak and rate of decay in responding. The predicted peak response rate is believed to provide a measure of the maximal motoric output of an animal; whereas the decay rate provides a motivational measure of how reinforcers invigorate subsequent bouts of responding (Phillips et al. 2017). Additionally, changes in the structure of response bouts can be examined. Animals naturally undergo 'break-and-run' type behaviour which is characterised by periods of responding, or response bouts, separated by pauses (Shull et al. 2001). The number of responses within a bout has been reported to be a measure of motoric integrity; whereas the refractory pausing between bouts provides a measure of an animals motivational state (Brackney et al. 2011). Analysis of these additional measures could potentially provide a broader view of the effects of an experimental manipulation than examining breakpoint alone. A summary of potential measures that can be taken during touchscreen PR testing is available in table 2.1.

Measure	***Definition***
Breakpoint	The number of responses in the final completed trial within a session
Post reinforcement pause	The latency from magazine exit following reward to the first target response in the subsequent trial
Decay rate	The exponent predicting the decline in response rates over the course of a PR session
Between Bout pausing	The mean length of the nonresponding period between response bouts
Predicted peak response rate	The predicted response rate at time point 0s, based upon the change over the course of a session.
Response bout length	The mean number of responses in a bout of responding
Reward collection latency	The mean latency to collect rewards following delivery
Nontarget screen Responses	The mean number of screen responses not directed at the stimulus per second

IR beam breaks	The mean number of infrared beam breaks per second
Magazine entries	The mean number of magazine entries made per second

Table 2.1: The dependant variables assessed during PR responding and the relevant constructs.

In the present study, performance on two schedule types was compared in response to a number of manipulations. Initially, the reward outcome value was manipulated. Firstly, this was achieved by increasing the magnitude of reward, which was hypothesised, based on previous reports, to increase breakpoint (Skjoldager et al. 1993; Eagle et al. 1999; Rickard et al. 2009). Secondly, the reinforcer was devalued through a pre-feeding procedure, which was predicted to decrease breakpoints (Skjoldager et al. 1993; Eagle et al. 1999). Subsequently, performance was assessed following systemic administration of dopaminergic compounds. Based on previous reports it was predicted that administration of the D_2/D_3 receptor antagonist raclopride would disrupt PR performance (Cheeta et al. 1995; Aberman et al. 1998). Finally, it was predicted that PR performance would be facilitated following systemic d-amphetamine administration (Poncelet et al. 1983; Mobini et al. 2000; Bensadoun et al. 2004).

2.2 Methods

2.2.1 Animals

Twenty-four male Sprague Dawley rats (Charles River, UK) were used in the current experiment. Animals were group housed (4 per cage) in a light- and temperature-controlled environment (lights on 1900-0700). Following at least seven days habituation to the facility, animals were placed on a programme of controlled feeding and maintained at no less than 85% of their free feeding body weight at the start of food restriction. No correction was applied to this 85% control weight to match the animals expected growth curve. Cages were changed twice weekly and drinking water was available *ad libitum* throughout. All testing took place 5-7 days/week, in the animals' dark phase. All experiments were regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 and following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB).

2.2.2 Apparatus

All testing took place within automated rat touchscreen operant chambers (Campden Instruments Ltd., Loughborough, U.K.) The chambers were contained within light and sound-attenuating boxes. The chambers consisted of black plastic walls in a trapezoidal shape (height: 30cm, length: 33cm, width: 25cm at screen, 13cm at magazine) and a perforated stainless-steel floor. Each chamber was fitted with a 15-inch touch-sensitive LCD screen, with a screen

resolution of 1,024 x 768) IR photocells were positioned <5mm from the screen to record responses. Therefore, no physical force against the touchscreen was necessary for a response to be recorded. On the opposing side was a magazine connected to a pellet dispenser that delivered standard 45mg dustless pellets (TestDiet, Indiana, USA). The food tray was fitted with a light emitting diode and an infrared (IR) beam that registered magazine entries. Front and rear IR beams were fitted to monitor the rats' activity within the chamber. Front IR beams were positioned 6cm from the screen and rear IR beams 5cm from the magazine. During all stages of testing, black plastic masks were fitted to the touchscreens that had five $9cm^2$ response apertures, at equal heights, spaced 1cm apart.

2.2.3 Pretraining

Initially, all animals were initially given a 20-minute habituation session. During this session, the boxes were active, but no stimuli were presented. Following this, rats underwent one day of screen press training. A white square stimulus was presented in the central aperture for 30s. A single response to this stimulus resulted in three food pellets being delivered. Stimulus offset and a short tone (1000ms, 3 kHz) accompanied reward delivery. Following a 5s inter-trial interval (ITI) the stimulus returned to the screen. If no response was made within 30s the trial ended, and a single food pellet was delivered, accompanied by stimulus offset and the tone. Each session was terminated following 100 rewards being delivered or 45 minutes having elapsed.

2.2.4 Fixed Ratio Training

Rats then underwent fixed ratio (FR) 1 training. During these sessions, a single response to the central stimulus was required for a single pellet reward delivery. Reward delivery was again accompanied by the tone. A 5s ITI was employed. Each session was terminated following 45 minutes or 100 trials being completed. All animals were required to complete 100 trials within the 45 minutes before moving on to the next stage of training. The subsequent training stage consisted of FR5 responding, where five responses were required for each reward delivery. The first four responses in a trial were accompanied with a shorter 'click' tone (10ms, 3 kHz) and a brief (500ms) stimulus offset. The fifth response to the stimulus completed the trial and resulted in delivery of reward and the longer duration tone. All other parameters were identical to the FR1 stage of training. Each session was terminated following 100 trials (i.e. 500 target responses) or after 45 minutes. Each animal was required to complete 100 trials within a session before being placed on a PR schedule of reinforcement.

2.2.5 Progressive ratio

Animals were randomly assigned to either a linear (PR4) or exponential (PREXP) schedule (n=12 each). On both schedules, the number of target responses required increased following completion of each trial. On the PR4 schedule, the response requirement began at one and increased by four on each subsequent trial (yielding response requirements of 1, 5, 9, 13, 17 etc.). The PREXP schedule increased according to the formula ($5 * e^{(0.2*n)} - 5$), where n is the trial number, yielding response requirements of 1, 2, 4, 6, 9, 12 etc., to the nearest whole number. If no response was made to the touchscreen within 180s, on either schedule, the session was terminated, otherwise sessions ended after 45 minutes elapsing. Together, a total of ten days of training were needed before probes were administered. Throughout this task the ITI was triggered following reward collection (i.e. entry and then exit from the magazine). As a consequence, this PR task was not self-paced.

2.2.6 Outcome Manipulations

Outcome manipulation probes were delivered in a within-subject cross-over design. Firstly, rats underwent a reward magnitude probe. On these days, rats received either a standard (single pellet) or an increased (three pellet) reward following each completed ratio. The groups were counterbalanced so that on each day equal numbers of PR4 and PREXP rats were in each condition. A baseline day was administered between test days, where rats were tested as normal and received a single pellet reward for each completed trial. On the prefeeding probe days, rats were randomly assigned to a prefeed or no pre-feed (control) condition. Rats within the prefeed condition were given 1 hr of free access to homecage lab chow prior to testing. Rats within the no pre-feed control condition were tested as normal with chow provided after the PR session was completed. Equal numbers of PR4 and PREXP rats were tested on both conditions on each test day. Again, a baseline day was given between test days to ensure no carry-on effects of prefeeding were observed upon PR performance.

2.2.7 Dopaminergic Manipulations

Pharmacological challenges were delivered in a within-subject Latin square design. All drugs were dissolved in physiological saline and delivered via intraperitoneal injections at a volume of 1 ml/kg of each rat's body-weight, 30 minutes prior to PR testing. Rats were returned to their home cages for the post injection period of 30 minutes. The D_2/D_3 receptor antagonist s(-)raclopride(+)-tartrate salt (Sigma-Aldrich, Dorset, UK) was administered at doses of 0, 0.03 and 0.3 mg/kg. Following a seven-day washout period, the indirect catecholamine agonist d-

amphetamine sulphate (Sigma-Aldrich, Dorset, UK) was administered at doses of 0, 0.1 and 1 mg/kg.

2.2.8 Behavioural Measures

The primary measure of interest was breakpoint (BP), defined as the number of target responses made in the last successfully completed trial for each subject. The mean post reinforcement pause (PRP) was defined as the latency between an animal removing its head from the magazine following reinforcement and the first touchscreen target response of the subsequent trial. Response rates were analysed as previously described (Phillips et al., 2017). Briefly, response rates per trial were calculated by dividing the number of responses made in each trial by the time taken to complete each trial, from the first response (therefore, excluding post reinforcement pauses). The first two trials in each session were excluded from the response rate analyses. The first trial was excluded as it only involved a single lever press, meaning it is not possible to calculate a response rate. The second trial was excluded as it only required two responses in the PREXP schedule. The low number of responses needed in this condition may have made comparison between groups problematic by inflating the response rate within this group. The following negative exponential function was then fitted to the mean response rates per condition: y = -a*exp(x*b); with *y* being the response rate and *x* being the trial number. The predicted peak response rate (a) and decay rate parameter (b) were extracted and analysed across conditions. Response bout analysis defined bouts as consecutive touchscreen target responses separated by no more than 5s. The mean number of responses in a bout was taken to represent a bout length. Following response bout completion, the pause until the subsequent target response was calculated. Only voluntarily terminated bouts were analysed and PRPs excluded from the bout pause analysis. Additional measures of motoric activity included the mean reward collection latency, the rate of IR beam breaks (beam breaks/sec), the rate of non-stimulus (blank) touchscreen responses (blank touches/sec) and the rate of magazine entries (magazine entries/sec). A description of each measure is available in table 2.1 and a visualisation of a number of measures can be seen in figure 2.1.

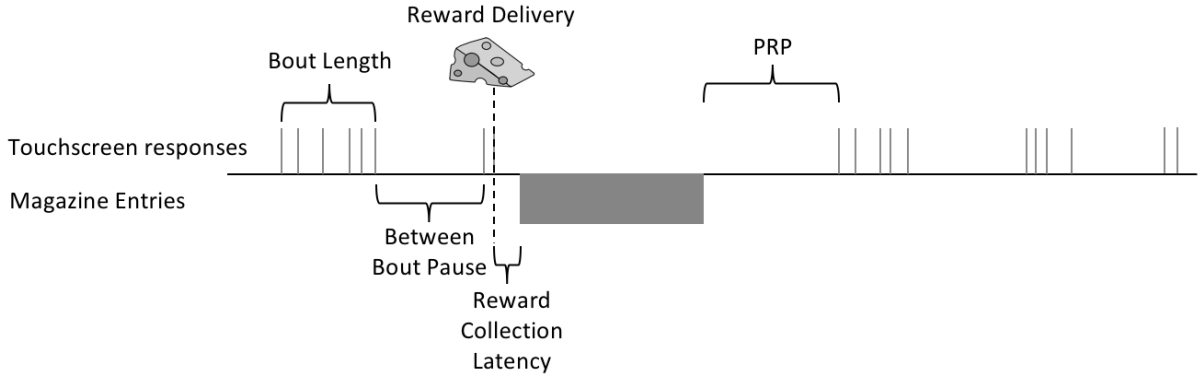


Figure 2.1: Visualisation of a PR trial including some of the measures used. Between bout pauses were defined as no response periods of 5s or greater. PRP: post reinforcement pause.

2.2.9 Statistical analysis

Analysis was conducted in SPSS Version 23 (IBM, Armonk, NY, USA) and the R software package (R Core Team, 2013). Graphs were produced using Prism (GraphPad, La Jolla, CA, USA) and the ggplot2 package in R (Wickham, 2009). To compare the effects of schedule at baseline, independent t-tests were used. Levene's test for equality of variance was employed and corrected where appropriate. For all other tests, repeated measures ANOVAs were employed. The Greenhouse-Geisser correction was applied for any violations of sphericity. All reported post-hoc testing was adjusted using the Bonferroni correction for multiple comparisons.

2.3 Results

2.3.1 Effect of reinforcement schedule on baseline PR performance

All measures were collapsed across the first five PR sessions. The mean breakpoint did not differ significantly according to schedule group ($t(22) = .051$, $p = .96$; figure 2.2A). The mean duration of the PRP also did not differ across reinforcement schedule groups ($t(22) = 1.024$, $p = .317$; figure 2.2B). Animals reinforced under the PR4 schedule did, however, make significantly more touchscreen responses per reward ($t(22) = 2.785$, $p < .05$; figure 2.2C). There were no differences between the mean number of IR beam breaks made per second ($t(22) = 1.441$, $p = .164$. Response rates appeared to differ between schedule groups (figure 2.2D). The predicted peak response rate was significantly higher in animals reinforced under the PREXP schedule ($t(22) = 3.067$, $p < .01$; figure 2.2E). The response rate decay was also significantly greater in rats tested under the PREXP schedule of reinforcement ($t(22) = 3.177$, $p < .01$; figure 2.2F). Reinforcement schedule did not affect the structure of response bouts

(bout length:($t(17.109) = 1.367$, $p = .189$); between bout pausing ($t(22) = 1.336$, $p = .195$)). Supplementary measures of motoric activity are available in Table 2.2.

	Reward Collection Latency		Magazine Entries/Sec		Nontarget responses/Sec		No. 45-min terminations	
	PR4	PREXP	PR4	PREXP	PR4	PREXP	PR4	PREXP
Reward magnitude								
1 Pell	1.11 ± 0.06	**1.36 ± 0.09#**	**0.07 ± 0.01#**	**0.05 ± 0.00#**	0.02 ± 0.00	0.03 ± 0.01	5	2
3 Pellets	0.97 ± 0.08	**0.94 ± 0.12#**	**0.10 ± 0.01#**	**0.08 ± 0.01#**	0.02 ± 0.00	0.03 ± 0.01	8	6
Pre-feeding								
No Feed	1.39 ± 0.09	1.51 ± 0.13	**0.06 ± 0.01***	**0.05 ± 0.00***	0.02 ± 0.00	0.02 ± 0.00	3	2
Prefeed	1.27 ± 0.05	1.56 ± 0.10	0.06 ± 0.01	0.04 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	1	0
Raclopride								
Vehicle	**1.23 ± 0.09***	**1.63 ± 0.15***	**0.08 ± 0.01***	**0.06 ± 0.01***	0.02 ± 0.00	0.03 ± 0.01	0	0
0.03 mg/kg	1.28 ± 0.06	1.58 ± 0.14	0.07 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	2	2
0.3 mg/kg	**1.44 ± 0.19***	**2.65 ± 0.72***	**0.05 ± 0.01†**	0.04 ± 0.00	**0.01 ± 0.00†**	0.02 ± 0.01	1	0
Amphetamine								
Vehicle	**1.44 ± 0.09***	**1.80 ± 0.12***	0.09 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	1	0
0.1 mg/kg	**1.40 ± 0.08***	**1.71 ± 0.16***	0.08 ± 0.01	0.06 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	1	2
1 mg/kg	1.35 ± 0.06	1.56 ± 0.09	0.10 ± 0.01	**0.09 ± 0.01†**	0.05 ± 0.01	0.05 ± 0.01	6	8

Table 2.2: Additional measures of motoric activity for both schedule types. Mean values ± SEM, of the reward collection latencies, rate of magazine entries (magazine entries per second), and the rate of nontarget (blank) screen responses (Nontarget responses/sec) and the number of rats (out of 24) failing to reach breakpoint for all experimental conditions. All behavioural measures were reported to two decimal places. Bold type signifies significant effects. *A significant group difference between schedule types, $p < .05$. # A significant effect of increasing the reward magnitude, $p < .05$. † A significant effect relative to the vehicle condition $p < .05$.

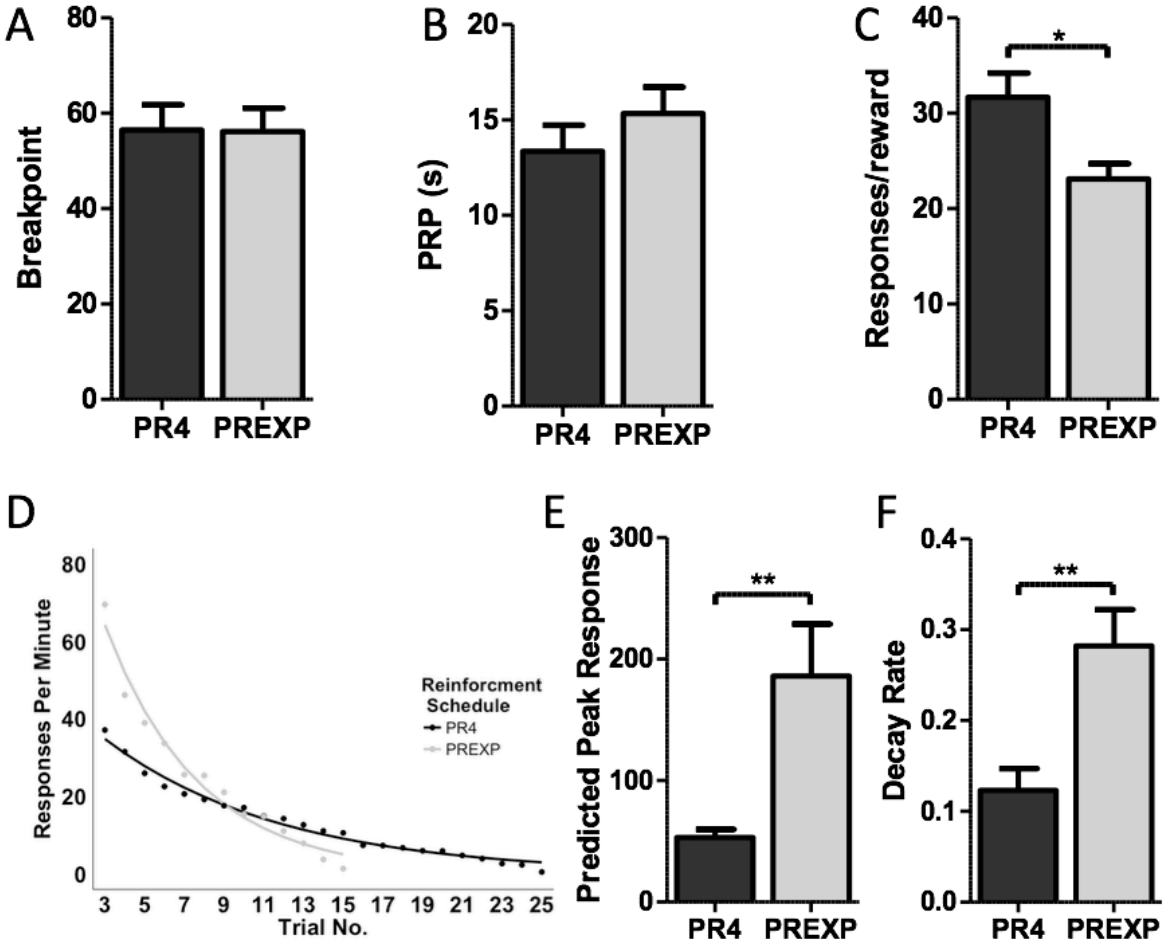


Figure 2.2: Effects of schedule of reinforcement on PR performance**. A** The mean breakpoint for both schedule groups**. B** The duration of the post-reinforcement pause (PRP). **C** The mean number of touchscreen responses made per reward was higher in animals reinforced with the PR4 schedule. **D** The group mean response rate for each trial, from the third trial onwards for both reinforcement schedule. **E** Reinforcing animals under a PREXP schedule significantly increases the predicted peak response rate. **F** Reinforcing rats under a PREXP schedule significantly increases the rate of decay in responding. Error bars represent ±SEM. * $p<.05$; ** $p<.01$.

2.3.2 Increasing the magnitude of the reward enhances PR performance

Increasing the magnitude of reward significantly increased breakpoint ($F(1,22) = 35.183$, $p < .001$; partial eta squared = .615; figure 2.3A). Breakpoints were significantly higher following three-pellet rewards in both schedule groups (both $p < .01$). Breakpoints did not significantly differ between schedule groups ($F(1,22) = 2.13$, $p = .159$). There was also no significant interaction between reward magnitude and schedule type ($F(1,22) = 2.584$, $p = .122$). Increasing reward magnitude did not affect PRPs ($F(1,22) = .388$, $p = .54$; figure 2.3B). The

duration of the PRP also did not differ between reinforcement schedule groups ($F(1,22) = 1.409$, $p = .248$). Again, there was no significant interaction between schedule and reward magnitude ($F(1,22) = 2.83$, $p = .107$). The rate of IR beam breaks was not significantly affected by reward magnitude ($F(1,22) = .311$, $p = .583$). There was also no effect of either reinforcement schedule upon the rate of IR beam breaks ($F(1,22) = 1.951$ $p = .176$), or any interaction between schedule and reward magnitude ($F(1,22) = .107$, $p = .583$). Increasing reward magnitude did not affect any additional measure of activity (Table 2.2).

Changing the magnitude of reward had did not affect response rates in either schedule group (figures 2.3C,D). The predicted peak response rate was not affected by increasing the magnitude of reward ($F(1,22) = 1.630$, $p = .215$; figure 2.3E). However, the effect of schedule upon the predicted peak response rate trended towards significance ($F(1,22) = 4.277$, $p = .051$). There was no interaction between schedule and reward magnitude ($F(1,22) = 1.285$, $p = .269$) upon the peak response rate. Increasing the magnitude of the reward did not significantly affect the rate of decay in responding ($F(1,22) = 4.193$, $p = .053$; figure 2.3F). The rate of decay differed between schedule groups ($F(1,22) = 9.494$, $p < .01$; partial eta squared = .301). The decay in responding was higher in the PREXP when reinforced with three-pellet rewards ($p < .05$). There was no significant schedule x reward magnitude interaction upon the response decay rate ($F(1,22) = .687$, $p = .416$). The length of response bouts was significantly increased by increasing the magnitude of rewards ($F(1,22) = 6.329$, $p < .05$; partial eta squared = . 223). The mean bout length was also significantly longer in PR4 animals ($F(1,22) = 7.095$, $p < .01$; partial eta squared = .244). However, there was no significant interaction between schedule and reward magnitude upon bout length ($F(1,22) = 2.536$, $p = .126$). The mean pause between response bouts was reduced by increasing the reward magnitude ($F(1,22) = 24.301$, $p < .001$; partial eta squared = .525). Pausing was not significantly affected by either schedule of reinforcement ($F(1,22) = 1.799$, $p = .194$) or by any interaction between schedule and reward magnitude ($F(1,22) = 3.315$, $p = .082$).

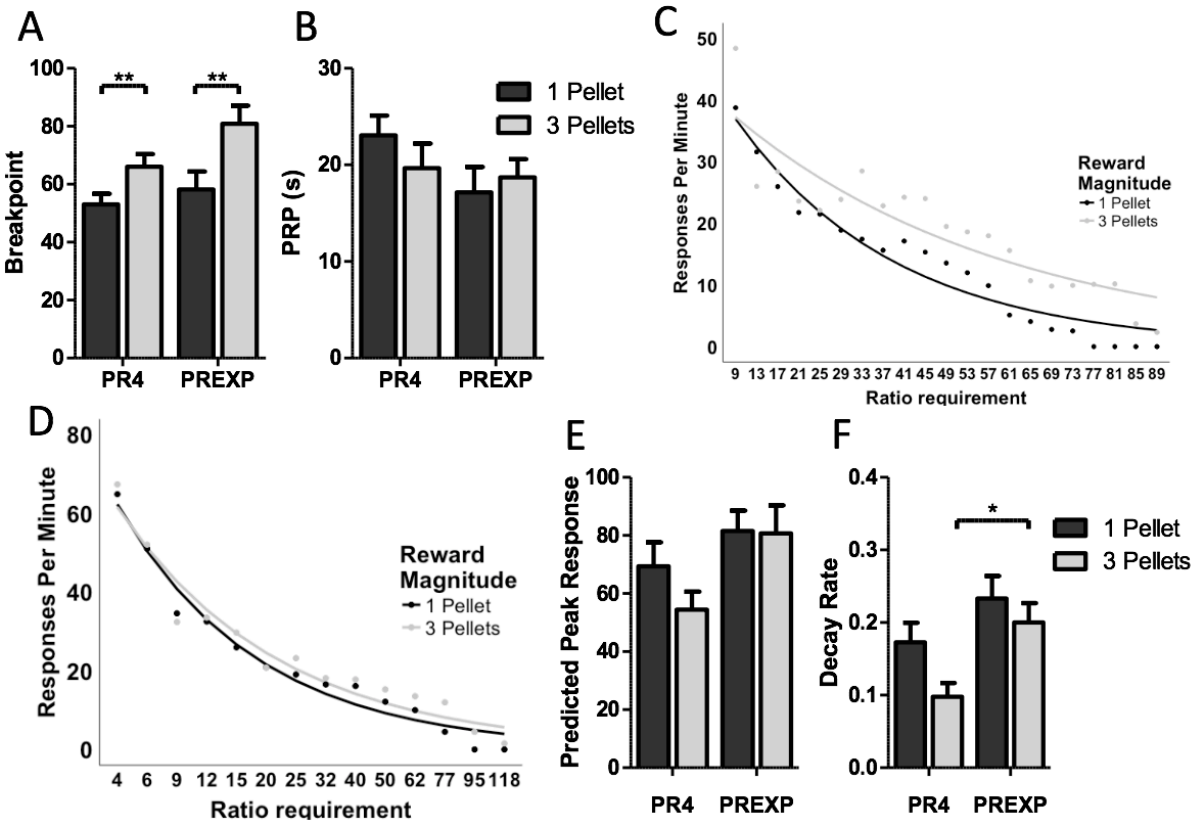


Figure 2.3: Increasing the magnitude of reward facilitates PR performance. **A** Reinforcing PR performance with 3 pellet rewards significantly increases breakpoints in both schedule groups. **B** Changing the magnitude of reward does not alter the post reinforcement pause (PRP). **C** The PR4 group mean response rate for each trial, from the third trial onwards **D** The PREXP group mean response rate for each trial, from the third trial onwards **E** Increasing reward magnitude does not affect the predicted peak response rate. **F** Increasing the magnitude of reward does not affect the decay in responding. The PREXP group show a greater decay rate when reinforced with 3 pellet rewards. Error bars represent ±SEM. * $p<.05$; ** $p<.01$.

2.3.3 Prefeeding with chow prior to testing disrupts performance under PR schedules of reinforcement.

Pre-feeding the rats with chow significantly reduced breakpoint ($F(1,22) = 22{,}796$, $p < .001$, partial eta squared = .509; figure 2.4A). Breakpoints were significantly lower following pre-feeding in both PR4 and PREXP schedule groups (both $p < .01$). Breakpoint did not differ between schedule groups ($F(1,22) = .179$ $p = .676$). There was also no interaction between schedule type and pre-feeding status ($F(1,22) = .041$ $p = .841$). The duration of the mean PRP was not affected by pre-feeding ($F(1,22) = .869$, $p = .361$; figure 2.4B). The duration of PRPs was significantly affected by reinforcement schedule type ($F(1,22) = 4.494$, $p < .05$, partial eta squared = .170); however, no effect survived multiple comparison adjustments in post-hoc

testing. There was no interaction between pre-feeding status and schedule type upon PRPs ($F(1,22) = .291$, $p = .595$). Similarly, pre-feeding had no effect on the number of IR beam breaks made per second ($F(1,22) = .964$, $p = .337$). The rate IR of beam breaks was not significantly affected by either reinforcement schedule ($F(1,22) = 1.187$, $p = .288$), or by any interaction between schedule and pre-feeding state ($F(1,22) = 1.243$, $p = .277$). Prefeeding had little effect on motoric activity (Table 2.2).

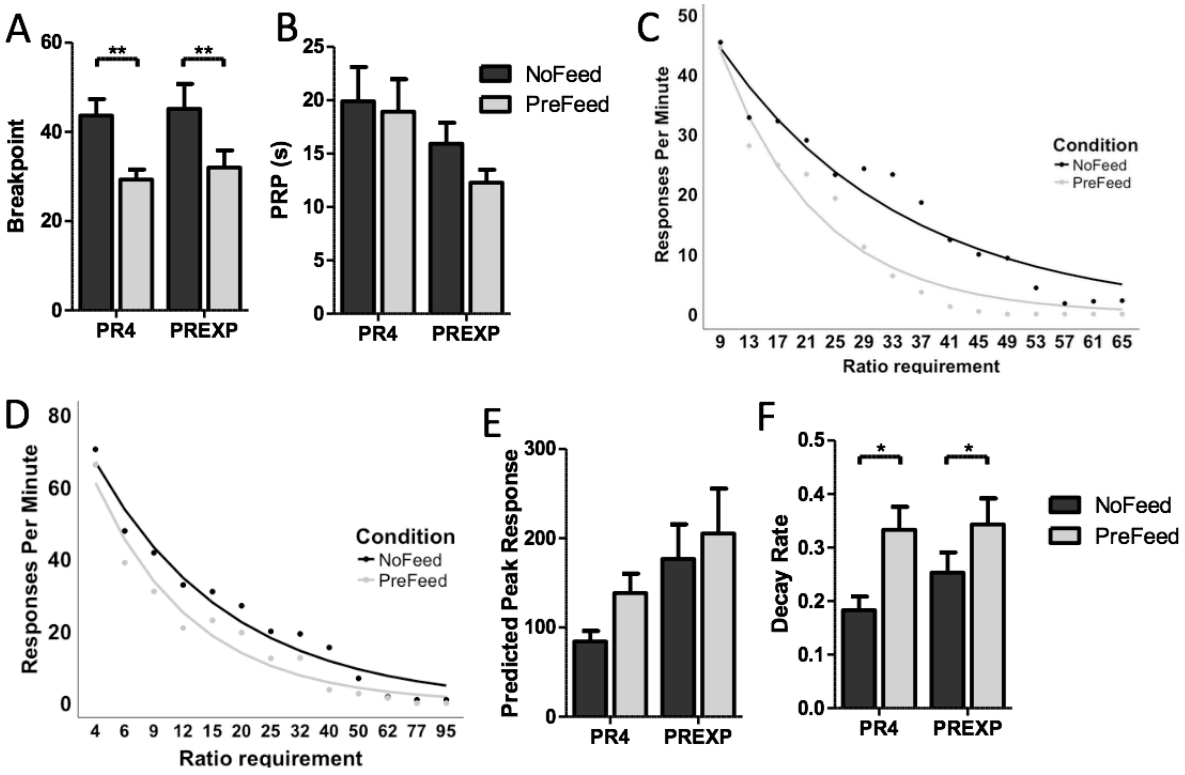


Figure 2.4: PR performance is supressed by prefeeding rats with homecage chow prior to testing. **A** Breakpoints are significantly lowered by prefeeding in both schedule groups. **B** Prefeeding with lab chow does not affect the duration of the mean post reinforcement pause (PRP). **C** The influence of prefeeding on the PR4 group mean response rate for each trial, from the third trial onwards **D** The influence of prefeeding on the PREXP group mean response rate for each trial, from the third trial onwards. **E** Prefeeding does not affect the predicted peak response rate **F** The decay rate was significantly increased after prefeeding with chow. Error bars represent ± SEM. * $p<.05$; ** $p<.01$.

Pre-feeding appeared to affect response rates in both the PR4 (figure 2.4C) and PREXP groups (figure 2.4D). Prefeeding the rats with chow had no effect upon the predicted peak response rate ($F(1,22) = 2.025$, $p = .166$; figure 2.4E). The peak response rate also did not differ significantly between rats reinforced with different schedules ($F(1,22) = 3.708$, $p = .067$).

There was no significant prefeeding x schedule interaction upon the predicted response rate (F(1,22) = .548, p = .467). The rate of decline in responding was, however, significantly affected by pre-feeding (F(1,22) = 9.839, p < .01; figure 2.4F). Post-hoc testing revealed a significant increase in the rate of decay of responding in both schedule groups following pre-feeding (both p < .05). The rate of decay was not significantly affected by reinforcement schedule (F(1,22) =1.749, p = .200). There was also no schedule x pre-feeding interaction observed upon the decay rate (F(1,22) = .011, p = .916). Response bouts were again significantly longer in PR4 animals (F(1,22) = 10.029, p <.01; partial eta squared = .313). However, bout length was not affected by either prefeeding (F(1,22) = .001, p = .989) or by any interaction between prefeeding and schedule type (F(1,22) = 2.186, p = .153). There were no significant effects found upon the pausing between response bouts (prefeeding state (F(1,22) = .187, p = .669); schedule type (F(1,22) = 1.907, p = .181); magnitude x schedule interaction (F(1,22) = 1.107, p = .304)).

2.3.4 Systemic administration of the D2/D3 receptor antagonist raclopride impairs PR performance

Two rats did not complete any trials following administration of the *D_2/D_3 receptor antagonist* 0.3 mg/kg raclopride, therefore, the data from these animals were removed from all raclopride analyses. Administration of raclopride significantly reduced breakpoints (F(2,40) = 14.113, p < .001; partial eta squared = .414; figure 52.5A). Breakpoints were significantly reduced by administration of 0.3 mg/kg compared to vehicle in both schedule groups (p < .01). However, breakpoints were not affected by reinforcement schedule type (F(1,20) = .075, p = .788). There was also no interaction between raclopride administration and schedule type (F(2,40) = 234, p = .792). The length of PRPs was also significantly affected by raclopride administration (F(1.498,32.962) = 8.955, p < .01; partial eta squared = .289; figure 2.5B). Post-hoc testing suggested that raclopride significantly increased pausing following administration of 0.3 mg/kg in the PR4 group (p < .05) but not the PREXP group. There was also a significant interaction between the dose of raclopride and reinforcement schedule (F(1.498,32.962) = 5.042, p < .05; partial eta squared = .186), suggesting that raclopride produced greater effects on pausing in animals reinforced under the PR4 schedule. There was also a significant effect of reinforcement schedule on pausing (F(1,20) = 12.523, p < .01); partial eta squared = .363). Post-hoc testing revealed that the mean PRP was significantly greater in the PR4 group following administration of both vehicle and 0.3 mg/kg raclopride. Raclopride administration significantly affected the rate of IR beam breaks (F(1.309,26.185) = 6.298, p < .01; partial eta squared = .239). Post-hoc tests revealed that 0.3 mg/kg raclopride reduced the rate of beam breaks, relative to

administration of 0.03mg/kg raclopride, in the PREXP group only ($p < .05$). The rate of beam breaks was not affected by reinforcement schedule ($F(1,22) = .145$, $p = .708$). There was also no interaction between the dose of raclopride and reinforcement schedule ($F(1.309,26.185) = .201$, $p = .723$). Motoric activity was largely unaffected by either dose of raclopride (Table 2.2).

Response rates following raclopride administration were analysed (figures 2.5C,D). The predicted peak response rate was not significantly affected by raclopride administration ($F(2,40) = .983$, $p = .383$; figure 2.5E). There was however, a significant effect of reinforcement schedule ($F(1,20) = 15.662$, $p < .01$; partial eta squared = .439). Post-hoc testing revealed that the PREXP group had a significantly higher predicted peak response rate following administration of vehicle and 0.3 mg/kg raclopride. Administration of raclopride also significantly affected the decay in response rate ($F(1.207, 24.142) = 5.860$, $p < .01$; partial eta squared = .227; figure 2.5F). Post-hoc testing did not reveal any significant differences between doses. The decay rate was not significantly affected by reinforcement schedule type ($F(1,20) = 1.448$, $p = .243$). There was also no interaction between the dose of raclopride and schedule type upon the decay rate of responding ($F(1.207, 24.142) = .023$, $p = .977$). Overall bout length was again longer in animals reinforced under the PR4 schedule ($F(1,20) = 6.334$, $p < .05$; partial eta squared = .241).The mean length of response bouts was not significantly affected by either raclopride administration ($F(1.245,24.899) = 2.139$, $p = .153$) or any interaction between schedule type and raclopride ($F(1.245,24.899) = 1.440$, $p = .249$). Raclopride administration did not significantly affect the length of pausing between bouts ($F(1.214,24.277) = 2.231$, $p = .145$). Pausing was not also not significantly affected by either schedule type ($F(1,20) = .203$, $p = .657$) or by any schedule x raclopride interaction ($F(1.214,24.277) = .448$, $p = .547$).

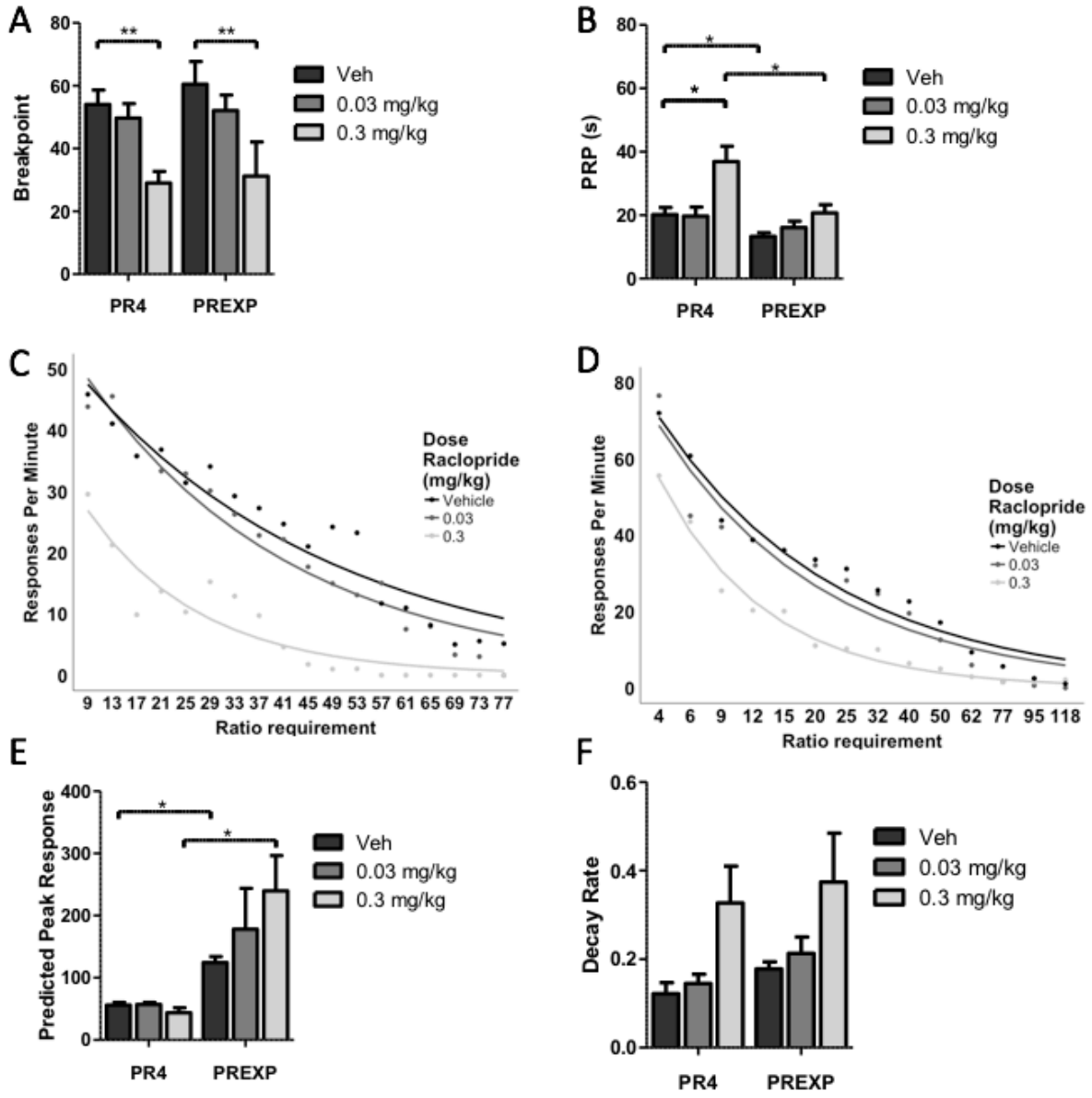


Figure 2.5: Systemic administration of the D2/D3 antagonist raclopride disrupts PR performance**. A** Raclopride administered at a dose of 0.3mg/kg significantly disrupts breakpoints reinforced under both PR4 and PREXP schedules. **B** 0.3mg/kg raclopride significantly increases post reinforcement pauses (PRPs) in the PR4 condition only. The duration of PRPs was also significantly higher in the PR4 condition. **C** Suppression of response rates by raclopride in the PR4 group for each trial, from the third trial onwards. **D** PREXP group mean response rates are suppressed following raclopride administration. **E** Raclopride administration does not significantly affect the predicted peak response rate. Rats reinforced with the PREXP schedule are estimated to have a significantly higher peak response rate. **F** Raclopride administration did not significantly affect the decay rate. Error bars represent ± SEM. * $p<.05$; ** $p<.01$.

2.3.5 Systemic d-amphetamine facilitates PR performance

The indirect catecholamine agonist amphetamine significantly increased breakpoints ($F(1.169,25.711) = 47.935$, $p < .001$; partial eta squared = .685; figure 2.6A). There was also a significant effect of reinforcement schedule ($F(1,22) = 5.072$, $p < .05$; partial eta squared = .187), as well as a significant interaction between amphetamine and schedule upon breakpoint ($F(2,44) = 6.488$, $p < .01$; atrial eta squared = .227). 1 mg/kg amphetamine significantly increased breakpoint compared to vehicle for animals on both schedules of reinforcement (both $p<.05$). However, breakpoints were significantly higher following administration of 1mg/kg of amphetamine in the PREXP group. This finding indicates that amphetamine produced a greater effect upon breakpoints in animals tested on an exponential schedule of reinforcement compared to those on a linear reinforcement schedule. Amphetamine also had a significant effect on the mean PRP duration ($F(2,44) = 13.451$, $p <.001$; partial eta squared = .379; figure 2.6B). Post-hoc testing revealed that 1 mg/kg amphetamine reduced the duration of PRPs relative to vehicle in both schedule groups. There were no significant effects of either reinforcement schedule ($F(1,22) = .520$, $p = .478$), or any significant interaction between dose of amphetamine and schedule upon PRPs. ($F(2,44) = 2.100$, $p = .135$). Amphetamine administration also significantly affected the number of IR beam breaks made per second ($F(1.440, 31.673) = 38.390$, $p < .001$; partial eta squared = .636). Post-hoc testing revealed that 1m/kg amphetamine increased the rate of beam breaks in both schedule groups relative to vehicle ($p < .01$). Reinforcement schedule had no effect on the rate of IR beam breaks ($F(1,22) = .316$, $p = .580$). There was also no significant interaction between the dose of amphetamine and schedule type ($F(1.440, 31.673) = 2.034$, $p = .158$). Amphetamine had little effect on other measures of motoric activity (Table 2.2).

Systemic administration of amphetamine appeared to enhance response rates (figures 6C, D). Amphetamine administration decreased the predicted peak response rate ($F(2,44) = 6.237$, $p <.01$; partial eta squared = .221; figure 2.6E). The predicted peak rate was reduced following 1 mg/kg amphetamine relative to all other doses ($p < .05$) in the PREXP group only. The predicted peak response rate was again significantly affected by schedule type ($F(1,22) = 7.44$, $p < .05$; partial eta squared = .253). Post-hoc testing revealed that the peak rate was significantly higher in the PREXP group following administration of vehicle and 0.1mg/kg amphetamine ($p<.05$). There was no significant interaction between drug administration and reinforcement schedule ($F(2,44) = 1.466$, $p = .242$). The rate of decay in responding was significantly reduced

by amphetamine administration ($F(2,44) = 25.548$, $p < .001$; partial eta squared = .537; figure 2.6F). Post-hoc testing revealed that 1mg/kg amphetamine reduced the rate of decay relative to both other doses in both schedule groups ($p < .01$). The rate of decay in responding did not differ between schedules of reinforcement ($F(1,22) = 2.128$, $p = .159$). There was no significant drug x schedule interaction on the rate of decay ($F(2,44) = .506$, $p = 606$). As with previous conditions, the mean length of response bouts was significantly greater in PR4 rats ($F(1,22) = 9.767$ $p < .01$; partial eta squared = .307) Bout length was not significantly affected by either administration of amphetamine ($F(2,44) = 2.112$, $p = .133$) or by any interaction between the dose of amphetamine and schedule type ($F(2,44) = 1.467$, $p = .242$). In contrast, amphetamine significantly reduced the length of the mean pause between response bout ($F(2,44) = 10.409$, $p < .001$; partial eta squared = .321). Pausing did not differ between schedule groups ($F(1,22) = 1.105$ $p = .305$). There was however a significant interaction between amphetamine and schedule on the length of the pause (($F(2,44) = 8.281$, $p < .01$; partial eta squared = .271). Post hoc testing revealed that 1mg/kg of amphetamine reduced the length of between bout pausing in PREXP rats ($p < .001$), but not PR4 conditions ($p > .05$).

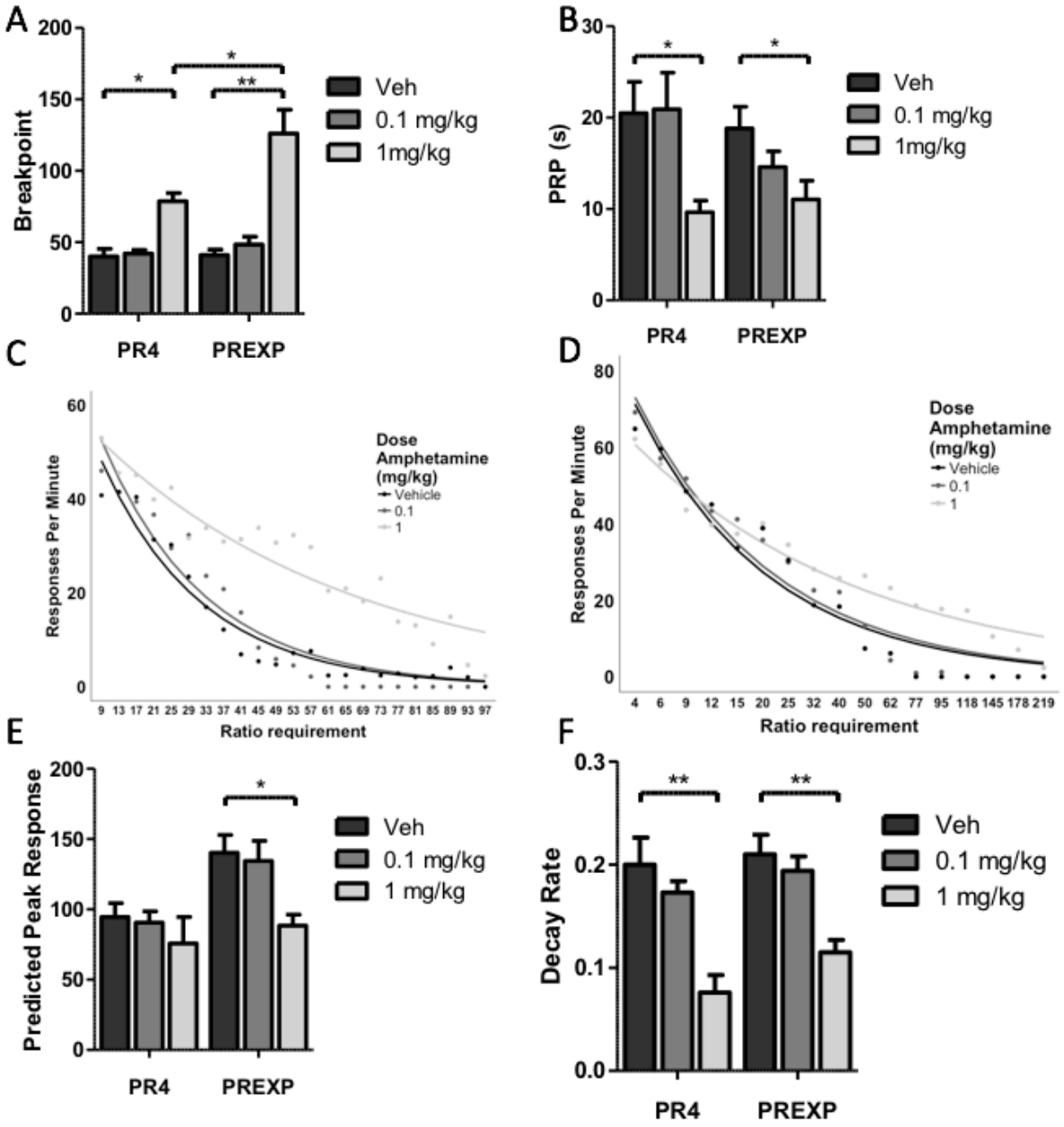


Figure 2.6: Facilitation of PR performance following systemic administration of d-amphetamine. **A** Administration of 1mg/kg d-amphetamine significantly increase breakpoints in both schedule groups. Breakpoints are significantly higher in the PREXP group relative to rats reinforced under the PR4 schedule following administration of 1mg/kg amphetamine. **B** The duration of the mean post reinforcement pause (PRP) is significantly reduced by 1mg/kg amphetamine, in both reinforcement schedule conditions. **C** Enhancement of response rates following administration of amphetamine in rats reinforced with the PR4 schedule. **D** Response rates are enhanced following administration of amphetamine in rats reinforced with the PREXP schedule **E.** Amphetamine significantly reduces the predicted peak response rate in animals reinforced under the PREXP schedule only. The decay rate of responding is significantly reduced in rats in both schedule groups. Error bars represent ±1 SEM. * $p<.05$; ** $p<.01$

2.4 **Discussion**

2.4.1 Progressive ratio as an assay of motivation

PR schedules are widely used across species, to probe motivated behaviour. Touchscreen versions of PR have been developed to assess motivation in mice (Heath et al., 2015), humans (Bland et al., 2016) and nonhuman primates (Weed et al., 1999). Maintaining high face validity between species may increase the likelihood of successful translation of findings (Bussey et al., 2012). Additionally, development of a rat touchscreen variant of progressive ratio will allow for assessment of motivation in this species within the same environment and using the same reinforcers earned in the assessment of more complex behaviours (Horner et al., 2013; Mar et al., 2013; Oomen et al., 2013). In the present study, a novel rat touchscreen PR task was assessed and found to be sufficiently sensitive for detecting changes in performance following outcome manipulations and systemic administration of dopaminergic drugs previously found to be efficacious in non-touchscreen versions of the schedule. The similarity in results across these different procedures further strengthens the use of measurement of responding under PR schedules of reinforcement to assay motivation. Together, this represents the successful validation of the task for use in the rat touchscreen operant system.

2.4.2 Effect of reinforcement schedule on PR performance.

Both linear and exponential schedules of reinforcement are widely used in PR tasks. The reinforcement schedule determines the frequency that rewards are delivered with respect to operant responding. Relative to the linear PR4 schedule, the PREXP schedule has an initially high frequency of reinforcement that then declines rapidly. In the absence of any additional manipulations, breakpoints were remarkably similar between the two schedules (figure 2.2A). This is in spite of the difference in the total number of screen responses needed to achieve these breakpoints (figure 2.2C). This finding indicates that prior history of reinforcement within a session has little influence upon breakpoint. Likewise, the duration of PRPs did not differ between the two schedule types. The duration of PRPs are strongly affected by work or effort requirements (Powell, 1968; Baron et al., 1992; Alling & Poling, 1995), suggesting a motivational component to the length of the pause, which may explain the lack of a difference in PRPs between schedule types. Additionally, there were no group differences in between bout pausing, in line with the interpretation of this variable as a measure of motivation to work for a given reward (Shull et al. 2001).

We also observed stark differences in the pattern of response rates between schedules. The initial predicted peak response rate was significantly higher in the PREXP schedule. The group differences observed are likely a reflection of the lower work requirements in the first few analysed trials in the PREXP condition (i.e. 4,6,9 vs 9,13,17 stimulus touches required in the linear PR4 schedule). The lower ratio requirement may allow the rats to respond at an initial faster rate. Furthermore, rats reinforced under the PREXP schedule also displayed a significantly greater rate of decay in responding. This may be a reflection of a more rapid progression in response requirements in the exponential schedule. This rapid increase may result in the faster decay in responding due to a process such as instrumental extinction. The two schedules differ in response requirements, particularly at later schedules. It would be useful to test whether animals trained on the different schedule types show different rates of extinction in the absence of reinforcement (as measured by total number of screen touches and response rates). This could allow for the investigation of whether PR4 and PREXP show different rates of instrumental extinction.

2.4.3 Outcome manipulations

Increasing the magnitude of rewards resulted in a significant increase in breakpoints, in line with previous reports (Skjoldager et al. 1993; Eagle et al. 1999; Rickard et al. 2009). Larger magnitude rewards increase the vigour of operant responding (Skjoldager et al. 1993). Breakpoints may represent the outcome of a cost/benefit decision making process (Salamone, Correa, et al. 2009). If an action or series of actions lead to a greater benefit (e.g., a larger food reward), then an organism should be more willing to overcome greater costs to obtain the goal. The rat touchscreen PR task was also sensitive to the effects of outcome devaluation through prefeeding. This is also in line with previous reports showing that both specific (Skjoldager et al. 1993) and nonspecific satiety (Eagle et al. 1999) results in a reduction in breakpoints. Prefeeding with chow would be expected to devalue the reinforcer and reduce the effort an organism is willing to expend to receive the reward.

The length of PRPs was not significantly affected either by changing reward magnitudes or prefeeding. PRPs have been shown to increase with the ratio requirements (Powell 1969; Baron et al. 1992). Increasing reward magnitudes, increases trial completion and therefore the average ratio requirement within a session. This would be expected to increase the length of the average PRP. This result may explain why larger magnitude rewards did not decrease pausing. PRPs were also unaffected by prefeeding rats with homecage chow. This matches previous findings (Skjoldager et al. 1993; Eagle et al. 1999) of prefeeding on pausing under

PR schedules. This is in contrast to the effects observed under FR schedules, where prefeeding animals has been reported to increase the duration of PRPs (Sidman & Stebbins 1954). Again, this may be as a result of prefeeding decreasing the total number of trials completed, and therefore decreasing the mean ratio requirement in these sessions. Together, this highlights a potential confound in evaluating performance based upon mean PRP across a PR session, without controlling for the total number of trials completed.

Neither increasing the reward magnitude nor prefeeding significantly altered the peak response rate. This is in agreement with the view that this variable reflects some measure of maximal motoric output (Phillips et al. 2017). Increasing the magnitude of reward also did not significantly affect the rate of decay in touchscreen responding. Previous reports suggest the efficacy of different food reinforcers in supporting PR performance does not appear to affect response rate decay (Kim et al. 2017). Therefore, it is not surprising that larger magnitude rewards do not affect the rate of decay, in spite of larger rewards supporting higher breakpoints. In contrast, reward devaluation through prefeeding significantly increased the rate of decay of responding. It is likely, therefore, that each food reward earned has a reduced ability to activate and support subsequent effortful behaviour resulting in an accelerated decay in response rates.

2.4.4 Dopaminergic manipulations

Effort-based responding is highly sensitive to dopaminergic manipulations (Salamone & Correa, 2012). Presently, systemic administration of raclopride and amphetamine decreased and increased breakpoints respectively. This is in line with previous reports in lever-based versions of PR (Poncelet et al., 1983; Cheeta et al., 1995; Aberman et al., 1998), as well as in the mouse touchscreen version (Heath et al., 2015). Amphetamine was able to produce a greater effect on breakpoints in animals reinforced under the PREXP schedule of reinforcement. This suggests that this schedule may have higher sensitivity for detecting the effects of manipulations that facilitate PR performance. Exponential PR schedules are commonly used in drug self-administration studies (Richardson & Roberts, 1996). The rapidly increasing response requirement in later trials reduces the risk of ceiling effects in time-limited sessions (Roberts et al., 1989). In a similar vein, exponential schedules allow higher breakpoints to be reached with fewer responses and fewer rewards earned. This may reduce the influence of motor fatigue and/or satiety affecting the enhancement of breakpoints.

Both raclopride and amphetamine affected the duration of the PRPs. Amphetamine has previously been reported to decrease the length of PRPs (Evenden & Robbins 1983), whereas

D_2 receptor antagonists increase pausing (Salamone 1986). The effects of dopaminergic compounds on PRP were in contrast to the lack of effects observed following the outcome modulations. The magnitude of the effects produced by the higher doses of raclopride and amphetamine appeared far larger than those produced by prefeeding and increasing reward magnitude. It may be the case that PRP as a measure is not as sensitive to changes in motivated behaviour as breakpoint, and larger effects are needed to detect significant changes in this measure. One notable effect is that raclopride appeared to produce a greater effect on pausing under the PR4 schedule of reinforcement (figure 2.5B). Breakpoints were roughly equal between groups following the high dose of raclopride (figure 2B). To achieve this breakpoint under a PR4 schedule, the mean response requirement is greater than that under the PREXP schedule, which begins small and undergoes larger increases in later trials. The length of PRPs is determined in part by the upcoming response requirement (Schlinger et al., 2008). Therefore, the larger response requirements in the PR4 group likely exacerbate the effects of raclopride on pausing, resulting in longer PRPs.

Another notable result was the effect of amphetamine on the pattern of response rates. The high dose of amphetamine reduced both the peak response rate and the rate of decay in responding. The reduced initial peak rate may be a reflection of the anxiogenic and/or appetite supressing effects of amphetamine (MacPhail & Gollub 1974; Lapin 1993). The reduction in the rate of decay may have been a result of amphetamine altering the rats' response to extinction. The low frequency of reinforcement relative to responding may result in extinction in later PR trials (Killeen et al. 2009). A slower decay in response rates may have reflected an increased resistance to extinction. However, if this were the case, it may have been expected that a greater effect upon the rate of decay would be observed in the PREXP group. The sharper increase in ratio requirements observed in the exponential schedule suggests a greater likelihood of extinction occurring relative to the linear schedule used in the PR4 group. As amphetamine reduced the rate of decay similarly in both groups, an increase in resistance to extinction is unlikely to be the sole explanation for a reduction in the rate of decay. A previous study, investigating within session changes in response rates reported that a similar dose of amphetamine (0.8mg/kg), increased the activating or motivational effects of reinforcers upon behaviour (Mobini et al. 2000).The reduced rate of decay observed presently may reflect an increase in the behavioural activation following each reinforcer. As a consequence, each reinforcer is able to support behaviour for longer, which may also underlie, at least in part, the increased breakpoints following the high dose of amphetamine.

2.4.5 Comparisons to other PR tasks

It is worth noting that, in the absence of any additional manipulations, breakpoints are lower in touchscreen PR than those observed in lever-press PR tasks. For example, across both linear and exponential schedule types, breakpoints in excess of 100 are typically observed in lever-responding rats (Skjoldager et al. 1993; Olarte-Sánchez et al. 2015; Bezzina et al. 2015). Therefore, the present findings of rats returning breakpoints in the region of ~45-55, is considerably lower than those seem with levers. The rate of operant responding is highly sensitive to physical characteristics such as the height of the lever (Skjoldager et al. 1993) and the required response force (Alling & Poling 1995). The touchscreen used in the present study use IR photocells to record screen touches (in fact, the rat is not strictly speaking required to 'touch' the screen). Therefore, touchscreen responding would be expected to require less physical effort than responding on a lever. The differences in breakpoint, therefore, cannot be explained in terms of force-requirements. One possibility is that the biophysical feedback from touchscreen responding is considerably less than that obtained by pressing a lever. In turn, there may be less salient cues to associate with reward. Pavlovian cues associated with reward are able to strongly influence instrumental behaviour (Rescorla & Solomon 1967). The reduced salience of cues associated with touchscreen responding relative to lever-pressing, may therefore reduce their invigorating effects upon responding (e.g. Saunders & Robinson 2011).

A separate possibility is the delay between response and reward. Increasing the delay from a response to a reward will shift behaviour to obtaining an immediately available, but less preferred reward (Thiébot et al. 1985). In the current touchscreen PR task, it is only possible to make a response every 0.5s. This is due to a brief stimulus-offset, added to provide visual feedback that a response has been made (see Methods). As a consequence, the rate of responding would be expected to be lower than a lever-based version of PR where rats are able to make multiple lever responses every second (e.g. Olarte-Sánchez et al. 2015). The longer time taken to complete each ratio may increase the costs associated with obtaining reward and result in animals ceasing responding earlier. Taken together this suggests that in spite of less physical effort being required to respond compared to levers, the touchscreen may be more 'cognitively effortful'. The reduced breakpoints and response rates in touchscreen PR may confer certain advantages: the avoidance of ceiling effects that may obscure potential faciliatory effects of interventions, particularly when using time-limited schedules, and a lower number of responses which may reduce the potentially confounding influences of satiation and motor fatigue upon performance. It should be noted that although the majority of animals reached a breakpoint (had a three-minute period without touching the screen within a 45-

minute session, table 2.2), a number of animals failed to do so. This was especially apparent following administration of amphetamine, where 14/24 rats failed to reach breakpoint. This represent a potential confound, since an animals breakpoint should represent the maximum number of responses emitted for a given reward, The inclusion of a time constraint suggests that the breakpoints reported in this study may not have been a true reflection of motivation. This further supports to concurrent analysis of response rate data to measure motivated behaviour, which may have been less affected by the time constraint.

2.4.6 Conclusions

Taken together, this study demonstrates the successful adaption and validation of PR for the rat operant touchscreen system. Like the mouse touchscreen- and traditional lever-based versions, the rat touchscreen PR variant is sufficiently sensitive to detect bidirectional changes in motivated behaviour following outcome manipulations and dopaminergic drugs. Furthermore, this study demonstrates that the use of exponential schedules of reinforcement may provide a greater sensitivity to detecting the effects of compounds that enhance PR performance. Additionally, this study demonstrates the utility of the complementary approach of studying within-session changes in behaviour in addition to cumulative parameters, such as breakpoint. Finally, effort-based motivated behaviour can now be assayed, with high face validity, across species.

Chapter 3. Validation of a test battery to probe effort-related decision-making in rats

3.1 Introduction

In spite of being widely used as assays of motivated behaviour, there are several confounds associated with the use of PR schedules. For example, manipulations that affect appetite or hedonic processes could also influence breakpoint (Pratt & Kelley 2004; Higgs et al. 2005; Maccioni et al. 2008). Therefore, it may not be clear whether a manipulation that disrupts PR performance is affecting effort exertion or the primary motivation for the food reinforcer. An alternative or complementary approach is to examine effort-related decision making processes (Salamone et al. 1991). In effort-related decision making tasks, rodents are given the choice between making an effortful response for a valued reward or making a lower effort action for a less preferred reward (Salamone et al. 1991; Salamone et al. 1994). These effort-related choice (ERC) tasks allow for better dissection of changes in motivated behaviour (Salamone, Correa, et al. 2009). A widely studied operant version of the ERC task involves a choice between lever-pressing under a fixed ratio (FR) schedule for sucrose pellets or consuming freely available chow (Salamone et al. 1991). Under low/moderate ratio requirements, rodents will typically choose to lever press for pellets. However, as the effort requirement for the preferred reward increases, animals will shift from lever pressing to consuming the freely available chow. Crucially, ERC can differentiate between perturbations that affect effort exertion and those that affect hedonic or appetitive processes. For example, a drug that specifically affects effort-exertion processes will reduce lever pressing and cause a shift in behaviour to the less preferred reward (Salamone et al. 1991). In contrast, a manipulation that disrupts hedonic processes would supress both lever pressing and chow consumption (Salamone et al. 2007).

In operant boxes, effort is typically manipulated by increasing the number of responses required for reward. However, by increasing the response requirement there is a concomitant increase in the delay from trial onset to reward delivery. Such delays represent a separate cost from effort which also affects behaviour. For example, increasing the time taken for a valued reward to be delivered will shift animals behaviour towards a less preferred but immediately available option (Thiébot et al. 1985; Evenden & Ryan 1996). As a consequence, any manipulation that affects PR or operant ERC performance, particularly at high ratios, could be affecting tolerance of delays rather than effort exertion. The effort discounting (EFD) task offers a way to account for the separate costs of delay and effort (Floresco et al. 2008). During

EFD, a given response results in a large food reward and a separate response yields a smaller reward. Throughout a session the response requirement for the large reward increases, whereas the response requirement for the low reward remains constantly low. Importantly, a delay can be added to the low reward, that matches the time taken to complete the equivalent large reward trial (figure 3.1C). As the delays are matched, only the effort requirements vary between the small and large rewards. Using this task, it has been shown that animals will shift behaviour, within a session, from the large reward to the small reward as the effort requirement increases. Furthermore, through the use of EFD it can be shown that certain pharmacological manipulations can affect effort independently of delay (Floresco et al. 2008).

A separate approach to control for potential delay confounds would be to manipulate effort requirements without changing the response requirements. Maze-based versions of ERC (Salamone et al. 1994; Pardo et al. 2012) and EFD (Bardgett et al. 2009) use a barrier obstacle, which an animal has to scale to obtain the large reward, whereas the small reward is available without the need to scale any barrier. The height of the barrier allows for the effort to be increased without substantially increasing the delay to reward; however, these tasks lack the advantages of automated operant testing. The use of a touchscreen stimulus as a manipulandum could allow for stimulus height to be increased within a session. Therefore, an effort discounting task could be developed that replaces an increasing ratio for the large reward with an ascending stimulus. As the height increases, rats would be expected to shift behaviour towards the small reward stimulus which remains at floor level (figure 3.1B). This approach would also remove the need for an animal to learn that a given number of responses is required for reward delivery. Therefore, a task using an ascending stimulus may provide a measure of effort-related choice in the absence of potential cognitive confounds.

The present chapter aimed to validate a battery of tasks to assess effort-based behaviour in rats. The validation of an effort-based battery of touchscreen tasks may aid discovery of novel treatments by helping facilitate the translation of results from rats to clinical populations (see chapter 1). Furthermore, the use of a touchscreen allows each task to be compared to the many other tests in the same context using the same food rewards. The present chapter describes an attempt to adapt and validate a battery of effort-based decision-making assays for use in rats as well as validate a novel rearing effort discounting (RED) task

3.2 **Methods**

3.2.1 Animals

A total of 34 male Sprague Dawley rats (Charles River, UK) were used in the current experiment. The twenty-four rats were tested on the ERC and EFD tasks, had previously been tested on the PR task (chapter 2). A separate cohort of ten naive rats were tested on the Rearing Effort Discounting (RED) task. All housing and husbandry procedures were identical to those described previously (chapter 2).

3.2.2 Apparatus

All testing took place within automated Bussey-Saksida touchscreen chambers as described in detail in chapter 2. Masks and stimulus sizes are outlined in figure 3.1A.

3.2.3 Effort-Related Choice

Initially, animals underwent FR5 testing identical to previous procedures (chapter 2), with no trial limit imposed. Sessions were terminated following 30 minutes. Once stable performance was reached, approximately 20g of homecage chow was scattered on the floor of the chambers and FR5 ran otherwise as normal. Following each session, the remaining chow was weighed to calculate the amount consumed. Rats were initially habituated to this FR5-Chow procedure for ten days. In total, ERC training took 14 days. Following this period, data were analysed from three consecutive FR5 sessions. Subsequently, rats were given three consecutive days each of FR10, FR20 and finally FR40. This escalating FR procedure was used to avoid potential extinction effects.

The primary measures of interest were the number of stimulus responses made and the amount of chow consumed. The post-reinforcement pause (PRP), the inter-response interval (IRI) and the latency to the first response were analysed as measures of task engagement. In order to assess general activity levels, the mean reward collection latency; the rate of IR beam breaks (beam breaks per second) and the rate of nontarget screen touches (blank touches per second) were recoded.

3.2.4 Effort discounting

During EFD, responding to either a left or right stimulus (balanced across subjects) resulted in delivery of the large reward (LR, 3 pellets). A response to the opposite stimulus resulted in delivery of the small reward (SR, 1 pellet). All reward delivery cues (tone and magazine light) were identical to those used previously (chapter 2).

Initially animals undertook discrimination training which consisted in presenting rats with forty free-choice trials where both the LR and SR stimuli were presented simultaneously. A single response to either stimulus resulted in reward delivery. If no response was made to either stimuli within 30s an omission was recorded and the next trial commenced. Sessions were terminated after completion of 40 trials or following 45 minutes elapsing. Rats were required to make 30 LR choices (i.e. 75%) within a session before moving onto the next stage of training. All animals reached this criterion within seven days.

Animals then underwent five days of EFD training. Throughout testing, sessions were split into four bins of ten trials. The number of responses needed for the LR delivery increased following each bin. The response requirement for the LR in each of the four bins was: FR1, FR6, FR12, FR18. Prior to the start of each bin, two forced-choice trials were randomly presented (one each for the SR and LR stimuli), that helped inform the rats of the upcoming response requirements. During forced-choice only one stimulus was presented, and animals had to complete both forced-trials before being presented with the ten free-choice trials. Once a response was made to the LR stimulus in a free choice trial, the SR stimulus was removed from the screen until the start of the subsequent trial. Sessions were terminated following 40 free-choice trials or 45 minutes.

In order to add an equivalent delay to the SR (figure 3.1C), the mean response times for LR trials during bins 2, 3 and 4 were calculated from the previous stage of testing. During the subsequent EFD sessions, reward delivery following SR choices was delayed according to the mean LR response times: bin 2- 6.64s: bin 3: 14.80s; bin 4: 25.50s. All other parameters were maintained from the previous sessions. Rats were tested on this delayed-EFD for five consecutive days. The primary measures of interest were the percentage of free-choice trials in each bin ending with a large reward choice, a small reward choice or an omission.

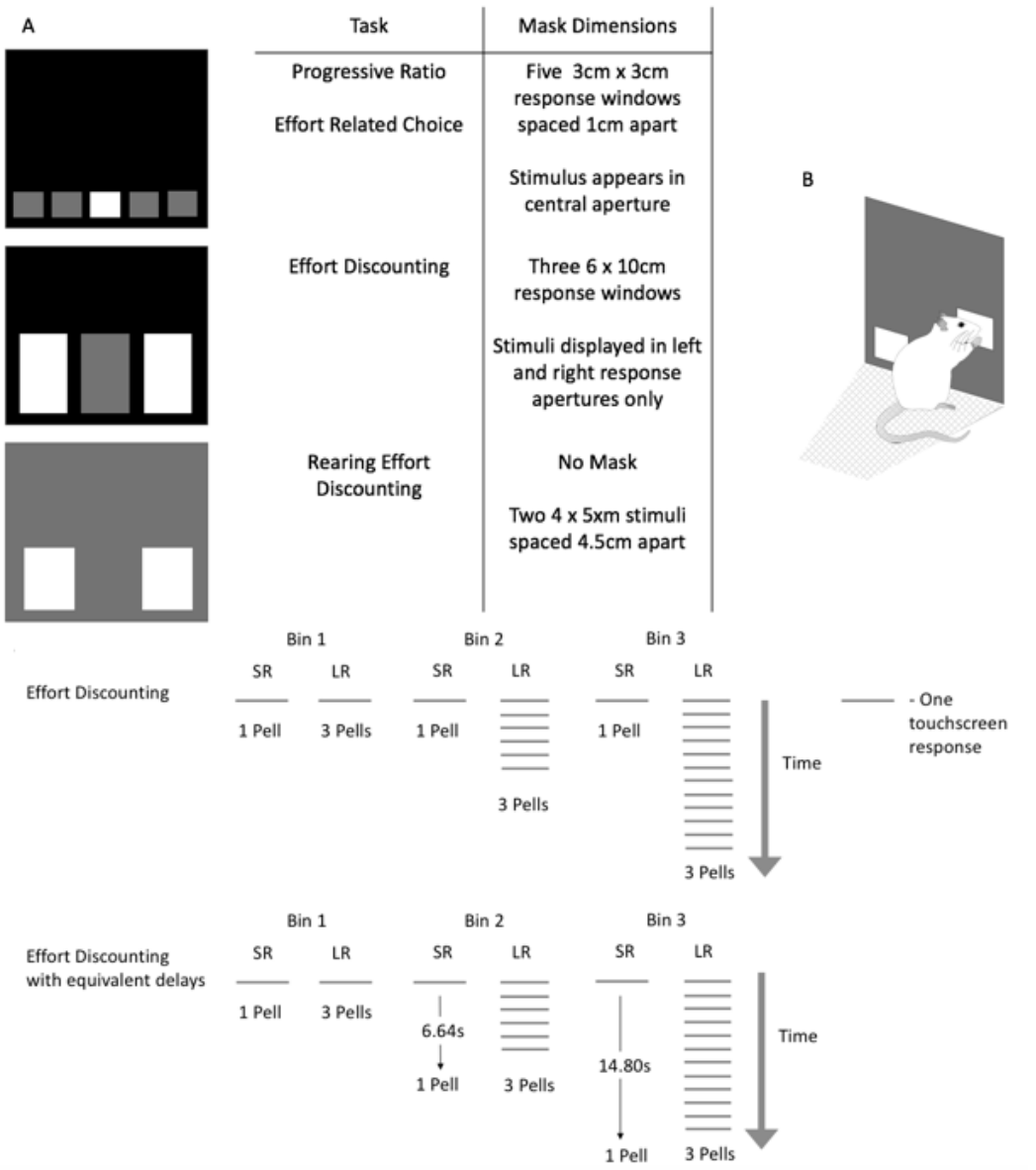


Figure 3.1: A Details of the mask dimensions and stimuli locations in the tasks used in the present study. **B** Visualisation of the rearing effort discounting task. **C** Overview of trials within the effort discounting task and the effort discounting with equivalent delays variant. Blue bars represent a touchscreen response to either the SR or LR stimuli. SR: small reward; LR: large reward; 1 Pell: a single pellet reward; 3 Pells: Three pellets given as a reward.

3.2.5 Rearing effort discounting

Rearing effort discounting (RED) is an adapted EFD task in which the repeated response requirement is replaced by a requirement for the animal to rear up to respond to the LR stimulus, which increases in height at the start of each bin. This allows for the effort to be increased without substantially increasing the time taken to complete a LR trial. This also reduces possible cognitive confounds as the effort required in any given trial is apparent to the

rat, unlike in the traditional EFD task during RED two white rectangular stimuli (see figures 3.1A,B) were presented, 4.5 cm apart. A response to one stimulus resulted in a single pellet delivery, whereas a single response to the other resulted in three pellets being delivered. The location of the small (SR, 1 pellet) and large (LR, 3 pellets) rewarded stimuli was counterbalanced across subjects, and remained constant for the duration of the study.

An overview of the training procedure is available in figure 3.2A. Following screen touch training (chapter 2), rats were trained to discriminate between the large and small rewards. Discrimination sessions consisted of 20 forced choice trials, followed by 20 free choice trials. At the start of each trial, the magazine light flashed (250ms on/off) until the rat entered the magazine (therefore, unlike all other tasks, this task was self-paced). Following magazine entry either the SR or LR stimulus was presented on the screen and remained presented until touched. Reward delivery(SR: 1 pellet, LR: 3 pellets) was accompanied by stimulus offset, magazine illumination and tone (as described in chapter 2). Ten SR and ten LR trials were presented in a random order, with no type presented more than two times in a row. Following these forced choice trials, each rat undertook twenty free-choice trials. Here, both LR and SR stimuli were both presented on the screen, a single response to either the LR or SR stimuli resulted respective reward delivery and trial completion. All other trial parameters were identical to the forced choice trials. Rats were required to select the LR stimulus on at least 15 out of 20 free-choice trials to complete the reward magnitude discrimination.

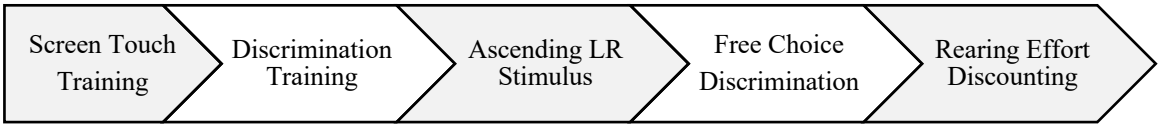
Rats were subsequently trained to respond to an ascending LR stimulus. During these sessions only the LR stimulus was presented. As before, the centre of the stimulus was 2.5 cm from the floor of the chamber, and a response resulted in delivery of three food pellets. Every two trials, the stimulus was raised by 2.5cm (but remained the same shape and size). This continued for a maximum of 20 trials when the centre of the stimulus was 25cm from the floor of the chamber. If no response was made to the stimulus within 300s the session ended, otherwise sessions were terminated after completion of the 20 trials. Four consecutive daily sessions of height training with these parameters were given. Subsequently, three additional sessions were administered with the response period reduced to 180s, 60s and 40s respectively.

Finally, animals underwent free choice discrimination training, to examine any influence of satiety on choice, in the absence of any escalating effort requirements. During free choice discrimination, forty free choice trials were presented. If no response was made to either stimuli

within 40s an omission was recorded and the next trial was initiated. All rats were given three consecutive daily sessions of this discrimination testing before undergoing the RED task.

Subsequently, rats were tested on the RED task. As before, this consisted of 40 trials separated into four bins of ten free-choice trials. During bin 1, both the SR and LR stimuli were located level with the floor of the chamber (stimulus centre 2.5cm from floor). However, during subsequent bins, the LR stimulus was elevated, whereas the SR stimulus remained fixed in the lower position. The centre of the LR stimulus was located at the following heights: bin 2- 7.5cm, bin 3 – 12.5cm, bin 4 – 17.5cm. Again, a single response to either stimuli was needed for reward delivery of their respective magnitudes. All other session parameters remained constant. Analysis was conducted by bin to examine the effects of increasing the response stimulus height on the discounting of large rewards. Following stable performance, rats were tested on several validation probes, which can be seen in figure 3.2B. As with the EFD analysis, the primary variables of interest were the percentage of trials within each bin that a subject chose either the large reward, the small reward, or omitted responding.

A



B

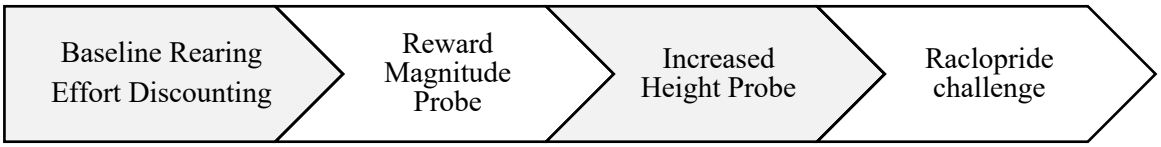


Figure 3.2: Experimental timeline of the rearing effort discounting task**. A** An overview of the training for Rearing effort discounting. LR: Large reward. **B** Outline of the validation procedures used during the Rearing effort discounting testing.

Reward magnitude probe.

During this probe, the heights of stimuli in bins 2 and 3 were increased (due to the lack of a difference in LR choices between bins 1 and 2 and bins 3 and 4 at baseline, see *results*). The height of the LR stimulus was as follows: bin 2 – 10cm, bin 3 - 15 cm (bin 4 remained at 17.5 cm). A response to the LR stimulus resulted in 6 food pellets being delivered; all other parameters remained constant. This was to examine if increasing reward magnitudes decreases

the rate of discounting. Rats received three consecutive days of increased magnitude training. Behavioural results were collapsed across the three days

Increased height probe

An increasing height probe was carried out in order to examine whether increasing the heights of the LR stimulus increased the rate of discounting. As with the standard RED, the LR and SR stimuli were located adjacent to each other during bin 1. The height of the LR stimulus during the remainder of the task was as follows: bin 2 – 12.5cm; bin 3 – 20cm; bin 4 – 25cm. Therefore, relative to the standard RED task the LR stimuli were located at equal during bin 1, 2.5 cm higher during bin 2, 5cm higher during bin 3 and 7.5 cm higher during bin 4. Rats received three consecutive days of increased height training. Behavioural results were collapsed across the three days

3.2.6 Drugs

S(−)-Raclopride(+)-tartrate salt (Bio-techne, Abington, UK) was administered via intraperitoneal injection at a dose of 0.1 mg/kg of each rat's body weight (volume: 1 mg/ml), 30-minutes prior to testing. This dose was chosen to avoid the potential motoric effects observed previously (chapter 2). Raclopride was dissolved within physiological saline and administered prior to EFD with equivalent delays condition and RED testing

3.2.7 Statistical analyses

All statistical analyses were conducted using SPSS (Version 23.0, IBM Corp, Armonk, NY, US). Graphs were produced using Prism (GraphPad, La Jolla, CA, USA). Repeated measures ANOVAs were used to analyse all results. When needed, a Greenhouse-Geisser correction was applied to adjust for violations in sphericity. All post hoc testing was corrected using Bonferroni adjustments for multiple comparisons.

3.3 **Results**

3.3.1 Increasing the work requirement shifts behaviour during Touchscreen ERC testing

Figure 3.3A shows how increasing the ratio requirement significantly reduced the number of screen responses made ($F(3,69) = 53.246$, $p < .001$; partial eta squared = .698). The number of target responses made under the FR5 schedule was significantly greater than all other conditions ($p < .001$); whereas the number of responses made under the FR40 schedule was significantly smaller than all other conditions ($p < .001$). There was no difference between the

FR10 and FR20 schedules ($p > .05$). Figure 3.3B suggests that increasing the work requirement significantly increased chow consumption ($F(3,69) = 34.907$, $p < .001$; partial eta squared = .603; figure 3.3B). The amount of chow consumed was significantly lower on the FR5 schedule compared to all other schedules ($p < .001$). Chow consumption on the FR40 schedule was significantly greater than the FR10 ($p < .01$). No other comparisons were significant. Increasing the ratio requirement significantly affected task engagement. Increasing the ratio also increased the mean inter-response interval (IRI; $F(1.13,25.996) = 11.165$, $p < .01$; partial eta squared = .327; figure 3.3C). The mean IRI was significantly shorter on the FR5 schedule compared to all other schedules ($p < .05$). The IRI of rats reinforced on a FR40 schedule was also significantly longer than the FR10 ($p < .01$). There were no other differences in the mean IRI. Increasing the ratio requirement did not significantly affect the latency to the first response within a session ($F(2.272, 52.259) = 1.386$, $p = .254$).

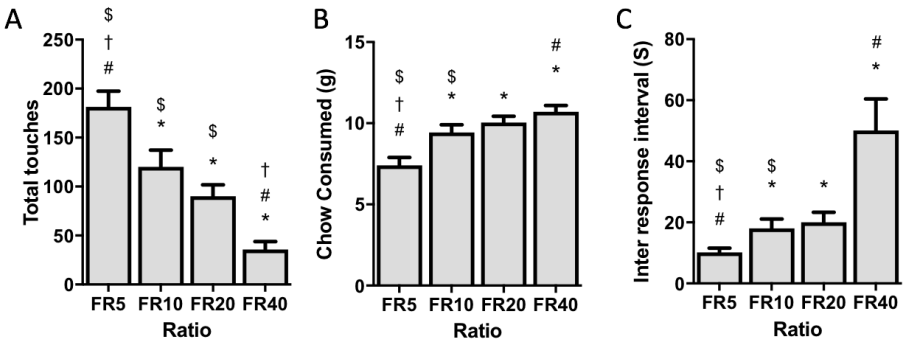


Figure 3.3: Increasing the ratio requirement affects ERC performance. **A** Increasing the number of responses for pellet delivery reduces target touches and **B** increases chow consumption. **C** The mean inter response interval is increased at the highest ratio. Symbols note a significant($p<.05$) post-hoc comparison * Significantly different from FR5; # significantly different from FR10; † significantly different from FR20; $ significantly different from FR40. Error bars display the SEM.

Changing the ratio requirement also appeared to reduce the activity levels of rats within the chambers. As shown in figure 3.4A, increasing the work requirement decreased the rate of IR beam breaks ($F(3,69) = 11.675$, $p < .001$; partial eta squared = .337). Beam breaks were significantly greater in the FR5 condition compared to the FR20 ($p < .01$) and FR40 conditions ($p < .001$). There were no significant differences between any other condition (all $p > .05$). The rate of nontarget screen touches was also significantly affected by the ratio requirement, as shown in figure 3.4B ($F(2.058, 47.326) = 10.378$, $p < .001$; partial eta squared = .311). The number of blank touches made per second was significantly higher in the FR10 schedule

relative to all other conditions (all $p < .05$). The majority of rats (17/24) failed to complete a trial in the FR40 condition. Therefore, PRP and reward collection latency were only analysed for the FR5, FR10 and FR20 schedules. The ratio requirement did not significantly affect either the duration of the PRP ($F(1.470, 20.864) = 2.490$, $p = .113$) or the reward collection latency ($F(2,42) = .128$, $p = .881$; figure 3.4C).

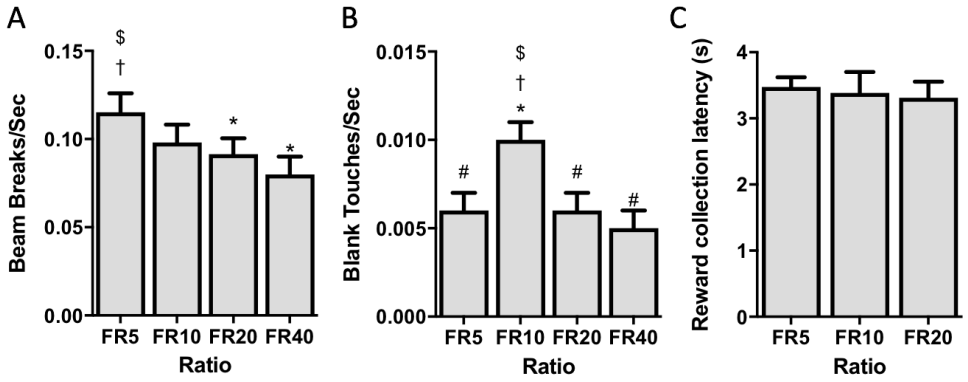


Figure 3.4: Effects of increasing the ratio requirement on measures of motoric performance **A** Increasing the ratio requirement decreases the rate of IR beam breaks. **B** The rate of blank (nontarget) screen responses is highest in the FR10/Chow condition. **C** Changing the response requirement did not affect the latency to collect rewards. Symbols note a significant post hoc comparison ($p<.05$). * Significantly different from FR5; # significantly different from FR10; † significantly different from FR20; $ significantly different from FR40. Error bars display the SEM.

These data indicate that increasing the ratio during the ERC task decreases operant responding and shifts behaviour towards the less preferred, freely available chow. This is in line with previous reports using levers in rats and the touchscreen in mice (Salamone et al. 1997; Heath et al. 2015). A reduction in the total target screen responses and mean IRIs suggest at higher ratios rats spend less time engaged in operant responding.

3.3.2 Effort discounting without programmed delays

Given the high rates of chow consumption in the ERC, it may be necessary to develop a touchscreen-based assay of effort-related decision making that does not include a freely available chow option. Therefore, rats were tested on a touchscreen effort discounting (EFD) task. Figure 3.5A suggests that increasing the large reward (LR) response requirement, reduced the percentage of LR choices ($F(3,69) = 66.922$, $p < .001$, partial eta squared = .744). The percentage of LR choices under each ratio differed significantly from all others (all $p < .05$). Concurrently, as seen in figure 3.5B, increasing the ratio requirement of the LR also resulted

increased the percentage of small reward (SR) choices ($F(3,69) = 55.960$, $p < .001$; partial eta squared = .709). The percentage of SR choices made within each bin differed between each other condition (all $p < .05$). However, as seen in figure 3.5C, increasing the work requirement for the LR did not affect the rate of omissions ($F(2.278, 52.386) = 1.804$, $p = .170$; figure 3B). Together, these results suggest that increasing the work requirement for the large reward option resulted in rats shifting their preference towards the small reward across the course of the session.

3.3.3 Effort discounting with equivalent delays

In this condition, a delay was added to the small reward to match the average time taken to complete a LR trial in each bin. This allowed for the effects of effort-requirements to be separated from the effects of escalating delays. Figure 3.5D shows how, in the presence of equivalent SR delays, increasing the effort needed to obtain the large reward again significantly decreased the percentage of LR choices made ($F(1.764, 40.574) = 56.873$, $p < .001$; partial eta squared = .712). The percentage of LR choices made in under each ratio differed significantly from each other ratio (all $p < .01$). Figure 3.5E shows there was also a significant effect on the percentage of SR choices made ($F(3,69) = 9.334$, $p < .001$; partial eta squatted = .289). Post hoc comparisons revealed that there was a significant increase in SR choices from bin 1 to bins 2 ($p < .001$), bin 3 ($p < .01$). However, there were no other significant differences between the percentage of SR choices between any other bins (all $p > .05$). This suggests, despite an initial increase in the small reward choices made, there were no increases following bin 2.

In contrast to the effects observed in the absence of delays, there was a significant increase in omissions over the course of a session, as seen in figure 3.5F ($F(1.662, 38.231) = 49.249$, $p < .001$; partial eta squared = .682; figure 3F). The percentage of choices that ended in omissions in bin 1 did not differ from bin 2 ($p = .135$). There was however a significant increase in omissions from bin 2 to bins 3 and 4 (both $p < .001$). The percentage of omissions also increased from bins 3 to 4 ($p < .001$) Together these results suggests, that in the presence of a delayed SR, increasing the ratio requirement for the LR decreases the preference for that reward option; however, instead of shifting behaviour towards SR choices, there was an increase in the rates of response omission.

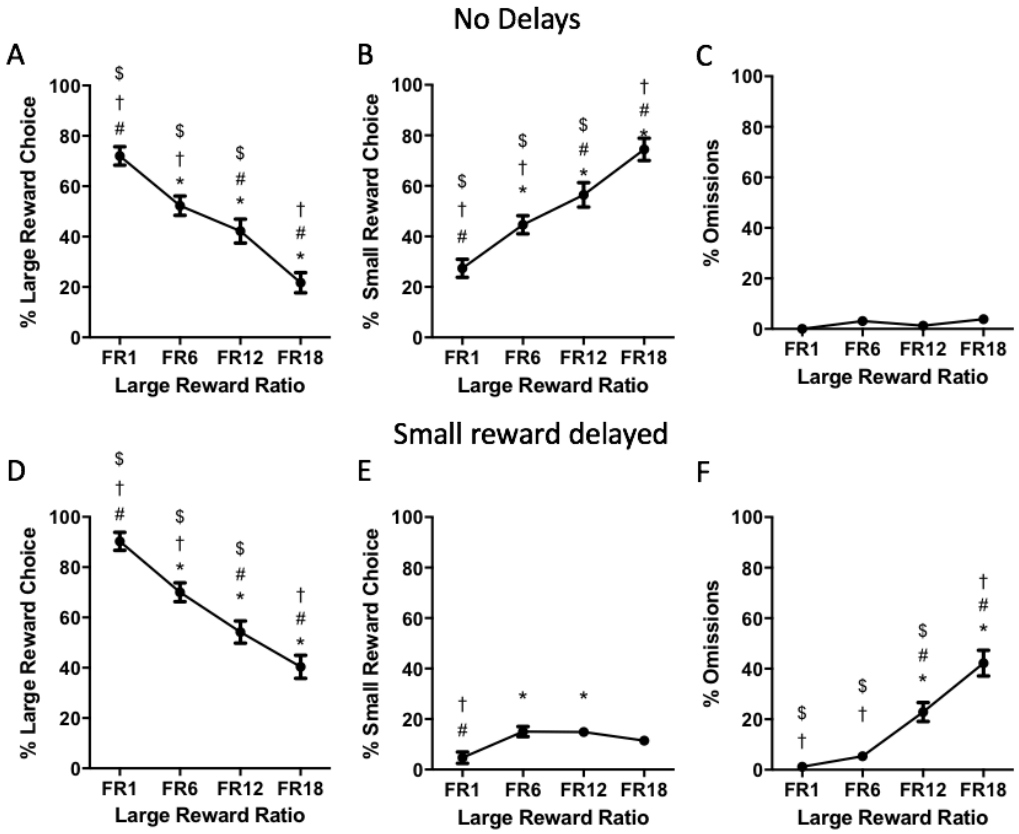


Figure 3.5: Touchscreen effort discounting performance in rats (**A-C**) Effort discounting without any reward delays and (**D-F)** effort discounting with an equivalent delay added to the small reward (to match the average time taken to complete a LR trial in each bin). **A** Increasing the response requirement for the large reward decreases large reward choices and **B** increases small reward choices. **C** In the absence of any delay, increasing the work requirements for the large reward did not affect omissions. With equivalent delays **D** increasing the work requirement decreased the choice of large rewards. **E** However, only an initial small increase in small reward choices was observed. **F** In the presence of delays, increasing the work requirement causes an increase in omissions. Symbols above data points note a significant post hoc comparison (p<.05). * Significantly different from FR1; # significantly different from FR6; † significantly different from FR12; $ significantly different from FR18 Error bars display the SEM.

3.3.4 Raclopride increases the rate of omissions during effort discounting

To test the effects of a dopaminergic challenge, 0.1mg/kg of raclopride was administered prior to the EFD with equivalent SR delays. Figure 3.6A shows how LR choices were significantly reduced both by increasing the response requirement ($F(3,69) = 51.014$, $p < .001$; partial eta squared = .689) and by raclopride administration ($F(1,23) = 32.487$, $p < .001$; partial eta

squared = .585). There was also a significant interaction between ratio and drug ($F(3,69)$ = 6.476, $p < .01$; partial eta squared = .220). Under both vehicle and raclopride conditions there was a significant decrease in LR choices across the session (i.e. between FR1 and FR18 conditions). Furthermore, whereas raclopride did not affect LR choices under FR1 ($p = .90$); raclopride reduced the percentage of LR choices compared to vehicle at all other ratio requirements (all $p < .001$). Together, this suggests that raclopride administration increased the rate of effort discounting.

As seen in figure 3.6B, the percentage of SR choices was also significantly affected by the LR ratio ($F(3,69) = 3.654$, $p < .05$; partial eta squared = .137). However, raclopride did not affect the percentage of trials ending in SR choices ($F(1,23) = 2.499$, $p = .128$). There was however, a significant interaction between LR ratio and raclopride on SR choices ($F(3,69) = 3.548$, $p < .05$; partial eta squared = .134; figure 3.6B). In the vehicle condition, SR choices were greater in bin 3 compared to bin 1 ($p < .05$). Following raclopride administration, there were no significant differences in SR choices between any bin (all $p > .05$). When comparing between drug conditions, SR choices were higher following administration of raclopride in bin 2 ($p < .01$) only (all other comparisons $p > .05$). Together, these data suggest that raclopride produced an initial increase in SR choices; however, this did not increase with escalating LR effort costs.

Figure 3.6C shows how the percentage of trials ending in omission was significantly affected by both LR response requirement ($F(1.894,43.567) = 48.362$, $p < .001$; partial eta squared = .678) and raclopride administration ($F(1,23) = 26.905$, $p < .001$; partial eta squared = .539). There was also a significant interaction between response requirement and drug on omissions ($F(3,69) = 11.616$, $p < .001$; partial eta squared = .336). In the vehicle condition, there was no difference in omission rates between bins 1 and 2 ($p = .807$); however, omission rates differed significantly between all other bins ($p < .05$). In the raclopride condition, omissions differed significantly between each bin (all $p < .05$). When comparing between drug conditions, raclopride significantly increased the percentage of omissions made in bins 2-4 ($p < .01$) but not bin 1 ($p < .05$). This result mirrors the effects of raclopride on LR choices (figure 3.6A) and suggests that raclopride increases the rate of omissions, but only under effortful conditions. Together, this highlights how, in the presence of delays, decreases the choice of high effort reward options and fails to increase the rate of delayed low effort choices.

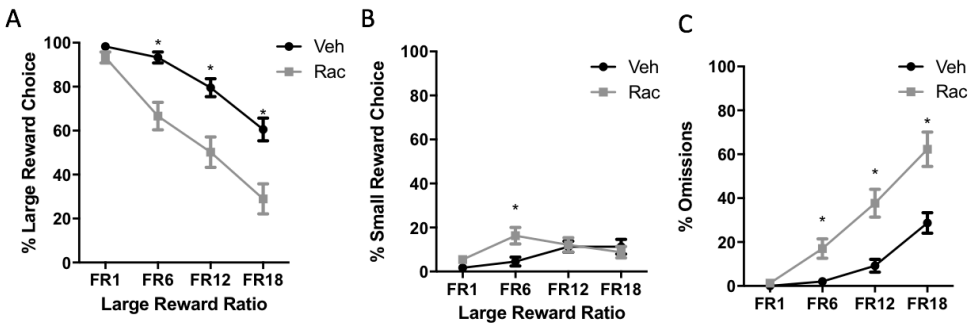


Figure 3.6: Effect of raclopride on effort discounting performance with a delayed small reward. **A** Raclopride and higher work requirements decrease large reward choices. **B** Raclopride produces only a small effect on small reward choices. **C** Raclopride increases the effects of ratio on omissions. Rac: Raclopride 0.1mg.kg, Veh: Saline vehicle. Symbols above data points note a significant (p<.05) post hoc comparison.* A significant difference between drug conditions. Error bars display the SEM.

3.3.5 Rearing Effort Discounting

The previous results demonstrate that the addition of a delay to the low-effort option, results in an increase in the rate of response omissions. We therefore sought to develop a task that allows effort to be manipulated in the absence of any substantial increase in the delay-to-reward. Maze-based ERC tasks use the height of a barrier to increase the effort needed to obtain a reward (Salamone et al. 1994). In the rearing effort discounting (RED) test, the height of the LR stimulus increased across a session, rather than the ratio requirement. During height training, all animals were able to successfully respond the maximum LR stimulus height (25cm), in the absence of a SR response option.

For the baseline RED procedure, the following LR stimulus heights were used: bin 1-2.5cm (floor level) bin 2- 7.5cm, bin 3 – 12.5cm, bin 4 – 17.5cm. Figure 3.7A shows that increasing the height of the LR stimulus, significantly decreases LR choices ($F(3,27) = 24.547$, $p < .001$; partial eta squared = .723). The percentage of the LR choices did not differ between bins 1 and 2 or bins 3 and 4 ($p > .05$); however, all other comparisons were significant (all $p < .01$). During baseline RED training, no omissions were made, meaning every non-LR choice was a SR choice. Therefore, increasing the height of the stimulus had an equal but opposite effect on the percentage of SR choices ($F(3,27) = 24.547$, $p < .001$; partial eta squared = .723; figure 3.7B). Together, these data demonstrate that increasing the height of the LR stimulus results in a shift towards the SR without affecting omission rates.

3.3.6 Effect of increasing the large reward magnitude on RED performance

Figure 3.7C shows how LR choices were significantly affected by both bin ($F(1.524, 13.720) = 11.963$, $p < .01$; partial eta squared = .571) and reward magnitude ($F(1,9) = 11.963$, $p < .01$; partial eta squared = .596). There was no significant interaction between reward magnitude and bin ($F(3,27) = .757$, $p = .528$; partial eta squared = .078). Overall, LR choices were greater when reinforced with 6 pellets compared to the standard 3 pellet rewards ($p < .01$). This suggests that increasing the magnitude of the reward facilitates selection of the high effort, LR option independently of the height of the stimulus. As seen in figure 3.7D, increasing the magnitude of the large reward option also significantly affected the percentage of SR choices ($F(1,10) = 15.240$, $p < .01$; partial eta squared = .629). There was also a significant main effect of bin on SR choices ($F(1.543, 13.890) = 11.007$, $p < .01$; partial eta squared = .550). Again, there was no interaction between bin and the large reward magnitude on SR choices ($F(1.565, 14.081) = .971$, $p = .382$;). Overall, having a larger LR significantly decreased small reward choices (3 pellets: 39.67 ± 5.97, 6 pellets: 27.42 ± 5.06; $p < .01$). The percentage of omissions was not affected by either reward magnitude ($F(1,9) = 2.250$, $p = .168$), bin ($F(3,27) = 2.096$, $p = .124$) or by any bin x magnitude interaction ($F(3,27) = 2.020$, $p = .135$).

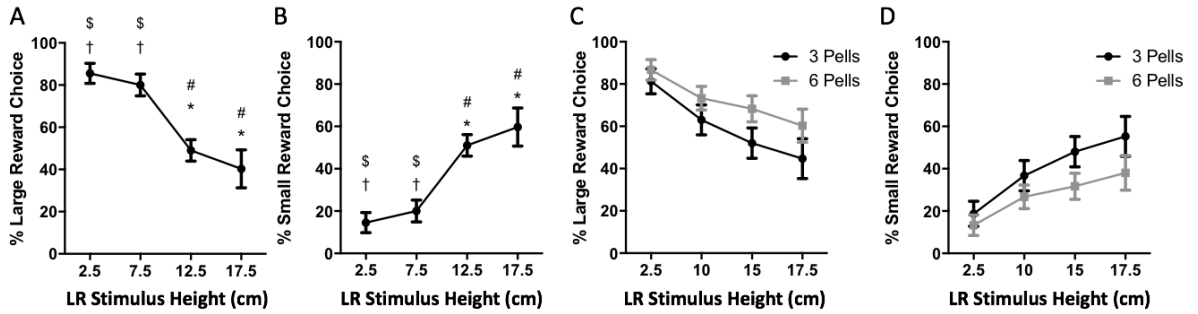


Figure 3.7: (**A-B**) Rearing effort discounting performance and (**C-D**) effects of increasing the magnitude of the large reward on rearing effort discounting. **A** Increasing the height of the large rewarded stimulus decreases responses and **B** increases choices of the small rewarded stimulus. **C** Increasing the magnitude of the large reward increases large reward and **D** decreases small reward choices. Symbols above data points note a significant post hoc comparison ($p<.05$). *Significantly different from bin 1; #significantly different from bin 2; †significantly different from bin 3; $ significantly different from bin 4. Error bars display the SEM.

3.3.7 The effects of increasing the height of the large rewarded stimulus on RED performance

Increasing the height of the LR stimuli decreased the percentage of LR choices, as can be seen in figure 3.8A ($F(1,9) = 31.14$, $p < .001$; partial eta squared = .776). There was also a significant effect of bin ($F(3,27) = 30.737$, $p < .001$; partial eta squared = .774) and a significant bin x height interaction ($F(3,27) = 23.978$, $p < .001$; partial eta squared = .727) on LR choices. The percentage of LR choices decreased from bin 1 to 4 in both height conditions (standard $p < .05$; increased height: $p < .001$). The percentage of LR choices differed between height schedules in bin 4 only ($p < .001$). As shown in figure 3.8B, increasing the height of the LR stimulus also affected the SR choices ($F(1,9) = 24.214$, $p < .01$; partial eta squared = .729). There were also significant effects of bin ($F(2,27) = 25.890$, $p < .001$; partial eta squared = .742) and a bin x height schedule interaction ($F(3,27) = 18.360$, $p < .001$; partial eta squared =.671). SR choices increased significantly from bin 1 to 4 in both height conditions (standard: $p < .05$; increased height: $p < .001$). Between height conditions, there was a significant difference in the percentage of SR choices in bin 4 only ($p <.001$). The percentage of trials ending in omissions was also affected by both height ($F(1,9) = 6.036$, $p < .05$; partial eta squared = .402) and bin ($F(1,966, 17.694) = 6.152$, $p < .05$; partial eta squared = .406). The bin x schedule interaction on omissions was also trending towards significance ($F(1.922,17.30) = 3.368$, $p = .06$; figure 3.8C). Overall the percentage of omissions was significantly increased by increasing the target height ($p < .05$).

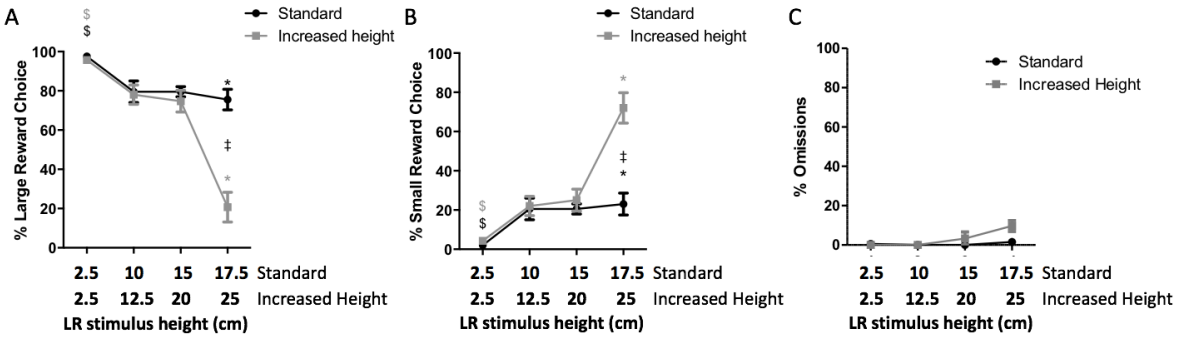


Figure 3.8: Effects of stimulus height on rearing effort discounting. **A** Increasing the stimulus height only decreases large reward choices at the final height. **B** Increasing the large reward stimulus height causes a shift in responding to the small reward stimulus in the final bin only. **C** Increasing the large stimulus results in a small increase in omissions in the final bin. Symbols above data points note a significant ($p<.05$) post hoc comparison. * Significantly different from bin 1; $ significantly different from bin 4; ‡ Significant difference between drug conditions. Error bars display the SEM

3.3.8 Effect of raclopride administration on RED performance

Subsequently, the effects of 0.1mg/kg of raclopride on rearing effort discounting was tested. As seen in figure 3.9A, the percentage of LR choices was significantly reduced by both bin ($F(3,27) = 5.323$, $p < .01$; partial eta squared = .507) and raclopride administration ($F(1,9) = 9.240$, $p < .05$; partial eta squared = .372). However, there was no interaction between raclopride and bin ($F(3,27) = 1.187$, $p = .333$; figure 7A). Concurrently, as seen in figure 3.9B, SR choices were significantly increased by both raclopride administration ($F(1,9) = 9.333$, $p < .05$; partial eta squared = .507) and bin ($F(3,27) = 4.321$, $p < .05$; partial eta squared = .324). However, there was no drug x bin interaction ($F(3,27) = .401$, $p = .753$; figure 7B). The percentage of omissions, which can be seen in figure 3.9C, were not significantly affected by either raclopride ($F(1,9) = 4.355$, $p = .067$) or bin ($F(1.119,10.069) = 2.591$, $p = .137$). There was also no significant interaction between raclopride and bin ($F(1.231, 11.082) = 2.771$, $p = .121$).

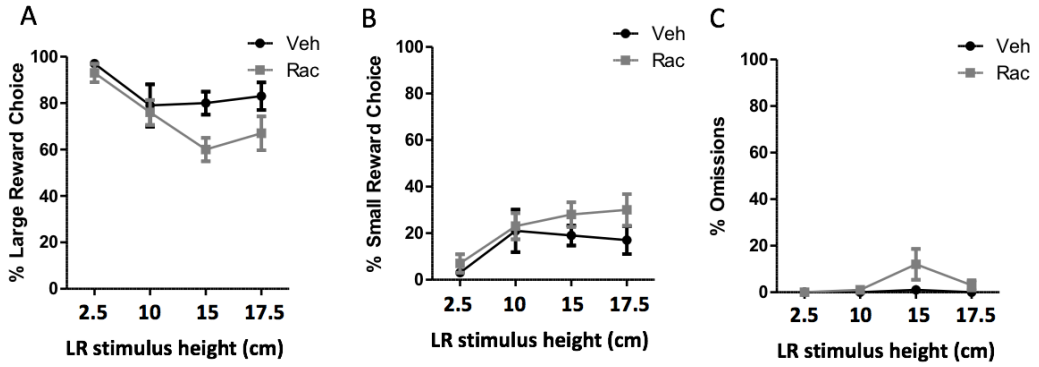


Figure 3.9: Effect of systemic raclopride on rearing effort discounting. **A** Raclopride has only a small effect on large reward choices **B** Raclopride does not affect small reward choices. **C** Raclopride produces a small increase in omissions. Veh: saline vehicle; Rac: Raclopride 0.1mg/kg. Error bars display the SEM

3.4 Discussion

The ability to accurately measure effort-based decision-making in rodents can help provide a translational means of measuring motivation. The use of choice tasks can offer a complimentary approach to progressive ratio assays, particularly when testing the effects of novel pharmacological interventions. Choice assays allow for dissociation of activational and directional components of motivation (Salamone & Correa 2012). Furthermore, a number of choice tasks may avoid the confounds of progressively increasing reward delays observed in PR (Wanat et al. 2010), that may confound interpretation of results. Finally, effort-based decision-making tasks have been developed for use in clinical populations (Treadway et al.

2009; Chong et al. 2015). The present chapter explored the potential of three effort-based decision-making tasks for use within the rat touchscreen operant system.

3.4.1 Effort related choice

Operant ERC tasks have been widely used to understand the neural and pharmacological substrates of effort-based behaviour (Salamone, Correa, et al. 2016; Bailey, Simpson, et al. 2016). In the present study, increasing the response requirement for food pellets resulted in a significant decrease in touchscreen responding for food pellets. This decrease in touchscreen responding was accompanied with a significant increase in the consumption of freely available chow. This behavioural shift is in line with the previous lever-based versions and the mouse touchscreen ERC task (Salamone et al. 1997; Cagniard et al. 2006; Heath et al. 2015). Additionally, increasing the response requirement increased the mean inter-response interval (IRI) and decreased the rate of IR beam breaks. This suggests that the rats disengaged from touchscreen responding as the response requirement increased. This behavioural shift is believed to be an outcome of a cost-benefit decision process (Salamone, Correa, et al. 2009). Increasing the response requirement increases the cost associated with the preferred reward, as the costs begins to outweigh the benefit of the preferred reward a shift occurs to the less preferred food reward. The point at which animals shift is sensitive to both pharmacological manipulations and individual differences in neurochemical correlates (Randall et al. 2012).

One issue of note was the baseline performance of rats during ERC responding, when tested under low effort requirements (i.e. FR5). In traditional versions of ERC, in the absence of any other manipulations, rats consume very little chow on a FR5 schedule (Salamone et al. 1991; Cousins & Salamone 1994). This is in contrast to the present study where rats consumed, on average, almost 7.5g of chow in 30 minutes of testing at this ratio. The number of touchscreen responses observed (~180) was also substantially lower than the number of lever presses typically seen in a 30-minute ERC session, which is typically above 1000 (Salamone et al. 1991; Cousins and Salamone 1994). Therefore, under low-effort conditions, rats already spend considerable periods of time consuming the freely available chow rather than engaging in operant responding. This is likely due to the nature of the touchscreen manipulandum (see chapter 2). Importantly however, the present results suggest that the rat touchscreen ERC is still sensitive to the behavioural shift that occurs when increasing the effort requirement; the baseline performance is not necessarily important for preclinical testing of interventions that affect performance.

3.4.2 Effort discounting

The EFD task, like the ERC task, allows for the dissociation of effort-based processes and hedonic/motoric confounds on effort-related decision-making. However, the EFD task is advantageous as it can also control for the effects of delays conferred by escalating response requirements (Floresco et al. 2008). In the absence of any delay attached to the small reward, rats displayed the expected shift in behaviour from the large rewarded (LR, 3 pellets) response, to the small reward (SR, 1 pellet), as the number of responses needed for the LR increased (figures 3.5A,B).

Subsequently, a delay was added to the SR in each bin that matched the time taken to obtain the LR. Initially, there was a small shift from the LR to the SR as the response requirement increased from one to six. However, as the requirement increased, an increase in response omissions, rather than an increase in the number of SR choices was observed (figures 3.5D-F). This effect was exacerbated by administration of raclopride, which further increased the effects of response requirement on omissions. In previous versions of EFD, administration of dopamine receptor antagonists increases the rate of effort discounting and facilitates the shift from LR to SR choices (Floresco et al. 2008). However, we presently observed an increase in the shift from LR choices to omissions with no effect on the rate of SR choices.

A likely explanation for this result is the duration of the SR delays imposed. These delays are determined from the time taken to obtain the LR in any given bin. The delay between making a response and receiving an appetitive reward represent a separate cost to the total number of responses made (Bailey et al., 2016). The delays imposed in the present study (FR6- 6.64s, FR12 - 14.80s, FR18 - 25.50s) were substantially longer than those reported in lever-based EFD paradigms (e.g. FR5 – 1.7s, FR10 – 2.8s, FR20 – 6.5s; (Floresco et al. 2008). As discussed previously (chapter 2), the response rates observed on touchscreen responding appear lower than those observed with levers. The delays imposed to the SR were calculated based on the time taken to complete the corresponding LR trial within the same bin. As responding is slower within a touchscreen system the costs associated with the delayed SR are higher than would be expected within an otherwise identical lever-based task. Therefore, rats do not have a low-cost option to shift behaviour towards, as, the costs associated with either the LR (effort costs) or SR options (delay costs) both increase across bins. This would explain the sharp increase in omissions observed, particularly in later bins. In the original lever-based version (Floresco et al., 2008), the shorter delays imposed on the SR mean in later trials rats are still presented with a relatively low-cost response option.

It should also be noted that dopamine receptor antagonists can increase the rate of delay- as well as effort-discounting (Wade et al. 2000; Floresco et al. 2008). Therefore, it is likely that the effect of raclopride was to reduce the rats' tolerance to perform either option, which resulted in the increase in omission rates. Therefore, it appears that EFD with equivalent delays, as currently tested, may require an alteration of the parameters used or an extended training schedule, for use within the touchscreen operant system. An alternative approach would be to utilise the condition without delays. Several studies do not include a condition using equivalent delays when using lever-based EFD tasks (Hosking et al. 2015; Robles & Johnson 2017). However, it may also be necessary to subsequently test any drug effects on a delay-discounting procedure to control for any delay-based mediating effects, which may unnecessarily inflate the number of animals needed for an experiment.

3.4.3 Rearing effort discounting

A separate approach to the study of effort related processes is to manipulate effort in the absence of altering the delay to reinforcement. In human and nonhuman primate studies this can be examined through increasing the force needed for a reward (Chong et al. 2015; Nougaret & Ravel 2015). In rodents, this has previously been achieved by imposing a physical barrier that must be scaled between the animal and a reward (Salamone et al. 1994; Pardo et al. 2012). This approach may also be advantageous as it reduces the cognitive demands placed on the decision making; as the degree of effort needed to obtain the reward is readily apparent, the animal is not required to learn at any given stage of a session how many responses are required for reward delivery.

The present study used a vertically ascending response stimulus to assess effort discounting in rats. Increasing the height of a stimulus associated with a large reward reduced the rats' preference for this reward and increased the responding for a lower smaller reward. In contrast to the delayed EFD task, increasing the effort needed for the large reward did not significantly increase the rate of omissions. Furthermore, the RED task is sensitive to manipulations that affect cost/benefit valuations. Increasing the benefits of performing the effortful actions made rats more resilient to the high effort costs. Conversely, increasing the costs associated with a goal decreased rats' preference for this action. This is in spite of the fact that all rats readily performed this action in the absence of a low effort alternative (see training).

However, there appeared to be a strong effect of repeated training on RED performance. Rats' preference for the LR in later bins, increased throughout the study. Within early stages of training, a strong discounting effect was observed (figures 3.7A,C); however, by the final stages, there was little discounting (figures 3.8B; 3.9A). This confound was especially apparent following the application of raclopride. In spite of an overall reduction in number of large reward choices and concomitant increase in small reward choices, raclopride did not appear to preferentially affect the high effort trials. The likely explanation is that due to repeated training effects, the stimulus heights employed were not sufficiently effortful. Following vehicle administration, the rats still selected the large reward option in over 80% of trials in the final bin (with a stimulus height of 17.5cm). Dopamine receptor antagonists, at moderate doses, produce little effect on low-effort operant responding (Salamone et al. 1997). Increasing the height of the large reward stimuli in the later bins may be necessary to demonstrate an interaction between dopamine receptor antagonism and effort. The practice effect on performance may limit the utility of the task for long-term studies of effort-based processes, or those examining chronic treatment effects. It may be possible to reduce the ceiling effects observed by decreasing the differences in the reward magnitudes between the low effort and high effort options (e.g. 1 vs 2 pellets) or by altering some other task parameter.

3.4.4 Conclusions

The present chapter describes the adaptation of two existing and one novel effort-based decision-making tasks for use in the rat touchscreen system. Each task had limitations. For example, rats performing on EFD with equivalent delays showed high rates of omission. This would make it difficult dissociate motivational effects from hedonic/motoric changes. When tested on the RED task rats demonstrated a strong practice effect, which limits its suitability to for repeated/longitudinal testing. ERC, in spite of low levels of baseline may represent the best task for assaying effort-related decision-making behaviours using the touchscreen in rats. In spite of a low number of trials completed at FR5, it is still possible to reduce this number by increasing the ratio requirement, suggesting the absence of any significant floor effects. Currently therefore, the ERC assay may be best suited to studying the effects of effort-related decision-making in the rat touchscreen system.

Chapter 4. Oxygen responses within the nucleus accumbens are associated with individual differences in effort exertion in rats

4.1 Introduction

Recently, the RDoC initiative (Insel et al. 2010; Cuthbert & Insel 2013) has put forward the idea that transdiagnostic biological domains such as motivation may afford a greater opportunity for therapeutic modulation than do existing psychiatric symptom clusters. Thus, an imperative is to gain a more foundational understanding of the neural substrates underlying motivated behaviours in healthy individuals, patient populations and animal models, using methodology that optimises translation between species. As previously discussed, activational components of motivation are disrupted across a number of disorders (Chapter 1). Within the RDoC matrix, approach motivation has been highlighted as a cluster of symptoms that are relevant to a number of neuropsychiatric disorders. One subconstruct of approach motivation is the "effort valuation/willingness to work", which could be assessed in rodents using a PR schedule of reinforcement. Through the use of PR, the neural substrates of effort-based behaviour have been widely explored (Bailey, Simpson, et al. 2016). Dopaminergic and excitotoxic lesions of the nucleus accumbens (NAc) core can profoundly alter PR performance (Aberman et al. 1998; Bowman & Brown 1998; Hamill et al. 1999), and has been linked with a apathy in a number of disorders (reviewed in chapter 1).

Concomitantly to measuring direct behavioural changes in effort motivation, neuroimaging and electrophysiological techniques can be used to assess its underlying neurophysiological correlates. Such an approach can help establish the so called 'neuro-cognitive' validity of tasks cross-species. That is that assays probe the same cognitive processes and are supported by equivalent neural structures (Bussey et al. 2012; Hvoslef-Eide et al. 2016). There are a number of functional imaging measures that can be used in rodent research to accurately measure direct neural (e.g. electrophysiology) or neurochemical (e.g. micro-dialysis/voltammetry) parameters during behaviour. However, in humans, the vast majority of functional imaging research is performed using the blood oxygen level dependant (BOLD) contrast measured by functional magnetic resonance imaging (fMRI, Ogawa et al. 1990). BOLD-fMRI is only a surrogate measure of neural activity, relying on a process of neurovascular coupling to infer neural processes from changes in oxygenated blood that are correlated with changes in neural activity (Logothetis et al. 2001; Logothetis & Wandell 2004). However, fMRI is a non-invasive

measure with high spatial resolution. Coupled with the wide availability of facilities, this makes fMRI a valuable tool in exploring the neural correlates of behaviour. BOLD-fMRI can be performed in rodents; however it typically requires rodents to be restrained and/or anesthetised (Li et al. 2016). Constant potential *in vivo* oxygen (O_2) amperometry can also be used to assess changes in regional brain O_2 levels in response to changes in neural activity (Lowry et al. 2010). Crucially, *in vivo* O_2 amperometry allows for recording of neurophysiological changes in animals performing complex behavioural tasks (e.g. McHugh et al. 2011; 2014; Li et al. 2015). Furthermore, unlike other preclinical imaging techniques, O_2 amperometry can provide an adequate (in terms of both validity and viability) proxy measure of the BOLD response in awake rodents (Lowry et al. 2010; Howe et al. 2013; Francois et al. 2016).

A series of studies have been performed to validate O_2 amperometry as a surrogate measure of neuronal activity. These studies broadly belong to two categories. Firstly, several experiments have demonstrated a tight correlation with electrochemically measured oxygen and direct electrophysiological recording (Li et al. 2011) and proxy hemodynamic measures of neuronal function including regional cerebral blood flow (Lowry et al. 1997) and fMRI-BOLD (Lowry et al. 2010). The second class of studies have focused on demonstrating the ability to detect well-established neural correlates of behaviour, such as reward prediction errors (Francois et al. 2012) across species (Francois et al. 2016). Taken together, this suggests that O_2 amperometry can be used to compare the neurophysiological correlates of behaviour to be across species

As reviewed above, the NAc has been widely implicated in effort-based behaviour. The orbitofrontal cortex (OFC) may also be a particularly relevant regional substrate that is not only implicated in a number of neuropsychiatric disorders associated with apathy (Menzies et al. 2008; Kanahara et al. 2013) but also mediates aspects of instrumental behaviour required for PR schedule performance (Cetin et al. 2004; Gourley et al. 2010; Münster & Hauber 2017). However, the effects of lesions to the mOFC and NAc produce different effects on effort-exertion (Bailey et al., 2016). The inclusion of a secondary region of interest, would also allow to the demonstration that any effects detected are at least partially regionally-specific, and not due to a confound caused by some global change in O_2 across the brain.

The aim of the present study was to assess the relative contribution of the NAc and OFC regions to rodent performance under a PR schedule of reinforcement, using O_2 amperometry. The

influence of effort requirements on NAc and OFC O_2 responses were assessed, as well as the association between individual differences in motivated behaviour and O_2 responses. Finally, O_2 responses to rewards delivered independently of behaviour were examined. It is hypothesised that O_2 responses, as a measure of neural activity would be greater in later, higher effort trials. Moreover, given the established role of the NAc in effort based-behaviour it is hypothesised that this association between trial effort and O_2 responses would be found in the NAc, but not the OFC.

4.2 Materials and Methods

4.2.1 Animals

Male Wistar rats (Charles River, UK) were the subjects used in this study. A total of twenty-one rats underwent behavioural testing. Two were subsequently excluded due to poor signal quality resulting in all trials containing multiple artefacts (see *4.2.4* below). Four rats were excluded due to all electrodes being either mis-located NAc electrodes or having unidentifiable co-ordinates (see *4.3.1*). Resulting in fifteen rats being included in the main (NAc) analysis. A further five rats were excluded from the OFC analysis due to incorrectly located or unidentifiable OFC electrodes. All animals were group housed (3-4 per cage) throughout the study, in a temperature (20-22°c) and light controlled (lights on 0700-1900) environment. All testing took place during the light phase. Animals were given at least seven days of acclimation in the facility prior to surgical implantation of oxygen recording electrodes. Following a two-week surgical recovery period, animals were placed on a schedule of controlled feeding and maintained at no less than 85% of their free feeding body weight measured post-surgery. No correction was applied to this 85% control weight to match the animals expected growth curve (weight at training: 585g ± 10.38; weight at testing: 612g ± 16.45). Water was freely available throughout the study. All experimental procedures were conducted at Eli Lilly and Company Limited in accordance with the Animals (Scientific Procedures) Act 1986 and following approval from the local Animal Welfare and Ethical Review Board (AWERB).

4.2.2 Carbon paste electrode construction and in vitro calibration

Carbon paste electrodes (CPEs) were constructed and calibrated *in vitro* as previously described (McHugh et al. 2011; Francois et al. 2012; 2014) from 8T (200 µm bare diameter; 270 µm coated diameter) Teflon®-coated silver wire (Advent Research Materials, Suffolk, UK). Probes had a surface area of 0.05mm^2. The Teflon insulation was slid along the wire to

create a ~ 2mm deep cavity, which was filled with carbon paste. Carbon paste was prepared by mixing 7.1 g of carbon graphite powder and 2.5 ml of silicone oil (both Sigma-Aldrich, O'Neill et al. 1982). All electrodes were soldered to gold connectors. Reference and auxiliary electrodes were also prepared from 8T Teflon®-coated silver wire by removing the Teflon® tip.

In vitro calibration took place within a three-electrode glass electrochemical cell (C3 cell stand, BASi), with an Ag/AgCl reference electrode and a BASi platinum auxiliary electrode. Calibrations were performed in a 15-ml phosphate buffered saline (PBS) solution with a pH of 7.4, saturated with gaseous nitrogen (N_2), atmospheric air (from a RENA air pump), or pure O_2. This provided a 3-point calibration of known concentrations of 0 μM (N_2 saturated), 240 μM (air saturated), and 1260 μM (O_2 saturated) oxygen. CPEs were chosen for implantation if their calibration curves were linear and the measured O_2 values from the saturated solutions were not greatly different from those expected (least square linear regression, $R^2 \geq 0.98$).

4.2.3 Surgical implantation and in vivo validation of carbon paste electrodes

Rats were anaesthetised with 4% isoflurane (1 L/min O_2) and then maintained on 2% isoflurane (1L/min O_2) throughout the surgical procedure. CPEs were implanted into the following regions: bilaterally into the NAc [from bregma: anteroposterior (AP), +1.4mm, mediolateral (ML), ±1.4 mm and from dura: dorsoventral (DV): -6.1 mm]; and unilaterally into the medial orbitofrontal (mOFC, from bregma : AP, +4.4mm; ML, +0.6mm and from dura: DV, -4.0mm) and the lateral orbitofrontal cortices (lOFC, from bregma: AP, +3.8mm; ML, +2.6mm and from dura: DV, -4.4mm). The reference electrode was implanted, posterior to bregma, into the left posterior cortex (from dura: DV, -1mm). The auxiliary electrode was secured to a screw positioned, posterior to bregma, above the right posterior cortex. All electrodes were secured with dental cement and the gold connectors inserted into a six-pin socket (Plastics One), which in turn was cemented into place. Animals received analgesics pre- and post-surgery (carprofen, 5 mg/kg, subcutaneous; Pfizer) as well as antibiotic (Convenia, 5mg/kg, subcutaneous; Pfizer) administration post-surgery to aid recovery. Following surgery, animals were placed in thermostatically controlled cages and allowed to regain consciousness.

In vivo electrode validation of the electrodes took place following a two-week post-surgery recovery period by inducing mild hyperoxia and hypoxia through administration of gaseous O_2 (BOC medical) and nitrogen (BOC gases) respectively. Gases were administered to the animal

via a polyurethane tube held ~2cm from rats' snouts for 30s. Three O_2 and three nitrogen challenges were administered (in alternating order). Validation was considered successful if a positive signal was observed after all O_2 challenges, but none of the nitrogen challenges.

4.2.4 Constant potential in vivo oxygen amperometry recording technique

Constant potential amperometry (CPA) was used to measure local event-related *in vivo* changes in tissue O_2, as previously described in detail (Lowry et al. 1996; Francois et al. 2012; 2014). Briefly, a constant negative potential (-650 mV) was applied to CPEs to allow for the electrochemical reduction of dissolved O_2 to occur at the electrode tip. Changes in the measured current are directly proportional to changes in tissue O_2 (Hitchman 1978). During each of the recording sessions, rats were tethered to a four channel potentiostat (Biostat, ACM Instruments) via a six-pin socket and a flexible six-core cable (both Plastics One). A PowerLab 8/30 Data Acquisition System was used for analogue/digital conversion, and data were collected using Chart v.5 software (AD Instruments) at a sample rate of 200 Hz. Changes in current at each CPE were recorded and analysed separately. The negative potential was applied for at least 5 minutes prior to the start of any behavioural testing.

Following each behavioural session, raw recordings from each electrode were visually inspected. Although no distinct criteria were applied, any trials displaying apparent artefacts (by visual inspection) were excluded from all analyses. No further attempt was made to improve the signal quality of these artefact trials. Event related changes in current were analysed according to previous reports (Francois et al. 2012; 2014). Linear interpolation was used to replace occasional missing data points and a biquad Butterworth filter (high pass 0.1 Hz) was used for artefact suppression. Time 0s was taken as the time of reward delivery on completion of each ratio from which changes in O_2 current were measured. To compensate for different baseline between channels, data were normalised to the mean current in the 1s period preceding reward delivery. A boxcar-averaging algorithm was used to down sample the data, keeping a single average from multiple 0.5s non-overlapping windows. In the case of the NAc, the signals from the bilateral electrodes were averaged, to create a single NAc response for each animal. As there were no differences in reward O_2 responses between medial and lateral OFC responses, and to increase the power of the analyses, data from these electrodes were averaged into a single curve for each animal. The area under the curve (AUC) and the peak change in O_2 response were extracted from each subject's mean O_2 current.

4.2.5 Behavioural Apparatus

Behavioural testing took place within standard rat operant chambers (Med-Associates, Vermont, USA). Chambers were housed within sound and light attenuating boxes. Each chamber consisted of a house light and two retractable levers either side of food magazine. Standard food pellets (45mg, BioServ) were delivered to the magazine via an automated dispenser. Experimental sessions were governed by programmes written with Med-PC software.

4.2.6 Progressive Ratio Testing

All behavioural testing took place following surgical recovery, 5 days per week. Rats were randomly assigned to either a right or left active lever; this would be the only lever presented throughout the experiment. Training began with one day of magazine training. Over a period of 30 minutes, food pellets were delivered independently of any behaviour with a variable interval of 60s (range 15-105s). Following magazine training, animals began fixed ratio (FR1) training. Each session began with a 30s of pre-session blackout, after which the house light was turned on and the lever presented. A single lever press was required for a food pellet to be delivered, and the lever retracted. Following reward delivery, the lever remained retracted for a 15s intertrial interval (ITI). The session was terminated after either 45 minutes or following 50 completed trials. All animals were required to complete all 50 rewarded lever-presses within a session before moving onto the next stage of training. During the next stage of training, five lever presses were required for reward delivery (FR5). As before animals were required to complete 50 trials (250 lever-presses) before moving onto the final stage of training. All other parameters remained identical to FR1 training. Finally, animals were placed on a progressive ratio (PR) schedule of reinforcement. Session parameters were identical to the FR1 and FR5 stages, apart from the ITI, which was increased to 30s. The response requirement on each trial was determined by the following formula: $(5 * e^{(0.2*n)}-5)$; where *n* is the trial number, resulting in response requirements of: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40 etc. As with the touchscreen PR task (chapter 2), this task was not self-paced as reward collection initiated the subsequent trial. All animals were initially trained for five days without any O_2 recordings, to ensure a stable behavioural performance. Then rats underwent five days of habituation to the tethering and recording procedures to ensure no adverse effects of the tethering procedure. During these sessions, PR and CPA recordings were performed as normal, however the data were not analysed. Following this period, animals received ten sessions of PR (1 session per day), from which O_2 responses were analysed. The primary behavioural measure of interest

was breakpoint, defined as the number of lever presses completed in the last successfully completed trial. Additional measures of behaviour included the rate of lever presses and the delay-to-reward (the mean latency from trial start to completion).

4.2.7 Whole Session O_2 *amperometry analysis*

As slow drifts in baseline over the course of a session may confound event-related analyses, global changes in O_2 levels following session start were assessed. Following the first lever presentation, the change in current was divided into 120s non-overlapping bins. As animals are typically only actively engaging in the task for a portion of the 45-minute PR session (i.e. the period prior to breakpoint being reached), the change in global O_2 levels was examined for the rats' mean active period. The mean active period across all session (latency to last completed ratio) for the rats included in the NAc analysis was 1041 ± 127.97s. The mean active period for the rats included in the OFC analysis was 1207 ± 170.33s.

4.2.8 Progressive Ratio O_2 *amperometry Analysis*

During the PR task, changes in the recorded current was assessed for a period of 30s following reward delivery (at the successful completion of every ratio). O_2 responses were averaged, within regions, and across PR sessions, to create a single reward response per region per subject. The influence of effort requirements on O_2 responses was assessed by dividing each PR session in half based on the number of trials completed. The first half of trials were deemed 'low effort' and the latter half deemed 'high effort' trials, regardless of the total number of trials completed (e.g. Wanat et al. 2010; Covey et al. 2016). O_2 reward responses following low and high effort trials were averaged across the ten sessions to give a single low and single high effort O_2 response per region per subject. Individual differences in motivated behaviour were obtained by dividing the animals into high and low performing rats based upon a median split of the mean breakpoint across the ten PR sessions. O_2 reward responses were then compared between these two groups of low and high performing rats. To account for differences in the number of trials completed between these two groups, a separate group comparison was conducted on the minimum number of trials completed by all subjects across all sessions. Behavioural stratification also took place based upon median splits the mean delay to reward and the mean response rate across the ten PR sessions.

4.2.9 Non-contingent reward delivery testing

It is possible that the vigorous, repetitive lever pressing that occurs during PR performance may confound the O_2 signal analysis. Therefore, following completion of PR testing, animals were exposed to a single session, where food pellets were delivered independently of any behaviour. Small (1 pellet) or large (3 pellets) rewards were delivered pseudo-randomly without any cue, according to a 120s variable interval schedule (range 90-150s).

4.2.10 Non-contingent reward O_2 amperometry analysis

For the NAc, O_2 were again examined for 30s following reward from a 1s pre-reward baseline. O_2 signals from OFC electrodes were analysed for 45s following reward, as O_2 levels remained elevated 30s post reward. O_2 signals in response to small and large rewards were analysed separately. Additionally, O_2 changes following small and large rewards were examined in the previously identified high and low performing groups. Finally, O_2 responses to single pellet rewards were compared in the first half and second half of trials. This was in order to examine, whether in the absence of any behaviour, there was any temporal-dependent change in the magnitude of OFC and NAc O_2 responses.

4.2.11 Histology

In order to confirm CPE placement, rats were euthanized and brains were rapidly removed and placed in 10% buffered paraformaldehyde solution prior to histological analysis (Covance, US). Brain sections (200 μm) were viewed microscopically to view location of electrode tips; any animal with improper electrode locations was excluded from the analysis.

4.2.12 Experimental design and Statistical analysis

Statistical analyses was based upon previous amperometry studies (Francois et al., 2012; 2014). In cases where O_2 data were excluded (due to excessive noise), the behavioural data were also excluded. Therefore, the stability in breakpoints across the ten PR sessions was assessed using a mixed model design, which allows for missing cases with a Sidak correction for multiple comparisons was applied during post hoc testing. All O_2 analyses were completed separately for the NAc and OFC regions. The temporal stability of the O_2 signal was assessed by averaging each subjects' global O_2 signal across sessions, to give a single global signal per subject. Repeated measures ANOVA was used to test for any significant within-session change in O_2 signals. When appropriate, a Greenhouse-Geisser correction was applied to correct for violations in sphericity. Examining the influences of work-requirement upon O_2 responses to reward was achieved by dividing each subjects' trials into early (low effort) and late (high

effort) trials based upon the number of trials each subject completed in a session. Each subject's O_2 reward responses were averaged across trial type and session to give a single O_2 reward responses per subject for both early and late trial types. The time course of these O_2 reward responses were analysed using a repeated measures ANOVA, with a Fishers correction applied to post hoc comparisons. The parameters of each subjects' O_2 response (AUC and peak value) for early and late trials were then compared via within-subject paired t-tests.

The effects of individual differences in PR performance and O_2 responses were tested by arbitrarily splitting animals into high and low performers based upon a median split of the mean breakpoints from across the ten PR sessions. The time courses were again analysed using a mixed model ANOVA, with a Fisher's post hoc test. The parameters of each subjects mean O_2 reward response from all trials was then compared by high and low responding groups via independent t-tests. The same between-subject approach was taken when assessing subjects' O_2 responses based upon their mean response rate and mean delay-to reward for each session, where parameters of the O_2 reward response were compared between groups created by median splits of the mean delay-to-reward and response rate. Behavioural measures were compared between high and low responders and between early and late trials and analysed via repeated measures ANOVAs. In the case of the non-contingent reward delivery testing, O_2 reward responses to one and three-pellet rewards were compared within and between high and low performing groups. Repeated measures ANOVAs were used to test for significance, with a Sidak correction applied to any post hoc test. For all statistical tests a significance criterion of $p < .05$ was adopted. All statistics were conducted using SPSS version 23.0 (IBM Corp) and graphs produced using Prism (GraphPad, La Jolla, CA, USA).

4.3 **Results**

4.3.1 Histology

Figure 4.1 shows the locations of the 15 NAC (figure 4.1A) and 10 OFC (figure 4.1B) carbon paste electrodes (CPEs).

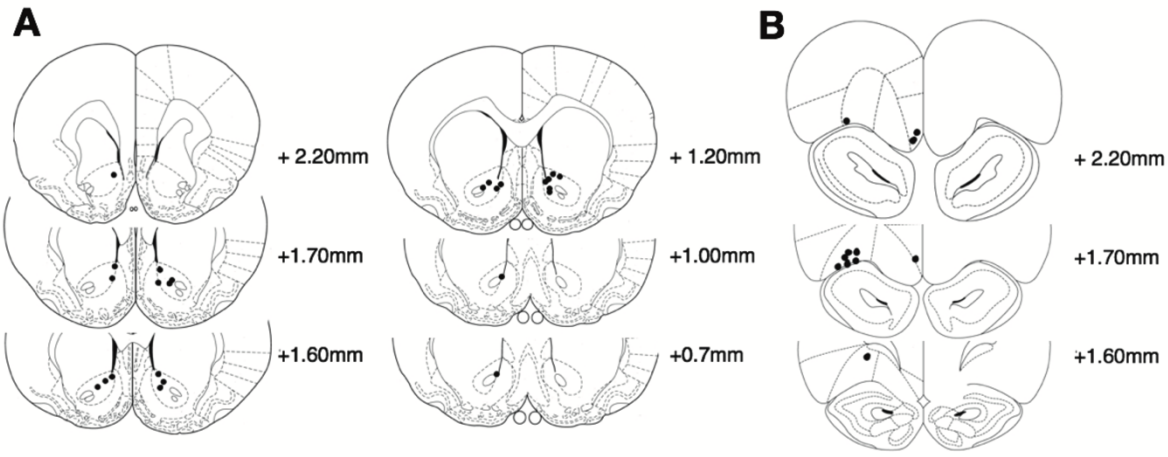


Figure 4.1: Reconstructions of CPE placements within: **A** the NAc and **B** the OFC. The location of CPE tips are marked by the black circles. Coronal slices are adapted from (Paxinos & Watson 2009)

4.3.2 Behavioural analysis

For analysis purposes, O_2 responses following reward delivery were collapsed across 10 PR sessions. Therefore, the stability in behavioural performance across these ten sessions was examined. As seen in figure 4.2A, there was a significant effect of session upon breakpoint ($F(1,9) = 2.248$, $p < .05$); however, post-hoc testing revealed no significant differences in breakpoint between sessions (all comparisons $p > .05$). Additionally, there was only a small degree of variance within-subjects; the mean range of trials completed (maximum trials completed within a session - minimum number completed) was 3.93 (SEM ± 0.45). Together, these analyses suggest that collapsing behaviour (and therefore O_2 responses) across the 10 PR sessions is a suitable approach.

To assess the relationships between effort and O_2 reward responses, O_2 signals were compared between early and late trials as well as low and high responding rats (figure 4.2B). Therefore, behavioural differences between these groups were examined. The mean number of completed lever presses between early and late trials were examined (figure 4.2C). There were significant

effects of trial type ($F(1,13) = 44.946$, $p < .001$; partial eta squared = .776) and group ($F(1,13) = 11.873$, $p < .01$; partial eta squared = .477). There was also a significant trial type x group interaction ($F(1,13) = 10.623$, $p < .01$; partial eta squared = .450). High responding animals made a significantly greater number of lever presses between early and late trials ($p < .01$), as did low responding rats ($p < .05$). High responding rats also made significantly more lever presses than low performing rats in both early ($p < 01$) and late trials ($p < 01$). Figure 4.2D shows the mean delay to reward in early and late trials for low and high responding groups. Delay to reward increased between early and late trials ($F(1,13) = 56.788$, $p < .001$; partial eta squared =.814), however there was no effect of response group ($F(1,13) = .065$, $p = .803$), nor any group x trial interaction ($F(1,13 = . 078$, $p = .780$). The mean rate of responding of high and low responding groups in early and late trials was also examined (figure 4.2E). The response rate decreased significantly between early and late trials ($F(1,13) = 217.578$, $p < .001$; partial eta squared =.944), however there was no effect of response group ($F(1,13) = 1.610$, $p = .227$), nor any group x trial interaction ($F(1,13 = 2.898$, $p = 0.113$)).

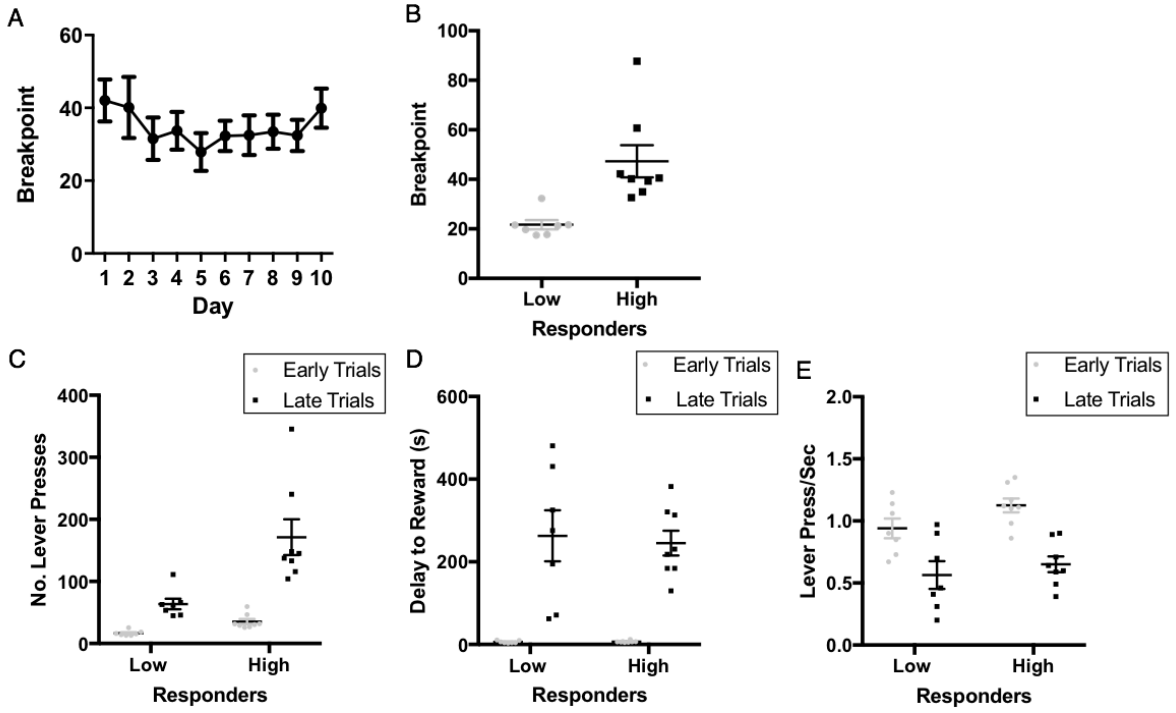


Figure 4.2: Overview of behavioural performance **(A-B)** and stratification of behavioural measures by trial type and response groups **(C-E)**. **A** The group level mean breakpoint across the ten PR sessions. **B** Division of high and low responders based upon a split of subjects' mean breakpoint. **C** The mean number of lever presses made were greater in late trials, and in high responding rats. **D** The mean delay to reward was significantly greater in late trials, but did not differ between groups. **E** The mean rate of lever pressing was reduced in late trials, but did not differ between groups. Error bars represent the SEM.

4.3.3 Within session O_2 signal stability

NAc O_2 recordings from a total of 143 PR sessions (15 rats, 5-10 sessions per subject) were analysed. There was no significant change in the NAc O_2 current during the mean period of activity ($F(1.2,16.9) = 2.033$, $p = 0.171$). For the OFC, O_2 recordings from a total of 83 sessions (10 rats, 5-10 session per subject) were analysed. There was no significant change in current over the mean active period ($F(1.4,12.7) = 1.465$, $p = .260$). This highlights the stability of the basal signal over time and suggesting that any event-related observations were unlikely to be confounded by slow drifts in baseline signal.

4.3.4 Early versus late trial stratification

NAc O_2 responses appeared greater in the late, higher effort trials (figure 4.3A). O_2 responses were significantly affected by both time post reward ($F(1,14) = 5.463$, $p < .001$; partial eta squared = .281) and trial type ($F(1,59) = 34.917$, $p < .001$; partial eta squared = .714). There was also a significant interaction between time and trial type ($F(59,826) = 39.408$, $p < .001$; partial eta squared = .738). In early trials, there was an initial increase in O_2 levels, the measured current was significantly greater than baseline for the first 4s post reward (all comparisons $p < .05$, figure 4.3A). There was subsequently a significant decrease in O_2 levels, which were significantly lower than baseline from 18s to the end of the 30s trial (all comparisons $p < .05$, figure 4.3A). In late trials, there was a significant positive response that was sustained for the duration of the 30s; all-time points were significantly greater than baseline (all comparisons $p < .05$). The magnitude of the O_2 signals was significantly greater in late trials compared to early trials from 6s post reward for the remainder of the 30s duration (all $p < .05$). For each subject, the peak value and the AUC of the mean NAc O_2 response was extracted. The peak O_2 value during late trials had a significantly greater peak response value ($t(14) = 4.870$, $p < .01$; figure 4.3B) and AUC ($t(14) = 5.863$, $p < .001$; figure 4.3C), relative to early trials. This suggests that completion of higher effort trials was associated with greater O_2 responses to reward.

As with the NAc, O_2 responses to rewards within the OFC appeared greater in later trials (figure 4.3D). O_2 levels were significantly affected by time post reward ($F(59,331) = 22.750$, $p < .001$; partial eta squared = .717). However, there was no effect of trial type (i.e. early vs late; $F(1,9) = 2.923$, $p = .12$). There was also no significant interaction between trial type and time post reward upon the change in OFC O_2 levels ($F(59,531) = 1.152$, $p = .213$). The peak

OFC O_2 response was significantly elevated in late trials ($t(9) = 3.636$, $p < .01$; figure 4.3E); although the difference in the AUC was not significant ($t(9) = 1.760$, $p = .112$, figure 4.3F).

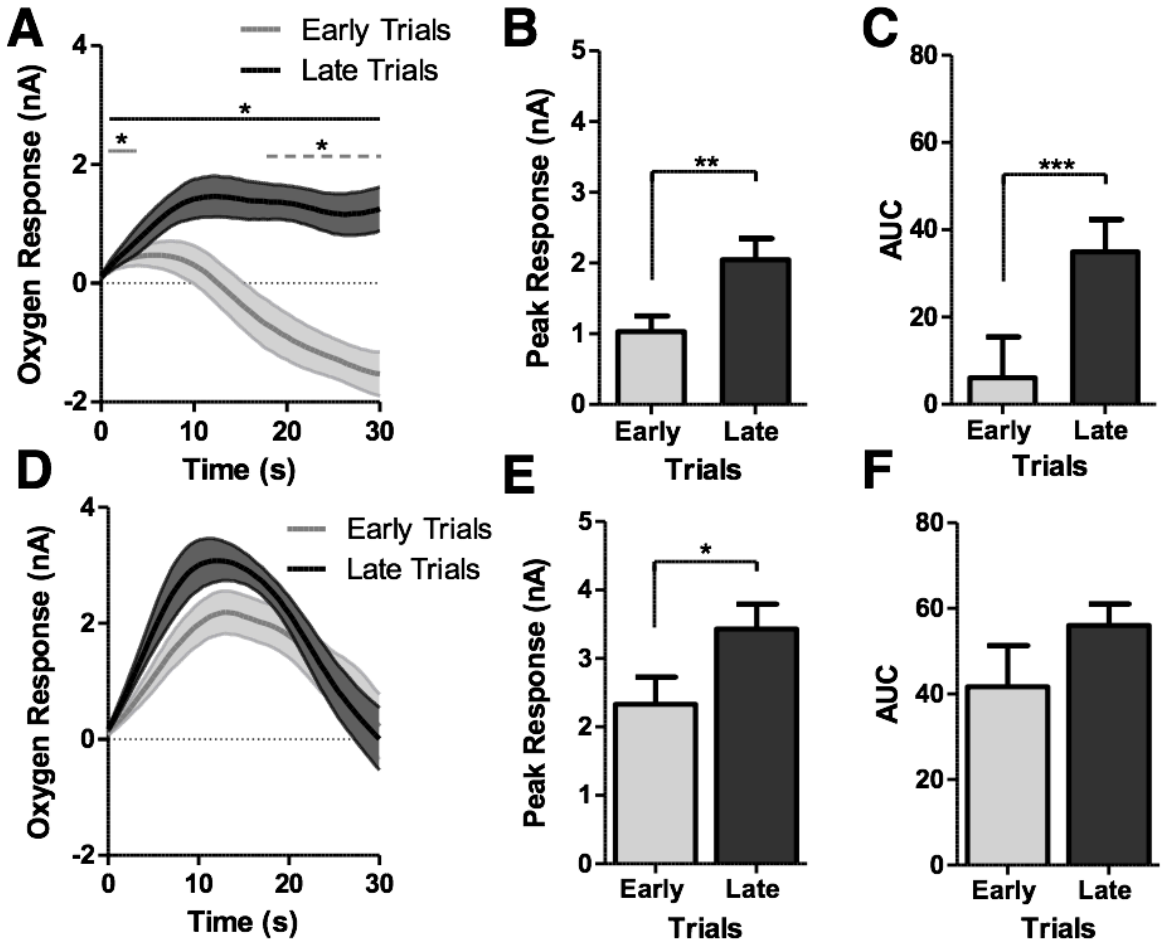


Figure 4.3: Tissue O_2 responses within the NAc (**A-C)** and OFC (**D-F**) during PR performance. **A.** Mean O_2 reward responses within the NAc following the first half ("early") and second half ("late") of PR trials across all subjects. The solid grey line represents a significant increase in O_2 levels in early trials, compared to 0s. The dashed grey line shows all time points that are significantly lower than 0s, in early trials. The solid black line shows all time points within late trials with significantly higher O_2 levels compared to the 0s timepoint. **B** The peak NAc O_2 reward response was significantly greater in late relative to early trials**. C** The area under the curve (AUC) of the NAc response was significantly elevated in late trials relative to the early PR trials. **D** The mean OFC O_2 reward response following the early and late PR trials across all subjects. **E** The peak value of the OFC response was significantly greater in late relative to early trials **F** The AUC of the OFC reward response did not differ between early and late trials. * $p < .05$; ** $p < .01$; *** $p < .001$. Error bars represent the SEM.

4.3.5 Low versus high responder stratification

To examine the relationships between the magnitude of O_2 responses and behavioural performance, correlations were conducted between the mean breakpoint and NAc O_2 signal parameters for each rat. Significant positive correlations were observed between mean breakpoint and the peak O_2 value (r = .606, p < .05; figure 4.4A) and between breakpoint and the AUC (r = .672, p <.01; figure 4.4B) of the NAc O_2 response, suggesting an association between individual differences in behaviour and NAc O_2 responses. This relationship was further examined by grouping subjects according to average breakpoint (n=7 low responders, n=8 high responders; figure 4.2A). There was no significant difference in the mean bodyweight of these groups (t(14) = 1.669, p = .119; high responders = 583 ± 29.89g; low responders = 640 ± 12.82g). The high responding group displayed significantly greater O_2 responses relative to the low performing group (figure 4.4C). The recorded current was significantly affected as a function of time (F(59,767) = 6.112, p < .001; partial eta squared = .320) as well as response group (F(1,13) = 18.396, p < .001; partial eta squared = .586). There was also a significant interaction between time and response group (F(59,767) = 2.908, p < .001; partial eta squared = .183). In low responding rats, there was a significant decrease in O_2 levels compared to baseline from 24s post reward to the end of the 30s (p < .05). In high responding rats, there was an initial positive O_2 response, before returning to baseline. The mean time course for high responders displayed a significant positive change in the measured current from 0.5s post reward until 18.5s post reward (all p < .05, figure 4.4C). Between subjects, high responding rats displayed a significantly greater O_2 signals, compared to low responders from 3.5s post reward for the remainder of the 30s analysed (all p < .05). Other O_2 signal parameters were also significantly different between groups. Both the peak O_2 response (t(13) = 4.288, p <. 05; figure 4.4D and the AUC (t(14) = 4.298, p < .01; figure 4.4E) were significantly greater in high performing rats.

However, this association may be confounded by differences in the number of trials completed between groups. Therefore, an additional between-subjects' analysis of the high and low response groups was limited to the first five trials of each session, the minimum number of trials completed by all animals across all sessions. The high response group still displayed a greater O_2 response, during these trials, compared to the low response group (figure 4.4F). The measured current in the first five trials was significantly affected by both time post reward (F(59, 767) = 5.562, p < .001; partial eta squared = .300) and response group (F(1,13) = 6.210, p <.05; partial eta squared = .323). There was also a significant interaction between time post reward and response group upon the measured current (F(59, 767) = 1.543, p <.01; partial eta

squared = .106). In low responding rats, no significant positive or negative change from baseline was observed. In high responding rats, there was an early significant increase in O_2 responses. The measured current was significantly greater than baseline for the first 15s analysed (all $p < .05$, figure 4.4F). No other time points differed significantly from baseline. High responding rats displayed significantly greater O_2 responses, compared to low responders from 5.5s to 16s post reward (all $p < .05$). Again, both peak O_2 response ($t(13) = 3.911$, $p < .01$; figure 4.4G) and AUC ($t(13) = 2.613$, $p < .05$, figure 4.4H) were significantly greater in high responding rats.

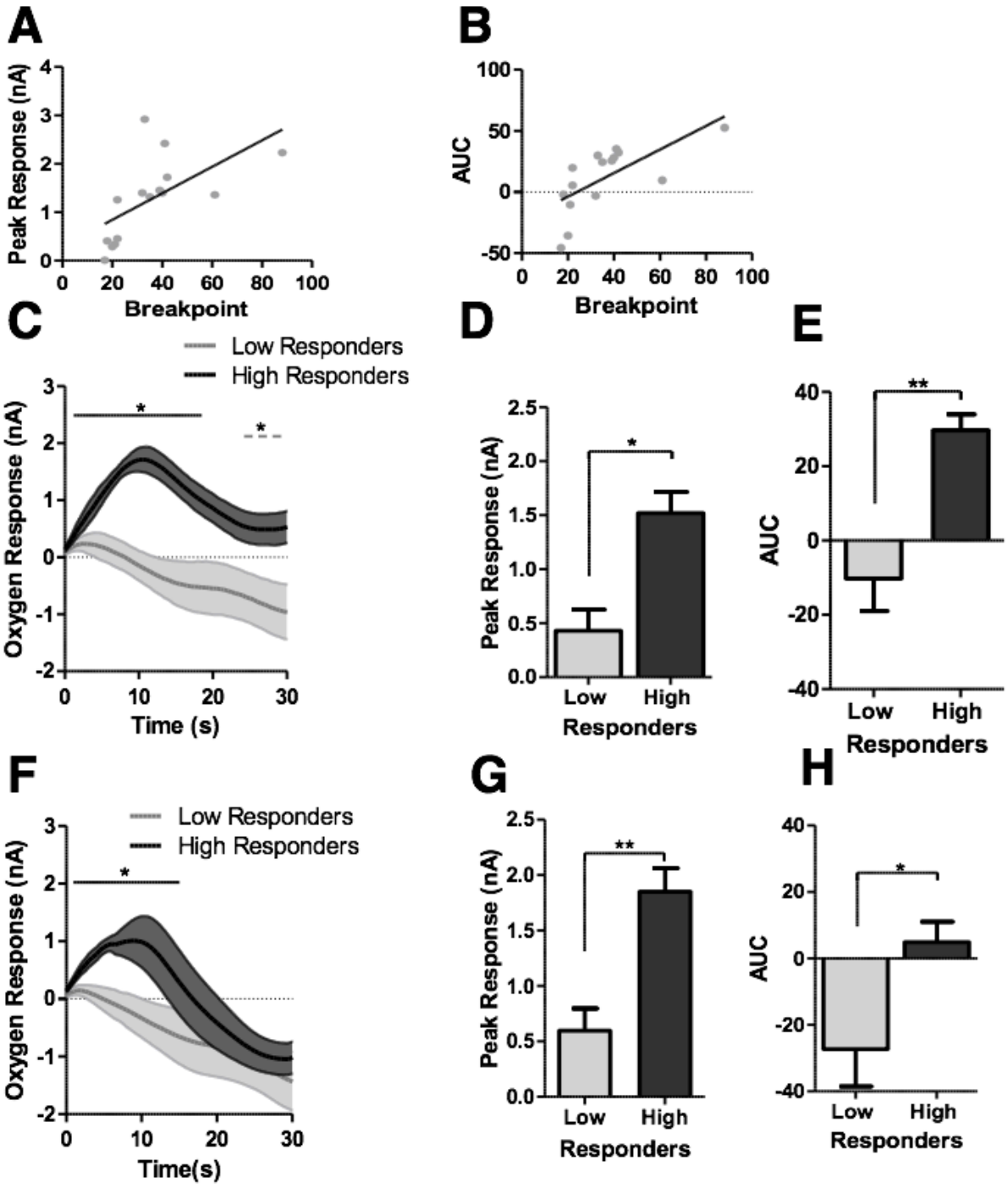


Figure 4.4: Associations between behavioural performance and NAc O_2 responses during PR performance. **A** The significant positive correlation between each rats' breakpoint and peak value of their respective O_2 response. **B** The significant positive correlation between breakpoint and the AUC of the NAc O_2 response. **C** The mean NAc O_2 reward responses in low and high performing rats. The dashed grey line shows all time points that had significantly lower O_2 levels relative to 0s, in low responding rats. The solid black line shows all time points with significantly higher O_2 levels compared to the 0s time point, within the high responding group. **D** The peak NAc O_2 response was significantly greater in high performing rats. **E** The AUC of the O_2 response was significantly greater in high performers **F** The mean O_2 response for high and low performing rats from the first five trials of each session. The solid black line shows all time points with significantly higher O_2 levels compared to the 0s time point, within the high responding group only. **G** High performing rats had a significantly higher peak NAc O_2 response in the first five trials. **H** The AUC of the O_2 response to reward in high performing rats was also significantly greater in the first five trials. * $p < .05$; error bars represent the SEM.

The time courses for the mean OFC responses following reward were also analysed. The measured current was significantly affected by time post-reward ($F(59,472) = 21.024$, $p < .001$; partial eta squared = .724), but not by response group ($F(1,8) = .143$, $p = .715$). There was, however, a significant interaction between response group and time ($F(29,472) = 1.469$, $p < .05$; partial eta squared = .281). There was no significant difference between groups at any time point (all comparisons $p > .05$). For the OFC analysis, subjects were again split into groups based on breakpoint. As shown in table 4.1, there were no significant differences in the OFC O_2 responses between high and low performing groups [AUC: ($t(8) = .813$, $p = .440$); peak response: ($t(8) = .475$, $p = .647$)], nor any correlation between the O_2 response and behavioural performance.

OFC	Low Responders	High Responders	Correlation with Breakpoint, *r (p-value)*
Peak Response	2.60 ± .40	2.92 ± .53	.431 (.214)
AUC	44.72 ± 10.05	53.00 ± 8.97	.435 (.209)

Table 4.1: The association between behavioural performance and parameters of the OFC O_2 reward response. There were no significant differences between low and high responding

groups (Values are means ± SEM) nor any significant correlation between breakpoint and either O_2 reward response parameter.

4.3.6 Delay and response stratification

Alongside increasing response requirements, the delay to reward concomitantly increases throughout a PR session (e.g. figure 4.2B), which may explain the differences in O_2 responses. To examine the relationship between delay and O_2 responses, rats were divided into groups, based on a median split of the mean delay to reward, creating longer (n = 8) and shorter delay (n = 7) groups. NAc O_2 responses did not significantly differ between these two groups (Peak response: $t(8.85) = .205$, $p = .842$; AUC: $t(9.77) = .305$, $p = .756$). Although this cannot exclude the influence of reward delay upon the magnitude of O_2 reward responses, it suggests the association between individual differences and O_2 reward responses was not fully mediated by differences in delay-to rewards. NAc O_2 signals were also analysed based upon the average rate of responding. There were no significant differences in NAc O_2 responses between rats with low (n = 7) and high (n = 8) response rates [peak O_2 response: $t(7.91) = .610$ $p = .559$; AUC: $t(13) = 1.578$, $p = .139$)].

The association between OFC O_2 responses and delay-to-reward and rates of responding were also examined. As with the NAc, there were also no significant group differences when rats were grouped by delay-to-reward [AUC:($t(8) = 1.532$, $p = .164$); peak response:($t(8) = 1.629$, $p = .142$)]; nor response rate [AUC:($t(8) = 1.282$, $p = .236$); peak response:($t(8) = 1.657$, $p = .136$)] . Table 4.2 summarises the lack of an association between the parameters of the O_2 response and the delay and response rate groups. Differences in the AUC and peak values of the O_2 responses between NAc and cortical regions are in line with previous reports (Francois et al., 2014).

NAc	*Short Delay*	*Long Delay*	*Slow Responders*	*Fast Responders*
Peak Response	1.22 ± .15	1.31 ± .41	1.12 ± .44	1.40 ± 18
AUC	13.40 ± 5.74	9.01 ± 12.47	-.01 ± 12.35	20.81 ± 6.16
OFC				

Peak Response	3.23 ± .41	2.21 ± .45	2.20 ± .44	3.24 ± .04
AUC	58.15 ± 9.31	37.42 ± 8.2	38.66 ± 8.80	56.91 ± 9.41

Table 4.2: The association between groups based upon median splits of delays-to-reward and rate of responding for both the parameters of the Nucleus accumbens (NAc) and orbitofrontal cortex (OFC) O_2 reward responses. There were no significant differences in the parameters of the O_2 reward response between these groups. Values are means ± SEM.

4.3.7 Non-contingent reward delivery

To control for nonspecific effects of arousal or that may have existed between response groups, O_2 responses to rewards delivered independently of behaviour were examined. Delivery of small (1 pellet) and large (3 pellets) rewards elicited strong positive NAc O_2 responses (figure 4.5A). O_2 responses to small and large rewards were examined based on the previously identified low and high PR response groups. There were significant main effects of both reward magnitude ($F(1,13) = 7.112$, $p < .05$; partial eta squared = .354) and group ($F(1,13) = 5.344$, $p < .05$; partial eta squared = .291) for the peak O_2 response, but no significant interaction between the two ($F(1,13) = 2.116$, $p = .138$, Figure 4.5B). There were, however, no significant effects of reward magnitude ($F(1,13) = 3.463$, $p = .086$); group ($F(1,13) = 161$, $p = .165$) nor any interaction ($F(1,13) = 2.258$, $p = .157$), for the AUC. Trials were then divided into first half and later half of the trials. NAc O_2 responses to single pellet rewards did not appear to change between early and late trials (figure 4.5C). Neither the peak NAc response ($t(14) = .570$, $p = .578$) nor the AUC ($t(14) = 1.605$, $p = .131$) significantly differed between early and late trials. Suggested that the previous differences between early and late trials were not as a consequence of the passage of time or the accumulation of rewards.

OFC O_2 responses to small and large reward delivery were also analysed as described above. Delivery of small and large rewards resulted in a O_2 response, lasting longer than previously analysed 30s period post reward delivery. O_2 responses following delivery of non-contingent food rewards was therefore analysed over a longer period of time (45s) (figure 4.5D). The peak OFC response was not significantly affected by reward magnitude ($F(1,8) = 2.492$, $p = .153$), nor by response group ($F(1,8) = .168$, $p = .692$). There was also no significant magnitude x group interaction effect on the peak OFC response ($F(1,8) = 1.915$, $p = .204$). There was, however, a significant main effect of reward magnitude ($F(1,8) = 15.910$, $p < .01$; partial eta

squared = .665), on the AUC of the OFC O_2 response (Figure 4.5E). However, the AUC was not affected by response group ($F(1,8) = .18$, $p = .682$), nor any significant magnitude x group interaction ($F(1,8) = .006$, $p = 942$). OFC O_2 responses to small rewards in early and late trials were also examined (figure 4.5F). As with the NAc, there were no significant differences between trial types in the magnitude of the OFC O_2 responses [peak response: ($t(9) = 1.281$, $p = .232$); AUC:($t(9) = .412$, $p = .690$)]. Since there were no differences in the delay to reward between early and late trials in this condition, this highlights that the previous observed effects of trial type on OFC responses (figure 4.3D-F), may have been as a function of the delay to reward.

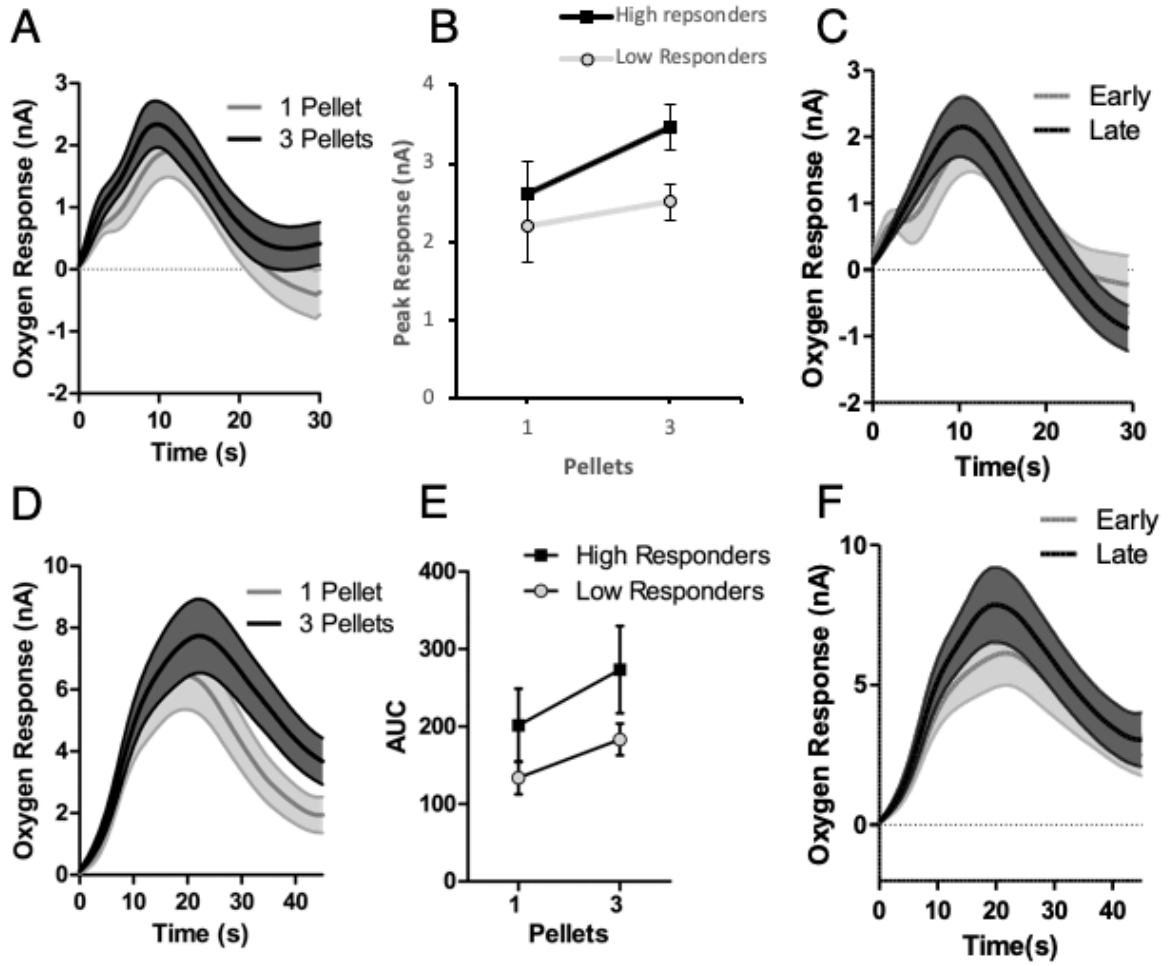


Figure 4.**5**: Tissue O_2 changes following uncued rewards within the NAc and OFC. **A** The mean O_2 responses to 1 ("small") and 3 pellet ("large") rewards within the NAC. **B** The peak NAc O_2 response of the responses to single and three pellet rewards for both low and high responding animals. **C** NAc O_2 responses to early and late single pellet reward trials. **D** The mean OFC O_2 responses to 1 and 3 pellet rewards. **E** The AUC of the OFC O_2 response to small

and large rewards for both low and high responding rats. **F** OFC O_2 responses to early and late single pellet reward trials. Error bars represent the SEM

4.4 **Discussion**

Using a highly translatable proxy measure of neural activity, as well as a translational behavioural assay of motivation, it was found that O_2 responses to reward within the NAc were predictive of individual differences in motivated behaviour. To our knowledge, no previous study has investigated the association between a functional imaging measure such as this and individual differences in effort-based behaviour. Furthermore, the assessment of changes in tissue O_2 allows for direct comparison of results with human BOLD-fMRI. Functional neuroimaging can be used to help establish equivalence of cognitive processes across species. Whereas there are several rodent imaging techniques that can directly measure electrophysiological and neurochemical correlates of behaviour, it is not typically possible to perform such techniques in humans. Given the widespread use of fMRI in humans, techniques such as O_2 amperometry, may better help bridge the translational divide, and facilitate basic research into clinical benefit.

In the present study, O_2 responses to rewards within both the NAc and OFC increased in magnitude as the effort exerted to obtain the rewards grew. These increases in O_2 responses were not observed in the absence of any work requirements, suggesting that neither accumulation of rewards nor the progression of time was sufficient for an increase in O_2 responses. Moreover, the magnitude of the O_2 responses within the NAc was associated with behavioural performance. Rats exerting greater amounts of effort displayed greater NAc O_2 responses to rewards. This association remained when controlling for the total number of trials completed, highlighting how subjects that show greater NAc activity in early, low effort trials, subsequently continue to overcome greater effort costs, and achieve higher breakpoints. Furthermore, these high performing rats also showed greater NAc O_2 responses to rewards delivered independently of any behaviour. This observation suggests that the previous results were not a confound resulting from differences in activity levels between groups. In contrast, O_2 responses within the OFC did not predict PR performance. Consequently, the pattern of activity observed within the NAc displayed at least some degree of regional specificity. Taken together, this suggests that PR performance is directly related to the neural responses to reward within the NAc.

4.4.1 Physiological basis of the measured O_2 signals

The physiological origin of the O_2 signal measured within the NAc has been previously discussed in detail (Lowry et al. 2010; Francois et al. 2012; 2014). The measured signal reflects changes in extracellular tissue concentrations of O_2 (Lowry et al. 1997). Increases in hemodynamic measures such as tissue O_2 concentrations or the BOLD contrast, occur in response to neuronal activation and/or changes in cerebral blood flow. This allows for the use of techniques such as CPA and BOLD-fMRI as proxy measures of neural activity. Tissue O_2 concentrations are highly correlated with induced changes in regional cerebral blood flow (Lowry et al. 1997), as is the BOLD signal measured with fMRI (Logothetis & Wandell 2004). Changes in cerebral blood flow appear primarily related to local synaptic activity (Mathiesen et al. 2000). Similarly, BOLD-fMRI is believed to reflect afferent inputs to an area rather than spiking outputs (Logothetis et al. 2001). Together, this raises the possibility that the present changes in O_2 levels, within the NAc, are driven by afferent inputs to this region. A major input to the NAc, are dopaminergic neurons projecting from the ventral tegmental area. This input pathway has been widely linked with, among other processes, effort exertion. Lesions to this pathway severely disrupt PR performance (Aberman et al. 1998; Sokolowski & Salamone 1998; Hamill et al. 1999). Furthermore, manipulation of this pathway either via optogenetic or pharmacogenetic tools can bidirectionally affect breakpoints (Fischbach-Weiss et al. 2018; Boekhoudt et al. 2018). As with the current O_2 results, the magnitude of phasic DA reward responses, measured with fast scan cyclic voltammetry (FSCV) are greater in later, higher effort PR trials (Wanat et al. 2010; Covey et al. 2016) whereas, DA responses to rewards following low-effort trials are negligible (Wanat et al. 2010). Together, this raises the possibility that the current O_2 results may reflect changes in DAergic neuron activity within the NAc, However, more appropriate methods (e.g. FSCV) should be used to confirm this.

In the present study, negative O_2 responses were observed on several occasions, as have been observed in previous studies (McHugh et al., 2014; Francois et al., 2014). O_2 changes in response to rewards were calculated as a relative change, compared to a 1s pre-reward baseline period. During the pre-reward period, it is likely that the animals would have been actively engaged in lever pressing. A degree of NAc activity, and therefore extracellular O_2, would be expected during this baseline period. The negative changes observed in the present study, may therefore represent a return to normal levels. In support of this, in the absence of any pre-reward effort, during the noncontingent reward paradigm, no negative O_2 responses were observed.

4.4.2 O_2 reward responses and individual differences in behaviour

It is noteworthy that high responders still show significant positive NAc O_2 changes in response to rewards in spite of the likely pre-reward neural activity. A greater neural response to reward may motivate future behaviour, enabling subjects to remain on task for longer and overcome greater effort requirements. Appetitive rewards, such as food, produce activational effects, that can increase the vigour and frequency of behaviour (Skjoldager et al. 1993) Likewise, enhanced NAc DA release has an activational effect on behaviour (Robbins & Everitt 1992; 2007). The increased NAc O_2 responses in high performing rats may, therefore, reflect a greater level of behavioural activation in response to food rewards.

In operant testing, effort is typically modulated by increasing the number of lever responses needed for reward. As a consequence, the delay from trial onset to reward delivery also increases. Reward-based DA responses have been shown to increase in response to escalating delays (Wanat et al., 2010). DA responses within the NAc have also been shown to signal reward prediction errors (RPE, Schultz et al., 1997). The increasing response requirements during PR may result in rewards becoming more unpredictable as a session progresses. The present findings could also be a reflection of greater RPEs in high effort trials. It is not clear, however, how either of these could account for the differences in NAc O_2 signals between low and high performing animals, since there was no difference in the mean delay to reward between these two groups. Although we sought to further examine the effects of longer delays as well as changes in RPEs, these should be fully investigated in future and separate studies. A control group with rewards yoked to delivery of rewards on a PR task (e.g. Wanat et al., 2010) would allow us to investigate the role of increasing delays on O_2 reward responses in the absence of any effort component. The contribution of NAc during expectancy of upcoming rewards has already been investigated using O_2 amperometry. (Francois et al., 2012, 2014). Interestingly, alterations in reward anticipation have previously been linked to motivational deficits in some clinical populations (Wolf et al., 2014; Barch et al., 2016), and therefore assessing changes in O_2 signals pre-reward delivery could also be of interest as a potential correlate of motivated behaviour.

Alongside regulating effort exertion, a number of studies have also investigated the role of NAc DA in Pavlovian-instrumental transfer (PIT). During PIT, cues associated with rewards are able to exert strong influences on behaviour, enhancing instrumental responding in their presence. Dopaminergic receptor blockade within the NAc disrupts PIT (Dickinson et al.,

2000), whereas intra-accumbens infusions of d-amphetamine enhances the Pavlovian influences on instrumental responding (Wyvell & Berridge, 2000). FSCV has been used to extend these findings, showing that a reward-paired cue that generates PIT is accompanied by a phasic DA response (Wassum et al., 2013). Moreover, the magnitude of the PIT effect was correlated with the magnitude of the phasic DA response (Wassum et al., 2013). When examining the effects of noncontingent reward delivery in NAc O_2 responses, it appears that only the high responding rats show an increased magnitude O_2 response following the three-pellet reward delivery (figure 4.5B). It is worth noting that high responders appeared to only show a greater magnitude reward response following three pellet rewards. Since, this probe was conducted after PR training, animals had only been exposed to single pellet rewards. The enhanced increase to these unexpectedly large rewards, may be a larger positive prediction error, which may reflect individual differences within the plasticity of the mesolimbic DA system. Furthermore, during this probe, rewards were delivered independently of any instrumental contingency, suggesting the O_2 responses may reflect Pavlovian influences upon reward. The present association between NAc O_2 responses and individual differences in PR performance may, therefore, be a reflection of individual differences in Pavlovian influences on behaviour or incentive motivation. In other words, the greater incentive motivation enabled the high performing rats to overcome greater effort costs to obtain more rewards under the PR schedule of reinforcement.

4.4.3 The role of the OFC in effort-related behaviour

The use of a control region is important to demonstrate that any results are not caused by some global change in tissue O_2 levels that may confound the results. This may be especially important when using a behavioural assay such as PR, which involves a large amount of vigorous, repetitive responding. In the present study, we used the OFC primarily as a control region. There was no association between behavioural performance and OFC O_2 reward responses, suggesting that the effects observed within the NAc display some degree of regional specificity. Previous reports have demonstrated that the medial and lateral regions of the OFC are functionally distinct (Noonan et al. 2012, for a review). Within the lateral OFC, excitotoxic lesions do not affect breakpoints in either rats (Kheramin et al. 2005) or mice (Gourley et al. 2010). In contrast, excitotoxic lesions to the medial OFC result in an increase in breakpoints in rats (Münster & Hauber 2017) and mice (Gourley et al. 2008), resembling the effects of excitotoxic lesions to the NAc (Bowman & Brown 1998). The medial OFC has strong projections to the NAc core (Hoover & Vertes 2011). In contrast, the lateral OFC projects

mainly to dorsolateral regions of the striatum (Schilman et al. 2008), an area that is not involved in supporting PR performance (Eagle et al. 1999). Together, these studies suggest the medial, but not the lateral, OFC regulates effortful instrumental responding. Presently, the majority of the CPEs were located within the lateral OFC (figure 5.1B), which may explain the lack of association between OFC O_2 recordings and behavioural performance.

OFC O_2 responses did, however, increase between early and late PR trials. It is unlikely that this effect represents RPE signals as OFC activity is not well correlated with RPEs (Hare et al. 2008). The lateral OFC has been widely implicated in modulating delay-based responding (Winstanley et al. 2006; Zeeb et al. 2010). Lesions to the lateral OFC reduce an animal's ability to tolerate delays for larger rewards (Mar et al. 2011) and levels of the DA metabolite DOPAC, increase within the lateral OFC, during a delay-discounting task (Winstanley et al. 2006). The increased O_2 reward responses in late trials may, therefore, have been a reflection of the greater delay-to-reward experienced in those trials. OFC O_2 signals were also modulated by reward outcome. Lateral regions of the OFC encode information regarding reward magnitude during reward receipt (van Duuren et al. 2007; 2008) suggesting, CPA can accurately capture OFC activity during processing of reward. The lack of a difference in the magnitude of OFC O_2 signals between the non-delayed early and late trials in the non-contingent reward delivery probe alongside the lack of an association with PR performance, further suggests that the differences in OFC O_2 signals between early and late trials is a reflection of the role of the OFC in delay-based behaviour.

4.4.4 Use in drug discovery

Drug discovery can be aided through the use of functional imaging to provide additional measures of compound efficacy beyond behavioural effects (Wise & Tracey 2006; Li et al. 2016). In the present study, through the use of a translatable imaging technique, it is possible to detect a neural correlate of PR performance. PR schedules as assays of motivation are useful for testing novel compounds due to ease of application and relatively high throughput. However, the unspecific nature of measures such as breakpoint may inflate the likelihood of false positives. If a compound that increased breakpoint also enhanced the NAc reward response, it would provide a stronger argument for a change in motivation than a behavioural change alone. Such approaches have been used in the cases of analgesic drug discovery (Duff et al. 2015; Wanigasekera et al. 2018). The examination of a drug effect upon neural activity could be used early in clinical trials (i.e. Phase II), to reduce the expensive Phase III failures.

However, it would be first necessary to test whether humans display the same association between NAc activity and effort-exertion, measured with fMRI. If this could be established, it would also strengthen the argument that the cognitive process of interest (i.e. effort expenditure) translates across species (Keeler & Robbins 2011)

4.4.5 Conclusions

Amperometric measurement of tissue O_2 changes, a highly translatable and valid proxy measure of BOLD-fMRI in behaving rodents, provided a novel insight into the role of the NAc function and individual differences in effort-related behaviour. The O_2 response to reward within the NAc is related to effort exerted under a PR schedule of reinforcement. This highlights the dynamic role neural signals within the NAc play in maintaining motivated behaviour. Furthermore, this study demonstrates *in vivo* O_2 amperometry can be used to probe the neural correlates of behaviour in rodents. Furthermore, through the use of such techniques, hypotheses can be derived that can subsequently be tested in humans, therefore facilitating cross-species research.

Within the RDoC framework (Cuthbert and Insel 2013) aberrant approach motivation has been identified as a transdiagnostic symptom of psychiatric disorders. One subconstruct of motivation within this framework is effort valuation/willingness to work, The use of PR schedules can be used, across species, to probe these subconstructs of motivated behaviour (Young & Markou 2015). The approach taken by the RDoC initiative emphasises the need to bypass diagnostic categories and first understand the neural substrates of behavioural constructs in both healthy and nonhealthy subjects. Therefore, the present study is in line with this approach. Through identifying a translatable imaging correlate of PR performance, future studies could use fMRI to determine whether there is an equivalent association between the BOLD response and PR performance in humans and whether this association is disrupted in clinical populations.

Part 2: Muscarinic antagonists as a novel target for symptoms of amotivation

Chapter 5. Effects of muscarinic receptor antagonism on effort-related behaviour

5.1 Introduction

Based upon a previous review of the literature, a number of compounds that enhance motivated behaviour in rodents also appear to have efficacy in reducing apathy in clinical samples (chapter 1). A number of these compounds, either directly or indirectly modulate dopaminergic activity. There is also a wealth of studies demonstrating that muscarinic acetylcholine receptor (mAChR) antagonists can facilitate dopaminergic activity. Based upon this, compounds that act as mAChR antagonists may facilitate motivated behaviour in rodents and could be a novel target for the treatment of apathy.

In rodents, motivated behaviour can be readily assessed by probing expenditure of effort for food rewards (Salamone & Correa 2012). However, few previous studies have investigated a role of mAChRs in effort-related behaviour. Systemic administration of the nonselective mAChR antagonist scopolamine has been reported to facilitate progressive ratio (PR) performance in an 'inverted-u' pattern (Stewart et al. 1974). However, this claim was based on visual inspection of behavioural data from six rats only, two of which did not show any increase in PR breakpoints. Moreover, a lack of control tests raises the possibility that this enhancement in breakpoint was driven by a confounding behavioural change. A separate study examined the role of mAChRs within the nucleus accumbens (NAc) on effort-related choice (ERC) behaviour (Nunes et al., 2013). Intra-NAc infusion of a nonselective mAChR agonist shifted behaviour from lever-pressing for a palatable reward to consumption of a less preferred, but freely available reward (Nunes et al., 2013). Co-administration of scopolamine subsequently reversed this motivational deficit. Together, these studies suggest that mAChR antagonists may facilitate effort-based behaviour. It should be noted that in spite of the evidence linking muscarinic antagonism to facilitation of motivated behaviour, these effects are contrary to what would be expected in Alzheimer's disease (AD). Apathy is one of the most prevalent psychological symptoms of AD (Landes et al. 2001). However, AD is also associated with a reduction in cholinergic tone (Coyle et al. 1983), with the most effective symptomatic treatments upregulating the cholinergic system (Seltzer et al. 2004). Additionally, muscarinic receptor agonists have been widely investigated as therapeutic options for AD (Langmead et

al. 2008). Together, this suggests that a separate approach to treating apathy may be needed in AD.

Another consideration is the potential role of mAChR subtypes. In line with the effects of M_1 and M_4 receptors upon dopaminergic function and behaviour reviewed earlier (chapter 1), it would be expected that antagonism of these receptors would facilitate motivated behaviour. Only one study has investigated the effect of preferential M_1 receptor antagonism on PR performance (Klinkenberg & Blokland 2011). Systemic administration of the preferential M_1 receptor antagonist biperiden did not affect breakpoint. However, PR sessions were limited to 1050 lever presses (Klinkenberg 2012). This could have created ceiling effects and may explain why biperiden did not increase breakpoints. No study has investigated a role of M_4 receptor ligands in effort-based behaviour.

The present chapter aimed to test the effects of mAChRs antagonists on motivated behaviour. Initially, the effects of systemic administration of several muscarinic receptor antagonists were tested on touchscreen PR performance in mice (Heath et al. 2015; Heath et al. 2016). Firstly, the effects of the non-specific mAChR antagonist scopolamine were examined. Then to investigate the role of the M_1 receptor subtype the centrally acting antagonist biperiden was tested. However, biperiden also produces some peripheral effects when administered systemically (Guthrie et al. 2000). Therefore, telenzepine, a M_1 antagonist that does not effectively cross the blood-brain barrier (Ichikawa et al. 2002; Pediani et al. 2016), was used to examine potential peripherally-mediated M_1 receptor effects on PR performance. Finally, the contribution of the M_4 receptor was tested through examining the effects of the preferential antagonist tropicamide.

Subsequently, the effects of the preferential M_1 receptor antagonist biperiden and the nonselective mAChR antagonist scopolamine were tested on a number of control tasks the examine the behavioural mechanisms underlying the changes in PR performance. To control for potential effects on satiety and motor output the drugs were tested prior to FR5 performance. Potential changes in appetite were examined by testing the effects of the compounds upon free milkshake consumption. To test whether the effects were sensitive to changes in outcome value, the compounds were tested following reinforcer devaluation through prefeeding prior to PR testing. Additionally, to control for any increases in perseverative-like responding, biperiden was tested under extinction conditions. Subsequently, both biperiden and scopolamine were tested upon a touchscreen ERC task to assess for any

changes in effort-related decision making (Heath et al. 2015). Finally, to examine the effects of biperiden upon dopaminergic function, biperiden and the indirect catecholamine agonist amphetamine were applied alone and in combination prior to PR performance.

5.2 **Materials and Methods**

5.2.1 Animals

Sixty-four male C57BL/6 mice (Charles River Laboratories, Margate, UK) were involved in this study and divided into four cohorts (n = 16 each, see table 5.1). Mice were 6-8 weeks old at the start of the study and group housed (4 per cage) in a temperature and light controlled facility (lights on 1900-0700). Following arrival at the facility, animals were given seven days to acclimatise with handling limited to routine husbandry. The mice were then placed on a schedule of controlled feeding and maintained at approximately 85% (and no less than 80%) of their free-feeding body weight. No correction was applied to this 85% control weight to match the growth curve (weigh at start of testing: 25.3g ± 0.4). Cages were changed once weekly and drinking water was available *ad libitum* throughout. All behavioural testing took place 5-7 days per week during the animals' dark phase. Two mice failed to complete the pretraining procedure and was removed from the study. Another mouse was culled due to ill-health part way through the study. All experiments were conducted in accordance with the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 and following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB).

5.2.2 Apparatus

All testing took place in standard mouse Bussey-Saksida touchscreen chambers (Campden Instruments Ltd, Loughborough, UK), The behavioural arenas were housed within light and sound attenuating chambers, which were fitted with a fan for ventilation and to reduce background noise. Each behavioural arena consisted of a stainless-steel floor and trapezoidal black plastic walls opening out to a 12.1-inch touch sensitive screen (resolution 800x x 600) at one end and a reward delivery magazine at the other. Entries into the magazine were monitored via an infrared (IR) beam. Magazines were also fitted with a light-emitting diode, that illuminated upon delivery of the liquid reward (Yazoo® strawberry milkshake; Friesland Campina UK, Horsham, UK) used in the study. IR beams were also fitted across the floor 6 cm in front of the touchscreen and 3cm from the magazine to monitor activity levels during testing. Black plastic masks consisting of five 4x4cm response apertures, spaced 1 cm apart were placed in front of each screen. During the current study, stimuli were only presented in the central aperture.

5.2.3 Behavioural training

Prior to behavioural testing, mice were exposed to the milkshake reinforcer in their home cages, to reduce any effects of neophobia. Subsequently, mice were habituated to the testing chambers. This consisted of a 20-minute exposure session, where the touchscreens were active, but no stimuli were displayed and there were no programmed consequences of behavioural output. 200µl of milkshake was placed in each magazine prior to the habituation session. Initial operant testing began with a single screen touch training session. During this session, a white square stimulus was presented in the central response aperture for 30s. Upon stimulus offset a reward tone (1000ms, 3kHz) was issued, the magazine was illuminated and 20µL of milkshake was delivered. Following reward delivery, entry into the magazine initiated a 5s inter trial interval (ITI) and extinguished the magazine light. Following the ITI the next trial began. If the mouse touched the stimulus during the 30s presentation period, the stimulus was immediately turned off and the tone, triple the usual reward (60µL of milkshake) was delivered and the ITI was immediately triggered. Each session was terminated following 30 trials

Animals then undertook fixed-ratio (FR) training. Each trial commenced with the presentation of the response stimulus in the central aperture. A single response to the stimulus resulted in stimulus offset, the reward tone and 20µL of milkshake being delivered to the magazine. Entry and subsequent exit from the magazine began the ITI, which was shortened to 4.5s, following which the next trial began immediately. Sessions were terminated following 30 rewards being delivered or 60 minutes elapsing. All animals were required to complete 30 trials within the 60-minute session before moving on to the next stage of training. The subsequent stage consisted of responding on a FR2 schedule. During this stage, two touches to the white square stimulus were needed for trial completion. Following the first response in each trial, the stimulus was briefly removed (500ms) and a short 'click' sound (10ms, 3kHz) was played. This brief offset and sound accompanied all subsequent stimulus responses. A session was again terminated following 30 trials (i.e. 60 stimulus responses) or 60 minutes elapsing. Once 30 trials were completed within a 60-minute session, animals moved onto FR3 training. During FR3 training, three stimulus responses were needed for reward delivery. All other session parameters remained the same as the FR2 training. FR3 training was followed by FR5 training, where five stimulus responses were required for reward delivery. Aside from changes in response requirements, all session parameters remained the same. Animals were required to complete 30 trials (150 stimulus responses) within 60 minutes for two consecutive FR5 sessions before beginning PR testing.

5.2.4 Progressive ratio (PR) Procedure

The parameters of the PR schedule (Heath et al., 2015) were identical to the FR schedule except that the response requirement for reward delivery increased by a linear +4 step following each trial, yielding response requirements of 1,5,9,13,17 etc. No trial limit was imposed. If no response was made to the touchscreen within 300s, sessions were terminated, otherwise sessions ended after 60 minutes. As with the rat touchscreen PR task (chapter 2), this task was not self-paced.

5.2.5 Uncapped Fixed Ratio Procedure

Uncapped Fixed Ratio-5 (FR5) testing was used to test for any changes in satiety/motor output changes. During FR5 testing, five responses were required for each reward delivery. No trial limit was imposed. Sessions were terminated following 60-minutes.

5.2.6 Food Consumption Procedure

The milkshake consumption test took place within the touchscreen chambers. Mice were given 60-minutes of free access to milkshake which was placed within a small bowl that was fixed to the floor of the chamber. The bowls were weighed before and after the session to determine milkshake consumption.

5.2.7 Prefeeding procedure

The prefeeding procedure involved giving mice 60-minute free access to either a bowl milkshake reinforcer (prefeed) or water (control), within the homecages, prior to PR testing. Subsequently, the bowls were removed and the drug administered (see *Drugs* section for details). Animals had no further access to the bowls prior to PR testing. All mice received both vehicle and drug following prefeeding with both water and milkshake (resulting in four experimental conditions per compound).

5.2.8 Extinction Procedure

In this paradigm, the white target screen stimulus was presented; however, responding did not yield reward delivery nor the presentation of reward associated cues such as the stimulus offset tone or the sound of the milkshake pump. However, the usual brief stimulus offset and 'click' tone accompanied stimulus responses. Sessions were terminated after 60-minutes or following 300s without any responses to the touchscreen.

5.2.9 Effort Related choice Procedure

During effort related choice testing (Heath et al. 2015) two pellets of standard lab chow (approximately 5g) were weighed and scattered on the floor of each touchscreen chamber. Animals were then tested on the FR5 schedule for 60-minutes. Following testing, the remaining chow (including spillage) was weighed to calculate consumption.

5.2.10 Behavioural Measures

During all PR tests, including the prefeeding tests and amphetamine tests, behavioural measures were calculated as described in the rat touchscreen PR4 and ERC programs (Chapters 2 and 3). During FR5 responding, response rates were calculated by fitting the parabolic function: $y = -b(x)^2+a$ to calculate the predicted peak response rate (a) and decay rate parameter (b, Phillips et al., 2017). Unlike the PR calculations it was not necessary to exclude the first trial from the response rate analysis. Otherwise all behavioural measures were taken as in the PR analysis. During extinction testing it was not possible to calculate any measure related to reward delivery (post reinforcement pause, reward collect latency). In the absence of discrete trials in the extinction procedure, the response rate analysis was calculated by taking the time to complete blocks of five responses.

5.2.11 Drugs

All compounds were dissolved in physiological saline and administered via intraperitoneal injections at a volume of 10ml/kg of body-weight, 30 minutes prior to testing. The following doses were tested: Scopolamine hydrobromide (Bio-techne, Abingdon, UK): 0.1 and 0.3mg/kg; biperiden hydrochloride (Sigma-Aldrich, Dorset, UK): 1 and 3mg/kg; telenzepine dihydrochloride (Bio-techne, Abingdon, UK): 3 and 10mg/kg; and tropicamide (Bio-techne, Abingdon, UK): 2.5, 10, 20mg/kg. Amphetamine (Sigma-Aldrich, Dorset, UK), 0.1, 1mg/kg. When applied in combination, amphetamine and biperiden were co-administered in the same syringe to reduce the number of injections needed. Drug routes, doses and vehicles were selected based upon previous behavioural effects in mice: scopolamine (Shannon et al. 1999; Podkowa et al. 2016; Witkin et al. 2014); biperiden (Witkin et al. 2014; Popelíková et al. 2018); telenzepine (Yano et al. 2009; Ztaou et al. 2016); tropicamide (Veeraragavan et al. 2011; Ztaou et al. 2016) and amphetamine (Heath et al. 2015).

5.2.12 Experimental design and statistical analysis

Table 5.1 outlines the experimental procedures undertaken by each cohort. During PR testing, all compounds were administered in a within-subject, Latin square design. Tropicamide was

also subsequently administered at a higher dose in a counterbalanced within-subject crossover design. At least one day of drug free baseline testing was given between drug administration days within the Latin square and cross-over experiments. A drug free washout period of at least five days was given between each Latin square experiment. Statistical analyses were conducted with SPSS version 23.0 (IBM Corp, Armonk, NY, US) and the R software package (R Core Team, 2013). Graphs were produced using Prism (GraphPad, La Jolla, CA, USA) and the ggplot2 package in R (Wickham, 2009). Repeated measures ANOVAs were used to analyse the results of all Latin square designs. When violations of sphericity were detected, the Greenhouse-Geisser correction was applied. Effect sizes were calculated as partial eta squared values. All reported post hoc testing was Bonferroni corrected to account for multiple comparisons. Cross-over designs were analysed with paired t-tests.

Cohort	**1**	**2**	**3**	**4**
Number of mice at testing	n = 16	n =16	n = 15	n = 15
Experimental procedures	Progressive ratio *Scopolamine* Prefeeding *Scopolamine* Effort Related Choice *Biperiden* *Scopolamine*	Progressive ratio *Biperiden* *Telenzepine* *Tropicamide*	Fixed Ratio-5 *Scopolamine* *Biperiden* Food Consumption *Scopolamine* *Biperiden* Prefeeding (n = 14) *Biperiden* Extinction (n = 14) *Biperiden*	Progressive ratio *Amphetamine x biperiden interaction*

Table 5.1: The experimental procedures and compounds given to each cohort of mice

5.3 Results

5.3.1 Nonselective antagonism of mAChRs *with scopolamine facilitates PR performance and increases nonspecific locomotor activity at higher doses*

To initially investigate the role of mAChRs in motivated behaviour, scopolamine was administered prior to PR performance. Figure 5.1A shows how systemic administration of scopolamine significantly increased breakpoint ($F(1.37,20.56) = 9.957$, $p < .001$; partial eta squared = .399). Scopolamine at doses of 0.1 mg/kg ($p < .05$) and 0.3 mg/kg ($p < .01$) increased breakpoint relative to vehicle. In contrast, scopolamine had no significant effect upon PRPs, figure 5.1B ($F(1.40,21.06) = 1.331$, $p = .279$). Scopolamine, as seen in figures 5.1C,D,

appeared to significantly increase activity levels. The rate of IR beam breaks was significantly increased following scopolamine administration ($F(2,30) = 5.562$, $p < .01$; partial eta squared = .284; Figure 5.1C). Scopolamine at 0.3mg/kg increased the rate of IR beam breaks relative to both other doses (both $p < .05$). Scopolamine administration also increased the rate of non-target screen touches ($F(2,30) = 10.12$, $p < .001$; partial eta squared = .403; Figure 5.1D). The rate of non-target responses was significantly elevated following 0.3mg/kg of scopolamine relative to vehicle and 0.1 mg/kg (both $p < .01$).

Analysis of response rates (Figure 5.1E) revealed that scopolamine did not significantly affect either the predicted peak response ($F(1.33,19.89) = 1.71$, $p = .209$) or the decay rate ($F(2,30) = 2.671$, $p = .086$; Figure 5.1F). Administration of scopolamine did also not significantly affect the mean length of response bouts ($F(2,30) = .229$, $p = .796$). There was however, a significant effect upon the duration of pausing between bouts ($F(2,30) = 4.775$, $p < .05$; partial eta squared = .241; Figure 1G). The mean pause duration was significantly shorter following 0.3mg/kg of scopolamine compared to the 0.1mg/kg condition ($p < .01$). Additional measures of activity are available in table 5.2. These data indicate that scopolamine can increase effort expenditure, but that it also appears to increase nonspecific locomotor activity levels at high doses.

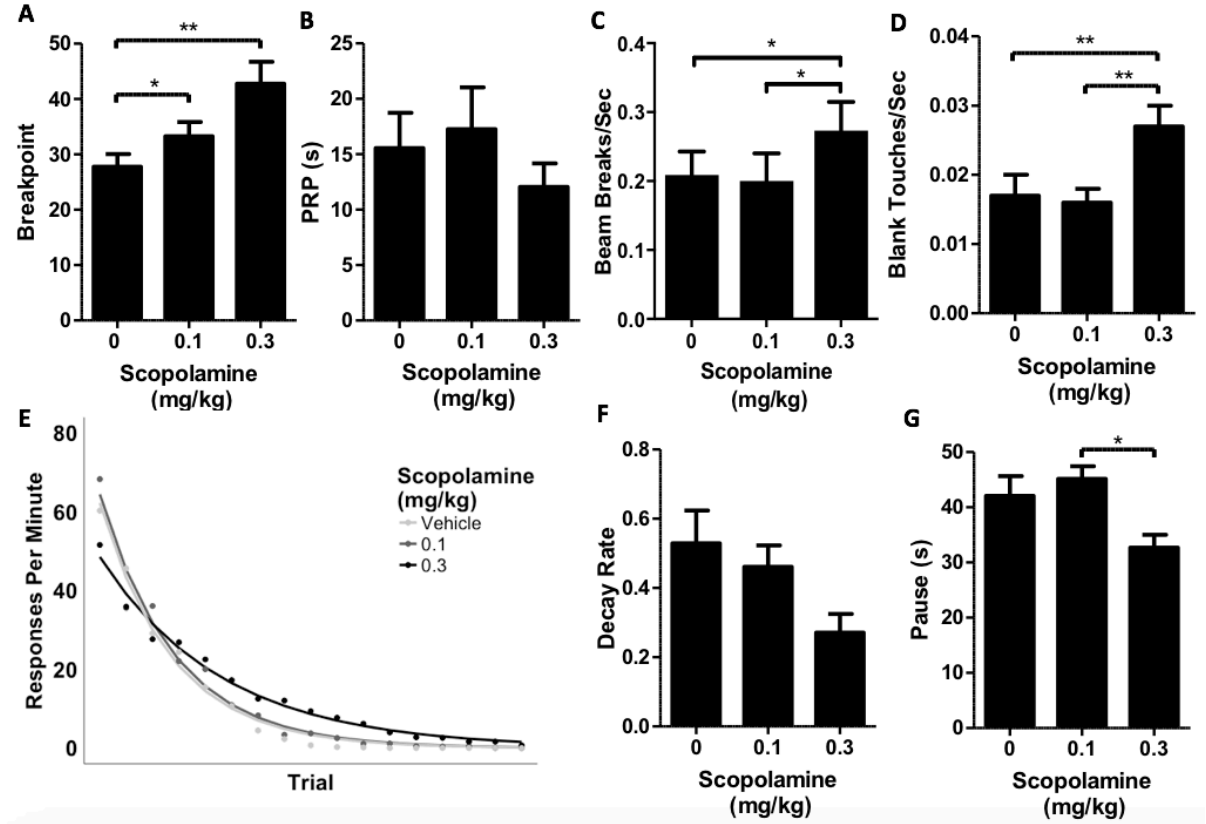


Figure 5.1: The effects of scopolamine on PR performance. **A** Systemic administration of scopolamine enhances breakpoint. **B** Scopolamine does not affect post-reinforcement pausing.

Scopolamine increases measures of general activity including the **C** rate of IR beam breaks and **D** blank screen touches. **E** Effect of scopolamine upon response rates. **F** Scopolamine did not affect the rate of decay in responding. **G** Administration of the high dose of scopolamine reduces the mean pause between response bouts. Error bars represent SEM. * $p < .05$; **$p<.01$.

5.3.2 Systemic administration of the M1 receptor antagonist biperiden enhances PR performance without affecting general activity levels.

It is possible that the effect of scopolamine upon PR performance was driven by actions at the M_1 receptor subtype. To investigate this, the preferential antagonist biperiden was tested. Figure 5.2A shows that breakpoints were significantly increased following systemic administration of biperiden ($F(2,30) = 18.168$, $p < .001$; partial eta squared = .548). Biperiden at 3 mg/kg significantly increased breakpoint compared to both vehicle and 1 mg/kg (both $p < .01$). As shown in figure 5.2B, biperiden also significantly decreased the duration of the mean PRP ($F(2,30) = 8.366$, $p < .01$; partial eta squared = .358). PRPs were significantly shorter following 3mg/kg relative to both vehicle ($p < .01$) and 1 mg/kg of biperiden ($p < .05$). Biperiden (figures 5.2C and 5.2D) did not significantly increase measures of nonspecific activity. Neither the rate of IR beam breaks ($F(2,30) = .927$, $p = .407$; Figure 5.2C), nor the rate of nontarget screen touches ($F(1.23, 18.48) = 1.266$, $p = .286$; Figure 5.2D) were significantly affected by drug administration.

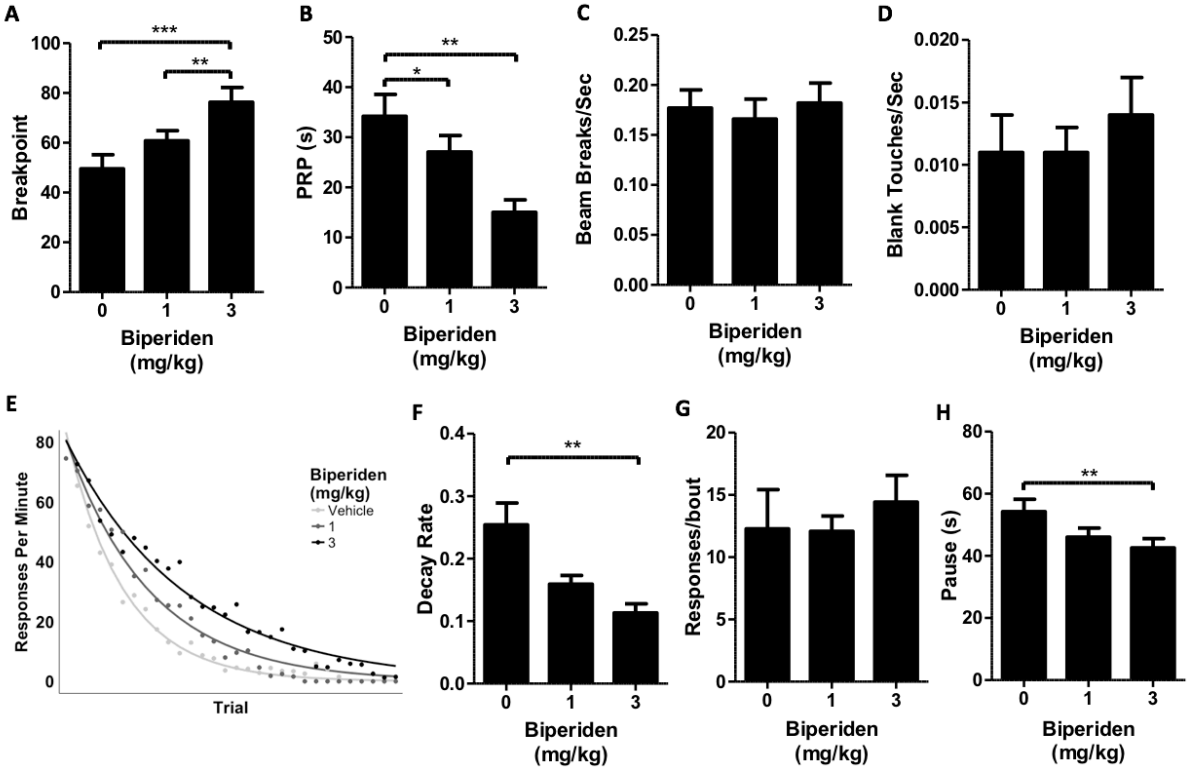


Figure 5.2: Enhancement of PR performance following administration of biperiden. **A** Breakpoint is dose-dependently increased by biperiden. **B** Biperiden reduces the length of the post reinforcement pause (PRP). Administration of biperiden does not affect measures of general activity, including **C** the rate of IR beam breaks and **D** the rate of blank screen touches . **E** Biperiden affects response rates during PR performance. **F** The highest dose of biperiden significantly reduces the decay rate of responding. **G** Administration of biperiden does not increase the mean length of a response bout. **H** Biperiden significantly reduces the length of pauses between response bouts. Error bars represent SEM. * $p < .05$; ** $p<.01$; *** $p<.001$.

Biperiden also affected the pattern of response rates (Figure 5.2E). The predicted peak response rate was not significantly affected by biperiden administration ($F(1.35, 20.23) = 2.05$, $p = .1.46$). However, as shown in figure 5.2F, biperiden significantly reduced the response decay rate ($F(2,30) = 9.576$, $p <.01$, partial eta squared = .390; Figure 52F). 0.3mg/kg of biperiden significantly reduced decay rate relative to the vehicle ($p <.01$). Biperiden did not significantly affect the mean length of response bouts ($F(1.480,22.203) =.515$, $p = .551$; Figure 5.2G). However, there was a significantly effect of biperiden upon pausing between bouts, figure 5.2H ($F(1.295,19.423) = 9.682$, $p <.01$; partial eta squared = .392; Figure 5.2H). 3 mg/kg of biperiden significantly reduced the duration of pausing between bouts relative to vehicle ($p <.01$). Supplementary measures of activity are available in table 5.2.

Scopolamine	*Veh*	*0.1mg/kg*	*0.3mg/kg*
Reward Collection Latency	1.32 ± .04	1.33 ± .05	1.42 ± .13
Magazine entries per second	0.02 ± .00	0.02 ± .00	**0.03 ± .00***
Biperiden	*Veh*	*1mg/kg*	*3mg/kg*
Reward Collection Latency	1.43 ± .06	1.48 ± .05	1.56 ± .09
Magazine entries per second	0.01 ± .00	0.01 ± .00	0.02 ± .00
Telenzepine	*Veh*	*3mg/kg*	*10mg/kg*
Reward Collection Latency	1.46 ± .07	2.26 ± .56	1.49 ± .06
Magazine entries per second	0.01 ± .00	0.02 ± .00	0.02 ± .00
Tropicamide	*Veh*	*20mg/kg*	
Reward Collection Latency	1.35 ± .03	1.31 ± .03	
Magazine entries per second	0.02 ± .00	0.02 ± .00	

Table 5.2: Effects of muscarinic antagonists upon supplementary measures of activity. Mean values ± SEM of reward collection latencies and rate of magazine entries during PR performance following systemic administration of Scopolamine, biperiden, telenzepine and tropicamide. * Significantly different to 0.01mg/kg of scopolamine ($p < .05$).

Together, these results suggest that biperiden facilitates PR performance without significantly affecting measures of general activity. These data also suggest that the previous effects of scopolamine upon motivation may have been driven by the M_1 receptor, whereas the effects on locomotor activity may have been driven by a different receptor subtype.

5.2.3 Systemic administration of telenzepine does not affect PR performance

As a control for potential peripheral effects, the M_1 receptor antagonist telenzepine, believed to have relatively poor brain penetrance (Ichikawa et al. 2002; Pediani et al. 2016), was administered prior to PR performance. Figure 5.3A shows how telenzepine did not significantly affect PR breakpoint ($F(2,30) = 2.772$, $p = .079$). The duration of the mean PRP was also not affected by administration of telenzepine ($F(2,30) = .624$, $p = .543$; Figure 5.3B). Neither the rate of IR beam breaks ($F(2,30) = 1.617$, $p = .215$), nor the rate of blank touches was affected by any dose of telenzepine administered ($F(2,30) = .503$, $p = .610$). Response rates were also unaffected following telenzepine administration (Figure 3.5C). Telenzepine had no significant effect on the predicted peak response rate ($F(2,30) = .172$, $p = .884$). The decay in response rates was also not affected by telenzepine ($F(1.28,19.17) = .595$, $p = .558$). Systemic administration of telenzepine produced no significant effect upon the mean length of a response bout ($F(2,30) = .432$, $p = .654$). Telenzepine also did not affect the duration of pausing between response bouts ($F(1.452,20.328) = .328$, $p = .655$). Taken together, these data indicate that telenzepine had no effect on effort-related behaviour. Therefore, the effects of biperiden are likely to be centrally-mediated.

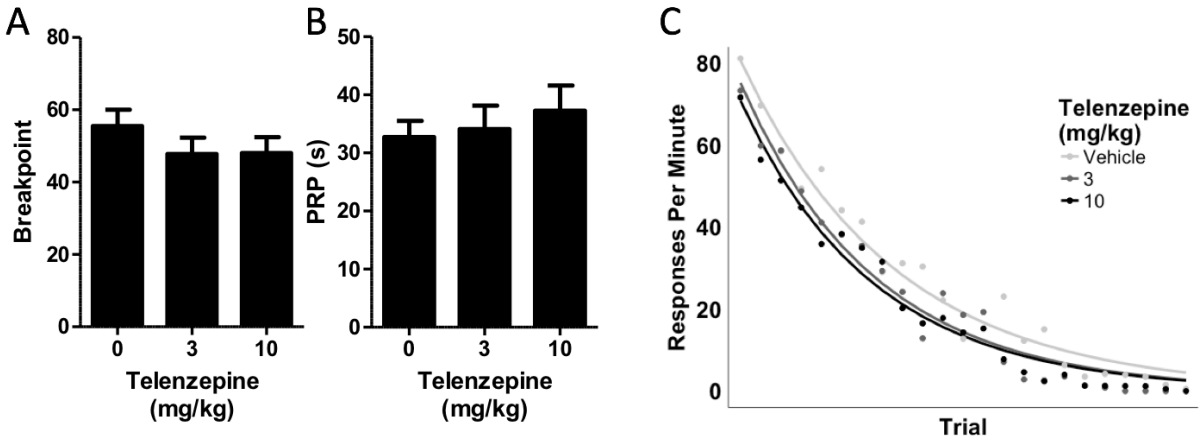


Figure 5.3: PR performance is unaffected by systemic administration of telenzepine. **A** Breakpoint is not affected by either dose of telenzepine. **B** The post reinforcement pause (PRP) is unaffected by telenzepine. **C** Telenzepine does not affect the decay in response rates. Error bars represent SEM.

5.2.4 Blockade of M4 receptors with tropicamide facilitates PR performance and at high doses increases some measures of activity.

To test for a potential contribution of the M_4-receptor subtype in facilitating motivated behaviour, tropicamide was administered at doses of 2.5 and 10 mg/kg. Neither dose significantly affected PR breakpoint, figure 5.4A ($F(2,30) = 2.007$, $p = .152$). There was however, a trend towards an effect on PRP ($F(2,30) = 3.268$, $p = .052$; Figure 4B). There were also no significant effects upon the rate of IR beam breaks ($F(2,30) = 1.950$, $p = .162$) or the rate of nontarget screen touches ($F(2,30) = 1.708$, $p = .198$). Tropicamide also failed to affect response rates (predicted peak rate: ($F(2,30) = .722$, $p = .494$); decay rate: ($F(2,30) = .814$, $p = .452$). There was however, a significant drug effect upon the mean number of responses within a bout ($F(2,30) = 3.582$, $p < .05$; partial eta squared = .193). However, post hoc testing did not reveal any significant comparisons. Tropicamide also affected pausing between response bouts ($F(2,30) = 3.335$, $p < .05$; partial eta squared = .182). Again, no post hoc comparisons were significant (all $p > .05$)

Given the strong trend towards an effect of tropicamide on several measures of PR performance, animals were subsequently administered the higher dose of 20 mg/kg. At this dose, figure 5.4C tropicamide significantly increased PR breakpoint ($t(15) = 2.218$, $p < .05$). Tropicamide failed to affect PRPs ($t(15) = 1.01$, $p = .328$; Figure 5.4D) or IR beam breaks ($t(15) = .567$, $p = .579$). There was however, a significant increase in nontarget touches, following tropicamide administration, figure 5.4E ($t(15) = 3.153$, $p < .01$). Tropicamide at 20mg/kg did not affect response rates (Figure 5.4F). Neither the predicted peak response rate ($t(15) = .507$, $p = .619$), nor the rate of decay in responding ($t(15) = 1.393$, $p = .184$) were significantly affected. 20mg/kg of tropicamide also failed to significantly affect the length of response bouts ($t(15) = 1.856$, $p = .083$). There was also no effect of drug administration upon pausing between bouts ($t(15) = .228$, $p = .823$). Supplementary measures of behaviour are available in table 5.2. These results suggest that antagonism of M_4 receptors may facilitate PR performance through an enhancement in nonspecific activity levels. The enhanced activity levels observed following scopolamine administration may have been driven through its actions at the M_4-receptor subtype

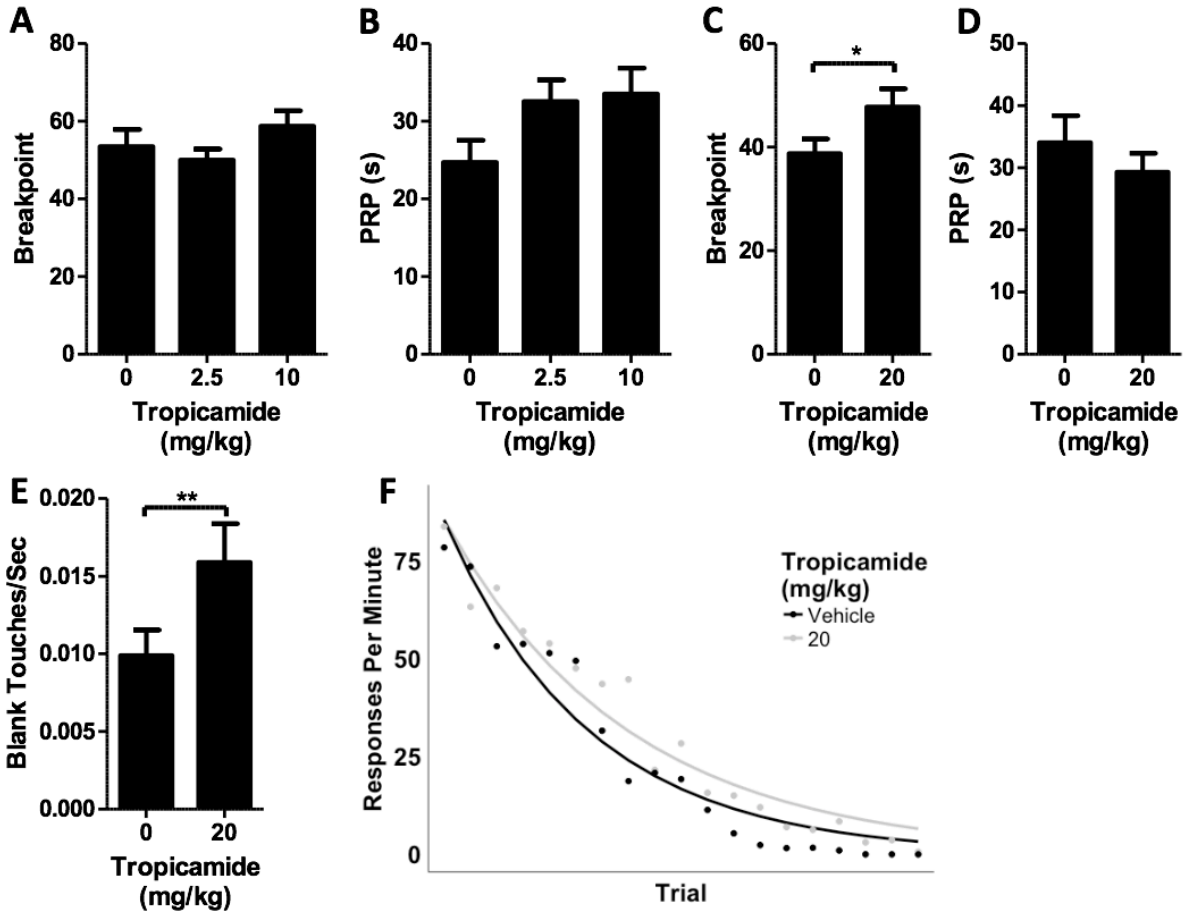


Figure 5.4: Tropicamide at high doses increases breakpoint but also nonspecific activity. Neither **A** breakpoint nor **B** the duration of the post reinforcement pause (PRP), were affected by either 2.5 or 10mg/kg of tropicamide. **C** The high dose of tropicamide increases breakpoint but **D** fails to affect PRP. **E** The rate of nontarget blank screen touches is significantly increased by tropicamide. **F** Tropicamide has no effect upon the decay in responding during PR performance**.** Error bars represent SEM. * $p < .05$; ** $p < .01$.

5.2.5 Scopolamine and biperiden do not affect performance on a FR5 schedule of reinforcement or alter free-feeding behaviour

The previous results demonstrate that PR performance can be enhanced through systemic administration of both scopolamine and biperiden. However, an increase in breakpoint could be driven by a number of behavioural mechanisms other than a change in motivation. Therefore, scopolamine and biperiden were tested on an uncapped FR5 schedule to investigate the potential drug effects on satiety and/or motor fatigue. Figure 5.5A shows how drug administration, under these low effort conditions, did not affect the number of trials completed ($F(2,28) = 2.180$, $p = .132$). There was however, a significant effect of drug administration on the duration of the mean PRP, as can be seen in figure 5.5B ($F(2,28) = 4.678$, $p < .05$; partial

eta squared = .250). PRPs were shortened following biperiden administration compared to scopolamine (p < .01). The mean reward collection latency was also affected by drug administration (F(1.316, 18.42) = 8.461, p < .01; partial eta squared = .377; Figure 5.5C). Biperiden administration significantly increased the mean reward collection latency relative to vehicle (p <.05). Figure 5.5D shows that there was also an effect of drug on the rate of IR beam breaks (F(2,28) = 3.920, p < .05; partial eta squared = .219). Scopolamine, but not biperiden, significantly increased the rate of IR beam breaks compared to vehicle (p < .05). Drug administration did not significantly affect the rate of nontarget (blank) screen responses (F(2,28) = .009, p = .991). There was also no significant effect of drug upon the rate of magazine entries (F(2,28) = 2.689, p = .085). Crucially, response rates, which can be used to index the onset of satiety (Phillips et al., 2017) also appeared unaffected (figure 5.5E). Neither the predicted peak response rate (F(2,28) = .180, p = .836), nor the decay in response rate (F(2,28) = .958, p = .396; Figure 5.5F) were affected by drug administration.

To further control for any nonspecific drug effects on appetite, the effect of scopolamine and biperiden administration on free strawberry milkshake consumption was tested. Drug administration, which can be seen in figure 5.5G, produced no effect on 60-minute milkshake consumption (F(2,28) = 1.388, p = .266). Together, these data indicate that the previously observed effects of scopolamine and biperiden on PR were unlikely to be driven by changes in satiety, motor fatigue or appetite.

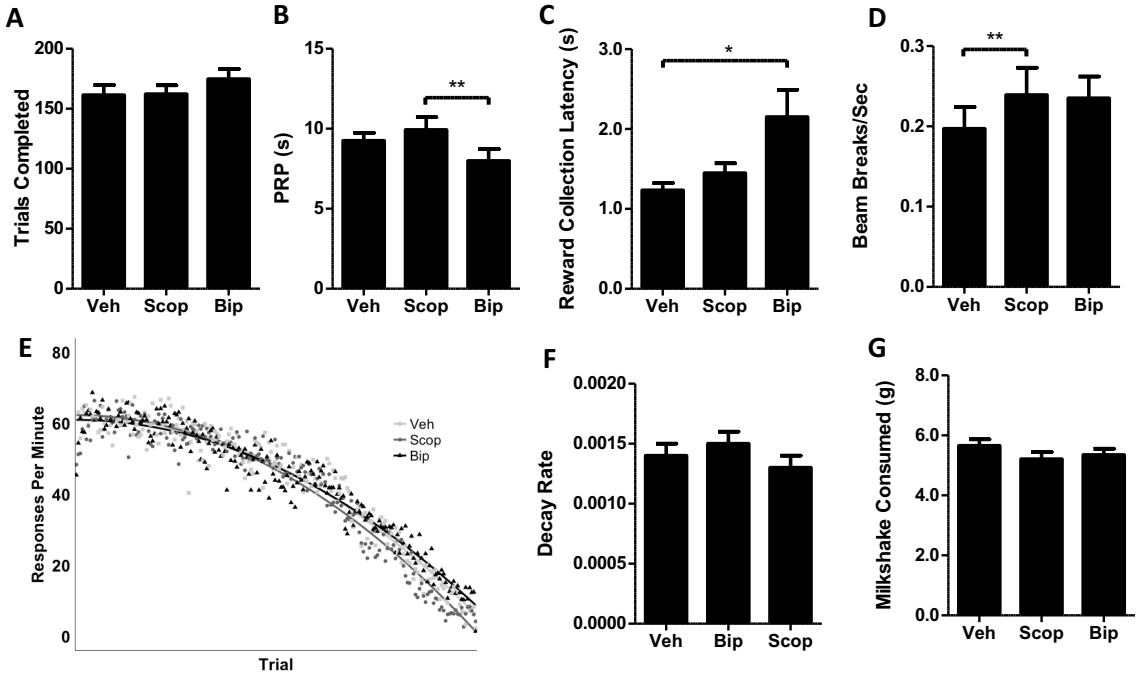


Figure 5.5: The effects of scopolamine and biperiden upon FR5 performance (**A-F)** and free consumption of milkshake (**G**). **A** Neither scopolamine nor biperiden affect the number of FR5

trials completed in a 60-minute session. **B** Neither scopolamine nor biperiden reduce the duration of post reinforcement pauses (PRPs) relative to vehicle, however, there is a significant difference between scopolamine and biperiden**. C** The latency to collect rewards is increased by biperiden administration**. D** The rate of IR beam breaks is significantly increased following administration of scopolamine but not biperiden**. E**. Neither biperiden nor scopolamine affect response rates reinforced under a FR5 schedule **F** The decay rate in FR5 responding is unaffected by systemic scopolamine and biperiden. **G** Neither scopolamine nor biperiden significantly affect milkshake consumption during a 60-minute free-feeding test**.** Error bars display represent SEM. Veh: Vehicle (saline); Scop: Scopolamine 0.3 mg/kg; Bip: Biperiden 3 mg/kg;* $p < .05$; **$p<.01$.

5.2.6 The effects of scopolamine and biperiden on PR are reduced in partially satiated mice.
In order to assess whether the effects of scopolamine and biperiden were sensitive to changes in outcome value, the effects of drugs were tested following prefeeding with milkshake. Initially, the interaction between scopolamine and prefeeding was assessed. Figure 5.6A shows how breakpoint was significantly reduced by prefeeding ($F(1,15) = 49.903$, $p < .001$; partial eta squared = .769) and increased by administration of scopolamine ($F(1,15) = 108.182$, $p < .001$; partial eta squared = .878). There was also a significant drug x prefeeding interaction upon breakpoint ($F(1,15) = 16.529$, $p < .01$; partial eta squared = .524). Scopolamine increased breakpoint following prefeeding with water ($p < .001$) and milkshake ($p < .05$). Breakpoints were also lower following prefeeding with milkshake in both drug conditions ($p < .001$; figure 5.6A). PRPs were not affected by prefeeding ($F(1,15) = .136$, $p = .718$; partial eta squared = .009), but were reduced by scopolamine ($F(1,15) = 5.753$, $p<.05$; partial eta squared = .277). The interaction between scopolamine and prefeeding was not significant ($F(1,15) = .812$, $p= .382$).

Figure 5.6B shows how the rate of IR beam breaks was significantly decreased by prefeeding ($F(1,15) = 5.544$, $p = .050$; partial eta squared = .232) but not affected by scopolamine ($F(1,15) = 3.662$, $p = .075$). There was however, a significant interaction between the drug and prefeeding ($F(1,15) = 10.106$, $p < .01$; partial eta squared = .403). Scopolamine significantly increased the rate of IR beam breaks following prefeeding with water ($p < .01$) but not milkshake ($p = .899$). Prefeeding with milkshake significantly reduced the rate of IR beam breaks in the scopolamine ($p < .01$), but not the vehicle condition ($p = .762$). Figure 5.6C shows how the rate of nontarget screen responses was also significantly reduced by prefeeding ($F(1,15) = 8.726$, $p < .01$; partial eta squared = .368) and increased by scopolamine ($F(1,15)$

= 14.186, $p < .01$; partial eta squared = .486). There was also a significant interaction between prefeeding status and scopolamine on the rate of nontarget screen touches ($F(1,15) = 6.043$, $p < .05$; partial eta squared = .287). Scopolamine increased nontarget responses following prefeeding with water ($p < .01$) but not following prefeeding with milkshake ($p = .124$). Prefeeding with milkshake reduced the rate of nontarget touches in the scopolamine ($p < .05$), but not vehicle condition ($p = .072$).

The decay in response rates was also examined. The decay rate was significantly increased by prefeeding ($F(1,15) = 8.538$, $p < .05$; partial eta squared = .363) and reduced by scopolamine administration ($F(1,15) = 9.095$, $p < .01$; partial eta squared = .377). There was also a significant interaction between drug administration and prefeeding state ($F(1,15) = 5.139$, $p < .05$; partial eta squared = .255). Scopolamine significantly reduced the decay rate in both prefeeding conditions ($p < .05$). The rate of decay was significantly higher following prefeeding with milkshake in both drug conditions ($p < .05$). Supplementary measures are available in table 5.3. Together, these results suggest that, the majority of the effects of scopolamine are abolished following prefeeding with milkshake. However, scopolamine was still able to significantly increase breakpoint in partially satiated mice.

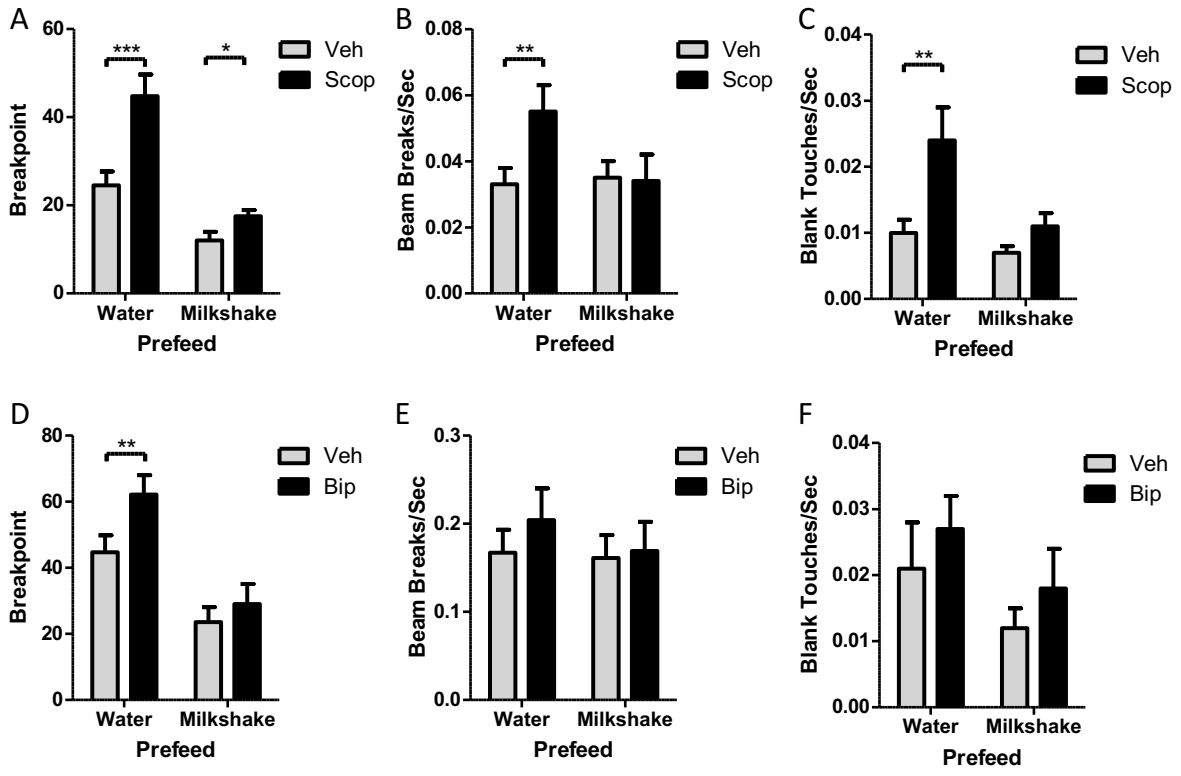


Figure 5.6: Interaction effects of prefeeding with water and milkshake and biperiden and scopolamine administration upon PR performance. **A** Prefeeding with milkshake attenuates the effect of scopolamine upon breakpoint. **B** The scopolamine induced increase in IR beam breaks and **C** blank screen touches are abolished by prefeeding the mice with milkshake **D**. The effects of biperiden upon breakpoint are abolished in mice prefed with milkshake**. E** Neither biperiden administration nor prefeeding affects the rate of beam breaks or **F** blank touches. Error bars display represent SEM. Veh: Vehicle (saline); Scop: Scopolamine 0.3 mg/kg; Bip: Biperiden 3 mg/kg; *p < .05; **p<.01.

The interaction between biperiden and behaviour was also examined through prefeeding. Figure 5.6D shows how prefeeding with milkshake had a significantly reduced breakpoint (F(1,13) = 48.481, p <.001; partial eta squared = .789). There was also a significant effect of biperiden administration (F(1,13) = 10.161, p <.01; partial eta squared = .439), as well as drug x prefeeding interaction (F(1,13) = 4.875, p <.05; partial eta squared = .273). Biperiden significantly increased breakpoint following prefeeding with water (p < .01), but not following milkshake (p = .211). Mice prefed with milkshake exhibited lower breakpoints in both drug conditions (both p < .001). Prefeeding also increased the mean PRP (F(1,13) = 6.896, p <.05; partial eta squared = .347). Biperiden administration, however, did not affect PRPs (F(1,13) = .155, p = .700). In addition, there was no interaction between biperiden and prefeeding observed upon PRP (F(1,13) = .283 p = .604).

As seen in figure 5.6E, the rate of IR beam breaks was not significantly affected by either prefeeding (F(1,13) = 3.027, p =.107), biperiden (F(1,13) = 2.470, p =.142; Figure 6E) or by any prefeeding x drug interaction (F(1,13) = 1.579, p = .233). Prefeeding did, however reduce the rate of nontarget screen touches (F(1,13) = 12.396 p <.01; partial eta squared = .488). There was also a significant effect of drug on the rate of nontarget responses, which can be seen in figure 5.6F (F(1,13) = 7.367 p <.05; partial eta squared = .362). There was no interaction between biperiden and prefeeding state (F(1,13) = .013, p =.910).

Finally, the effects of biperiden and prefeeding on response rates were examined. Prefeeding had no significant effect on the rate of decay (F(1,13) = 1.197, p =.294). There was also no effect of systemic administration of biperiden (F(1,13) = .033, p =.859). There was, however, a significant interaction between drug and prefeeding state (F(1,13) = 11.093, p <.05; partial eta squared = .331). Biperiden reduced the rate of decay in responding following prefeeding with milkshake only (p<.01). Supplementary measures are available in table 5.3. Together,

these effects suggest the behavioural effects of biperiden are abolished in partially satiated mice.

Reward Collection Latency (s)	Scopolamine		Biperiden	
	Veh	0.3 mg/kg	Veh	3mg/kg
Water	1.59 ± .20	1.50 ± .08	1.31 ± .07	1.30 ± .06
Milkshake	1.35 ± .04	1.69 ± .10	1.41 ± .08	1.96 ± .40
Magazine entries per sec	**Scopolamine**		**Biperiden**	
	Veh	**0.3 mg/kg**	**Veh**	**3mg/kg**
Water	0.01 ± .00	0.03 ± .00	0.01 ± .00	0.01 ± .00
Milkshake	0.01 ± .00	**0.02± .00***	0.01 ± .00	0.01 ± .00

Table 5.3: Effects of muscarinic antagonists upon supplementary measures of activity following prefeeding with either water or milkshake. Mean values ± SEM of PR performance following prefeeding with either water or milkshake and administration of scopolamine or biperiden. Comparisons only reported in cases of a significant drug x prefeed interaction *Significantly different from vehicle (Milkshake condition only, $p < .05$).

5.2.7 Scopolamine and biperiden do not significantly affect effort related choice behaviour in intact mice

When examining the effects of scopolamine and biperiden on effort-related choice behaviour, drug administration produced no significant effects on chow consumption ($F(1.092,15.439) = 1.552$, $p = .323$; Figure 5.7A). There was however, a significant effect of drug administration on the number of FR5 trials completed ($F(2,30) = 4.320$, $p < .05$; partial eta squared = .224; Figure 5.7B). Neither compound significantly affected the number of trials completed relative to vehicle administration (both $p > .05$); however, mice treated with biperiden completed more trials compared to scopolamine ($p < .05$). PRPs were not affected by drug administration ($F(1.516,17.337) = .956$, $p = .396$). Drug administration also had no effect on either the rate of IR beam breaks ($F(2,30) = .251$ $p = .780$) or the rate of nontarget screen touches ($F(2,30) = .706$, $p = .502$). Together, this suggests that neither biperiden nor scopolamine affect effort-related choice behaviour.

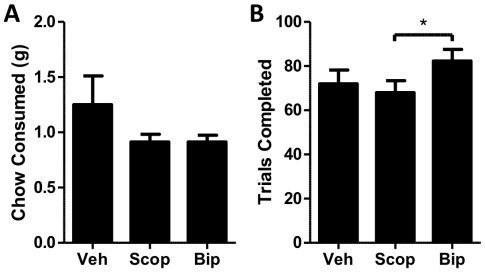


Figure 5.7: The effects of scopolamine and biperiden of effort related choice (ERC) behaviour. ERC performance is unaffected by drug administration. Nether scopolamine nor biperiden affected the amount of chow consumed **A** or the number of trials completed **B** relative to vehicle. Error bars display represent SEM. Veh: Saline Vehicle; Scop: Scopolamine 0.3 mg/kg; Bip: Biperiden 3 mg/kg; * $p < .05$.

5.2.8 Biperiden does not affect extinction behaviour

The previous results suggest biperiden enhances motivation. It is possible that these effects may have been driven by an increase in perseverative-like stimulus responding during a period without reinforcement. Therefore, the effects of biperiden on responding in extinction conditions (during which the reinforcer is removed) were evaluated. One animal was removed following an outlier analysis (with the number of touchscreen responses being greater than +2 standard deviations above the mean). Figure 5.8A shows that Biperiden did not significantly affect the total number of target touches ($t(12) = 1.773$, $p = .102$). Biperiden also did not affect the decay in responding (figure 5.8B). Neither the predicted peak response rate ($t(12) = 1.139$, $p = .277$) nor the decay rate ($t(12) = 1.808$, $p = .096$; figure 5.8C) were significantly affected by biperiden. Therefore, the previous effects of biperiden upon effort output are dependent on the mice receiving reinforcement and cannot be fully explained by an increase in target perseveration.

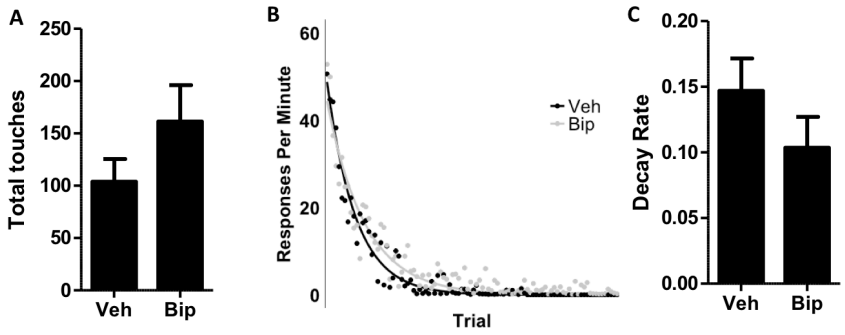


Figure 5.8: Effects of biperiden under extinction conditions. **A** Biperiden does not significantly affect the total number of target screen touches. **B** Biperiden does not affect the change in

response rates. **C** The decay rate in responding is not significantly affected by biperiden administration. Veh: Saline vehicle, Bip: Biperiden 3mg/kg. Error bars display the SEM.

5.2.9 Biperiden facilitates the effects of amphetamine upon PR performance

In order to examine whether biperiden interacts with dopaminergic function the potential faciliatory effects of biperiden on amphetamine were tested on PR performance. Sub-effective and effective doses of amphetamine (0.1 and 1mg/kg respectively, Heath et al. 2015) were applied in isolation and in combination with two sub-effective doses of biperiden (0.3 and 1mg/kg) which were also applied in isolation. As shown in figure 5.9A, drug administration significantly affected breakpoint ($F(3.016,42.227) = 14.093$, $p < .001$; partial eta squared = .516). 1mg/kg of amphetamine increased breakpoint relative to vehicle ($p<.01$). When 1mg/kg of amphetamine was applied in combination with the sub-effective 1mg/kg of biperiden, breakpoints were increased relative to all other conditions (all $p<.05$), demonstrating an additive effect. No other doses or combination significantly affected breakpoint relative to vehicle.

PRPs were also significantly affected by drug administration ($F(3.428, 47.993) = 2.773$, $p < .05$; partial eta squared = .163); however, there were no significant differences between any drug condition (all $p > .05$). Neither the latency to collect rewards ($F(1.899, 26.591) = 1.330$, $p = .280$) nor the rate of nontarget screen touches ($F(2.834) = 1.294$, $p = .290$) were significantly affected by drug administration. When examining the rate of IR beam breaks, which can be seen in figure 5.9B, there was a main effect of drug ($F(1.987, 27.696) = 11.337$, $p < .001$; partial eta squared = .447). 1mg/kg of amphetamine increased breakpoints relative to vehicle ($p< .05$). The high dose biperiden/amphetamine combination increased beam breaks relative to vehicle ($p<.05$); however, this combination failed to increase the rate of beam breaks relative to 1m/kg of amphetamine alone. Together, these results suggest a dose of biperiden that does not significantly affect motivated behaviour, is able to enhance the effects of amphetamine on motivation, but not nonspecific locomotor activity.

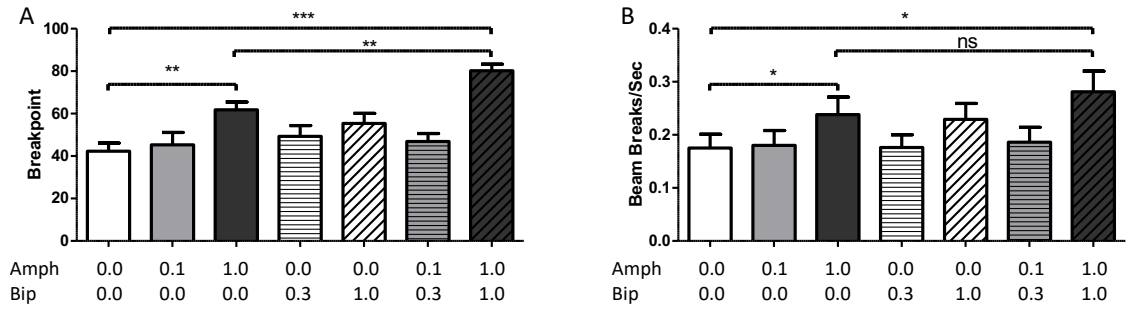


Figure 5.9: The effects of biperiden in facilitating the behavioural effects of amphetamine upon PR performance. The dose of amphetamine is represented by the graph fill, whereas the dose of biperiden is indicated by the graph pattern. **A** 1mg/kg of biperiden facilitates the enhancement in breakpoints caused by amphetamine but does not affect breakpoint when administered in isolation. **B** 1mg/kg of biperiden does not enhance the general effect of amphetamine upon IR beam breaks. Amph: amphetamine; Bip: biperiden. All doses in mg/kg. ns $p > .05$; * $p < .05$; **$p<.01$; ***$p<.001$

Visual inspection of breakpoints (figure 5.9A) suggests that the 1 mg/kg dose of biperiden alone, produced a nonsignificant increase in breakpoint. This suggests that the effect of biperiden with amphetamine may have been additive rather than synergistic. The presence of a significant interaction between treatments would suggest the presence of synergistic effects (Slinker 1998). Therefore, breakpoints following neither treatment (i.e. vehicle), both treatments alone (i.e. 1 mg/kg of amphetamine and 1m/kg amphetamine) and the treatments in combination were examined. When examining breakpoints there were main effects of both amphetamine, figure 5.10 ($F(1,14) = 49.182$, $p < .001$; partial eta squared = .778) and biperiden ($F(1,14) = 19.119$, $p < .001$; partial eta squared = .577). However, there was no significant interaction between the two ($F(1,14) = 1.107$, $p = .311$; figure 5.10A). This suggests the effect of biperiden upon amphetamine was additive, rather than more-than-additive, or synergistic.

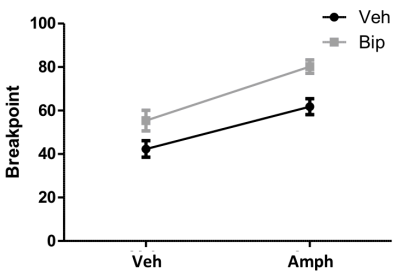


Figure 5.10: The additive effect of biperiden and amphetamine on PR performance. No significant interaction was observed between biperiden and amphetamine upon breakpoint, suggesting the effects were additive not synergistic. Amph: Amphetamine 1mg/kg; Bip: Biperiden 1mg/kg. Veh: Saline vehicle. Error bars represent the SEM.

5.3 Discussion

Apathy remains a large-scale clinical problem with few effective treatments. Preclinical identification of targets is a key step in developing new treatments for such symptoms. Progressive ratio (PR) schedules can probe effort-based behaviour in a way believed to model activational processes commonly disrupted in clinical populations. Touchscreen-based PR tasks in particular are useful assays of motivation as they maintain a high degree of face-validity across species (Weed et al. 1999; Heath et al. 2015; Bland et al. 2016). PR schedules are highly sensitive to pharmacological manipulations; however, are also produce relatively unspecific measures of behaviour. Therefore, it is important to follow-up positive results in PR with a number of control tasks, to ensure that a change in behaviour is drive by changes in motivation rather than a confounding construct. Through this process, this chapter demonstrates that muscarinic acetylcholine receptors (mAChRs) can affect motivated behaviour in mice, and antagonists of the M_1 subtype in particular may provide a new target in the treatment of apathy.

5.3.3 Effects of mAChR antagonists upon PR performance

Several compounds (biperiden, scopolamine and tropicamide) significantly increased breakpoint, the typical primary measure of PR performance. However, the overall pattern of behaviour was markedly different. For example, the M_1 selective biperiden appeared to produce relatively selective effects on motivation, by increasing breakpoint in the absence of any significant changes in locomotor measures. In contrast, the nonselective muscarinic antagonist scopolamine and the M_4 receptor antagonist tropicamide produced concomitant increases in nonspecific activity (such as the rate of IR beam breaks and nontarget screen touches). Therefore, it is possible that the effects on breakpoint induced by scopolamine and tropicamide may have been driven by changes in locomotor activity.

It is possible to obtain complementary measures of behaviour during responding under a PR schedule of reinforcement. One such measure is the post reinforcement pause (PRP). The length of PRPs reflect, in part, the upcoming work requirement (Felton & Lyon 1966) and decrease with an increased motivational state (Powell, 1969). A reduction in the length of PRPs

may suggest an animal is more motivated to engage with a task to obtain rewards. Biperiden, but neither scopolamine nor tropicamide, reduced the length of PRPs. Similar results were observed when examining the pattern of response rates. Over the course of a PR session, the rate of operant responding decreases (Killeen et al. 2009). By examining trial-by-trial changes response rate it is possible to extract additional parameters of behaviour. The predicted peak rate of responding is believed to reflect the maximal energy output of an animal (Kim et al. 2017; Phillips et al. 2017). In contrast, the rate of decay in responding may reflect the excitatory influence of reinforcers upon subsequent bouts of behaviour (Phillips et al. 2017). Biperiden alone reduced the rate of decay in responding without affecting the predicted peak response rates, again suggesting a motivational effect in the absence of changes to reward value. In contrast, neither scopolamine nor tropicamide produced significant effects upon response rates, suggesting fewer motivational effects on instrumental behaviour.

Operant responding is also characterised by bouts of successive responses separated by pauses (Shull et al. 2001). Biperiden decreased the length of pausing between bouts, without altering the mean number of responses within a bout. This result again suggests that biperiden increased task engagement in mice, attenuating the effects of increasing work requirements on response rates as well as pausing. Together these results highlight the advantages of analysing multiple measures of behaviour other than breakpoint. Not only do such analyses provide a richer source of data of the effects of manipulations on behaviour but may also reduce the need for additional control tasks and could ultimately reduce the number of experimental animal procedures conducted.

5.3.4 Effects of biperiden on a battery of control tasks

A drug induced enhancement in PR performance could be driven by a number of behavioural mechanisms. In order to examine potential confounds, previous reports have used a number of control tasks to understand the psychological mechanisms underlying a change in behaviour (Bailey, Williamson, et al. 2016). We therefore tested the effects of mAChR blockade on a battery of control tasks, to investigate whether the effects observed on the PR schedule were driven by behavioural changes other than an increase in goal-directed motivation.

PR performance is sensitive to the effects of satiation. The cholinergic system has been implicated in the regulation of satiety processes. For example, the slowing of feeding towards the end of a feeding bout is accompanied by an increase in striatal acetylcholine levels (Mark et al. 1992). Certain appetite suppressing drugs also cause an increase in ACh within the

striatum (Rada & Hoebel 2000). It is possible that mAChR antagonists reduce the effects of endogenous ACh on satiety, allowing higher breakpoints to be achieved. A separate possibility is that the drugs attenuated motor fatigue that may affect PR performance. To account for these possibilities, we tested the effects of scopolamine and biperiden upon FR5 performance. At the effective doses, neither biperiden nor scopolamine significantly affected the number of FR5 trials completed, suggesting the effects observed upon PR performance was not mediated by a change in motor fatigue. Furthermore, neither compound affected the decay in response rates, which reflect, in part, the onset of satiety (Kim et al. 2017). In humans, anticholinergic drugs, including scopolamine, can cause 'dry-mouth' symptoms (Scully CBE 2003; Drevets & Furey 2010). It is possible that such symptoms may cause an increase in the seeking of a liquid reinforcer such as milkshake. To further investigate this possibility, the effects of scopolamine and biperiden upon free consumption of the strawberry milkshake reinforcer were tested. Neither drug significantly increased milkshake consumption during 60-minute free-feeding, suggesting that the effects observed on PR were unlikely driven by appetitive changes.

Interpreting enhancements in PR performance can also be confounded by changes in general arousal that do not reflect changes goal-directed behaviour (Bailey et al. 2015). Goal directed behaviour involves an animal forming action-outcome contingencies (Dickinson & Balleine 1994). If the effects of a drug are restricted to goal-directed behaviour, then the effects should be sensitive to changes in reward value. The effects of biperiden on PR performance were replicated in the absence of satiation (prefeeding with water). In contrast, biperiden produced no behavioural effects in mice prefed with milkshake. This result suggests the behavioural changes induced by biperiden were goal-directed. Scopolamine was still able to produce a small, but significant increase in breakpoint in satiated mice. However, the effect upon breakpoint was significantly attenuated by prefeeding. This effect of prefeeding suggests, that scopolamine may facilitate PR performance through means other than an increase in locomotor activity. It should be noted that a dual-reinforcer procedure is necessary to determine whether a behaviour is goal-directed or habitual (Dickinson & Balleine 1994). The present study does however, suggest that the effects are sensitive to changes in outcome value.

Biperiden also did not significantly affect responding in the absence of reinforcement. This finding suggests that the behavioural actions of biperiden on PR performance are unlikely to explained by any increase in perseverative or compulsive-like responding. In the present study, we did not test the effects scopolamine under extinction; however, previous reports have

suggested that higher doses of the drug can reduce extinction behaviour (Morley & Russin 1978; McKim 1970).

5.3.5 Scopolamine and Biperiden fail to increase effort related choice

In spite of the effects observed on PR performance, neither biperiden nor scopolamine significantly affected ERC performance. One possibility is that the effort required in a FR5 schedule is too low to detect the facilitative effects of the compounds. In line with this, previous studies investigating the facilitation of ERC behaviour have typically either attempted to reverse a motivational deficit following pharmacological pre-treatment (Nunes, Randall, Hart, et al. 2013), or have used PR-choice tasks with escalating work requirements (Randall et al. 2012). A higher ratio employed in an ERC task may be more sensitive to detect any enhancements in motivated behaviour following administration of mAChR antagonists.

5.3.6 Biperiden facilitates the effects of amphetamine

The behavioural effect of amphetamine on breakpoint, but not locomotor activity, was facilitated by co-administration of biperiden. It is possible that such an effect could be simply additive or synergistic (i.e. more-than-additive). Previous reports have suggested that the nonselective antagonist scopolamine can facilitate the locomotor enhancing effects of amphetamine (Carlton 1961). Moreover, in a direct test of synergism, scopolamine and cocaine were shown to have more-than-additive effects upon locomotor activity when applied in combination (Thomsen 2014). However, it is not clear whether these effects also occur for effort expenditure or occur through actions at the M_1 receptor.

When examining drug combinations there are multiple ways of approaches to assessing possible synergistic effects (Foucquier & Guedj 2015). Some approaches require multiple combinations of the two drugs at differing ratios (Tallarida 2011). An alternative approach is to use null hypothesis testing and a two-way ANOVA to test for the presence of a significant interaction between the two drugs when applied alone and in combination (Slinker 1998). In the present study, there was no significant interaction between biperiden and amphetamine suggesting an additive effect. This result, coupled with some separate behavioural effects of amphetamine (Bailey et al., 2015), suggests that effects of biperiden on effort exertion is occurring through a separate physiological pathway than amphetamine. This suggests that the previous reports of synergism between scopolamine and stimulants may be dependent on actions at receptors other than the M_1 subtype. However, in the absence of a direct electrophysiological or neurochemical investigation, the effects of biperiden on dopamine

function are unknown. Future studies may wish to combine biperiden administration with such techniques to fully investigate the effects on dopaminergic function. It should also be noted that amphetamine also facilitates other neurotransmitter systems including serotonin and in particular noradrenaline (Kuczenski & Segal 1992; Kuczenski & Segal 1997; Rothman et al. 2000). The present results may therefore represent a facilitation of nondopaminergic catecholamine function.

This finding does however, raise the possibility of using combination therapy of muscarinic antagonists and stimulants such as amphetamine. Amphetamine has been previously proposed as a treatment for apathy (Clark & Mankikar 1979), however the long term use of psychostimulants as therapeutics are associated with a number of side effects (reviewed in Stotz et al. 1999). The co-administration of biperiden may allow for lower doses to achieve the same therapeutic benefits reducing the severity of side effects associated with either drug alone.

5.3.7 Compound selectivity and penetrance

Muscarinic receptor subtypes are molecular targets for a number of disorders (Langmead et al. 2008). However, due to similarity in the structure of muscarinic binding pockets, it is not possible to produce highly selective ligands that act upon the orthosteric binding sites (Foster et al. 2014; Thal et al. 2016). Therefore, orthosteric muscarinic ligands, at best, have preference for one receptor subtype. Biperiden has been reported to have preference for the M_1 receptor over other types (Syvälahti et al. 1987; Bolden et al. 1992; Kimura et al. 1999), and is commonly used as a M_1 antagonist in the literature (e.g. Salin-Pascual et al. 1993; Klinkenberg & Blokland 2011; Roldán et al. 1997; Popelíková et al. 2018). Aside from the M_1 subtype, biperiden also acts upon M_4 receptors (Bolden et al. 1992; Witkin et al. 2014). The M_4 receptor antagonist tropicamide failed to produce the large PR performance observed following scopolamine and biperiden administration. Tropicamide is a potent M_4 receptor antagonist, but has poor selectivity for the receptor (Lazareno et al. 1990; Croy et al. 2016). There are currently a lack of highly selective of M_4 receptor antagonists, that cross the blood-brain barrier. However, tropicamide can be used to produce M_4 receptor-mediated effects on behaviour (Betz et al. 2007; Veeraragavan et al. 2011; Aliane et al. 2011; Ztaou et al. 2016). The effects of tropicamide upon PR performance are therefore in-line with hypothesis that biperiden and scopolamine enhance PR performance through actions at the M_1 receptor subtype, however the use of genetic knock-out mice would be needed to conclude this conclusively.

In the present study, all compounds were administered systemically. mAChRs are also expressed in peripheral tissues, including the gut, heart and bladder (Caulfield 1993). It is possible, therefore, that any drug effects may be driven by actions at peripheral receptors. Scopolamine is a highly potent antagonist at all mAChR subtypes that readily crosses the blood-brain barrier (Witkin et al. 2014), and produces centrally mediated effects when administered systemically (Klinkenberg & Blokland 2010). Biperiden also readily penetrates into the brain (Battaglia et al. 2001; Sudo et al. 1999), and is prescribed as a centrally acting treatment in PD and to treat extrapyramidal symptoms of antipsychotic treatment in Schizophrenia (Gjerden et al. 2009). Conversely telenzepine, believed to have difficulty crossing the blood-brain barrier (Ichikawa et al. 2002; Pediani et al. 2016), had little effect on PR performance, suggesting the effects of biperiden and scopolamine upon PR performance likely occurred through centrally located receptors.

As previously discussed (chapter 1), within the NAc, M_1 and M_4 receptors are situated upon medium spiny neurons (MSNs). M_4 receptors are expressed mainly upon the D_1 type striatonigral MSNs, whereas M_4 receptors are expressed upon both striatonigral and the D_2 type striatopallidal receptors (Goldberg et al. 2012). Suppression of the striatopallidal pathway activity, facilitates PR and ERC performance (Randall et al. 2012; Carvalho Poyraz et al. 2016). Conversely, compounds that would be expected to enhance striatopallidal MSN excitability, disrupt PR performance (Mingote et al. 2008; Bari & Pierce 2005). Blockade of the G_q-coupled M_1 receptors would be expected to reduce neuron activity. It is possible that the present facilitation of PR performance by biperiden and scopolamine was mediated by striatopallidal M_1 receptors. However, further study using disconnection lesions (Mingote et al. 2008) or cell specific receptor knock out mice (Jeon et al. 2010) would be needed to investigate this.

5.3.8 Conclusions

Motivational impairments are a particularly deleterious symptom common to many neuropsychiatric and neurodegenerative diseases, yet no therapeutic options are currently available. The present results indicate that targeting the M_1 mAChR can increase motivation, in intact mice, in the absence of a number of potential mediating confounds. Biperiden in particular may represent a viable drug for the treatment of disrupted motivation.

Chapter 6. Evaluation of ageing as preclinical model of apathy

6.1 Introduction

The work described in the previous chapter suggested that such muscarinic acetylcholine receptor (mAChR) antagonists appear to facilitate effort-based behaviour in intact mice (Chapter 5). In order to strengthen the preclinical evidence for muscarinic receptors as pharmacological targets, it is useful to test the pharmacological effects of these compounds in preclinical models of apathy. Rodent models, which are commonly used in central nervous system (CNS) drug discovery, aim to recreate an aspect or aspects of the human condition in a preclinical preparation (van der Staay 2006). Therefore, a key stage in preclinical drug discovery is evaluating and characterising behavioural profiles in potential animal models, which could subsequently be used to test the efficacy of novel therapeutics. As discussed previously, apathy can be modelled in rodents by changes in effort-related behaviour (Salamone & Correa 2012). Recent preclinical studies have identified a number of potential novel targets that can rescue motivated behaviours in deficit models (Simpson et al. 2011; Randall, Lee, Podurgiel, et al. 2014; Mingote et al. 2008). The models used include pharmacological (Randall, Lee, Nunes, et al. 2014; Farrar et al. 2007); genetic (Simpson et al. 2011) and developmental manipulations (Amitai et al. 2017).

There is strong evidence that non-pathological ageing is associated with neurophysiological changes and an associated decline in a number of cognitive processes (Morrison & Baxter 2012; Samson & Barnes 2013). Age-related changes in hippocampal and prefrontal structure and function, in rodents, have been widely noted (for reviews see, Small et al. 2011; Gray & Barnes 2015; McQuail et al. 2015) Behavioural changes have also been associated with ageing. Relative to young rats, older rats display impairments in spatial memory (Barnes 1979; Gallagher et al. 1993), working memory (Dunnett et al. 1988; Dunnett et al. 1990), attention (Muir et al. 1999) and executive function (Barense et al. 2002). These cognitive changes in rats match those seen in normal human ageing (Robbins et al. 1994; De Luca et al. 2003; Mani et al. 2005). This has led some to use aged rodents as naturalistic models of cognitive impairment to test the efficacy of novel compounds (Ingram et al. 1994; Ye et al. 2000; Foley et al. 2004; Cui et al. 2008).

There is also evidence to suggest that motivated behaviour is also susceptible to age-related decline. Studies in human subjects have noted an increase in rates of apathy in healthy older age-groups (Onyike et al. 2007). Furthermore, a longitudinal study of subjects aged 58-85,

reported a significant increase in questionnaire-measured apathy scores over a five year period (Brodaty et al. 2010). This finding of an increase in apathy with age has since been replicated (Guercio et al. 2015). Although less researched than other cognitive domains, two studies have also reported an age-related decrease in motivated behaviour in rodents. Motivation is often probed in laboratory animals, through examining effort-based behaviour. Studies involving both mice (Bordner et al. 2011) and rats (Blokland & Raaijmakers 1993; Amancio-Belmont et al. 2017) have reported lower (progressive ratio) PR breakpoints in older groups of rodents. In the present study, we sought to confirm and explore any age-related changes in effort-related behaviour. Furthermore, ageing is the major risk factor for neurodegenerative disorders (Hindle 2010), which are commonly associated with motivational impairments. Aged rodents may model the 'baseline' that a treatment aims to restore. Therefore, it is important to explore whether age-related changes in physiology prevent a given pharmacological treatment from working. For example, there are reports of age-related reduction in mAChR expression and function in both rodents and humans (Morin & Wasterlain 1980; Rinne 1987; Biegon et al. 1989; Schwarz et al. 1990; Tayebati et al. 2004). Therefore, it is also important to investigate whether this interferes with the effects of mAChR antagonists on behaviour.

In experiment 1, alongside changes in PR performance, age-related changes in locomotor activity, a well-established behavioural change associated with age (e.g. Gage et al. 1984; Willig et al. 1987; Emerich et al. 1993; Scimonelli et al. 1999), was tested. Subsequently, in experiment 2, any age-related effects on motivation were tested within a touchscreen operant system. Additionally, the aged rats in experiment 2 underwent a modified food restriction procedure in order to better control for age-related change in weights. Finally, the effects of scopolamine, a nonselective mAChR antagonist, on PR performance and effort-related choice (ERC) performance were tested in both young and aged rats.

6.2 Experiment 1

6.2.1 Methods

Animals and ageing procedures

Male Sprague-Dawley rats (Envigo, UK) were used in the present study. Table 6.1 provides a summary of the ages and number of each group of rats. Across all cohorts and in both experiments, aged rats were approximately 12 months older than the young controls.

The rats in experiment 1 (cohorts 1 and 2) were transferred from the breeding facility (Envigo) to the testing facility (Eli Lilly) at 15 months of age, along with the young control groups at

three months of age. Following a week of acclimatisation, they were placed on a schedule of controlled feeding and maintained at no less than 85% of their free feeding weight. No correction was applied to this 85% body weight to match the expected growth curve. All animals were housed in groups of 3-4 in a temperature (20-22°c) and light controlled (12hr light/dark cycle; lights on 0700-1900) environment. Young and aged rats were group housed separately (i.e. no young and aged rats were mixed). Rats were housed in transparent plastic individually ventilated cages, with the bedding changed once weekly. Water was available *ad libitum* throughout the study. All testing took place within the animals' light phase. All experiments were regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 and following ethical review by the local Animal Welfare and Ethical Review Body (AWERB) at Eli Lilly and Co.

Behavioural Apparatus

Lever-based Operant chambers

Progressive ratio testing took place in standard operant chambers (Med-Associates) as described in detail in chapter 4.

Open field arenas

Spontaneous locomotor activity was assessed in 40 x 40 cm open field areas, surrounded by 30 cm Perspex walls. Testing took place within a dark room containing four infrared tables (100 x 100 cm). Four open fields were placed on each table. Locomotor activity was monitored by overhead infrared cameras (Sanyo VCV-3412P, Tracksys Ltd., UK). Cameras relayed data to a computer which ran the analysis software (Ethovision XT v8.5; Noldus, Netherlands). Rats were allowed to freely explore the arenas for 120 minutes. Locomotor activity, as distance travelled, was measured in 5-minute bins for the total 120-minutes.

Behavioural Assays

The lever-based PR pre-training and training took place as described in detail in chapter 4. R rats were trained to respond on the $(5 * e^{(0.2*n)} - 5)$ exponential PR schedule for ten sessions . Following this, breakpoints were analysed in response to the standard one pellet reward.

Statistics

Repeated measures ANOVAs and paired t-tests were used to evaluate age-related differences in all tasks. Greenhouse-Geisser corrections were applied to any violations of sphericity. Partial

eta squares were reported for all significant results. Bonferroni corrections were applied for any post-hoc tests.

6.2.2 Results

Aged rats display reductions in motivated behaviour and locomotor activity

Figure 6.1A shows that during the PR testing, breakpoints were significantly lower in aged rats ($t(38) = 2.610$, $p < .05$). This suggests that young animals are willing to exert significantly greater effort for equivalent food rewards. The effects of age on spontaneous locomotor activity can be seen in figure 6.1B. The distance travelled within each 5-minute bin was affected by both age ($F(1,38) = 46.107$, $p < .001$; partial eta squared = .542) and time ($F(23,897) = 71.480$, $p < .001$; partial eta squared = .647). There was also a significant age x time interaction on the distance travelled ($F(23,897) = 3.254$, $p < .001$; partial eta squared = .077). The young rats travelled significantly greater distances in the first 16 bins. Together, this suggests that the aged group showed both reduced PR performance and spontaneous locomotor activity

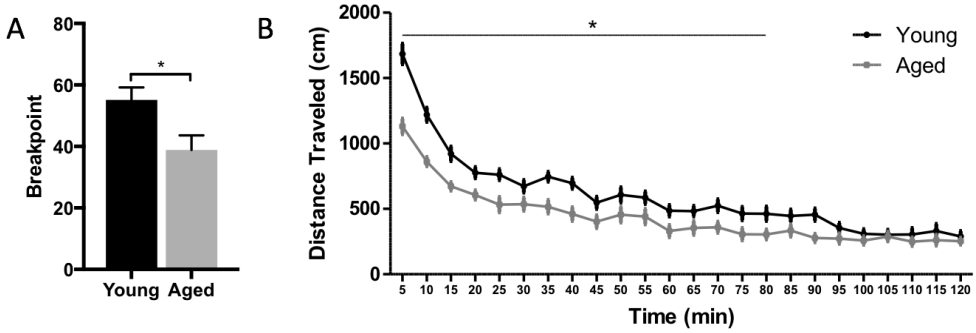


Figure 6.1: Effects of ageing on motivation, and locomotor activity in cohort 1. **A** Aged animals show reduced breakpoints on a PR schedule of reinforcement. **B** Aged rats travel less distance in an open field. Young: 6-8 months; Aged: 18-20 months old. Error bars are ± SEM. * A significant difference between aged and young animals $p < .05$.

Replication of the age-related deficit in motivated behaviour

As with the first cohort, breakpoints were lower in the aged rats ($t(35) = 3.290$, $p < .01$; figure 6.2A). Figure 6.2B shows again, reduced spontaneous locomotor activity in aged rats. The open-field distance travelled decreased with time ($F(11.298, 395.417) = 61.421$, $p < .001$; partial eta squared = .637). Aged rats again showed lower levels of locomotor activity ($F(1,35) = 39.422$, $p < .001$, partial eta squared = .530). There was also a significant age x time interaction on the distance travelled ($F(11.298, 395.417) = 6.998$, $p < .001$; partial eta squared = .167). Aged rats travelled significantly shorter distances in the first four bins (20 minutes),

as well as bins 10, 11 and 21. Together, this suggests that young animals initially show significantly higher levels of locomotor activity; however, over time this age-related difference dissipates.

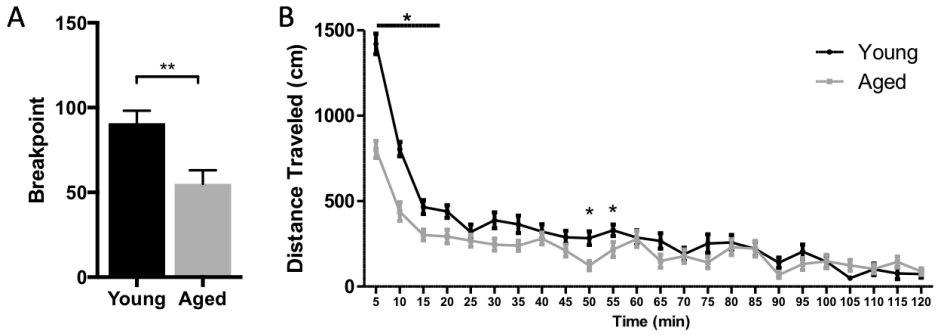


Figure 6.2: Replication of the effects of ageing on motivation and locomotor activity in an independent cohort (cohort 2) of rats. **A** Aged animals show reduced breakpoints on a PR schedule of reinforcement. **B** Aged rats travel less distance in an open field. Young: 3-4 months. Aged: 15-16 months old. Error bars are ± SEM. * A significant difference between aged and young animals $p < .05$; ** $p < .01$

Group differences in body-weight

Body-weights, as shown in table 6.1, were greater in aged rats. In cohort 1, the mean weight during testing was significantly greater in aged rats ($t(38) = 14.891$, $p < .001$). Similar differences were observed in cohort 2, where aged rats also had significantly greater mean body-weights ($t(23.263) = 46.009$, $p < .001$).

6.2.3 Discussion

The present results suggest that ageing in the absence of any specific pathology is associated with a reduction in both effort-based behaviour and spontaneous locomotor activity. It was important to replicate this effect, as the behavioural consequences of ageing may be sensitive to effects such as differential life experience. The replication of the age-related deficit raises the possibility that ageing may be a useful model to test the effects of mAChR antagonists on motivation. However, aged rats were notably heavier than the young controls. It is possible that this could confound results, through a reduction in the primary motivation for food rewards. PR performance, at least within-subjects, declines with increasing body-weight (Ferguson & Paule 1997). Therefore, an attempt should be made to better control for age-related increases in body-weight.

6.3 Experiment 2

6.3.1 Methods

Animals and ageing procedures

The purpose of experiment 2 was firstly to examine whether the age-related decline in PR performance remained in aged animals that were exposed to long-term controlled feeding. To achieve this, aged rats were placed on a schedule of controlled feeding from adulthood and maintained on this schedule throughout the ageing process.

All rats (male, Sprague-Dawley) in experiment 2 were initially housed in an external holding facility (Agenda Life Sciences, Hatfield, UK), having been transferred from the breeding facility (Envigo, UK) at four weeks old. All animals (both young and aged) were placed on a schedule of controlled feeding at three months of age with body-weights regularly monitored. Young and aged were then transferred to the testing facility (Cambridge) for behavioural testing (at approximately three and fifteen months of age respectively, as in experiment 1). Therefore, all aged rats had restricted feeding for at least twelve months prior to behavioural testing (see table 6.1, Young cohort weight at start of training: 408g ± 4.5, start of testing 412g ± 5.5; aged cohort start of training 477g ± 5.7, start of testing 485g ± 5.6).

There were also some housing and husbandry differences in experiment 2. Rats were group housed in standard open top plastic cages (2-3 per cage) in a light- and temperature-controlled environment (lights on 1900-0700). All testing took place 5-7 days/week, in the animals' dark phase. Bedding was changed twice weekly. All experiments were regulated under the Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 and following ethical review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB).

		Young		**Aged**	
		Age at test (*N*)	**Weight (g)**	**Age at test (*N*)**	**Weight (g)**
Experiment 1	Cohort 1	6-8 (23)	457.59 ± 8.63	18-20 (17)	564.65 ± 7.64
	Cohort 2	3-4 (20)	292.01 ± 2.82	15-16 (17)	690.76 ± 7.72
Experiment 2	Cohort 3 (PR)	4-7 (15)	404.80 ± 8.27	16-19 (19)	471.84 ± 5.70

Cohort 4 (ERC)	7-9 (19)	432.67 ± 11.61	19-21 (18)	484.56 ± 6.14

Table 6.1: Details of each experimental cohort. Age in months, is accompanied by the numbers in each group. The mean bodyweight of each cohort and the SEM in parentheses. PR Progressive Ratio; ERC: Effort related Choice

Behavioural Apparatus

In experiment 2 all testing took place in standard Bussey-Saksida rat touchscreen chambers described in detail previously (Chapter 2).

Behavioural Assays

Touchscreen progressive ratio schedule

Standard touchscreen pretraining was administered as described in chapter 2. Animals were then trained on the PREXP ($5 * e^{(0.2*n)} - 5$) schedule described previously (chapter 2).

Effort-Related Choice task

ERC pretraining was performed as previously described (chapter 3), to a separate cohort of young and aged rats. Due to the low number of trials completed previously a FR3 schedule, as opposed to a FR5 schedule, was used alongside the freely available chow. Animals initially received four FR3 with chow habituation sessions. Subsequently animals received three consecutive sessions each of FR3, FR9 and FR15 ERC tests. Performance from the three sessions at each ratio were collapsed for data analysis. All behavioural measured were analysed as described previously.

Drugs

Scopolamine hydrobromide was dissolved in physiological saline and administered at a volume of 1 mg/ml, via IP injection, 30 minutes prior to testing. Doses of 0.1 and 0.3 mg/kg (PR) and 0.3 mg/kg (ERC) were utilised. All rats received a single habituation injection of saline prior to administration of any compounds. Scopolamine was administered in a within-subject Latin square design (PR) and cross over-design (ERC) to aged and young rats.

6.3.2 Results

Group differences in body-weight

As in experiment 1 the mean body-weights during both PR testing ($t(32) = 6.881$, $p < .001$) and ERC testing ($t(20.878) = 3.987$, $p < .01$). However, as shown in table 6.1, the magnitude in the age-related differences in weights was substantially smaller in experiment 2.

Aged rats fail to show any motivational deficits on touchscreen PR

Initially, the previous effect of ageing on PR performance was examined using a touchscreen operant system. Figure 6.3A shows that, unlike the previous experiment, there was no significant difference in breakpoint between the two age groups ($t(32) = .332$, $p = .742$). PRPs were also not affected by the age of the rats ($t(32) = .686$, $p = .231$). Measures of general activity, which can be observed in table 6.2 motoric activity were also largely unaffected by age.

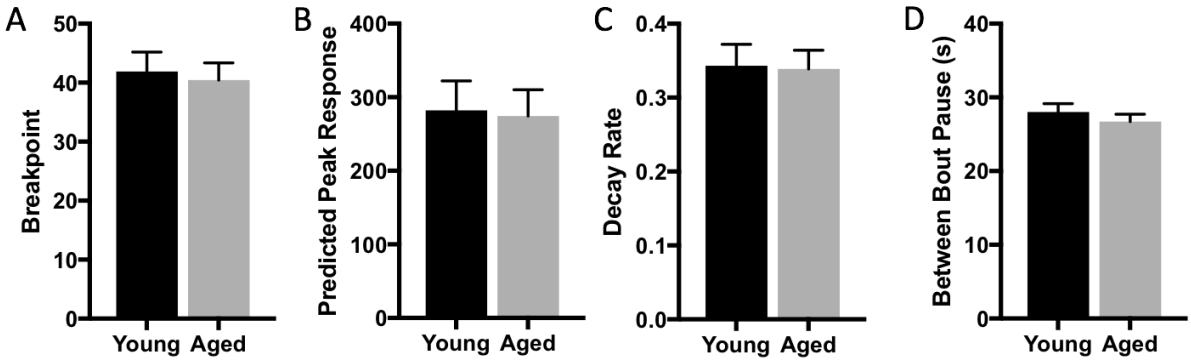


Figure 6.3: Effects of ageing on touchscreen PR performance. **A** Aged rats do not any deficit in PR. **B** The predicted peak response rate was not affected by age. **C** The decay rate did not differ between groups. **D** The pausing between response bouts was not affected by age. Young: 4-7months; Aged 16-19 months old. Error bars are ± SEM.

Analysis of response rates suggests that age did not affect the pattern of responding (figures 6.3B-D). The peak response rate (figure 6.3B) did not differ between the age groups ($t(32) = .145$, $p = .886$). The rate of decay in responding (figure 6.3C) was not significantly affected by the age group ($t(32) = .102$, $p = .919$). The average length of response bouts was also not affected by the age group ($t(32) = .265$, $p = .279$). Figure 6.3D shows that the mean length of the inter-bout pausing did not differ between age groups ($t(32) = .853$, $p = .400$). Together, these data suggest that this cohort did not display age-related differences in motivated behaviour or activity levels.

Progressive ratio	***Young***	***Aged***

Reward Collection latency (s)	1.57 ± .14	1.45 ± .12
IR Beam Breaks per sec	.10 ± .01	.09 ± .01
Nontarget Touches per sec	.03 ± .01	.04 ± .01
Magazine entries per sec	.06 ± .00*	.04 ± .00*

Table 6.2: Effects of ageing on measures of motoric activity during PR performance. Young: 4-7months; Aged 16-19 months old. * significant group difference ($p < .001$) All values are expressed as means ± SEM.

Scopolamine facilitates PR performance in both young and aged rats

The effects of scopolamine administration was subsequently tested in both young and aged rats. Figure 6.4A shows how breakpoints were significantly increased following scopolamine administration ($F(1.567, 50.144) = 8.940$, $p < .001$; partial eta squared = .218). There was also a significant effect of age group ($F(1,32) = 4.523$, $p < .05$; partial eta squared = .124). However, there was no interaction between age and scopolamine on breakpoint ($F(1.567, 50.144) = 8.940$, $p < .001$; partial eta squared = .218). As seen in figure 6.4B, post reinforcement pausing was not significantly affected by scopolamine ($F(2,64) = .111$, $p = .895$; partial eta squared = .126). There were also no effects of age ($F(1, 32) = .327$, $p = .571$), nor any age x scopolamine interaction on the duration of PRPs ($F(2,64) = .982$, $p = .380$). Measures of motoric activity are available in table 6.3

	Young			**Aged**		
Dose Scopolamine (mg/kg)	**Veh**	**0.1**	**0.3**	**Veh**	**0.1**	**0.3**
Reward Collection latency (s)	1.66 ± .18	1.76 ± .20	1.53 ± .56	1.50 ± .16	1.60 ± .17	2.47 ± .50
IR Beam Breaks per sec	.09 ± .01	.14 ± .02	.13 ± .02	.08 ± .01	.09 ± .01	.13 ± .01
Nontarget Touches per sec	.01 ± .00	.01 ± .00	.01 ± .01	.01 ± .00	.01 ± .00	.02 ± .00
Magazine entries per sec	.05 ± .01	.08 ± .01	.08 ± .00	.05 ± .01	07 ± .01	.09 ± .01

Table 6.3: Effects of ageing and scopolamine on measures of motoric activity during progressive ratio performance. All values are expressed as means ± SEM. Young: 4-7months; Aged 16-19 months old.

The predicted peak response rate was not affected by either scopolamine ($F(1.000, 32.001) = 1.805$, $p = .189$) or age ($F(1,32) = 1.747$, $p = .196$). There was also no interaction between age and dose on the peak response rate ($F(1.000, 32.001) = 1.735$, $p = .185$). Scopolamine did however, significantly increase the rate of decay in responding, as seen in figure 6.4C ($F(1.322,33.145) = 4.245$, $p < .05$; partial eta squared = .117). There was no effect of either age on the rate of decay in responding ($F(1,32) = 2.274$, $p = .141$) or any interaction between age and dose ($F(2,64) = 2.358$, $p = .103$). The mean length of response bouts was significantly reduced by administration of scopolamine ($F(2,64) = 4.594$, $p < .05$; partial eta squared = .126). However, there were no effects of age ($F(1,32) = .185$, $p = .670$) or any age x scopolamine interaction on bout length ($F(2,64) = .917$, $p = .405$). The length of pausing between bouts was not affected by neither scopolamine ($F(2,64) = 1.943$, $p = .152$), age group ($F(1,32) = 1.707$, $p = .201$), nor any interaction between age and dose ($F(2,64) = 1.393$, $p = .256$) on the length of pausing between response bouts. These data replicate the effects of scopolamine on PR performance previously reported in mice, of enhancing effort expenditure on a PR schedule of reinforcement. There was no evidence of any differential effects of scopolamine with age, as scopolamine enhanced motivated behaviour equally in both young and aged rats. This suggests that the efficacy of the drug is not affected by a potential age-related decline in muscarinic receptors.

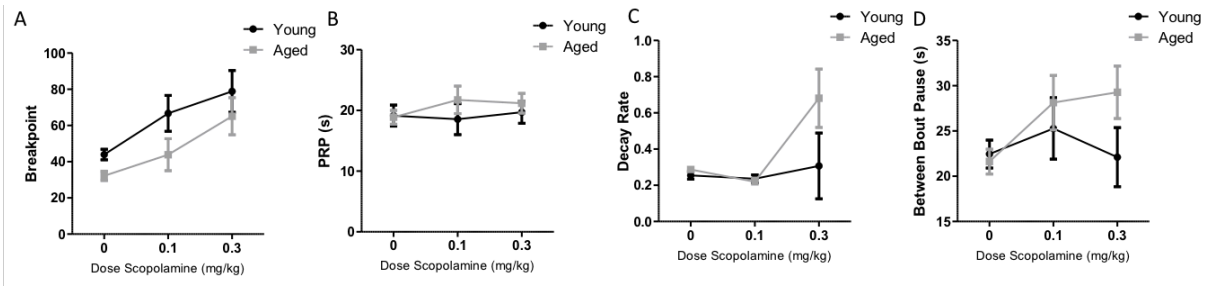


Figure 6.4: Scopolamine facilitates PR performance in young and aged rats. **A** Scopolamine dose-dependently increases breakpoint. **B** Neither scopolamine nor age affect the post reinforcement pause (PRP). **C** Scopolamine, at high doses, can increase the rate of decay in responding. **D** Neither age nor scopolamine significantly affect the length of pausing between response bouts. Young: 4-7 months; Aged 16-19 months old. Error bars are ± SEM.

Aged rats do not show any motivational deficits on touchscreen ERC

The previous results suggest that in long-term food controlled aged rats, effort exertion does not differ from young controls. We intended to extend these findings by investigating whether a separate facet of motivated behaviour, effort-related decision making, was affected by ageing.

Performance of a separate group of young and aged rats was compared on the previously validated effort related choice (ERC) task, in a new cohort of animals.

The ratio requirement for the food pellet reward was increased across sessions from FR3 to FR9 and FR15. Figure 6.5A shows that increasing the ratio requirement significantly reduced the number of trials completed ($F(1.087,32.623) = 281.100$, $p < .001$; partial eta squared = .904). In contrast, the number of trials completed did not differ between age groups ($F(1,30) = 1.794$, $p = .191$). There was however, a significant interaction between age and ratio on the number of trials completed ($F(1.087,32.623) = 4.383$, $p < .05$; partial eta squared = .127). In both young and aged rats, the number of trials completed within each ratio condition differed significantly from each other condition (all $p < .05$). However, there was no difference, between age groups, in the number of trials completed at any ratio (all $p > .05$).

Increasing the ratio requirement, as seen in figure 6.5B, significantly increased the amount of chow consumed ($F(2,60) = 27.722$, $p < .001$; partial eta squared = .471). Overall, aged animals also consumed significantly more chow than the young group ($F(1,30) = 4.268$, $p < .05$; partial eta squared = .125). There was no interaction between the ratio requirement and age group ($F(2,60) = .747$, $p = .478$). Figure 6.5C shows that increasing the ratio requirement significantly increased the duration of the mean PRP ($F(1.492,44.746) = 34.137$, $p < .001$; partial eta squared = .532). The duration of PRPs was not affected by either age ($F(1.30) = .159$, $p = .693$) or by any interaction between age and ratio ($F(1.492,44.746) = .816$, $p = .417$). Measures of general activity also did not differ between age groups, as seen in table 6.4. Together, these results suggest that ageing did not affect effort-related decision making, with young and aged rats equally affected by increasing the costs associated with a high value reward.

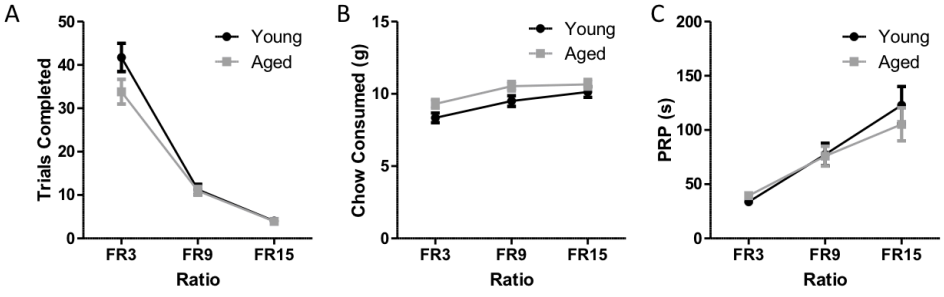


Figure 6.5: Age does not affect effort-related choice performance. **A** Increasing the ratio requirement decreases the number of trials completed. **B** Increasing the ratio requirement increases chow consumption. **C** The length of post reinforcement pausing (PRP) increases at higher ratios. Young: 7-9 months; aged 19-21 months old. $p < .05$; Error bars are ± SEM.

	Young			Aged		
Ratio	***FR3***	***FR9***	***FR15***	***FR3***	***FR9***	***FR15***
Reward Collection latency	2.69 ± .79	1.87 ± .26	1.55 ± .14	3.43 ± .69	1.70 ± .23	1.50 ± .12
IR Beam Breaks per sec	.12 ± .01	.08 ± .01	.07 ± .01	.09 ± .01	.06 ± .01	.05 ± .01
Nontarget Touches per sec	.001 ± .00	.001 ± .00	.001 ± .00	.002 ± .00	.003 ± .00	.002 ± .00
Magazine entries per sec	.04 ± .00	.01 ± .00	.01 ± .00	.03 ± .00	.01 ± .00	.01 ± .00

Table 6.4: Effects of ageing and ratio requirement on measures of motoric activity during effort-related choice performance. All values are expressed as means ± SEM. Young: 7-9 months; Aged 19-21 months old

Scopolamine interferes with effort-related choice behaviour in young and aged rats

The effect of scopolamine (0.3 mg/kg) was tested on the ERC assay using an FR3 schedule of reinforcement. Figure 6.6A shows scopolamine administration significantly reduced the number of trials completed ($F(1,30) = 9.774$, $p < .01$; partial eta squared = .240). However, the number of trials completed was not affected by either age ($F(1,30) = .656$, $p = .424$) or by any interaction between age and drug ($F(1,30) = .305$, $p = .583$). In contrast, scopolamine administration produced no significant effect on chow consumption, as shown in figure 6.6B ($F(1,30) = 2.766$ $p = .106$). Chow consumption was not affected by age group ($F(1,30) = .234$, $p = .632$) or by any interaction between age and scopolamine ($F(1,30) = .200$ $p = .658$). Figure 6.6C suggests that there were no significant effects on the duration of PRPs [scopolamine ($F(1,30) = 1.586$ $p = .221$); age ($F(1,30) = 1.284$ $p = .269$); age x scopolamine interaction ($F(1,30) = .048$ $p = .827$)]. The effects of scopolamine administration on motoric measures can be seen in table 6.5.

	Young		***Aged***	
Dose Scopolamine (mg/kg)	*Veh*	*0.3*	*Veh*	*0.3*
Reward Collection latency	4.76 ± 3.53	2.35 ± 1.50	7.43 ± 4.02	5.24 ± 1.27
IR Beam Breaks per sec	.07 ± .01	.09 ± .02	.07 ± .01	.09 ± .01

Nontarget Touches per sec	.001 ± .00	.002 ± .00	.001 ± .00	.002 ± .00
Magazine entries per sec	.02 ± .00	.02 ± .00	.02 ± .00	.02 ± .00

Table 6.5: Effects of ageing and scopolamine on measures of motoric activity during effort-related choice performance. All values are expressed as means ± SEM. Young: 7-9 months; Aged 19-21 months old

Together, these results suggest that scopolamine supresses touchscreen responding equally in both young and aged rats. In spite of facilitating unspecific activity levels (indexed by the rate of beam breaks and nontarget screen responses) scopolamine reduced the number of trials completed and the amount of chow consumption.

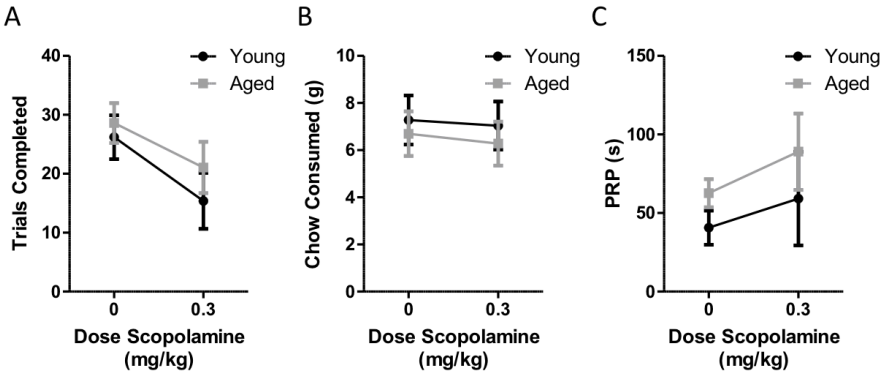


Figure 6.6: Effects of scopolamine and ageing on an ERC task. **A** Scopolamine reduces the total number of trials completed in both young and aged rats. **B** Neither scopolamine nor ageing affect the amount of chow consumed. **C** Scopolamine increases the length of the post reinforcement pause (PRP). Young: 7-9 months; Aged 19-21 months old ERC: Effort related choice Error bars are ± SEM.

6.3.3 Discussion

When tested on the previously validated touchscreen-based assays of motivation (chapters 2 and 3), aged rats did not show any evidence of a motivational deficit compared to younger control animals. Aside from differences in the housing/husbandry conditions (see *methods*), the major difference between experiments 1 (Eli Lilly) and 2 (Cambridge) was the control of feeding behaviour throughout the ageing process. When aged animals underwent long term (>12 months) controlled feeding, no motivational deficit was observed. The effects of these additional feeding controls can be observed by a reduction in size of the group differences in body-weights between the experiments (table 6.1). This raises the possibility that the effects

observed in experiment 1 were driven primarily by a reduction in appetite or the value of food rewards.

It should also be noted that a separate possibility is that differences in the results of experiments 1 and 2 arise from the operant systems used. In the young cohorts, breakpoints were substantially higher when tested on the lever-based PR system. Measures of responding (such as breakpoint) are substantially lower in rats tested on touchscreen-based tasks (reviewed in chapter 2). The lower baseline performance may have reduced the sensitivity of the task to detect an age-related change in PR performance. Differences in the rates of responding between touchscreen-based and lever-based operant systems may have obscured an age-related effect. As shown in table 6.6, there was a tendency across all PR conditions for young animals not to reach a breakpoint within the 45-minute session (i.e. not to have undergone 180s without a screen response).in other words, the previous results may have reflected in parts differences in rates of responding, rather than differences in effort exertion per se. The use of a longer touchscreen PR session may have help investigate such an age related-effect.

	Young	Aged
PR Baseline	11/75	4/95
Veh	0/15	0/19
0.1 mg/kg scopolamine	3/15	1/19
0.3 mg/kg scopolamine	6/15	5/19

Table 6.6: Number of sessions ending with rats failing to reach breakpoint during touchscreen progressive ratio training and during the scopolamine experiment.

It should also be noted that in the attempt to better control for differences in body-weight aged animals were food restricted for a significantly longer period of time. It is possible that the chronic food restriction conceals any deficit in effort exertion by an increase in the motivation for food. However, if this were the case it may be expected that the aged animals would consume significantly more freely available chow in the ERC assay, however this was not the case. This could be further strengthened by including free-feeding probes in any subsequent experiments.

Effect of scopolamine on effort-based behaviour in rats

In spite of a lack of an age-related deficit in motivation, it was still important to test the effects of mAChR antagonism on effort-based behaviour. Firstly, to extend our previous results in mice (chapter 5) to a separate species using tasks reinforced with a different (dry) food reward. Secondly, as some changes in mAChR function with age have been noted (Morin & Wasterlain 1980; Rinne 1987; Biegon et al. 1989; Schwarz et al. 1990; Tayebati et al. 2004), it is useful to test whether mAChR antagonism was able to affect behaviour. Scopolamine dose-dependently enhanced PR performance. This result replicates those found previously and suggests that age related changes in mAChR function or expression do not substantially affect the efficacy of mAChR antagonists on behaviour.

Subsequently, the effective dose of scopolamine was found to interfere with ERC performance, indexed by the number of touchscreen trials completed. This result was unexpected given that scopolamine facilitated effort exertion in PR and previously scopolamine did not suppress the number of trials completed by mice (chapter 5). There are other examples of pharmacological interventions facilitating PR performance but interfering with effort-related decision making. For example, amphetamine increases PR breakpoints (Poncelet et al. 1983). In contrast, amphetamine supresses trials completed in an FR/choice ERC task (Cousins et al. 1994), albeit at higher doses than those effective in PR. This suppression is likely to be a consequence of the appetite supressing effects of amphetamine (MacPhail & Gollub 1974). Similar effects may be occurring following scopolamine administration. Scopolamine can supress consumption of dry foods, likely due to the peripheral actions given rise to 'dry-mouth' type symptoms (Drevets & Furey 2010; Hodges et al. 2009). This may limit the rats' ability to consume large amounts of food pellets in ERC. This is less likely to affect PR performance given the low amount and frequency of rewards earned. Furthermore, liquid reinforcers are less susceptible to these peripheral effects of scopolamine administration (Hodges et al. 2009), which could explain why we previously (chapter 5) did not find a suppression of FR5 performance reinforced with milkshake. In line with this explanation, there was a trend for scopolamine to decrease the amount of chow consumed.

A related possibility is that scopolamine affects decision-making processes. Scopolamine is often used to disrupt cognition in rodents (Klinkenberg & Blokland 2010). Alongside effort, the costs of an action can be altered by altering the delay to reinforcement (Thiébot et al. 1985; Winstanley et al. 2003) or the probability of a reward being delivered (Richards et al. 1999). Increasing the delay or decreasing the probability of reinforcement will shift responding to a less preferred reward choice (Winstanley et al. 2003; Richards et al. 1999). Crucially,

scopolamine also interferes with both delay-based and probability-based decision making. A number of pharmacological interventions have been shown to potentiate the effects of increasing costs on behaviour, increasing the rate of discounting (e.g. Winstanley et al. 2003; 2006; Floresco et al. 2008). Such manipulations typically have little effects when the costs associated with the large reward are low. In contrast, scopolamine affects large reward choices, even in the absence of any costs (Mendez et al. 2012). Furthermore, scopolamine has been shown to affect the time taken to make a choice in a rodent gambling task (Silveira et al. 2015). Together, these results suggest that scopolamine may interfere with a rat's ability to choose between multiple actions. Such an effect could explain the reduction in ERC trials completed in the present study. Conversely, in PR where there is no choice to be made, disruptions to decision-making may not interfere with performance.

6.4 General Discussion

6.4.1 Ageing as an inconsistent model of apathy

Across species, ageing is associated with a decline in a number of cognitive processes (Morrison & Baxter 2012; Samson & Barnes 2013). Concomitant declines in motivated behaviour with age, have also been reported across species (Onyike et al. 2007; Bordner et al. 2011; Blokland & Raaijmakers 1993). The present chapter sought to establish and explore a potential age-related decline in motivated behaviour in rats, in order to test to efficacy of putative motivation enhancing compounds. Motivated behaviour was initially tested by examining lever pressing for food under a PR schedule of reinforcement. Aged rats had significantly lower breakpoints than animals 12 months younger, indicating lower motivation to work for food rewards. This deficit was subsequently replicated in a separate group of aged rats. However, both cohorts also had significantly different between-group bodyweights. It is possible that any age-related deficit was confounded by differences in weight. Therefore, an attempt was made to better control for weight differences by controlling the feeding of the aged rats throughout adulthood. Although the aged rats in the subsequent experiment still had significantly higher body-weights than the young controls, the magnitude of the difference was smaller. With this additional control, aged rats did not display any differences in motivated behaviour compared to their young controls.

The life-extending effects of long-term controlled feeding in rats have been long known (McCay et al. 1935). Further research has revealed a dose-dependent effect of calorific restriction on life-expectancy in rats (Weindruch et al. 1986). Reducing the calorific intake of

rodents has been shown to reduce physiological processes associated with age such as development of tumours and myocardial degeneration (Tannenbaum 1942; Yu et al. 1982). In addition, long-term calorific restriction reduces age-related neurophysiological changes in rodents (Joseph et al. 1983; Levin et al. 1981). Alongside the many physiological benefits, calorific restriction has been shown to reduce age-related behavioural changes. Life-long calorific restriction improves performance in assays of spatial memory (Stewart et al. 1989; Adams et al. 2008; Yang et al. 2014) as well as improving motor coordination (Ingram et al. 1987). Together, these studies indicate that food restriction reduces the rate of neurocognitive ageing in rats (Gallagher et al. 2011). In the present study, it is possible that the long-term controlled feeding in experiment 2 preserved the cognitive processes regulating motivation, preventing the decline in motivated behaviours seen in aged rats in experiment 1. An alternative possibility is that the age-related PR performance did not reflect changes in motivation, but instead reflect changes in appetite or satiety. The long-term controlled feeding may have prevented or reduced the changes in the primary motivation for food, which may have confounded the previous PR results. Whether due to the neuro-protective effects of food restriction or differences in appetite, these studies highlight the potential confounding effects of food restriction in ageing studies. It should also be noted that previous reports of age-related changes in PR performance (Blokland & Raaijmakers 1993; Bordner et al. 2011; Amancio-Belmont et al. 2017) do not report any methods for controlling for differences in body-weight between the groups. The lack of appropriate controls may be the reason why these previous studies, like experiment 1, report age-related differences in PR performance.

A separate approach to investigate the effect of ageing on apathy would be to use a within subject design to investigate changes in effort exertion across a rodent's lifespan. PR may be especially suited to this, as PR is believed to have fewer effects of repeated testing than other rodent assays (Yhnell et al. 2016). Whereas, this would be a useful approach to investigate age-related changes in motivation, this would not fulfil the primary aim of this chapter in characterising a deficit model, against which to test the potential pro-motivational effects of mAChR antagonists.

6.4.2 *Conclusions*

A series of experiments suggest that the previously reported age-related decline in PR performance in rodents is not a robust effect. Aside from concomitant declines in locomotor activity could potentially confound PR performance, long-term controlled feeding appears to abolish the motivational deficit observed in aged rats. However, this may in part be due to the

limitations of the methodology employed. From the data presented in this chapter it is not possible to conclude whether the failure to replicate the initial age-related deficit was due to differences in the food restriction procedures or the differences in the operant systems employed. None of the previous studies reporting age-related deficits in PR performance in rodents (Blokland & Raaijmakers 1993; Bordner et al. 2011; Amancio-Belmont et al. 2017)
, have reported either efforts to control for differences in body weight or response rates between groups. Taken together, these results indicate that aged rats may not be a suitable model against which to test the pro-motivational effects of muscarinic receptor antagonists, with further research needed to investigate potential mediating factors that may underlie differences in breakpoint.

Chapter 7. Biperiden rescues a model of antipsychotic-induced amotivation

7.1 Introduction

Previous studies investigating novel therapeutic targets for motivational impairments have tested the efficacy of compounds in rescuing the deficits induced by pharmacological manipulations (e.g. Farrar et al. 2007; Sommer et al. 2014; Yohn, Collins, et al. 2016). Numerous studies have implicated dopamine in supporting effort-based behaviour (Salamone 1988; Salamone et al. 1991; Salamone & Correa 2012, reviewed in chapter 1). Systemic administration of dopamine receptor antagonists can disrupt performance on progressive ratio (PR) schedules (Cheeta et al. 1995) and effort related choice (ERC) tasks (Salamone et al. 1991; Salamone et al. 1994), in the absence of any significant effects upon appetite or motoric output (Salamone 1986; Salamone et al. 1991). A number of studies have subsequently tested the efficacy of compounds in reversing the motivational deficits induced by dopamine receptor blockade (Worden et al. 2009; Nunes et al. 2010). Among the compounds used to induce the initial deficit, the dopamine D_2 receptor antagonist haloperidol is particularly relevant given its use as an antipsychotic treatment. There is evidence to suggest that typical antipsychotics, including haloperidol exacerbate symptoms of apathy in patients with schizophrenia (Artaloytia et al. 2006) and affect motivation in healthy subjects (Saeedi et al. 2006; Mas et al. 2013). Systemic haloperidol has been used as a deficit model in both lever- and maze-based ERC tasks (Farrar et al. 2007; Mott et al. 2009). In these studies, co-administration of an adenosine A_{2A} receptor antagonist was able to rescue the deficit in effort-related decision making.

Previous results (Chapter 5) suggest that the M_1 receptor antagonist biperiden can facilitate motivated behaviour in intact mice. However, the evidence for therapeutic potential of this compound would be strengthened by also reversing a deficit. Therefore, in the present chapter the efficacy of biperiden in reversing a haloperidol-induced deficit in PR performance was examined. Given the previous results, it was hypothesised that biperiden would reverse the PR deficit induced by haloperidol. Scopolamine was also included as a positive control. Previously, neither scopolamine nor biperiden affected ERC performance. However, as discussed, this may have been due to a ceiling effect. Therefore, the effects of scopolamine and biperiden following haloperidol administration on ERC performance were also tested.

7.2 Materials and methods

7.2.1 Animals

Sixteen male C57BL/6 mice were used in this study. Mice were aged 8 weeks at the beginning of behavioural training. Animals were maintained at no less than 85% of their free feeding body-weight. No correction was applied to this 85% control weight to match the expected growth curve (weigh at start of testing: 23.3g ± 0.3). All husbandry, housing and feeding procedures and ethical considerations were identical to those previously described in detail (chapter 5).

7.2.2 Apparatus

All testing took place within Bussey-Saksida mouse touchscreen chambers (chapter 5).

7.2.3 Behavioural procedures

The experimental timeline can be seen in figure 1. Initially, animals underwent touchscreen PR pretraining as described previously (chapter 5). Once stable PR performance has been reached animals underwent pharmacological PR challenge.

Subsequently animals trained upon ERC. This consisted of three consecutive days of uncapped-FR5 sessions (as described earlier, chapter 5), followed by seven consecutive days of ERC sessions where approximately 5g of chow was placed in the chambers and an otherwise normal uncapped FR5 session was run. Following this, the drugs were administered as detailed below. All behavioural measures for PR and ERC were analysed as detailed earlier (chapter 5).

7.2.4 Drugs

All compounds were administered via intraperitoneal (IP) injections at a volume of 10ml/kg of each animal's bodyweight. 0.1mg/kg haloperidol (Bio-techne, Abingdon, UK) was dissolved in 0.2% tartaric acid and administered 40 minutes prior to testing. This dose was chosen at it can reduce effort output in the absence of any significant motoric effects in mice (Pardo et al. 2013; Correa et al. 2016). In the vehicle only condition, 0.2% tartaric acid was used as a vehicle. The pH was checked prior to administration. 0.3 mg/kg scopolamine hydrobromide and 3mg/kg biperiden hydrochloride (both Bio-techne, Abingdon, UK) were dissolved in physiological saline and administered 30 minutes prior to testing. Doses were selected based on those used previously (chapter 5).

7.2.5 Experimental design

Compound administration was performed identically for both PR and ERC tests, in a within-subject design. On each testing day mice received two injections. Initially mice received an

injection of either vehicle (0.2% tartaric acid) or haloperidol. These were followed approximately 10 minutes later with an injection of either vehicle (saline), scopolamine or biperiden. This created the following conditions: Vehicle/vehicle, haloperidol/vehicle, haloperidol/scopolamine and haloperidol/biperiden. Injections were administered to alternating IP sites to minimise distress to the mice. A single baseline day without drugs was administered in between all test days. The behavioural measures and statistical analyses took place for PR and ERC as previously described (see chapter 5).

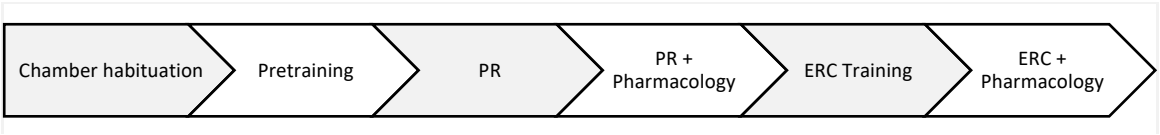


Figure 7.1: A timeline summarising the order of the experimental procedures. PR: Progressive ratio. ERC Effort related choice

7.3 Results

7.3.1 Biperiden and scopolamine reverse a haloperidol-induced deficit in PR performance

Figure 7.2A shows a main effect of drug administration on breakpoint ($F(3,45) = 14.680$, $p < .001$; partial eta squared = .495). Administration of haloperidol significantly reduced breakpoints relative the vehicle only condition ($p < .001$). Co-administration of either scopolamine ($p < .05$) or biperiden ($p < .01$) with haloperidol, significantly increased breakpoint compared to haloperidol alone. No other comparisons were significant (all $p > .05$). In contrast to the effects on breakpoint, PRPs were not significantly affected by drug administration ($F(3,45) = .304$, $p = .822$ figure 7.2B). The latency to collect rewards was however, significantly affected by drug administration ($F(3,45) = 4.144$, $p < .05$; partial eta squared = .216; figure 7.2C). Reward collection latencies were significantly longer in the haloperidol/scopolamine condition relative to vehicle ($p < .01$). No other comparisons were significant.

Overall, the rate of IR beam breaks, as shown in figure 7.2D, was significantly affected by drug administration ($F(2.235,33.521) = 4.245$, $p < .05$; partial eta squared = .221); however, there were no significant differences between any condition (all $p > .05$). The rate of nontarget screen touches was also affected by drug administration ($F(3,45) = 4.484$, $p < .01$; partial eta squared = .230; figure 7.2E). Administration of haloperidol significantly reduced the rate of nontarget touches compared to the vehicle only condition ($p < .01$). This reduction was significantly reversed by biperiden ($p < .05$) but not scopolamine ($p = .08$). The rate of magazine entries was

also significantly affected by drug administration ($F(1.799,26.986) = 11.126$, $p < .001$; partial eta squared = .426). The rate of magazine entries was significantly higher in the haloperidol/scopolamine condition relative to all other conditions (all $p < .05$). No other comparisons were significant.

The pattern in responding was also somewhat affected by haloperidol. There was no effect upon the predicted peak response rate ($F(1.013,15.193) = 1.355$, $p = .263$). However, the decay rate in responding was significantly affected ($F(1.399,27.388) = 4.438$, $p < .05$; partial eta squared = .228), although there were no significant differences between any condition. The average response bout length was not affected by drug administration, although there was a trend towards an effect ($F(1.974,29.613) = 2.631$, $p = .089$; figure 7.2F). The mean pause between response bouts was significantly affected by drug administration ($F(1.895,28.422) = 3.828$ $p < .05$; partial eta squared = .203); however, again there were no significant differences between groups. These results suggest that nonspecific antagonism of mAChRs, as well as a more selective M_1 receptor antagonist can reverse a deficit in motivation arising from dopamine receptor blockade.

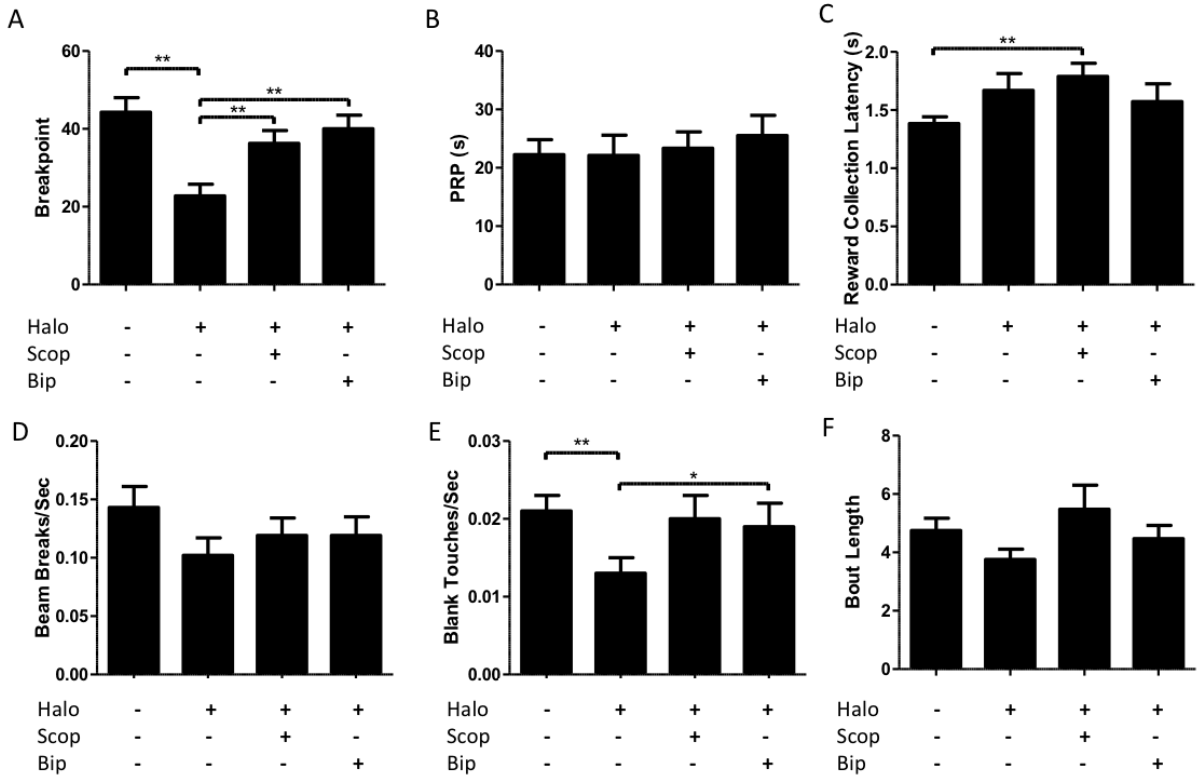


Figure 7.2: Scopolamine and biperiden reverse a haloperidol induced deficit in PR performance. **A** Haloperidol reduces breakpoint; co-administration of either scopolamine or biperiden is able to reverse the effects of haloperidol on breakpoint. **B** No drug combination affects post reinforcement pause (PRP). **C** Reward collection latency is significantly increased

by a combination of haloperidol and scopolamine. **D** The rate of IR beam breaks were not affected by drug administration. **E** The rate of nontarget (blank) screen responses was reduced by haloperidol and partially rescued by co-administration of biperiden. **F** The mean bout length was not affected by any drug condition. Halo: Haloperidol 0.1mg/kg; Scop: Scopolamine 0.3 mg/kg; Bip: Biperiden 3mg/kg. Error bars display the SEM. *$p < .05$, **$p < .01$.

7.3.2 Biperiden, but not scopolamine, reverses a haloperidol induced deficit in effort-related choice performance

Effort -related choice (ERC) behaviour may offer a better test of motivation (Markou et al. 2013) and can differentiate between directional and activational components of motivation. However, in our previous attempts to use a touchscreen-based ERC (chapters 3,5,6), rodents showed low baseline levels of operant responding. Therefore, it was initially important to demonstrate that mice display a significant preference towards responding for milkshake over the freely available chow. As shown in figure 7.3, during baseline ERC performance (the mean performance from the final two sessions prior to drug administration) mice consumed significantly more milkshake from FR5 responding than chow ($t(15) = 5.324$, $p < .001$), suggesting a significant preference towards operant responding for milkshake.

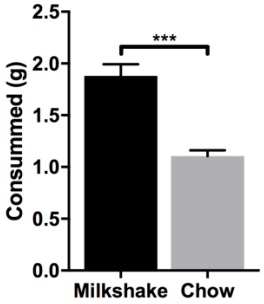


Figure 7.3: Mice show a significant preference towards FR5 responding for milkshake during baseline ERC testing. Error bars display the SEM. ***$p < .001$

Drug administration, as shown in 7.4A, significantly affected the number of trials completed ($F(3,45) = 10.386$, $p < .001$; partial eta squared = .409). Haloperidol significantly reduced the number of trials completed relative to vehicle ($p < .01$). Co-administration of biperiden ($p< .01$), but not scopolamine ($p > .05$) with haloperidol, was able to increase the number of FR5 trials completed compared to administration of haloperidol alone. Additionally, when receiving biperiden as treatment, mice made significantly more trials compared to when receiving scopolamine ($p < .01$). Chow consumption, as shown in 7.4B, was also affected by drug administration ($F(3,45) = 4.861$, $p< .01$; partial eta squared = .245). Both biperiden and scopolamine reduced the chow consumption following haloperidol administration ($p <.05$). In

contrast, as seen in figure 7.4C, drug administration did not significantly affect the total weight of food consumed, (F(1.805,27.705) = 2.879, p = .078), suggesting no changes in overall appetite.

PRPs were significantly affected by drug administration (F(3,45) = 10.386, p < .001; partial eta squared = .409; figure 7.4D). Haloperidol significantly increased the duration of the mean PRP (p < .01). The effect of haloperidol upon PRPs was reversed by both scopolamine and biperiden (p <.01). There was also a significant effect of drug administration on the latency to collect rewards (F(3,45) = 11.011, p < .001; partial eta squared = .423, figure 7.4E). Haloperidol increased the reward collection latency relative to vehicle only condition (p <.01). Reward collection latencies following co-administration scopolamine and biperiden condition were also significantly longer compared to the vehicle only condition (both p < .05). The rate of nontarget touches was not significantly affected by drug administration (F(3,45) = 42.668, p = .059). The rate of IR beam breaks, as shown in figure 7.4F, was significantly affected by drug administration (F(3,45) = 3.988, p < .05; partial eta squared = .210); however, no difference between any condition was significant (all p > .05). There was also a significant effect of drug on the rate of magazine entries (F(3,45) = 11.366, p < .001; partial eta squared = .431). Haloperidol administration reduced the rate of magazine entries relative to vehicle (p<.01). This was reversed following co-administration of biperiden (p <.01) but not scopolamine (p > .05). The rate of magazine entries in the scopolamine condition was lower than both the vehicle only and biperiden conditions (p < .05). Together, these data demonstrate that biperiden but not scopolamine, was able to reverse the deficit in ERC performance induced by haloperidol.

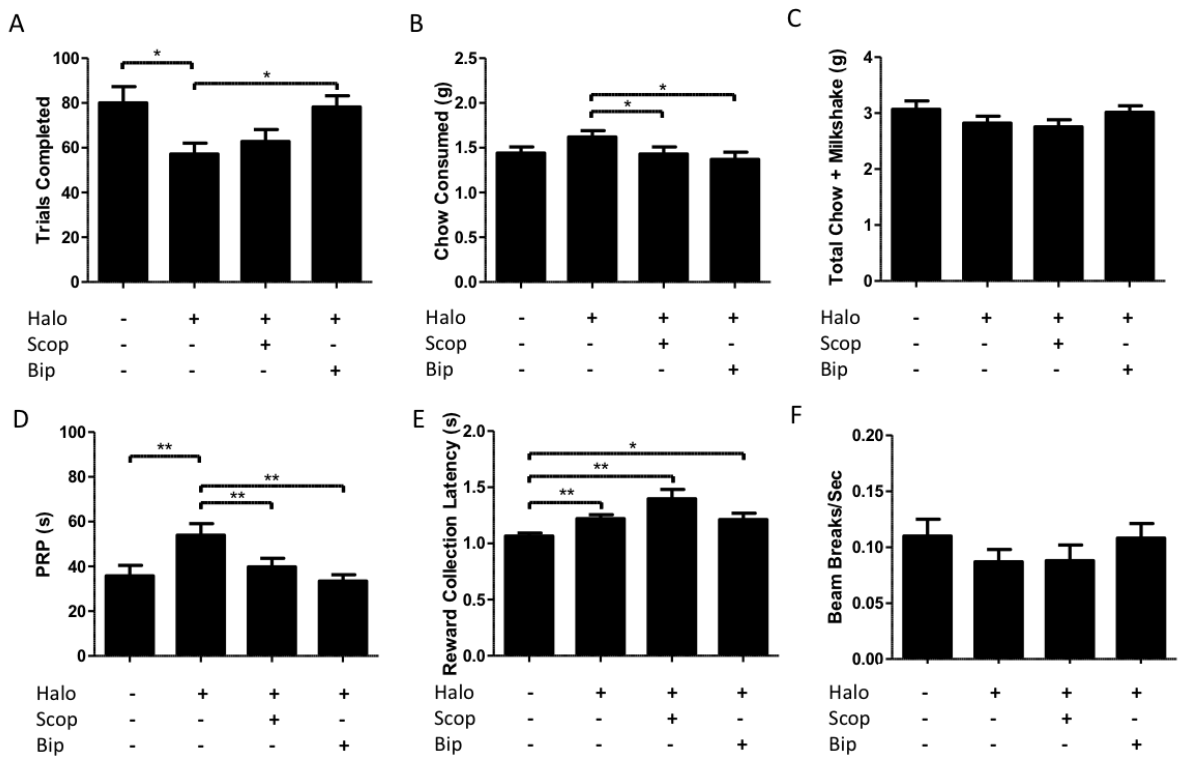


Figure 7.4: Biperiden rescues a haloperidol induced deficit in effort-related choice performance. **A** Co-administration of biperiden, but not scopolamine alongside haloperidol increases the number of touchscreen trials completed. **B** Administration of both scopolamine and biperiden decrease the amount of freely available chow consumed, **C** No drug combination significantly affects the overall amount of food consumed **D** The duration of post reinforcement pauses (PRP) was significantly increased by haloperidol and reversed by co-administration of either scopolamine or biperiden. **E** Haloperidol increases the reward collection latencies, which were not rescued by co-administration of either drug. **F** No drug combination significantly affected the rate of IR beam breaks. Halo: Haloperidol 0.1mg/kg; Scop: Scopolamine 0.3 mg/kg; Bip: Biperiden 3mg/kg. Error bars display the SEM. *$p < .05$, **$p < .01$

7.4 Discussion

In order to strengthen the evidence of a therapeutic benefit for biperiden, the effects of mAChR antagonists upon a dopaminergic deficit model of impaired effort-related behaviour were tested. Effort-based behaviour, measured by assays such as progressive ratio (PR) and effort-related choice (ERC) allows for motivational deficits to be modelled within a preclinical setting (Salamone et al. 2015; Salamone, Yohn, et al. 2016). Subsequently, the efficacy of novel therapeutics in reversing these deficits could be tested. The preferential M_1 antagonist biperiden was able to reverse the effects of the dopamine receptor antagonist haloperidol on

both PR and ERC performance, strengthening the preclinical evidence for possible therapeutic potential.

7.4.1 Haloperidol as a model of amotivation

Initially, it was important to demonstrate that haloperidol was able to induce a motivational deficit upon touchscreen-based assays of motivation, in mice. It was also important to examine whether haloperidol produced significant locomotor changes, as at high doses, dopamine D_2 receptor antagonists can impair motor output (Simón et al. 2000). It is possible that the subsequent actions of a compound were simply restoring motoric output, as mAChR antagonists have also been reported to increase locomotor activity (Meyers & Wilchin 1969; Chintoh 2003). At the present dose there was no evidence of any substantial motoric deficits following haloperidol administration prior to PR testing. In itself haloperidol did not significantly slow reward collection latencies or cause changes in structure of response bouts seen as a measure of motoric integrity (Brackney et al. 2011; Phillips et al. 2017). Dopamine receptor antagonists have also been observed to affect feeding behaviours (Clifton et al. 1991). A disruption of such processes could manifest as a disruption of PR performance resembling amotivation (Maccioni et al. 2008). However, haloperidol did not affect the total amount of food consumed in the ERC assay, instead the effect was to shift behaviour from touchscreen responding towards consumption of freely available chow. This result suggests that the suppression in responding was not as a consequence of a reduction in appetite. Haloperidol also significantly increased the PRP suggesting a reduction in touchscreen engagement. Together, these results suggest that haloperidol was effective at disrupting motivated behaviour on both the PR and ERC assays.

It should be noted that only moderate impairments were induced in both assays. This dose of haloperidol has been used previously to induce a deficit in lever-based PR in rats (Olarte-Sánchez et al. 2013), lever-based ERC in rats (Farrar et al. 2007) and maze-based ERC in mice (Pardo et al. 2012). Presently, haloperidol reduced breakpoint by approximately 50%, which is comparable to the effects of equal doses in rats (Olarte-Sánchez et al. 2013). However, haloperidol administration resulted in a ~ 30% reduction in touchscreen responses within the present ERC assay, previous studies have reported a ~90% reduction in lever-pressing (Farrar et al. 2007). This difference may be a result of the general lower total number of responses and rate of responding typically observed in touchscreen operant systems relative to lever-based chambers (chapter 2). Indeed, the ~30% reduction in high-effort choices is similar to the magnitude induced by this dose of haloperidol on maze versions of ERC tasks in both rats and

mice (Mott et al. 2009; Pardo et al. 2012). Taken together, these results suggest administration of haloperidol was successful at inducing a motivational deficit.

Previous studies have used other compounds to induce a motivational deficit. The vesicular monoamine transport inhibitor, tetrabenazine has been widely used as a model of amotivation (Nunes, Randall, Hart, et al. 2013; Randall, Lee, Nunes, et al. 2014; Yohn, Lopez-Cruz, et al. 2016). The physiological actions of tetrabenazine are more widespread than haloperidol, affecting D_1 receptor signalling, as well as the monoamines noradrenaline and serotonin (Pettibone et al. 1984). It could be argued that tetrabenazine, as a manipulation, induces a more physiological state of amotivation, one that may be particularly relevant to the motivational disturbances arising from depression (Nunes, Randall, Hart, et al. 2013). Haloperidol was chosen to induce amotivation given the link between motivation and the D_2 receptor in particular (reviewed in chapter 1). Furthermore, unlike other, more selective D_2 receptor antagonists, the effects of haloperidol on effort-related behaviour have been well validated (Salamone 1986; Salamone et al. 1991). Finally, haloperidol has been shown to induce motivational impairments in humans, suggesting a translational model of apathy (Mas et al. 2013).

7.4.2 Biperiden reverses a haloperidol induced deficit in effort related behaviour

We previously demonstrated that the M_1 receptor antagonist biperiden was effective at enhancing motivated behaviour in intact mice. The evidence for a potential therapeutic benefit of biperiden was extended by successfully reversing the PR deficit in an antipsychotic induced model of amotivation. Co-administration of biperiden with haloperidol was able to reverse breakpoint close to baseline levels (figure 7.2A). This result strengthens the previous argument that mAChR antagonists facilitate motivated behaviour. Previously, it was demonstrated, at least in rats, that PR performance is related to neural activity within the NAc (Chapter 4). In humans, aberrant ventral striatal structure and function has been highlighted as a major contribution to apathy (e.g. Kirschner et al. 2016, see chpter 1 for a review). Subsequent studies may wish to examine the effects of biperiden upon NAc activity during PR responding.

Biperiden was also found to rescue the effects of haloperidol upon effort-related decision making. ERC assays may be less susceptible to non-motivational confounds (Salamone et al. 2002) and closer resembles some of the decision making assays used in humans (Treadway et al. 2009; Chong et al. 2015; Young & Markou 2015). This result demonstrates biperiden reverses a dopaminergic deficit in effort-related decision-making. This matches the previous

findings of compounds such as dopamine reuptake inhibitors (Sommer et al. 2014) and adenosine A_{2A} antagonists (Farrar et al. 2007; Mott et al. 2009) at rescuing the effects of dopamine receptor antagonism. There is a previous report of scopolamine, infused into the nucleus accumbens (NAc), partially reversing the motivational deficits induced by intra-NAc infusion of pilocarpine, a nonselective mAChR agonist (Nunes, Randall, Podurgiel, et al. 2013). However, the present finding is the first example of a subtype-preferential mAChR antagonist reversing a dopaminergic deficit in motivation. This is particularly relevant give the previously discussed association between dopamine and apathy. Therefore, biperiden may be a useful therapy for apathy in hypodopaminergic conditions.

7.4.3 Scopolamine fails to reverse the deficit in effort related decision making

Whereas scopolamine, like biperiden, reversed the induced deficit in PR breakpoints, the compound did not reverse the effects of haloperidol upon ERC performance. It is noteworthy that an effective dose of scopolamine on PR performance did not facilitate ERC behaviour. As discussed previously (chapter 6) scopolamine disrupts decision making processes (Mendez et al. 2012), which may affect ERC performance. A separate possibility is that scopolamine affects feeding behaviour. Whereas, there is some evidence of systemic scopolamine supressing food consumption in rats (Hodges et al. 2009), scopolamine at the present dose did not significantly affect overall food consumption (Figure 7.4C).

We previously found (chapter 5) that scopolamine enhances locomotor activity, in line with previous reports (Meyers & Wilchin 1969; Chintoh 2003; Shannon & Peters 1990). An enhancement in locomotor activity would likely facilitate PR performance (Bailey et al. 2015). It is possible therefore, that the reversal of the PR deficit was partially mediated by an increase in such activity; whereas it is possible ERC performance may be less sensitive to such changes. It is also possible that the actions of biperiden, occurred by facilitating motor activity, which can be observed at higher doses (Sipos et al. 1999). This is not necessarily problematic, given high rates of fatigue that are associated in neurodegenerative and psychiatric conditions (Friedman & Friedman 1993; Karlsen et al. 2001; Chaudhuri & Behan 2000; Lou et al. 2003). Indeed, many of the other classes of drugs investigated as therapeutic targets for disorders of motivation, such as dopamine reuptake inhibitors and both 5-HT_{2C} and adenosine A_{2A} receptor antagonists also increase locomotor activity (Marriott 1968; Correa et al. 2004; Fletcher et al. 2006; Marin et al. 2011; Browne et al. 2017). Therefore, a reduction in fatigue alongside motivational effects may be of clinical benefit; however, caution should be adopted that the reversal of a deficit is not simply and additive effect on motor output.

7.4.4 Biperiden as a therapeutic tool

Numerous studies have reported an association between aberrant dopamine function and motivation in disorders including PD (Chong et al. 2015), HD (André et al. 2010) and antipsychotic-induced apathy (Artaloytia et al. 2006). Together these studies highlight the potential clinical significance of the efficacy of biperiden in reversing a dopaminergic deficit. The significance of present results are further enhanced by the fact that biperiden is already used as an antiparkinsonian treatment, as well as a treatment for the extrapyramidal side effects associated with antipsychotic medication (Gjerden et al. 2009). This suggests that the compound can be safely administered to these patient groups, which both display high rates of apathy (Foussias et al. 2014; Pedersen et al. 2009). Although there are no reports of the effects of biperiden upon motivational function in Parkinson's disease, there are reports that biperiden may reduce negative symptoms in schizophrenia (Tandon et al. 1992). However, as no specific measures of motivation were reported, this reduction in overall negative symptoms may be a consequence of the mood-enhancing effects of biperiden (Fleischhacker et al. 1987) or by affecting some other non-motivational symptom.

There are also a number of problems that could arise from the use of mAChR antagonists as a therapy. Aside from peripheral side effects (Guthrie et al. 2000), the use of biperiden in schizophrenia has been reported to worsen symptoms such as psychosis (Tandon et al. 1992). Furthermore, there are reports of abuse liability (Espi Martinez et al. 2012). However, other studies have suggested little evidence for biperiden abuse in clinical populations (Gjerden et al. 2009). Another important consideration may be the potential adverse effects upon cognitive function. M_1 receptor agonists and PAMs have also been evaluated as targets for cognitive enhancement (Friedman et al. 1999; Foster et al. 2014). For example, activation of M_1 receptors improves cognitive performance across species, including humans (e.g. (Brandeis et al. 1990; Nathan et al. 2013; Lange et al. 2015). Conversely, biperiden and scopolamine can impair cognition, across species (Safer & Allen 1971; Squire 1969; Glick & Jarvik 1969; Talpos et al. 2014; Sambeth et al. 2015; Klinkenberg & Blokland 2011). There is also some evidence that long term biperiden use may adversely affect cognitive functioning (Ogino et al. 2014). The putative clinical utility of targeting mAChRs for pro-motivational purposes should therefore be tempered by the potential cognitive impairment that could result from treatment. However, the dose of biperiden found to be effective in PR here is lower than those required to observe cognitive disruptions across several preclinical assays (Klinkenberg & Blokland 2011; Talpos et al. 2014; Malikowska et al. 2017). Furthermore, biperiden is currently used as a treatment in

PD and for the extrapyramidal side effects associated with antipsychotic treatment in Schizophrenia (Gjerden et al. 2009), suggesting that it can be administered in a clinically beneficial manner.

7.4.5 Conclusions

The present results highlight that biperiden can successfully rescue a preclinical model of apathy arising from dopamine receptor blockade. The more selective mAChR antagonist biperiden demonstrated greater efficacy in reversing the effects of haloperidol than the nonselective compound scopolamine. However, it is not clear whether this is due to differences in the selectivity profile of the compounds or some other factor. Together, the present results strengthen the preclinical evidence for a potential therapeutic benefit of biperiden, particularly in disorders associated with reduced dopamine function.

Chapter 8. General discussion

8.1 Overview

In recent years, there has been an increased focus on additional symptoms of neurodegenerative and psychiatric conditions. These include cognitive impairments such as memory, attention and executive functions (Marder 2006; Harvey 2008; Husain & Mehta 2011). Alongside these symptoms, impairments in motivation have been shown, across disorders, to be predictive of several key outcome measures such as patient quality of life, disease progression and caregiver burden. The aim of this present work was to facilitate the discovery of new approaches for the treatment of apathy. This was attempted by two separate approaches. Firstly, to refine the approaches used to measure motivated behaviour in rodents to maximise the likelihood of results translating across species. Secondly, to identify and interrogate a new pharmacological target for apathy.

8.2 Part 1: Facilitating cross-species translation.

The repeated failure of the effects of drug treatments to translate from rodents to humans is a major obstacle in the development of new CNS medicines (Geerts 2009; Cummings et al. 2014). There are likely a number of contributions to this "translational gap", ranging from inherent species differences and inappropriate experimental design to a lack of formal training in behavioural neuroscience (Zahs & Ashe 2010; Knopp et al. 2015; Bespalov & Steckler 2018). One key issue is the valid measurement of the constructs of interest in rodent preparations. Constructs such as memory and executive function can be readily measured in rodents (Keeler & Robbins 2011); however, the ability to model other clinical symptomology is more problematic. For example, attempts at modelling symptoms of psychosis, or depression in rodents has been problematic (Bergner et al. 2010; Forrest et al. 2014). Moreover, this has given rise to the term "-like" behaviour, which has been criticised as potentially masking a lack of validity (Garner 2014). In contrast, numerous studies have reported that motivation, through measuring effort, can be accurately measured in rodents (Salamone, Yohn, et al. 2016). Furthermore, a review of the literature (chapter 1) suggests that a number of pharmacological effects of drugs on effort-based behaviour translate from rodents into the clinic.

It may be possible to further refine the preclinical study of motivation to facilitate the likelihood of cross-species translation. One such approach has been to develop touchscreen-based assays for rodents (Hvoslef-Eide et al. 2016) that closer resemble the automated batteries used in nonhuman primates and humans (Owen et al. 1993; Weed et al. 1999). This approach can allow

near-identical tasks to be administered across species (Nithianantharajah et al. 2015). Effort-based behaviour can be readily assessed in a number of species (Cagniard et al. 2006; Treadway et al. 2009; Varazzani et al. 2015). However, the majority of previous pharmacological studies have been conducted in rats (reviewed in chapter 1). We therefore sought to develop and validate a battery of touchscreen-tasks to assess effort-based behaviour and decision making in rats. The PR task in particular seemed well suited to application within the rat operant touchscreen systems demonstrating sensitivity to detect changes in both outcome value and dopaminergic challenge. The key outcome of this study is that highly similar PR touchscreen assays can now be administered in rats, as well as mice, nonhuman primates and humans. Another observation arising from this study was the demonstration that multiple measures can be taken during operant responding to inform of changes in motivation. Through the use of such complementary measures, we were able to dissociate the effects of outcome manipulations and dopaminergic drugs which produce equivalent effects upon behaviour. Such an approach is especially relevant in the study of motivation where motoric changes could easily confound results (Bailey et al. 2015). Furthermore, a number of these measures can also be assessed in human participants (Williams et al. 2011).

However, the assays of effort-based decision-making, appeared less suited for use in rat touchscreen systems, in their current design. For example, high rates of low-effort chow consumption in effort-related choice (ERC) and omission rates in effort discounting (EFD), suggest that, at least with the current parameters, these tasks may be too effortful. This problem was further demonstrated through the use of raclopride, which increased the rates of omissions, rather than reward discounting. It is possible, that altering task parameters or training procedures may improve performance in these tasks. In particular, it may be necessary to reduce the ratio requirements for the high-effort options. A novel rearing effort discounting (RED) task, displayed initial promise by demonstrating sensitivity to alterations to the costs and benefits of high effort choices. However, there was a clear practice effect from repeated task exposure, which reduced the rate of discounting. As a consequence, the actions needed for the high value reward were not sufficiently effortful to cause a reliable shift to the low value reward. Again, systemic administration of the D_2/D_3 receptor antagonist raclopride highlighted this problem. It would be expected that dopamine antagonists exert greater effects at later, higher effort trials (Floresco et al. 2008), which was not observed.

Due to problems with theses effort-based decision-making tasks, an alternative approach would be to initially screen for potential effects on behaviour using a PR task, and subsequently

follow-up any positive results using a battery of control tasks (e.g. Bailey, Williamson, et al. 2016). However, it should be noted that this approach would require the use of a greater number of experimental animals, which may be ethically and financially disadvantageous.

The use of functional imaging could also facilitate translational research. Such approaches could be used to demonstrate that a behavioural assay is engaging equivalent neural circuitry across species (Keeler & Robbins 2011). *In vivo* oxygen amperometry was chosen as it can provide a viable and valid proxy measure of fMRI-BOLD, the most widely used functional measure in humans. We found that the degree of NAc activity evoked by a food reward was correlated with individual differences in motivated behaviour as measured by PR performance. To date, no study has combined fMRI (or any other imaging measure) with PR responding in humans. However, it would be possible to apply event-related fMRI and PR to investigate whether this relationship between performance and NAc activity can also been observed in humans. This would further strengthen the argument for the use of PR schedules as preclinical assays of motivation It would also be useful to determine whether such a signal is reduced in apathetic populations. Furthermore, functional imaging of hemodynamic responses can be used to demonstrate that a compound is exerting an expected central pharmacological effect (Wise & Tracey 2006; Li et al. 2016). By using a translational technique such as oxygen amperometry, such drug effects could subsequently be compared across species. Combining functional imaging with pharmacology has been proposed as a method to reduce expensive clinical trial failures (Wager & Woo 2015; Duff et al. 2015). Finally, imaging could be used to validate preclinical models of amotivation, by demonstrating similar deficits to those observed within clinical populations.

There are several potential limitations arising from this study. Amperometry, like fMRI, provides only a surrogate measure of neural activity. There are a number of techniques available that allow for direct measurement of neural or neurochemical activity such as microdialysis or electrophysiological recordings. Such measurements may be better suited to the study of pharmacological effects on motivated behaviour. This is because a number of drugs and disease states can alter the neurovascular coupling processes that allow for haemodynamic measures to be proxies of neural activity (D'Esposito et al. 2003; Wise & Tracey 2006; Diukova et al. 2012). Furthermore, imaging provides only a correlate of behaviour, and does not demonstrate a causal association. Neuromodulatory techniques such as optogenetics could be used to demonstrate a causal link between the activity of a given population of neurons and behaviour. However, none of the direct measures can be readily

performed in humans. Therefore, a major strength of this study is the translatability of the results. Nevertheless, it would be useful for future studies to also use more direct measures of neurochemical function, particularly when testing the effects of pharmacological interventions.

8.3 Part 2: Muscarinic acetylcholine receptors as novel targets for apathy

We subsequently aimed to investigate a potential novel target for the facilitation of motivated behaviour in rodents. Muscarinic acetylcholine receptors (mAChRs) were chosen based upon a review of the literature demonstrating an established interaction with the dopaminergic system (Chapter 1). Scopolamine, a nonselective mAChR antagonist, facilitated PR performance, but also appeared to increase locomotor activity. Subsequently, through administration of more-selective mAChR antagonists, we found that the effects upon effort-output were likely mediated through the M_1 receptor; whereas the effects on locomotor activity were likely to be occurring through the M_4 receptor subtype. Subsequently, through the use of a battery of control tasks, we found that the effects of the M_1 receptor antagonist biperiden were goal-directed and unlikely to be caused by changes in satiety, appetite, fatigue or perseverative behaviour.

One major limitation of the present study was the selectivity of the compounds used. Due to the physiological nature of muscarinic receptors, it has not been possible to produce highly selective ligands. Compounds with greater selectivity for both the M_1 and M_4 receptor subtypes exist (Sheffler et al. 2009; Croy et al. 2016). However, these drugs have poor brain penetrance. The compounds used in the present study were selected as having a balance between selectivity and an ability to exert centrally-mediated behavioural effects. In spite of the moderate selectivity profiles, a number of other studies support the study's conclusion of a relationship between M_1 receptors and motivated behaviour. For example, a more selective M_1 antagonist is able to reverse the motoric effects associated with high doses of haloperidol (Kharkwal et al. 2016). Additionally, through the local administration of a number of muscarinic receptor antagonists, with varying degrees of subtype selectivity, it affinity for the M_1 receptor predicted the efficacy in preventing haloperidol-induced catalepsy (Erosa-Rivero et al. 2014). However, even on the basis of these studies alone it should not be conclusively claimed that the effects of biperiden upon motivation are driven by actions at the M_1 receptor. Combining the effects of drugs with mAChR knock-out mice (Wess et al. 2007) would allow this to be fully investigated.

Having demonstrated an effect on motivation, we subsequently aimed to test the effects of muscarinic receptor antagonists upon a deficit model. Previous reports had suggested that aged rodents display deficits in PR performance relative to young controls (Blokland & Raaijmakers 1993; Bordner et al. 2011; Amancio-Belmont et al. 2017). Initially, we replicated this age-related deficit; however, in subsequent cohorts, aged rats showed no difference in motivation. This was likely a consequence of a change in feeding protocols that better controlled for the bodyweight differences between the age groups. It is possible that the long-term feeding schedule reduced age-related changes in feeding behaviours. Therefore, the initial results of an age-related decline in PR performance may have been confounded by group differences in appetite or satiety. It is also possible that such a confound occurred in the previous reports, which did not attempt to control for differences in weight. Together, this finding suggests that aged rats are not a suitable preclinical model of apathy. Aside from the lack of a behavioural deficit displayed by the aged rats, there are a number of problems that would have arisen using aging as a model, including differential stress reactivity (Buechel et al. 2014) and the effects of life experiences (Gallagher et al. 2011) could also confound results.

Given the lack of a deficit in aged animals, we subsequently tested the effects of biperiden and scopolamine in a well-validated model of impaired motivation. Haloperidol was chosen as it has been shown to be effective across a number of assays of effort-related behaviour, including both operant and maze-based effort-related choice (Salamone et al. 1991; Salamone et al. 1994) and effort discounting assays (Bardgett et al. 2009). Different classes of drugs, including adenosine receptor antagonists and psychostimulants are able to ameliorate the effects of haloperidol on effort-related behaviour (Bardgett et al. 2009; Mott et al. 2009). The use of such reversal studies has been suggested to be a useful test of potential therapeutic efficacy of compounds (Farrar et al. 2007). We found that biperiden was able to reverse the behavioural effects of haloperidol upon PR and an effort-related choice (ERC) assay. This finding suggests that muscarinic receptor antagonism may be a particularly useful therapeutic approach for disorders of motivation arising from hypodopaminergic sates or following dopamine antagonist treatments. Haloperidol has also been reported to produce psychomotor slowing (Salamone et al. 1993), mirroring the effects of fatigue or anergia frequently reported in clinical populations (Friedman & Friedman 1993; Lou et al. 2003). However, such symptoms may be more reflective of depression, which is a separate construct from apathy (Levy et al. 1998).

8.4 Enhancing motivation in healthy subjects

The focus of the present studies was to investigate a novel pharmacological target for clinical apathy. We found that biperiden was effective at facilitating motivation in intact mice in addition to those displaying a deficit. It is possible that motivation, as a cognitive process, could be enhanced pharmacologically in heathy subjects (Turner et al. 2003). There are already reports of otherwise heathy people using psychostimulants such as methylphenidate for perceived pro-motivational effects (Ilieva & Farah 2013). Participants self-report that the effects on motivation were greater than many perceived effects on other cognitive domains (Ilieva & Farah 2013). The effects of psychostimulants on motivation, in healthy subjects, are in line with a laboratory study suggesting that amphetamine increases effort expenditure in heathy subjects (Wardle et al. 2011). The use of compounds to enhance motivation in the general population may raise ethical considerations (Kjærsgaard 2015). The ethical considerations of pharmacological enhancement of cognition in the healthy population have been debated elsewhere (Farah et al. 2004; Porsdam Mann & Sahakian 2015). It is likely that putative pro-motivational drugs deserve a similar level of scrutiny.

8.5 General limitations

Throughout the present studies, effort was used as a way of operationalising motivation for measurement in rodents. Previous studies have suggested that apathy, as seen in clinical populations, seems to reflect deficits in behavioural activation (Salamone, Yohn, et al. 2016). However, in clinical cases, it is likely that apathy may arise from deficits in a number of processes including reward anticipation and decision making (Husain & Roiser 2018). Exertion of effort as measured by PR performance likely only captures an aspect of apathy as displayed by patient groups. Even within preclinical research there are multiple assays that are used to probe different aspects of motivational disruptions (Markou et al. 2013; Barnes et al. 2017). Development of novel treatments for apathy may also wish to examine the effects of compounds upon other tasks such as assays for 'cognitive effort' (Cocker et al. 2012) emotional/affective constructs (Robinson 2018) and reward anticipation (Barch et al. 2016).

An additional concern is that the assays used were measuring some construct unrelated to effort. In the majority of studies used, a touch sensitive screen was used as a manipulandum. As discussed in a previous chapter (chapter 2), no physical effort is needed for a response to be recorded. Therefore, the extent to which a touchscreen-based assays can capture effort-based behaviours may be questioned. However, the present work (chapter 2), along with a previous study (Heath et al., 2015) suggests that the use of touchscreen can support the energetic, repetitive responding performed by rodents during effort-based assays. Furthermore, the

cognitive processes governing effort are at least partly independent from motoric processes (Salamone & Correa, 2012). Therefore, the use of touchscreen as a manipulandum is unlikely to have confounded the present results.

In the present studies, time-limited PR sessions were used. The selection of session length and no-response periods was somewhat arbitrary but based on previous reports (Wirtshafter & Stratford 2010; Klinkenberg & Blokland 2011; Enkel et al. 2014; Heath et al. 2015). However, the choice of these parameters could easily affect breakpoint, particularly when investigating the effects of putatively performance enhancing compounds. A further potential issue arises from the behavioural measures adopted. Many of the supplementary measures of performance other than breakpoint adopted in this thesis have been less commonly used. For example, the rate of IR beam breaks and nontarget screen touches were used as measures of general activity. The justification for this has been the observation that pharmacological manipulations affect these measures similarly to traditional measures of locomotor activity such as the open field and inactive lever-press (Marriott 1968; Pijnenburg et al. 1975; Hillegaart & Ahlenius 1987; Heath et al. 2015). However, it is only assumed, rather than known that these measures equate to one another. Furthermore, the basis for the response bout-analysis adopted in the present set of studies is somewhat arbitrary. A 5s period without responses was taken the signify the end of a bout. Previous investigations of response bouts have used a 2s no-response period to define the end of a bout (Ko & Wanat 2016; Boekhoudt et al. 2018). However, these have used lever-based operant systems. The rate of responding is considerably lower within the touchscreen system compared to lever-based system (chapter 2). Therefore, based upon differences in breakpoints and response rates observed between the two systems, increasing the period to 5s seemed justified. A separate approach may have been to define the end of a bout by the presence of a separate action (such as a nontarget touch or IR beam break). However, based upon visual inspection of rodents performing PR, it was noted that during the sustained repetitive responding, rodents would occasionally make nontarget contact either side of the response aperture or move in such a way that their hindquarters may have triggered an IR beam break. In these cases, the use of such an action-based definition may have confounded measurement of response bouts

Another general limitation with the current set of studies is the reliance on systemic pharmacology. Whereas, this is not necessarily problematic when investigating the therapeutic potential of muscarinic receptor antagonists, it cannot inform us as to the location of the actions of these drugs. Based on a review of the literature (reviewed in chapter 1), it is likely that the

effective compounds were acting upon muscarinic receptors within the nucleus accumbens core. However, local administration studies should be used to confirm this. Previous studies have also used disconnection lesions to determine the output pathway of pro-motivational drugs (Mingote et al. 2008) This approach could also be used to identify regional differences within the NAc. The involvement of the core and shell subregions in effort-based behaviour is still not entirely clear (Bailey, Simpson, et al. 2016). Furthermore, it is not known whether compounds that enhance motivation are preferentially affecting on one region or another (Mingote et al. 2008). Being able to identify the location of a compounds actions would likely aid therapeutic development, by allowing more effective compounds to be developed (Anon 2010).

8.6 Conclusions

Through the study of effort exertion in rodents, it may be possible to identify novel pharmacological interventions that target motivation-related impairments. Such impairments are largely refractory to current treatment approaches. The work described presently outlines ways to facilitate cross-species assessment of motivated behaviour by validating translational touchscreen testing methods for motivational processes and identifying a translational neural signature of motivated behaviour. We were also able to demonstrate that, contrary to previous reports, healthy aging in rats is not reliably associated with any motivational impairments. Invalidation, through demonstration of such negative results is crucial for scientific progress (Popper 1959; Rosenthal 1979). Furthermore, through the use of touchscreen tests of motivation we identified that the antiparkinsonian drug biperiden was able facilitate motivated behaviour in both healthy mice and those displaying a deficit. Future studies can now investigate the therapeutic potential of this compound within clinical populations.

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