MUSCARINIC RECEPTOR CONTRIBUTIONS TO COST/BENEFIT DECISION-MAKING ON THE RAT GAMBLING TASK

by

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Abstract

The cholinergic system, encompassing the muscarinic and nicotinic receptor systems, plays a modulatory role in a variety of executive processes. However, its role in decision making is still unclear. Disorders characterized by disturbed muscarinic receptor functioning, such as schizophrenia, exhibit impaired performance on measures of real-world cost/benefit decision making, but whether this contributes to the choice deficits observed in the disorders is currently unknown. To address muscarinic receptor contributions to such processes, we investigated the effects of the broad-acting muscarinic receptor agonist oxotremorine (0.01, 0.03. 0.1 mg.kg) and antagonist scopolamine (0.01, 0.03, 0.1 mg.kg) on rodent Gambling Task (rGT) performance. Like the clinically administered Iowa Gambling Task (IGT), rodents must evaluate the costs and benefits of four nosepoke options that are each associated with the delivery of a different amount of reward, as well as different probabilities of receiving reward or a punishment time-out in which no reward can be earned. Rats quickly learn to select the advantageous options characterized by smaller rewards with lower penalties, and to avoid the large, high penalty reward options. Although systemic administration of oxotremorine had no effect, the highest dose of scopolamine impaired optimal performance by increasing choice of the option associated with the smallest reward and the lowest risk. This shift in choice is similar to that previously observed following administration of amphetamine, and suggests the drugs induce a hypersensitivity to loss. Given the functional connectivity of muscarinic and dopaminergic systems in the brain, and the antipsychotic-like profile of muscarinic agonists in amphetamine-induced animal models of schizophrenia, we then attempted to attenuate amphetamine's choice impairments by

prior administration of oxotremorine. Amphetamine (1.0 mg.kg) produced its characteristic choice impairments, despite pretreatment with oxotremorine. The results of this study suggest muscarinic receptor blockade can impair cost/benefit decision-making under conditions of risk and uncertainty, and prescribe a novel role to acetylcholine as a modulator of the decision process. Future work is required to pinpoint the mechanism driving amphetamine's effect on the rGT, as cholinergic signaling through muscarinic receptors does not appear to be involved.

Preface

Dr. Catharine Winstanley conceived the experiment, with input from Dr. Mohammed Shoaib and myself. I carried out subsequent behavioural experiments and conducted all statistical analyses. Research for this thesis was approved by the UBC Animal Care Committee, application number A13-0011.

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List of Abbreviations

g; Gram

h: Hour

kg: Kilogram

mg: Milligram

mg.kg: Milligram per kilogram

min: Minute

ml: Millilitre

s: Second

5-CSRT: 5-choice serial reaction time task

Ach: Acetylcholine

ACTH: Adrenocorticotropic hormone

AD: Alzheimer's Disease

BF: Basal forebrain

cAMP: Cyclic adenosine monophosphate

CB_1: Cannabinoid receptor subtype 1

DA: Dopamine

DAT: Dopamine transporter

HPA: Hypothalamic-pituitary-adrenal

IGT: Iowa Gambling Task

ITI: Inter-trial-interval

KO: Knock out

LDT: Laterodorsal tegmental nucleus

mPFC: Medial prefrontal cortex

NAc: Nucleus accumbens

NE: Norepinephrine

OFC: Orbitofrontal cortex

PPI: Prepulse inhibition

PPT: Pedunculopontine nucleus

rGT: Rodent Gambling Task

SNc: Substantia nigra pars compacta

VMPFC: Ventromedial prefrontal cortex

VTA: Ventral tegmental area

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Introduction

Maladaptive decision making is observed across psychiatric conditions, suggesting this executive function relies on the proper coordination of multiple brain systems (Lee, 2013). Research into the neurobiological basis of decision-making has benefited from the use of sensitive psychological assessment tools capable of capturing the processes underlying "real-world" decision making in a laboratory setting. One such metric that has been used to great effect in clinical populations is the Iowa Gambling task (IGT). In this paradigm, participants choose cards from four decks, each of which leads to varying amounts of monetary gain or loss as determined by set probabilistic schedules (Bechara et al., 1994). Decks associated with larger per-trial rewards also lead to disproportionately greater losses than those associated with smaller wins. Hence the optimal strategy is to avoid the tempting "high-risk, high-reward" decks and instead pick cards that yield incremental gains over time but lower penalties, thereby maximizing earnings on the task. Maladaptive choice patterns are observed on the IGT in disorders with distinct etiologies, including but not limited to ADHD, problem gambling, Alzheimer's Disease, and schizophrenia (Garon et al., 2006; Goudriaan et al., 2005; Sinz et al., 2008; Sevy et al., 2007).

Interestingly, both Alzheimer's Disease (AD) and schizophrenia are characterized by dysregulation of cholinergic signaling, but whether this contributes to the decision-making deficits observed in the disorders is currently unknown. Acetylcholine is a fast-acting, point-to-point neurotransmitter in the periphery, but in the central nervous system it plays a neuromodulatory role, altering neuron excitability, mediating presynaptic neurotransmitter release, and coordinating the firing of neuronal assemblies (Picciotto et

al., 2012). Acetylcholine signals through two distinct receptor classes- the metabotropic muscarinic receptor and the ionotropic nicotinic receptor. Muscarinic receptors are of five subtypes (M1, M2, M3, M4, M5) with distinct presynaptic, inhibitory (M2, M4) and post-synaptic, excitatory (M1, M3, M5) roles (Barak, 2009). In contrast, nicotinic receptors function as excitatory, nonselective cation channels where they promote neurotransmitter release presynaptically and depolarize neurons postsynaptically (Jones et al., 2012; McGehee et al., 1995). Muscarinic and nicotinic receptors are found across the brain, and receive diffuse cholinergic tone via projection neurons originating from basal forebrain (BF), pedunculopontine (PPT), and laterodorsal (LDT) tegmental nuclei, as well as cholinergic interneurons such as those observed in the striatum (reviewed by Karczmar, 2007). Acetylcholine has been implicated in a number of processes including attention, learning, memory, and stress, and so it is unsurprising that dysregulation of this system is implicated in both AD and schizophrenia. Specifically, AD is characterized by degeneration of the cholinergic projections originating from the BF, whereas decreased M1 and/or M4 receptor density in the hippocampus, caudate-putamen, and prefrontal cortex is believed to contribute to the disturbed mesocorticolimbic dopamine signaling observed in schizophrenia (Schliebs, 2005, Scarr et al., 2007; Crook et al., 2000, 2001; Dean et al., 1996, 2002). Additionally the α_7 nicotinic receptor, given its genetic linkage to schizophrenia and its reduced presence in the post-mortem brain, is a primary target in recent efforts to develop drugs that improve the disorder's cognitive symptoms (Mathew et al., 2007; Guan et al., 1999; Marder, 2006). Though work with clinical populations has unmasked some of the systems and brain regions involved in decision making, it is difficult to directly assess cholinergic contributions to these processes (Ernst & Paulus,

2005). Aside from a select literature demonstrating that nicotine abusers show elevated levels of impulsive decision making, work with healthy and clinical populations has been unable to elucidate the role of acetylcholine in the decision process (Friedel et al., 2014; Bickel, Odum, & Madden, 1999).

When studied in clinical populations, measures such as the Iowa Gambling Task are essential to understanding the activation patterns, pharmacological treatments, and genetic polymorphisms associated with impaired decision making (Koechlin & Hyafil, 2007; Clark, 2010). However, use of human subjects precludes a deep understanding of how neurotransmitters, such as acetylcholine, interact with other systems to guide behaviour. To address this issue directly a number of rodent models have been developed, thereby allowing for a direct investigation of the anatomical and chemical substrates underlying various forms of decision making. These so-called "cost/benefit" decision-making paradigms typically present subjects with the choice of small reward, low cost, and large reward, high cost options, where "cost" is defined as any experimental manipulation that impedes access to the larger, preferred reward (Floresco et al., 2008). Under normal conditions, rats prefer a larger amount of sucrose or food reward, but as the costs associated with the high reward option increase, discounting of this option occurs and rats incrementally shift choice to the smaller reward. To date, tasks have been developed to assess how the decision process is affected by delay costs (choice of immediate, small reward versus large reward presented at increasing delays), effort costs (choice of easily earned, small reward versus larger rewards only earned by exerting more physical or cognitive effort), and risk costs (choice of guaranteed, small reward versus large reward presented with decreasing probability) (Evenden & Ryan, 1996;

Salamone, Cousins, & Bucher, 1994; Floresco, Tse, & Ghods-Sharifi, 2008; Cocker et al., 2012; St.Onge & Floresco, 2009).

Combined, the tasks have revealed areas of the corticolimbic circuitry that subserve decision making regardless of the costs associated with the large reward, but have also highlighted brain loci and neurotransmitters mediating specific forms of cost/benefit decision-making. For example, dopamine fluctuations in the nucleus accumbens signal various aspects of the decision process (i.e., expected reward magnitude), and across tasks it appears the nucleus accumbens serves to bias responding towards selection of larger, riskier reward options (Day et al., 2010; Gan et al., 2010; Winstanley et al., 2006; St. Onge et al., 2012; Cardinal et al., 2001; Floresco, Tse, & Ghods-Sharifi, 2008). This can be contrasted with the orbitofrontal cortex (OFC) and the anterior cingulate of the medial prefrontal cortex, which are sensitive to delay and effort (physicial and cognitive) costs, respectively (Winstanley et al., 2004; Rudebeck et al., 2006; Hosking et al., 2014). Cholinergic agents have subsequently been assessed on some of these tasks, and the results suggest a complex role for the cholinergic system in the decision process. Initially, it was reported that systemic administration of nicotine increases “impulsive” choice of small, immediate rewards in the delay-discounting task; however, recent investigations suggest this effect can instead be explained by nicotine-induced impairments in reward magnitude sensitivity (Dallery & Locey; 2005; Kolokotroni et al., 2011; Locey & Dallery, 2009, 2011; Mendez et al., 2012). Nicotine has no effect on choice when assessed on the probability-discounting task, and nonspecific nicotinic receptor antagonism does not induce a choice shift on either task (Mendez et al., 2012). Thus, it appears manipulating cholinergic tone at the nicotinic

receptor has no effect on decision making that is characterized by delay or probability cost judgments. In contrast, while muscarinic receptor agonism has no effect on choice, nonspecific muscarinic receptor blockade (with scopolamine) produces robust shifts in choice on the delay and probability discounting tasks, biasing choice towards the small, immediate and small, guaranteed rewards, respectively (Mendez et al., 2012). Unfortunately, these are the only data available on muscarinic receptor involvement in decision making, and more studies are required to investigate the robustness of this effect across tasks associated with different cost/benefit contingencies.

The tasks described above involve discriminating between two response options that differ in reward magnitude (small versus large reward) and a single cost (small versus large delay, effort, or probability, respectively). These tasks assess pure forms of decision making, and certainly do not model real-life decision-making associated with varying magnitudes of reward, uncertainty, and punishment. The original IGT is a useful tool because it mimics the complexity of everyday decisions. Indeed, performance of the task requires evaluating multiple options based on their risk-reward ratio, monitoring outcomes, flexible planning following these outcomes, and the restraint to avoid options that are immediately rewarding (de Visser, 2011). In order to study the neurobiological processes contributing to this type of cost/benefit decision-making, our lab has developed a rodent analogue of the IGT (Zeeb et al. 2009, 2013; Zeeb and Winstanley 2011). Like the original human task, rats are presented with four options (pellet holes replace cards) that are each associated with a different magnitude of reward, but also different probabilities of receiving the reward or a specific punishment "time-out" period in which no reward can be earned. The duration of each session is fixed, and so the optimal

strategy is to choose small, low penalty rewards that yield the most pellets across the session whilst avoiding the tempting, large rewards whose selection often results in lost playing time. Thus, selection of P2 yields the most reward per unit time, which is associated with a 10s timeout period that occurs 20 % of the time (80 % chance of reward). P1 is the next best option (5s time-out, 90 % chance of reward), whereas the two disadvantageous options are both associated with larger immediate gains, 3 and 4 sucrose pellets, but longer timeout periods (P3, 30s timeout, 50 % chance of reward; P4, 40s timeout, 40 % chance of reward) (Zeeb et al., 2009; Baarendse et al., 2013). Like healthy participants on the IGT, rats readily choose the P2 option associated with the greatest long-term gains across a session.

Performance on the rGT is sensitive to manipulations that are known to affect decision making. Lesions of the orbitofrontal cortex, amygdala, or functional disconnection of these two areas prior to task exposure retards acquisition of the task, and lesions of the amygdala made post-training increase choice of disadvantageous options (Zeeb et al., 2011; Zeeb et al., 2013). These impairments mirror the IGT deficits of clinical patients with lesions to the amygdala or ventromedial prefrontal cortex (VMPFC) (encompassing the OFC), which is fitting given the original IGT was designed to assess patients with VMPFC damage (Bechara et al., 1994; Bechara et al., 1999; Brand et al., 2007). Subsequent pharmacological manipulations have implicated dopaminergic, serotonergic, and adrenergic systems in rGT performance, and most recently the cholinergic system has been probed on the task (Zeeb et al., 2009; Baarendse et al., 2013; Silveira & Winstanley, unpublished observations). Systemic administration of the nicotinic receptor agonist nicotine or the receptor antagonist mecamylamine has no effect

on the rGT choice profile (Jones et al., unpublished observations). However, the muscarinic system has not been studied, and the existing literature (reviewed above) suggests that while decision making is not sensitive to changes in acetylcholine availability at the nicotine receptor, muscarinic receptor blockade biases choice toward low cost, low reward options (Mendez et al., 2012). The corticolimbic circuitry expresses various muscarinic receptor subtypes, and the muscarinic receptor system functionally interacts with other transmitter systems (such as dopamine-described below) previously implicated in cost/benefit decision-making (Karczmar, 2007; Picciotto et al., 2012; Fobbs & Mizumori, 2014). Furthermore, acetylcholine has been associated with the signaling of probabilistic outcomes, and so it is possible that muscarinic receptor activation or blockade contributes to decision processes measured by the rGT (Yu & Dayan, 2005). Thus, the first aim of this investigation is to study the effect of broad-acting muscarinic receptor activation and blockade on rGT performance.

A number of studies indicate that administration of the psychostimulant drug amphetamine impairs decision making on the rGT by shifting choice away from the optimal P2 choice towards the less advantageous P1 (Zeeb et al. 2009, 2013; Baarendse et al. 2012; Young et al. 2011), Recall that P1 delivers the smallest amount of reward, but is the most likely to produce a reward on any given trial. Thus, the shift induced by amphetamine has been interpreted as a hypersensitivity to loss. Amphetamine's primary mechanism of action is to enhance extracellular levels of DA and NE via multiple pathways, including but not limited to: inhibition of the dopamine transporter (DAT), the norepinephrine transporter, and the vesicle monoamine transporter 2; inhibition of monoamine oxidase activity; as well as via DAT internalization (Partilla et al., 2006;

Rothman et al., 2001; Sandoval et al., 2001; reviewed by Sulzer, 2011). However, amphetamine also increases synaptically available serotonin via weak blockade of the 5-HT transporter, and in reality amphetamine potentiates the release of various neurotransmitters (Ritz & Kuhar, 1989; Hutson et al., 2014). Given the pronounced role played by DA in reward-related learning, as well as the signaling of uncertainty, it seemed likely that the choice impairment caused by amphetamine on the task reflected the drug's ability to potentiate DA's actions (Wise & Rompre, 1989; Fiorillo, Tobler, & Schultz, 2003). This notion received further support when administration of the D_2 receptor antagonist eticlopride was shown to increase optimal choice on the task (Zeeb et al., 2009). However, recent data demonstrate quite conclusively that amphetamine's effects on choice, unlike this drug's effects on motor impulsivity, cannot be attenuated by co-administration of either the D_1 receptor antagonist SCH23390 or the D_2 receptor antagonist eticlopride (Zeeb et al. 2013). Furthermore, amphetamine's choice impairments cannot be reproduced by administration of the selective DA reuptake inhibitor GBR12909, or by various DA agonists, strongly suggesting that this deficit is not mediated by direct alterations in dopamine signaling.

Administration of amphetamine, methamphetamine, or apomorphine is commonly used in animal studies to model the hyperactive mesolimbic dopamine-signaling central to the etiology of schizophrenia (Jones et al., 2011). Interestingly, a number of studies indicate that muscarinic receptor agonists can attenuate the behavioural effects produced by hyperdopaminergic agents, leading to the suggestion these compounds may have antipsychotic properties. For example, prepulse inhibition (PPI) of the acoustic startle reflex is a validated model used to evaluate sensory information-processing deficits in a

number of neurological conditions, including schizophrenia (Swerdlow et al., 2008; Swerdlow et al., 1994). Administration of the non-selective D_1/D_2 receptor agonist apomorphine produces PPI deficits that are reversed by D_2 receptor antagonists (i.e haloperidol and clozapine), as well as by the muscarinic receptor agonists xanomeline and oxotremorine (Jones et al., 2000; Stanhope et al., 2001). Similarly, methamphetamine-induced hyperlocomotion in mice can be reversed by muscarinic agonism, and monkeys treated with xanomeline (a selective M1/M4 muscarinic receptor agonist) are resistant to the stereotypies and unrest produced by acute doses of d-amphetamine and apomorphine (Maehara et al., 2008; Andersen et al., 2003). Furthermore, recent studies demonstrate that xanomeline can reverse amphetamine-induced abnormalities in latent inhibition, another commonly used model of sensorimotor gating deficits in schizophrenia (Barak & Wiener, 2011; Gray & Snowden, 2005). Collectively, these data suggest a functional interaction between the cholinergic and dopaminergic systems that may be of relevance with respect to the manifestation of sensory-processing deficits in schizophrenia.

Given the work described above, it is perhaps unsurprising that muscarinic and dopaminergic systems are anatomically and functionally connected in the reward-related circuitry of the brain. The dopamine neurons of the substantia nigra (SNc) and the ventral tegmental area (VTA) project to the striatum and nucleus accumbens, respectively. The specific firing rates of these cells regulate different forms of dopamine transmission, which in turn mediate processes such as goal-directed behavior and the signaling of rewarding or alerting stimuli (Floresco et al., 2003; Schultz, 1998). Dopaminergic cells of the midbrain express muscarinic and nicotine receptors, and in this way cholinergic

projections from the PPT and LDT of the mesopontine can regulate dopamine cell firing (Weiner et al., 1990; Charpantier et al., 1998; Floresco et al., 2003; Sziraki et al., 2002). Indeed, electrical activation of either the LDT or PPT induces a M5 receptor-dependent sustained elevation in basal NAc and striatal dopamine efflux, and muscarinic receptor agonism in the VTA or SNc increases DA efflux in the NAc and striatum, respectively (Forster & Blaha, 2003, Forster et al., 2000; Yeomans et al., 2001; Miller & Blaha, 2005). And although they only compromise 1-3% of all striatal neurons, cholinergic interneurons exert an important regulatory role over striatal DA transmission (Threlfell et al., 2010, 2012; Laplante et al., 2011, 2012). Knockout (KO) mice lacking the various M1-M5 receptors have also been particularly useful in delineating the contributions of muscarinic receptor subtypes to DA regulation. Thus, M1 KO mice display a two-fold increase in striatal DA concentrations and enhanced locomotor activity, suggesting M1 receptor activation normally exerts an inhibitory influence on DA release in this region (Gerber et al., 2001; Miyakawa et al., 2001). Similarly, M4 receptor KO mice exhibit heightened basal and amphetamine-induced levels of DA in the NAc, and M5 receptor KOs are hypersensitive to the stimulatory effects of amphetamine challenge (Tzavara et al., 2004; Gomeza et al., 1999; Schmidt et al., 2010; Wess et al., 2007). Given this ability of the muscarinic receptor system to regulate DA in regions involved in cost/benefit decision-making, along with the antipsychotic-like profile of muscarinic agonists in animal schizophrenia models, it is possible the cholinergic system is contributing to the decision-making deficits caused by amphetamine on the rGT. Thus, the second aim of the current investigation is to assess whether prior muscarinic receptor agonism can moderate amphetamine's choice profile on the rGT.

This thesis investigates cholinergic contributions to cost/benefit decision-making. Specifically, it aims to delineate the role of the muscarinic receptor system, and its interactions with the dopaminergic system, in decision making characterized by risk and uncertainty. Thus, rats were systemically treated with the broad-acting muscarinic agonist oxotremorine followed by the muscarinic antagonist scopolamine, and performance on the rGT was assessed. These experiments compliment recent work investigating nicotinic receptor contributions to rGT performance, and prescribe a novel role to acetylcholine as a modulator of the decision process. We subsequently investigated whether the muscarinic agonist oxotremorine could attenuate the choice impairment caused by amphetamine on-task. The goal of this experiment is to understand the elusive mechanism driving amphetamine's effect on the rGT, and is the first study of its kind to investigate cholinergic-dopaminergic interactions in the regulation of cost/benefit decision-making.

Materials and Methods

Subjects

Subjects were 16 male, Long-Evans rats (Charles River Laboratories, St. Constant, Quebec, Canada) weighing 275-300g at the start of testing. Two weeks following arrival, rats were food-restricted to 14g of rat chow per day and maintained at 85% of their free-feeding weight. Water was available *ad libitum.* All subjects were pair-housed in a climate-controlled colony room under a 12h reverse light-dark cycle (21° C; lights off at 8am). Behavioral testing took place 5 days per week. Housing and testing conditions were in accordance with the Canadian Council of Animal Care, and experimental protocols were approved by the UBC Animal Care Committee.

Behavioral Apparatus

Testing took place in eight standard five-hole operant chambers, each of which was enclosed in a ventilated, sound-attenuating chamber (Med Associates Inc, Vermont). Chambers were fitted with an array composed of five equidistantly spaced response holes along one wall. A stimulus light was located at the back of each hole, and nose-poke responses into these apertures were detected by vertical infrared beams. On the opposite wall, sucrose pellets (45 mg; Bioserv, New Jersey) were delivered to a food magazine via an external pellet dispenser. The food magazine was also fitted with a tray light and infrared sensors to detect food collection. A house light illuminated the chamber. The operant chambers were operated by software written in Med-PC by CAW, running on an IBM-compatible computer.

Behavioral Testing

Habituation and training.

Habituation to the operant chambers took place over two daily sessions, during which the chambers were turned on and sucrose pellets were placed in the response holes and food magazine. Following habituation, rats were trained to nose-poke an illuminated response hole within 10s to earn a reward. During these 30-minute sessions, the spatial location of the stimulus light varied among the five array holes on each trial. Once subjects completed these sessions with at least 80% correct trials and less than 20% omissions, they received seven forced choice sessions. These sessions were identical to the rGT, except that only one response hole option was presented on each trial. The forced choice sessions aimed to prevent spatial biases from developing by ensuring that subjects had equal experience with all four options.

The rGT.

The rGT has been described previously (Zeeb et al., 2009), and a schematic of the task is provided in Figure 1. Subjects began each trial by nose-poking in the illuminated food tray. This response extinguished the tray light and resulted in a 5s inter-trial interval (ITI), during which all lights in the chamber were extinguished. If subjects withheld responding during the ITI, holes 1,2,4, and 5 of the array were illuminated for 10s. A response in any illuminated hole turned off all stimulus lights and led to either onset of the tray light and delivery of a reward, or the start of a time-out 'punishment' period. Rewarded trials led to illumination of the food tray and immediate delivery of the appropriate number of sucrose pellets. If a trial was punished, no reward was delivered

and the stimulus light within the chosen hole flashed at 0.5 Hz until the punishing timeout elapsed, at which point the tray light was illuminated. In the case of rewarded and punished trials, a response in the food magazine initiated the next trial. If rats failed to make a nose poke in one of the illuminated holes within 10s, the trial was counted as an omission. Following these trials, the tray light was re-illuminated and subjects could begin a new trial. A response made in one of the five response holes during the ITI was punished by a 5s timeout period and recorded as a premature response. The time-out period was marked by illumination of the house light, after which the food tray light was re-illuminated and subjects could commence a new trial. Perseverative responses made at the array, both after reward delivery and during punishing timeouts, were recorded but not punished.

The reinforcement schedules were designed so that the optimal strategy was to select the two-pellet choice (P2), as this option results in the most reward earned per unit time. As a consequence of their probability of winning and losing, along with the duration of their punishing time-outs, selection of other options (one-,three-, or four-pellet options) yielded less reward per unit time (Fig 1) (Zeeb et al., 2009). The location of the pellet options (P1-4) was counterbalanced across animals. Thus, half of the rats (n = 8) were tested on version A (holes 1,2,4, and 5 of the operant chamber corresponded to pellet options P1, P4, P2 and P3, respectively) and the other half (n = 8) were tested on version B (holes 1,2,4 and 5 corresponded with pellet options P4, P1, P3, and P2). To establish baseline levels of performance on this task, subjects were tested daily five times a week. This continued for 66 sessions, at which point a statistically stable pattern of behaviour was observed across three sessions.

Drugs

After stable baseline performance was established, the effects of acute challenge with oxotremorine M and scopolamine hydrobromide were assessed. This was followed by an examination of whether administration of oxotremorine M could block the previously reported effects of amphetamine on rGT performance (Zeeb et al., 2009). Oxotremorine M and scopolamine hydrobromide were purchased from Tocris Bioscience (Ellisville, MO), whereas d-amphetamine hemisulfate was purchased under exemption via Health Canada from Sigma-Aldrich UK (Dorset, England). All doses were calculated as the salt and dissolved in 0.9% saline in a volume of 1 mg.kg. In keeping with previous reports, oxotremorine and scopolamine were injected subcutaneously 30 minutes before behavioural testing commenced (Jones & Shannon, 2000; Mirza & Stolerman, 2000). In the oxotremorine-amphetamine co-administration portion of the study, oxotremorine was injected sub-cue 30 minutes prior to testing, and was followed by an i.p injection of amphetamine 20 minutes later (Zeeb et al., 2009).

All drugs were prepared fresh daily, and the different doses were administered according to a balanced Latin Square design (four doses A-D: ABCD, BDAC, CADB, DCBA; Cardinal and Aitken 2006, p. 329). Drug injections were given on a 3-day cycle, starting with a baseline session. Subjects then received a drug or saline injection prior to testing, and on the following day were not tested. Animals were tested drug-free for a minimum of one week between compounds to prevent carryover effects. Subjects experienced three Latin squares in the following order with the listed doses: oxotremorine (0, 0.01, 0.03, and 0.1 mg.kg), scopolamine (0, 0.01, 0.03, and 0.1 mg.kg), and

amphetamine with oxotremorine pretreatment (saline/saline, saline/1.0 mg.kg amphetamine, 0.1 mg.kg oxotremorine/saline, 0.1 mg.kg oxotremorine/1.0 mg.kg amphetamine).

Data Analysis

Percentage choice of each pellet option (number of choices of each option (P1-P4)/total number of choices made x 100) was the main variable analyzed, along with percent premature responses (number of premature responses/total number of trials x 100) and percent omissions (number of omissions/total number of trials x 100). These percentage variables were subjected to an arcsine transformation to limit the effect of artificially imposed ceiling (McDonald, 2009). The total number of trials completed, number of perseverative responses made, and latencies to respond and collect reward were also analyzed.

Data analysis was conducted using SPSS for Mac (version 20.0; SPSS, Chicago, IL, USA). Pharmacological challenge data (oxotremorine, scopolamine) were analyzed with a two-way, repeated measures ANOVA with drug dose (four levels, vehicle plus three doses of compound) and/or choice (four levels, P1-P4) as within-subjects variables. If variables were not separated by choice (for example, premature responses and omissions), session or dose was used as the only within-subjects factor. If these analyses produced significant main effects of dose or dose x choice at the $p \leq 0.05$ level, further ANOVAs comparing individual drug doses with vehicle were performed, and values for individual choice options were compared post-hoc with saline values using paired samples t –tests. To analyze whether administration of oxotremorine blocked the

behavioural effects of amphetamine on the rGT, a repeated measures ANOVA with three variables was performed: antagonist (two levels: present/absent), dose (two levels: saline, amphetamine), and choice (four levels, P1-4). Choice was not included in this analysis if a measurement was not separated by the four choice options (for example, trials omitted or completed). A significance level of $p \leq .05$ was used for all analyses.

Results

rGT Baseline performance

Following seven forced choice sessions, rats significantly favored the objectively best option, P2, followed by P4, P3, and then P1 (Choice: $F_{3,45} = 6.126$, $p = .006$). As per previous reports, preference for P2 was established relatively early, but became more pronounced until a stable baseline was achieved (Zeeb et al., 2009; Zeeb et al, 2013a; Zeeb et al., 2013b; Zeeb & Winstanley, 2011, Baarendse et al., 2013). Rats completed about 90-100 trials per session and the number of omissions and perseverative responses made throughout training remained low. The number of premature responses made during each session was maintained at approximately 20%.

Oxotremorine

An acute dose of oxotremorine did not affect choice behaviour at any of the doses tested (Figure 2A: Dose x Choice: $F_{9,135} = 1.442$, NS). Premature responding significantly increased at the middle dose, but decreased at the highest dose of the drug (Figure 2B: Dose: $F_{3,45} = 8.179$, $p < .001$; sal *vs* 0.01 mg.kg: $F_{1,15} = 2.250$, NS; sal *vs* 0.03 mg.kg: $F_{1,15} = 6.319$, $p = .024$; sal *vs* 0.1 mg.kg: $F_{1,15}$ 5.564, $p = .032$). Oxotremorine also increased choice latency at the highest dose (Dose: $F_{3,45} = 9.085$, $p < .001$; sal *vs* 0.1 mg.kg: $F_{1,15}= 8.628$, $p = .010$), and slightly increased the percentage of omissions made, although this just failed to reach statistical significance (Dose: $F_{3,45} = 2.75$, $p = .053$; sal *vs* 0.01 mg.kg: $F_{1,15} = 0.212$, NS; sal *vs* 0.03 mg.kg: $F_{1,15} = 2.430$, $p =$ NS; sal *vs* 0.1 mg.kg: $F_{1,15} = 6.646$, $p = .021$). Oxotremorine had no effect on the other variables analyzed (Table 1: all Fs < 1.203, NS).

Scopolamine

Choice behaviour.

In contrast to oxotremorine, the muscarinic receptor antagonist scopolamine altered choice behaviour on the rGT (Figure 3A: Dose x Choice: $F_{4,135} = 2.517$, $p = .048$). The highest dose impaired decision making by significantly increasing choice of the one-pellet option (sal *vs* 0.1 mg.kg: $F_{3,45} = 2.804$, $p = .050$; P1: t(15) = 2.625, $p = .019$; P2: t(15) = 1.520, NS; P3: t(15) = 0.133, NS; P4: t(15) = 1.074, NS). The increased choice of P1 appeared to result from a shift in preference from P2, although the decline in choice of P2 did not reach significance.

Other behavioural measurements.

In general, acute treatment with scopolamine induced a motor slowing along with a decrease in motor output (Table 1). At the 0.03 and 0.1 mg.kg doses, premature responding significantly decreased (Figure 3B: Dose: $F_{3,45} = 4.299$, $p = .022$; sal *vs* 0.03 mg.kg: $F_{1,15} = 22.908$, $p < .001$; sal *vs* 0.1 mg.kg: $F_{1,15} = 5.397$, $p = .035$), and omissions increased (Dose: $F_{2,45} = 39.920$, $p < .001$; sal *vs* 0.03 mg.kg: $F_{1,15} = 29.525$, $p < .001$; sal *vs* 0.1 mg.kg: $F_{1,15} = 78.590$, $p < .001$). The number of trials completed also decreased and collect latencies increased at the highest dose (Trials completed- Dose: $F_{1,45} = 5.721$, $p = .016$; sal *vs* 0.1 mg.kg: $F_{1,15} = 6.518$, $p = .022$; Collect latency- Dose: $F_{2,45} = 8.269$, $p = .004$; sal *vs* 0.1 mg.kg: $F_{1,15} = 9.794$, $p = .007$). All doses of scopolamine increased choice latencies ($F_{3,45} = 44.848$, $p < .001$).

D-amphetamine and Oxotremorine Pre-administration

Choice behaviour.

Amphetamine significantly impaired choice behaviour on the rGT (Figure 4A: Dose x Choice: $F_{3,45} = 9.340, p < .001$) by shifting preference away from the advantageous P2 option towards P1 (P1: t(15) = -3.891, $p < .001$; P2: t(15) = 3.497, $p = .003$; P3: t(15) = 1.600, NS; P4: t(15) = -1.452, NS). Despite its similarity to scopolamine, this pronounced effect of amphetamine was not blocked by prior administration of oxotremorine (Antagonist x Dose x Choice: $F_{3,45} = 0.624, p = 0.603$). As reported above, administration of 0.1 mg.kg oxotremorine had no effect on choice behaviour (Antagonist x Choice: $F_{3,45} = 0.247, p = .863$).

Other behavioural measurements.

Co-administration of oxotremorine and amphetamine tended to increase the percentage of omissions made (Table 1: Antagonist x Dose: $F_{1,15} = 4.317, p = .055$; saline/saline *vs* oxotremorine/amphetamine: t(15) = -2.558, $p = .022$). Either drug administered alone had no effect on omissions (all $Fs < 6.44$, NS), but amphetamine alone significantly decreased the number of trials completed (Dose: $F_{1,15} = 6.455, p = .023$; saline/saline *vs* saline/amphetamine: t(15) = 2.498, $p = .025$; Antagonist: $F_{1,15} = 1.029$, NS; Antagonist x Dose: $F_{1,15} = 0.257$, NS). Amphetamine, oxotremorine, or co-administration of the two agents had no effect on premature responding (Figure 4B), choice latency, collect latency, or perseverative responding following reward (Table 1: all $Fs < 3.610$, NS).

Discussion

Our findings suggest decision making on the rGT can be perturbed by manipulations of cholinergic signaling through muscarinic receptor blockade, and provide some of the first direct evidence that the cholinergic system plays a role in decision making characterized by risk and uncertainty. Although the muscarinic agonist oxotremorine had no effect on choice preference, the muscarinic receptor antagonist scopolamine impaired decision making and produced a choice profile similar to that observed with amphetamine (current data, Zeeb et al., 2009). However, prior administration of the muscarinic agonist oxotremorine did not attenuate the choice impairment produced by amphetamine. Hence, although antagonism of muscarinic receptors and administration of amphetamine result in similar choice impairments on the rGT, these behavioural effects are mediated by independent mechanisms.

Muscarinic Receptor Modulation of rGT Decision Making

The neurotransmitter acetylcholine is unique for its widespread presence in both the central and peripheral nervous systems, and so it is important to dissociate a true effect of scopolamine on decision making from more general impairments in motor ability and basic function (i.e., appetitive (hypothalamic) behaviour, respiration, GI and endocrine activities– see Karczmar, 2007 for a review). Scopolamine increased omissions, decreased trials completed, and increased latencies to make a choice and collect reward, suggesting the drug decreased motor output. This is a common feature of cholinergic antagonists, which increase motor function and locomotor activity at intermediate doses, but induce comatose states at higher doses (Barak, 2009).

Scopolamine only moderately impaired motor ability, and rats completed enough trials to permit a valid discussion of the drug's effect on choice performance. Importantly, oxotremorine did not alter the choice profile despite producing locomotor effects, indicating that the choice shift observed with scopolamine is unlikely to be related to impaired motor function. Aside from basic motor effects, muscarinic antagonists decrease motivational behaviour towards food rewards, and in both the delayed and probability-discounting tasks scopolamine shifts choice to the smaller reward even when the larger reward is guaranteed (Pratt & Kelley, 2004; Mendez et al., 2012). However, impaired appetitive behaviour cannot easily explain the change in choice induced by scopolamine observed here, as P1 is the second-most advantageous option in terms of total pellets earned per session, and is the most likely to deliver reward on any given trial. In sum, the data suggest muscarinic modulation of decision making on the rGT is dissociable from the role this system has in regulating basic motor and consummatory functions.

The assorted cholinergic nuclei of the basal forebrain are the main source of cortical acetylcholine, with medial septal and diagonal band nuclei sending intense projections to limbic structures (including the amygdala and hippocampus), and nucleus basalis of meynert radiations projecting to the entire cortex (Picciotto et al., 2012). This widespread cholinergic enervation allows acetylcholine to modulate distinct forms of memory and executive function, some of which are likely recruited during optimal rGT performance (Croxson et al., 2011; Chudasama & Robbins, 2004; McGaughy et al., 2002). For example, attentional processing and working memory impairments are commonly observed following administration of scopolamine and similar muscarinic antagonists (Mirza & Stolerman, 2000; Robbins et al., 1998; 2002). However,

impairments in either of these domains would likely manifest as an indiscriminate flattening of the choice profile, rather than a bias towards P1. As such, it would appear that muscarinic receptor modulation can affect decision making in ways that are dissociable from the ability to attend to and recall the contingencies associated with each choice option.

In contrast, it has been previously shown that modulating cholinergic tone at the nicotinic receptor had no effect on decision making on the rGT (Jones et al., unpublished observations), a finding possibly explained by functional and regional expression differences in the two cholinergic receptor subtypes. Five G protein-coupled muscarinic receptors have been identified, three of which (M1, M3, M5) couple to Gq/G11-type G-proteins and increase intracellular calcium levels, whereas M2 and M4 receptors couple to Gi/o proteins, thereby inhibiting cAMP levels and prolonging potassium channel opening (reviewed by Wess, 2003). M1, M3, and M5 receptors are located postsynaptically, while M2 and M4 receptors are typically identified presynaptically where they function as autoreceptors on cholinergic neurons, or as heteroreceptors regulating the release of other neurotransmitters. Although the muscarinic subtypes are found throughout the brain, some subtype localization exists. For example, M1 receptors are the most abundant subtype in the cortex and hippocampus, in line with their purported role in learning and memory, whereas M4 autoreceptors are enriched on striatal interneurons (Reviewed by Jones et al., 2012). In general, the metabotropic nature of these receptors produces slower and more sustained synaptic responses. In contrast, the ionotropic ligand-gated nicotinic receptors, of which the α7 and α4β2 subunits are most abundant, mediate fast synaptic transmission throughout the nervous system (McGehee et

al., 1995; Corriveau & Berg, 1993). These nicotinic receptors are located pre- and postsynaptically, regulate the release of numerous neurotransmitters, and like muscarinic receptors are diffusely located throughout the brain, albeit in smaller concentrations (reviewed by Picciotto et al., 2000).

The methodology used here limits our ability to identify the neural loci in which muscarinic (but not nicotinic) receptor blockade can modulate choice behaviour on the rGT, a task made no simpler considering that the cholinergic system interacts with many, if not all, regions of the limbic and corticostriatal pathways implicated in cost/benefit decision-making. Although speculative, the basolateral amygdala (BLA) seems a likely candidate, given markers for acetylcholine in this area are among the highest in the rodent brain, and M1 and M2 receptors have been identified on BLA pyramidal and GABAergic interneurons, respectively (Ben-Ari et al., 1977; McDonald & Mascagni, 2011; Muller et al., 2013). The amygdala appears to bias choice towards options that produce the largest gains in the long-term, and in both humans and rodents lesions of the area enhance choice of options that are disadvantageous in the long run (Bechara et al., 1999; Ghods-Sharifi et al. 2009; Winstanley et al., 2004; Floresco et al., 2008). Fittingly, lesions of the BLA impair normal acquisition of the rGT, and increase risky choice if lesions occur following acquisition (Zeeb et al., 2011). Although the increase in choice of the disadvantageous P3 option caused by BLA lesions is different from the increase in P1 produced by scopolamine, both shift choice away from the most advantageous outcome (P2). Interestingly, inactivating the infralimbic area of the prefrontal cortex also impairs optimal choice on the task (Zeeb, Baarendse, Vanderschuren and Winstanley, unpublished observations). Given that computational models have implicated cortical

acetylcholine in uncertainty processing (Yu & Dayan, 2005), future studies targeting corticolimbic circuitry will be required to fully understand the mechanism by which muscarinic receptor blockade affects rGT performance.

The results obtained on the rGT mirror previous research highlighting a role for the muscarinic, but not nicotinic, system in distinct forms of cost/benefit decision-making (Mendez et al., 2012). In this study which investigated broad-acting agonists and antagonists of muscarinic and nicotinic receptors, only scopolamine produced robust choice impairments on the delay discounting and probability discounting tasks- paradigms in which rats must choose between small certain rewards and larger rewards that are delayed or increasingly unlikely, respectively. Similar to the choice shift observed in this investigation, scopolamine increased preference for the small immediate reward when the large reward was presented at increasing delays or probabilities. Across tasks, it therefore appears that cholinergic tone in the brain is optimized for decision making, whereby increasing cholinergic transmission via muscarinic or nicotinic receptor agonism has no effect (although results of nicotine on the delay discounting task are mixed- see Kolokotroni et al., 2011; Dallery & Locey, 2005; Anderson & Diller, 2010). However, the decision process is sensitive to decreases in cholinergic tone at the muscarinic receptor. The similarity in scopolamine's effects across divergent tasks suggests cholinergic regulation of decision making is independent of the nature of the costs associated with the larger rewards (Floresco et al., 2008). This notion is supported by a recent investigation demonstrating choice of large, costly rewards is linearly related to nicotinic receptor expression, and that many of these associations are observed across the delay and probability discounting tasks (Mendez et al., 2013).

Cholinergic Contributions to Amphetamine-induced Deficits in rGT Performance

Similar to the effect observed with scopolamine, systemic administration of 1 mg.kg amphetamine produced a substantial shift in choice, decreasing choice of P2 and increasing choice of P1 (Zeeb et al., 2009; Zeeb et al., 2013; Baarendse et al., 2012). However, amphetamine's effects were not blocked by prior administration of oxotremorine, suggesting the similar profiles produced by scopolamine and amphetamine are mediated via distinct mechanisms.

Efficient cost/benefit decision-making, as observed on the rGT, is subserved by different components of the dopamine mesocorticolimbic system, and phasic dopamine signalling in the terminal regions of this system –including the medial prefrontal cortex and nucleus accumbens- is linked to various aspects of the decision-making process (i.e expected reward magnitude, delay-related costs). Interestingly these regions show dissociable fluctuations in DA transmission during performance of the probability discounting task and delay-discounting tasks (Day et al. 2010; Gan et al., 2010; St.Onge et al., 2012; Winstanley et al, 2006). Cholinergic inputs from the pedunculopontine and laterodorsal tegmental nuclei act through muscarinic and nicotinic receptors to regulate the excitability of midbrain dopamine neurons originating from the substantia nigra pars compact neurons and VTA, respectively (Forster & Blaha, 2003; Forster & Blaha, 2000). These neurons, in tandem with cholinergic striatal interneurons, directly modulate DA neurons involved in motivation, movement, and attention set shifting (Oldenberg & Ding, 2011; Threlfell et al., 2012; Threlfell et al., 2010). In line with this modulatory role,

oxotremorine application increases DA release in the mPFC and NAc, likely via stimulation of M1/M4 receptors, and attenuates amphetamine-induced DA release in the NAc (Gronier et al., 2000; Ichikawa et al., 2002; Grilli et al., 2008; Gomeza et al., 1999; Jeon et al., 2010). Importantly, deficits in PPI and increased locomotion are observed following intra-accumbal infusions of dopaminergic agonists, and so the ability of oxotremorine to attenuate amphetamine-induced DA release in the NAc may be related to its antipsychotic-like profile (Swerdlow et al., 1990; Heidbreder & Fenton, 1998). Indeed, while depletion of cholinergic NAc interneurons produces PPI deficits and heightens the locomotor effects of amphetamine, oxotremorine reverses the enhanced amphetamine-induced accumbal DA release in cholinergically denervated rats (Laplante et al., 2011; Mattson et al., 2007). Although this proposed mechanism for oxotremorine's antipsychotic like-effects is speculative, it might explain why oxotremorine failed to block amphetamine's impairment on the task, as lesions of the NAc do not appear to influence rGT choice (Hosking and Winstanley, unpublished observations). Although this results seems at odds with the well-known contributions of accumbal dopamine to cost/benefit decision-making, previous reports suggest certain forms of decision making rely on accumbal DA more than others (Winstanley et al., 2005).

Oxotremorine is a nonselective muscarinic receptor agonist, and one of the difficulties in studying this system is that the orthosteric binding site is conserved across receptors, making it difficult to develop ligands that preferentially bind to specific receptors (Jones et al., 2012). A nonspecific agonist such as oxotremorine will bind to the five muscarinic subtypes (M1-M5) that are differentially located throughout the brain, and which have excitatory (M1, M3, M5) and inhibitory (M2, M4) roles in cholinergic

neurotransmission (Barak, 2009; Yeomans, 1995). It would be interesting to replicate this experiment with more selective muscarinic agents, as the ability of muscarinic agonists to attenuate dopamine-induced behaviour is contingent on the specificity of the agent used. For example, the selective M1/M4 receptor agonist xanomeline dose-dependently reverses apomorphine-induced deficits in prepulse inhibition -an effect not observed following administration of the nonspecific muscarinic receptor agonist pilocarpine (Stanhope et al., 2001).

However, the inability of oxotremorine to attenuate amphetamine's effects on the task, paired with the inability of selective dopamine agents to influence rGT performance, may further suggest amphetamine's effects are not directly dopaminergic (Zeeb et al.,2013; Zeeb et al., 2009; Baarendse et al., 2013). In light of these findings it is interesting to consider what other neurotransmitter might be mediating amphetamine's choice profile on the rGT. Amphetamine exerts direct effects on monoamine levels, which go on to indirectly affect other systems, including the glutamate, opioid, and endocannabinoid systems (Hutson et al., 2014, for a review). The endocannabinoid system seems to be an interesting target to pursue, as antagonism of the CB_1 receptor can attenuate the effects of dopaminergic agents on locomotor activity, relapse to drug seeking, and most recently impulsive action and impulsive choice (Tzavara et al., 2009; De Vries et al., 2003; Wiskerke et al., 2011).

Cholinergic Modulation of Impulsive Action

Impulsivity is a nonunitary construct, and is generally divided into distinct categories referred to as impulsive choice and action, respectively (Evenden, 1999;

Winstanley, Eagle & Robbins, 2006). The latter category reflects an inability to withhold a motor response, and in rats is commonly assessed with the 5-choice serial reaction time task (5-CSRT). Similar to the 5-CSRT, responses made prior to illumination of the choice holes (during the five second inter-trial-interval) are considered premature, and previous work with the rGT has shown task measures of impulsive action and decision making are dissociable at a pharmacological level (Baarendse et al, 2013; Zeeb et al., 2009).

Oxotremorine had bi-directional effects on premature responding, increasing these responses at the middle dose, while decreasing this form of impulsivity at the highest dose. As noted above, oxotremorine increases dopamine efflux in the nucleus accumbens, a region in which manipulations that elevate dopaminergic tone reliably increase premature responding (Gronier et al., 2000; Ichikawa et al., 2002; Grilli et al., 2008; Economidou et al., 2012; Pattij et al., 2007; Pezze et al., 2007; Cole & Robbins, 1989). It is therefore possible that oxotremorine's ability to increase accumbal dopamine is mediating the heightened impulsive action at the middle dose, but at the highest dose general locomotor slowing precludes responding (Mirza & Stolerman, 2000). In contrast, scopolamine dose-dependently decreased premature responding, an effect observed previously on the 5-CSRT following systemic or high dose infusion of scopolamine into the mPFC (Mirza & Stolerman, 2000, Chudasama & Robbins, 2004). However, other investigators have reported no effect of scopolamine on 5-CSRT premature responding, or increased responding if task parameters are made more attentionally taxing (i.e., bursts of white noise during the ITI), suggesting the effects of the drug are intricately tied to task parameters and complexity (Jones & Higgins, 1995; Ruotsalainen et al., 2000). These differential findings highlight that although both the rGT and 5-CSRT are sensitive

measures of impulsive action, they may reflect different facets of this subcategory. Indeed, waiting to respond to a brief 0.5s flash in a response hole in the 5-CSRT may drive an urgency to respond that could be lacking when waiting for four response holes which simultaneously remain lit and active for 10s.

Surprisingly, in contrast to previous observations using the rGT and 5CSRT (Zeeb et al., 2013; Zeeb et al., 2009; Baarendse et al., 2013), amphetamine did not increase premature responding in the current study. Interestingly, oxotremorine did not influence premature responding when the dose prefaced a saline injection, even though the same dose decreased impulsive action earlier in the study. This may indicate that the effect of oxotremorine is not very robust, or that the act of injecting the animals twice somehow altered the response to the muscarinic agonist. Certainly a comparable dose of oxotremorine in mice increases HPA axis reactivity within 20 minutes (Rhodes et al., 2008; Rhodes et al., 2005), and if a similar time course occurs in the rat brain, this increased HPA activity would be present at the time of the saline injection. Although speculative, it is possible the heightened ACTH levels exacerbated the stress associated with the second injection, and indeed manipulations of acute stress increase premature responding on similar measures of impulsive action (Sun et al., 2010). The opposing roles of stress and oxotremorine on premature responding may have cancelled each other out, explaining the seemingly inconsistent effect of oxotremorine on impulsive action observed here.

Future Directions

The current study implicates undisturbed cholinergic tone as a necessary requirement for optimal rGT performance. However, further studies are required to delineate the mechanisms underlying this effect. Although it seems unlikely the choice shift induced by scopolamine is due to its actions on the periphery, this can be ruled out definitively via administration of scopolamine methylbromide. This analogue of scopolamine does not readily cross the blood-brain barrier, and is 2-4 times less potent than scopolamine in stimulating Ach release in the frontoparietal cortex (Moore et al., 1992). Use of this drug along with scopolamine has been useful in delineating central versus peripheral actions of the drug on tests of attention, and could be assessed on the rGT to determine whether increased latencies (to make a choice, to collect reward) reflect motor or motivational impairments (see Jones & Higgins, 1995; Ruotsalainen et al., 1995).

The next logical step following the current investigation is to determine where scopolamine is mediating its choice effects. Scopolamine reduces cholinergic tone at the muscarinic receptor, and one method of reducing cholinergic tone at both muscarinic and nicotinic receptors is via selective lesions of cholinergic projection neurons and interneurons. Lesions of BF cholinergic neurons via infusion of 192-IgG saporin have been used to assess the importance of cortical acetylcholine in tests of attention, memory, and learning. This antibody binds to the low-affinity nerve-growth factor present exclusively on cholinergic BF neurons (p75), and recently antibodies have been developed to target cholinergic interneurons in the striatum (Baxter & Bucci, 2013; Laplante et al., 2011). Although this method has been commonly used in the literature to

deplete cortical Ach levels, it does not specifically target the muscarinic receptor, and thus lacks the receptor and spatial specificity required for our purposes. Instead, the brain loci mediating the scopolamine impairments can be investigated by systematic infusion of the drug into regions associated with cost/benefit decision-making. As described above, the amygdala, nucleus accumbens, and regions of the prefrontal cortex (OFC, infralimbic cortex) would be primary targets for such investigation.

More generally, it would be interesting to assess how cholinergic tone in the brain fluctuates during rGT performance, and specifically if fluctuations in Ach correlate with specific aspects of the task. For example, during the probability-discounting task DA levels in the mPFC change relative to the amount of reward received, whereas NAc transmission is associated with received rewards, the uncertainty associated with these rewards, and the voluntary selection of the options leading to reward (St.Onge et al., 2012). Cortical Ach efflux increases with the onset of attentional tasks such as the 5-CSRT, and it would be interesting to see if a similar effect exists on the rGT given acetylcholine's proposed role in uncertainty processing (Passetti et al., 2000; McGaughy et al., 2002; Yu & Dayan, 2005).

Conclusion

The results of this study suggest modulation of the muscarinic receptor system can affect decision making under conditions of risk and uncertainty. Specifically, we show muscarinic receptor antagonism with scopolamine impairs optimal choice on the rGT and produces a profile similar to that observed with amphetamine. This study adds to growing interest surrounding cholinergic contributions to decision-making (Fobbs &

Mizumori, 2014), and may help to understand the decision-making deficits observed in schizophrenia and Alzheimer's Disease. Future work is required to pinpoint the elusive mechanism driving amphetamine's effect on the rGT, as cholinergic signaling through muscarinic receptors does not appear to be involved.

Table 1. Effects of oxotremorine, scopolamine, and oxotremorine/amphetamine co-administration on behavioural rGT measures. Data are expressed as the mean ± SEM. *$p \leq .05$, **$p \leq .001$ indicates a significant difference compared to saline according to a paired samples *t* test. $^{\#}p \leq .05$ indicates a significant difference compared to saline-saline according to a paired samples *t* test.

	Dose (mg/kg)	Omissions (%)	Choice Latency (s)	Collect Latency (s)	Trials completed	Persev. Reward	Persev Punishment
Oxotremorine	saline	0.10 ± 0.07	1.17 ± 0.14	0.90 ± 0.07	90.38 ± 7.83	1.13 ± 0.46	121.06 ± 16.66
	0.01	0.16 ± 0.09	1.11 ± 0.11	0.96 ± 0.07	96.08 ± 8.34	1.88 ± 0.81	112.31 ± 16.99
	0.03	0.33 ± 0.13	1.08 ± 0.10	0.92 ± 0.07	90.34 ± 8.89	1.81 ± 0.89	115.81 ± 14.94
	0.1	0.74 ± 0.27	1.45 ± 0.13*	1.06 ± 0.13	89.38 ± 8.22	1.00 ± 0.43	101.88 ± 17.28
Scopolamine	saline	0.04 ± 0.04	0.90 ± 0.10	0.85 ± 0.06	92.15 ± 8.93	1.31 ± 0.62	139.94 ± 16.72
	0.01	0.83 ± 0.51	1.15 ± 0.11*	0.87 ± 0.07	93.69 ± 8.97	1.50 ± 0.90	160.63 ± 16.85*
	0.03	3.97 ± 1.24**	1.78 ± 0.22**	0.94 ± 0.08	86.94 ± 6.55	1.50 ± 0.55	182.5 ± 33.77
	0.1	13.00 ± 2.30**	2.57 ± 0.22**	1.21 ± 0.14*	68.34 ± 3.05*	2.44 ± 1.10	113.32 ± 23.81
Oxotremorine/ amphetamine	sal-sal	0.26 ± 0.22	0.90 ± 0.08	0.90 ± 0.07	97.76 ± 8.97	3.81 ± 1.74	117.13 ± 17.34
	sal-amp	2.25 ± 1.47	1.11 ± 0.14	0.91 ± 0.15	70.09 ± 7.81$^{\#}$	6.06 ± 2.29	69.63 ± 10.29$^{\#}$
	oxo -sal	0.11 ± 0.08	0.95 ± 0.10	0.89 ± 0.07	91.16 ± 8.19	2.25 ± 0.70	103.38 ± 14.42
	oxo-amp	7.84 ± 3.31	1.36 ± 0.23	2.96 ± 1.39	66.71 ± 6.38	6.25 ± 1.57	53.38 ± 9.30

Figure 1. Schematic of the Rodent Gambling Task. Each trial began with the illumination of the traylight. A nosepoke in the tray extinguished the traylight and initiated a 5-second inter-trial interval (ITI), during which all lights in the chamber were off. Following the ITI, stimulus lights were illuminated in apertures 1, 2, 4 and 5, each of which had a different schedule of reward/punishment associated with it. If the animal nosepoked one of the apertures within 10 seconds, the animal was rewarded or punished according to the schedule associated with that aperture. The size of reward and duration of punishment for each option are indicated on the schematic; the p-value in brackets beneath each of those indicates the probability of a win or loss on any given trial. On a rewarded trial, the traylight was illuminated and the requisite pellets dispensed. A response at the tray then initiated a new trial. On a punished trial, the light in the chosen aperture flashed at a frequency of 0.5Hz for the duration of the timeout period; all other lights were extinguished. At the end of the timeout, the traylight was once again illuminated and the animal could initiate a new trial. A nosepoke at an aperture during the ITI was scored as a premature response and initiated a 5-second timeout period during which the houselight was illuminated. Failure to make a response at an aperture within 10 seconds of the stimulus lights being illuminated was scored as an omission; the stimulus lights were extinguished, the traylight was once again illuminated, and the animal was able to initiate a new trial. Adapted from Neuropsychophamacology.

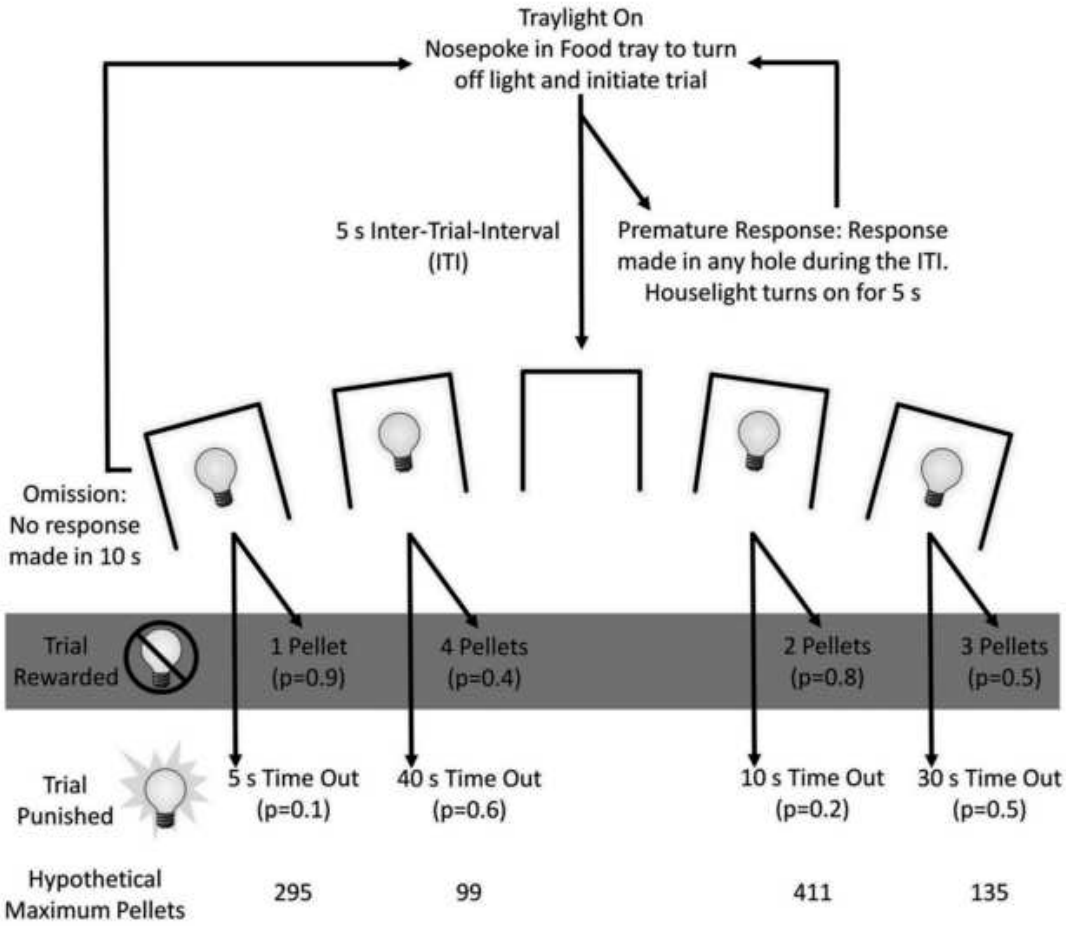
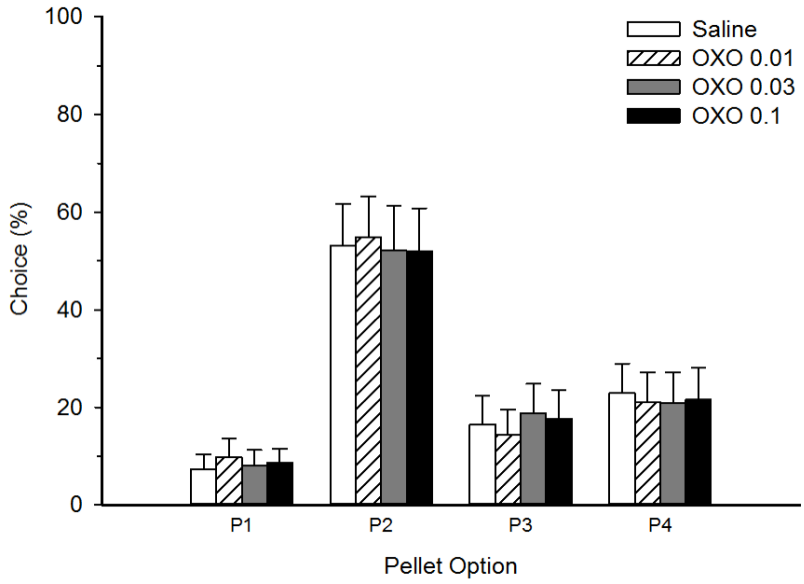


Figure 2. Choice performance (A) and premature responding (B) on the rGT following systemic administration of oxotremorine. Oxotremorine increased premature responding at the 0.03 mg.kg dose, but decreased premature responding at the 0.1 mg.kg dose. Oxotremorine had no effect on the choice profile. Data are shown as mean + SEM. *$p \leq$ .05 indicates a significant difference compared to saline according to a paired samples *t* test.

A



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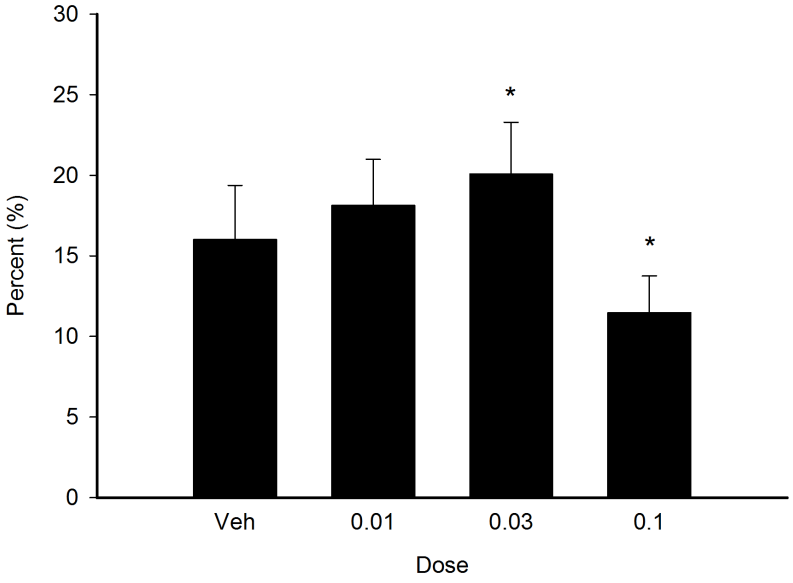


Figure 3: Choice performance (A) and premature responding (B) on the rGT following systemic administration of scopolamine. Scopolamine increased choice of P2 at 0.1 mg.kg. Scopolamine also decreased premature responding at the 0.03 and 0.1 mg.kg doses. Data are shown as mean ± SEM. *$p \leq .05$ indicates a significant difference compared to saline according to a paired samples *t* test.

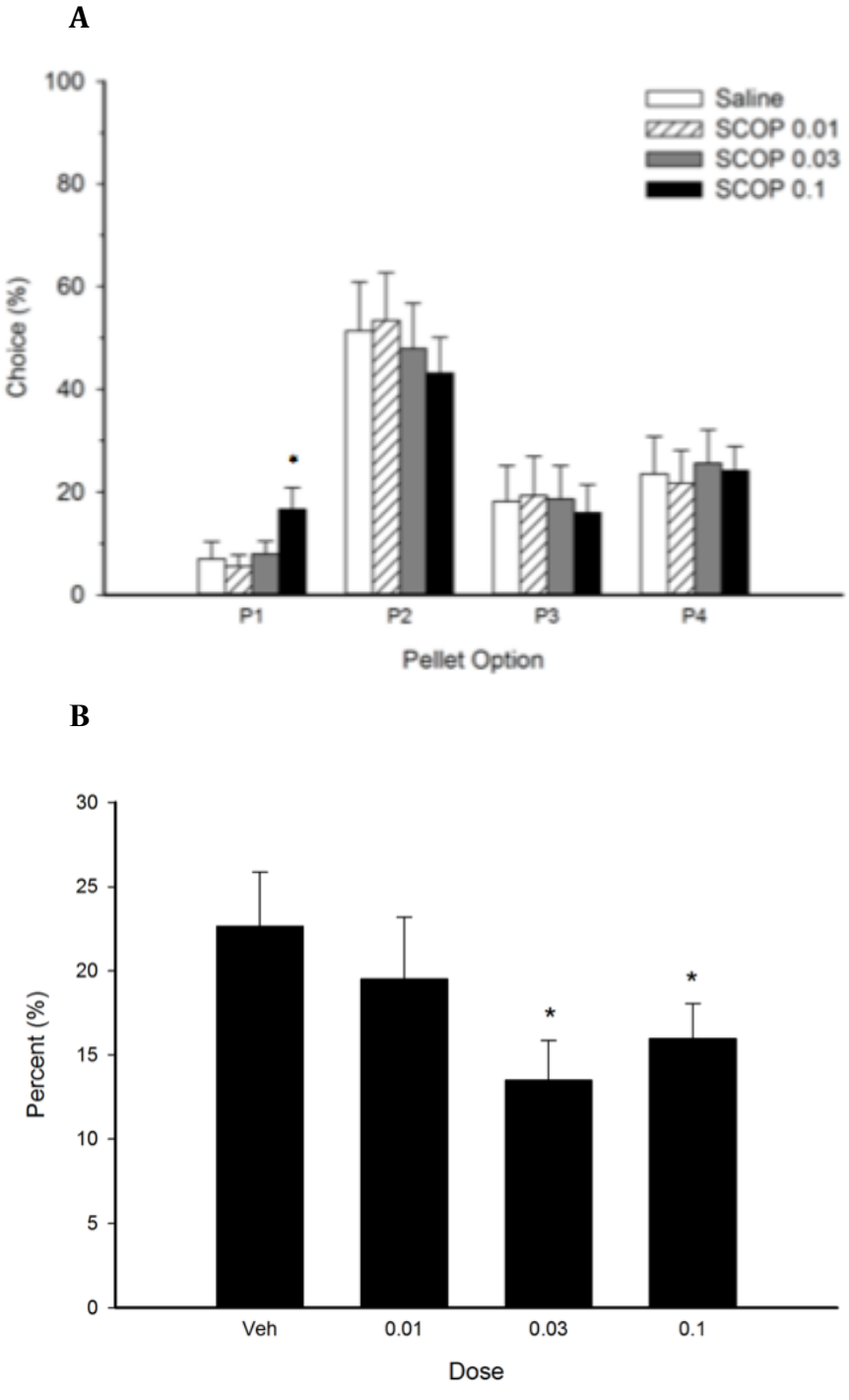
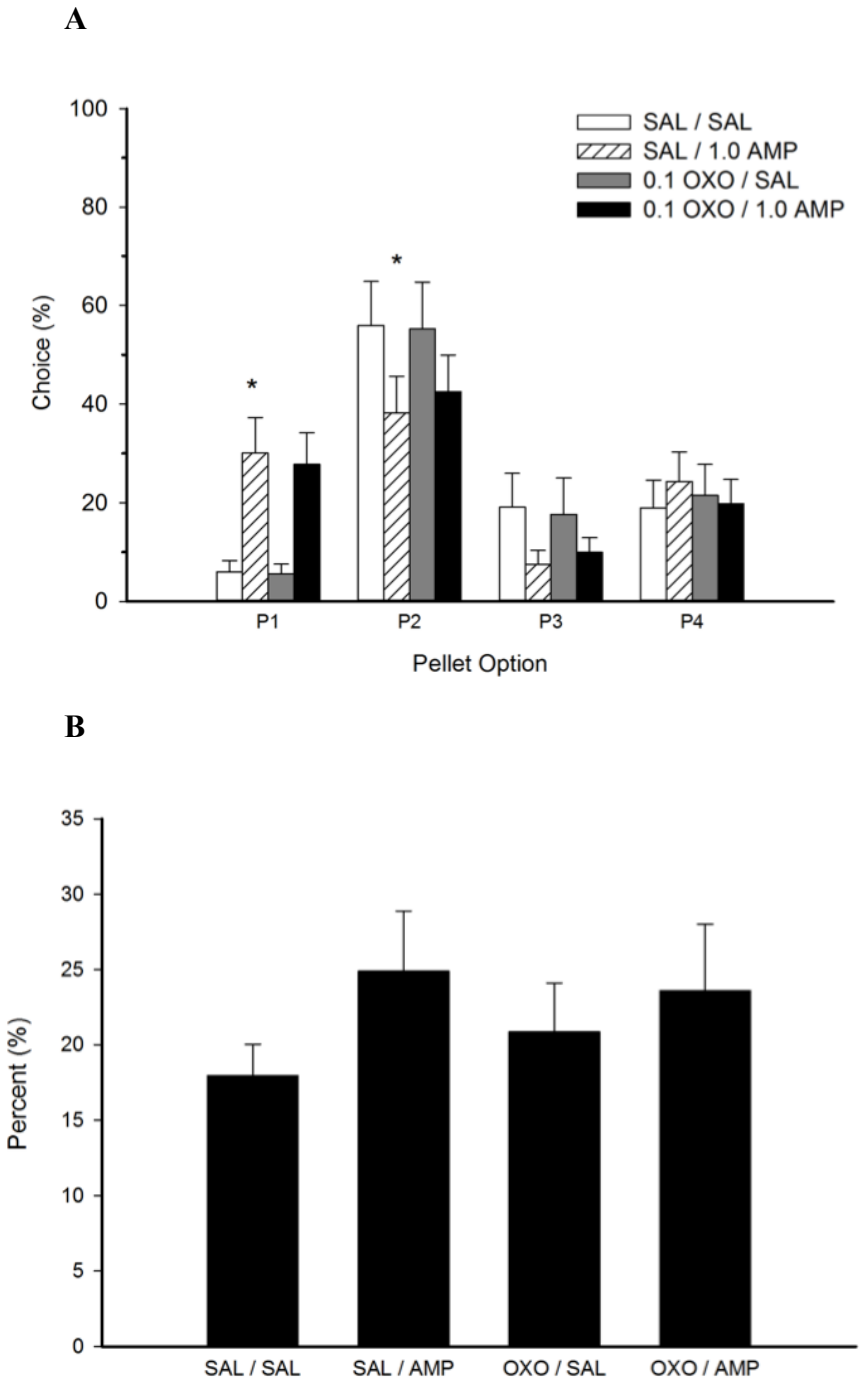


Figure 4: Choice performance (A) and premature responding (B) on the rGT following systemic administration of amphetamine, oxotremorine, and amphetamine with oxotremorine pretreatment. 1.0 mg.kg amphetamine increased choice of P1 and decreased choice of P2. Pretreatment with oxotremorine had no effect on amphetamine's choice profile. Amphetamine had no effect on measures of premature responding. Data are shown as mean ± SEM. *$p \leq .05$ indicates a significant difference compared to saline according to a paired samples *t* test.



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