THE ROLES OF NICOTINIC AND MUSCARINIC CHOLINERGIC RECEPTORS IN RISKY AND IMPULSIVE DECISION MAKING

A Dissertation

by

IAN ALFREDO MENDEZ

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

December 2010

Major Subject: Psychology

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Receptors in Risky and Impulsive Decision Making

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Approved by:

Co-Chairs of Committee,	Antonio Cepeda-Benito
	Barry Setlow
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ABSTRACT

The Roles of Nicotinic and Muscarinic Cholinergic Receptors in

Risky and Impulsive Decision Making. (December 2010)

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Co-Chairs of Advisory Committee: Dr. Antonio Cepeda-Benito
Dr. Barry Setlow

Psychopathological conditions in which decision making is impaired are common and include schizophrenia, attention deficit hyperactivity disorder, and addiction, among others. This dissertation aimed to investigate the role of cholinergic signaling in risky and impulsive decision making. Rats were trained in either a "probability discounting" task in which they chose between small guaranteed and large probabilistically delivered food rewards (a measure of risky decision making), or a "delay discounting" task in which they chose between small immediate and large delayed food rewards (a measure of impulsive decision making). Rats were also divided into high and low "risk-taking" or "impulsive" groups on the basis of their performance in the tasks.

Experiments 1 and 2 examined the effects of cholinergic drugs on performance in the probability and delay discounting task, respectively. In Experiment 1, acute administration of the acetylcholinesterase inhibitor donepezil decreased choice of the large risky reward in "risk-taking" rats. Acute administration of nicotine increased choice of the large risky reward in both groups, whereas administration of the nicotinic

receptor antagonist mecamylamine decreased choice of the large risky reward in "risk-taking" rats. In Experiment 2, nicotine increased choice of the large delayed reward and mecamylamine shifted impulsive choice in a non-specific manner in "impulsive" rats. The muscarinic receptor agonist oxotremorine decreased choice of the large delayed reward in "non-impulsive" rats and increased choice in "impulsive" rats, while treatment with the muscarinic receptor antagonist atropine increased impulsive choice in all rats.

In Experiment 3, another group of rats was used to examine correlations between baseline performance in both discounting tasks and nicotinic receptor density levels in several brain regions. Impulsive choice was positively correlated with α4β2 receptor levels in ventral hippocampus and nucleus accumbens shell, and α7 receptor levels in the basolateral amygdala, such that greater impulsivity was associated with higher receptor levels. Additionally, risky choice was negatively correlated with α4β2 receptor levels in nucleus accumbens shell, such that greater risk was associated with lower receptor levels. These experiments suggest that cholinergic receptors are involved in cost-benefit decision making and that they may prove a useful target for treatment of psychopathological conditions in which decision-making deficits are present.

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INTRODUCTION AND LITERATURE REVIEW

Cost-benefit decision making is an executive function characterized by the ability to make choices among reward options for which the outcomes differ in both cost and magnitude. Optimal cost-benefit decision making yields actions that return maximum rewards with minimal costs. Variations in the costs associated with a reward, however, may cause that reward to become more or less advantageous. To decide if selection of a reward is more or less advantageous, proper integration and evaluation of costs and rewards, as well as appropriate conflict resolution, are required. Encoding stimulus values, integration and consideration of gains and losses, and resolution of response conflict (all processes necessary for cost-benefit decision-making) are associated with frontal cortical areas (Beer et al. 2006; Critchley and Rolls 1996; Elliott et al. 2003; Wittfoth et al. 2009). The ventral tegmental area and the nucleus accumbens of the mesolimbic system have also been associated with processes necessary for cost-benefit decision making, including processing of reward and punishment, encoding of stimulus salience and reward value, and when working in concert with cortical areas, prediction of future rewards and punishments (O'Doherty 2004; Tom et al. 2007; Weller et al. 2007). The amygdala has also been shown to play a role in reward prediction; however, this role may be limited to predicting reward gain, and not reward loss (Tom et al. 2007; Weller et al. 2007).

Poor cost-benefit decision making results in impulsive and/or risky behavior,

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This dissertation follows the style of Psychopharmacology.

which can negatively impact the finances, social relationships, and health of the individual. Psychopathological conditions in which decision making is impaired are common and include schizophrenia, Alzheimer's disease, attention deficit hyperactivity disorder, Parkinson's disease, and addiction (Clark and Robbins 2002; Coyle et al. 1983; Euteneuer et al. 2009; Kalivas and Volkow 2005; Thompson et al. 2007; Weiler et al. 2009; Yip et al. 2009). Several neurotransmitter systems have been implicated in cost-benefit decision making, including dopamine, serotonin, and norepinephrine (Dallery and Locey 2005; Hoffman et al. 2006; Mobini et al. 2000; Winstanley et al. 2006). The cholinergic system has been linked with either the etiology and/or treatment of psychopathological conditions associated with altered cost-benefit decision making; however, in contrast with other neurotransmitter systems, little is known about its direct roles in cost-benefit decision making.

Acetylcholine is found in neurons projecting from the nucleus basalis (particularly in the nucleus basalis of Meynert) to neocortical areas, from the adjacent medial septum and diagonal band of Broca to the hippocampus, from the pedunculopontine nucleus in the brainstem to the ventral tegmental area, and in interneurons within the striatum (Butcher 1994; Squire 1986). Cholinergic neurons in the pedunculopontine nucleus that project to the midbrain activate both nicotinic and muscarinic acetylcholine receptors (AChRs) in the ventral tegmental area. (Good and Lupica 2009). Both nicotinic and muscarinic AChRs are found on dopamine neurons that project from the ventral tegmental area to the nucleus accumbens, and when activated, are suggested to underlie increases in reward salience and incentive

motivation for primary and secondary reinforcers (Picciotto et al. 2008; Zhang and Sulzer 2004). Nicotinic and muscarinic AChRs within the prefrontal cortex are implicated in executive function, and their activation has been suggested to improve cognitive performance (Cutuli et al. 2008; Furey et al. 2000; Furey et al. 2008; Ricciardi et al. 2009). Current research now suggests a role for both nicotinic and muscarinic AChRs in some of the underlying component psychological processes necessary for cost-benefit decision making, including executive functions within the PFC and incentive motivation within the mesolimbic system (Cutuli et al. 2008; Furey et al. 2008; Picciotto et al. 2008; Ricciardi et al. 2009). However, as mentioned above, there is very little direct evidence concerning the role of acetylcholine in cost-benefit decision making.

Research using cost-benefit decision making tasks in human smokers has begun to suggest a possible relationship between changes in nicotine levels and impulsive and risky decision making. Impulsive decision making is often assessed using delay discounting tasks, in which subjects are required to choose between a small immediate reward and a large reward with delays to its delivery (Evenden and Ryan 1996a). An increased preference for the immediate, less advantageous reward is suggestive of enhanced impulsivity. Research using delay discounting tasks has shown that long-term nicotine smokers will select the small immediate reward over the larger delayed reward more often than control subjects (Bickel et al. 1999; Field et al. 2006). The probability discounting task can assess risky decision making by requiring subjects to choose between a guaranteed small reward and a larger reward with varying probabilities of

delivery (Mobini et al. 2002). The probability discounting task has also been used to investigate risky decision making in nicotine smokers; however, these studies have produced mixed findings, reporting that smoking may increase, decrease, or have no effect on the preference for large, risky rewards over smaller guaranteed rewards (Mitchell 1999; Reynolds et al. 2004; Yu and Dayan 2005).

Although these correlational studies have shown an association between nicotine use and changes in cost-benefit decision making, it is not clear whether nicotine use (through smoking) causes changes in cost-benefit decision making or whether abnormal cost-benefit decision making is a predisposing factor for nicotine use. Because of the lack of understanding regarding the causality of this relationship, intrigue remains about whether or not changes in the activation of cholinergic receptors can affect cost-benefit decision making. To date only one study has investigated the causality of the relationship between changes in cholinergic activity and cost-benefit decision making. In this study, (Dallery and Locey 2005), the effects of several doses of nicotine (0.03, 0.1, 0.3, and 1.0 mg/kg, s.c.) on delay discounting were assessed in rats. Acute nicotine dose-dependently increased impulsive choice (increased choice of the small, immediate reward), while chronic exposure (once a day for 65 days) to each of the doses tested increased impulsive choice equally across all doses used. Furthermore, increases in impulsive choice were reported up to 30 days after discontinuation of nicotine treatment.

Decision making deficits observed in psychopathological conditions may be driven by alterations in cholinergic systems (Decker et al. 1997; Dellu et al. 1991; Diaz del Guante et al. 1991; Potter et al. 2006); therefore, the cholinergic system may provide

a target for pharmacological treatment of psychopathological conditions in which altered decision making is prominent. Clinical trials investigating the effects of cholinergic supplementation on cognitive deficits associated with numerous psychopathological conditions are currently underway (Kadir et al. 2008; Lemay et al. 2004; Winhusen et al. 2005). However, to better understand how alterations in cholinergic systems may contribute to decision making deficits, it will be necessary to define the role of the cholinergic system in normal decision making. The overall goal of this dissertation was to determine the role that cholinergic receptors play in cost-benefit decision making

Two sets of experiments used a behavioral pharmacology approach to investigate the role of nicotinic and muscarinic AChRs in cost-benefit decision making. Experiment 1 aimed to determine if experimental modulation of the cholinergic system could affect decision making for outcomes that involve possible risk of reward loss. To address this question, the effects of both nicotinic and muscarinic non-selective receptor agonists (nicotine and oxotremorine, respectively) and antagonists (mecamylamine and atropine, respectively), as well as an acetylcholinesterase inhibitor (donepezil), on performance in the probability discounting task were assessed. Experiment 2 aimed to determine if experimental modulation of nicotinic and muscarinic AChRs could affect decision making for outcomes involving delays to reward delivery. To address this question, identical methods as those used in Experiment 1 were used; however, impulsive decision making was assessed by testing animals in the delay-discounting task. Experiment 3 investigated relationships between baseline cost-benefit decision making and intrinsic nAChR subtype levels. This was accomplished by using radioligand receptor binding to

examine the relationship between baseline performance on both the probability and delay discounting tasks and nAChR subtype (α4β2 and α7) levels in brain regions implicated in cost-benefit decision making in previous experiments.

GENERAL METHODS

Subjects

The subjects were male Long-Evans rats that weighed 250-275g upon arrival (Charles River Laboratories, Wilmington, NC, USA). Experiment 1 utilized 15 rats, while Experiment 2 and 3 used separate cohorts of 16 rats each. Rats were housed individually in a climate-controlled vivarium (25^{o} C) in the Department of Psychology at Texas A&M University. Rats had food and water available *ad lib* (except as noted below). Testing was done during the light cycle of a 12-hour light/dark schedule (lights on 0800-2000). Animal testing was conducted according to the "Principles of Laboratory Animal Care" (National Academy of Sciences, USA) and met all NIH and institutional animal care and use guidelines. Rats were allowed to acclimate to vivarium conditions for at least one week before the start of data collection. Prior to testing in the discounting and instrumental responding tasks, all animals were food restricted to 85% of their free feeding weight. Upon completion of these tasks, rats were returned to an *ad lib* feeding schedule.

Behavioral Apparatus

Decision making and instrumental responding were assessed in eight identical standard rat behavioral test chambers (31 X 25 X 31 cm) with aluminum front and back walls, acrylic side walls, and a floor composed of steel rods (0.4 cm diameter, spaced 1.1 cm apart), and located in sound-attenuating cubicles (Coulbourn Instruments, Allentown,

PA, USA). A recessed food delivery trough (4.1 X 3.2 cm) equipped with a photobeam to detect head entries was placed in the center of the front wall of the chambers (2.2 cm above the floor). For the discounting tasks, a standard retractable response lever was placed on each side of the food trough (11 cm above the floor), and they were extended and withdrawn as described in the task behavioral protocol. When testing instrumental responding in these chambers, only 1 retractable response lever was extended, and the side that had the extended lever was counterbalanced across groups. The chambers were interfaced with a computer running Graphic State 3.01 software (Coulbourn Instruments) to control stimulus deliveries and record data.

Activity and exploratory measures were collected in eight identical activity monitoring chambers (Versamax System, Accuscan Instruments, Columbus, OH, USA). Each chamber (40×40×30 cm) contained an array of photobeams raised 0.5 cm above the floor to detect movement in the horizontal plane. The activity chambers were connected to a computer running Versamap software (Accuscan Instruments) which recorded photobeam breaks.

Behavioral Protocols

Probability Discounting Task

Test sessions for the probability discounting task were run once a day. Each session was 60 minutes long and consisted of five blocks of 18 trials each. Each 40-second trial began with a 10 second illumination of the food trough and house lights. A nose poke into the food trough during this time extinguished the food trough light and

triggered extension of either a single lever (forced choice trials) or both levers simultaneously (choice trials). Trials on which rats failed to nosepoke during this time window were scored as omissions. A press on one lever (either left or right, counterbalanced across animals) resulted in a single food pellet (the small reward) being delivered immediately following the lever press. A press on the other lever resulted in delivery of two food pellets (the large reward). During the first block of trials, the large reward was delivered with 100% probability following selection of the large reward lever. During each of the four subsequent blocks, the probability of large reward delivery was systematically decreased (75, 50, 25, 0%). Each 18 trial block began with eight forced choice trials used to expose the rats to the reward probabilities in effect for that block (4 for each lever), followed by 10 choice trials. Once either lever was pressed, both levers were immediately retracted. Food delivery was accompanied by re-illumination of both the food trough and house lights, which were extinguished upon entry to the food trough to collect the food or after 10 seconds, whichever occurred first.

To determine baseline responding, animals were run on this task until five consecutive sessions of stable responding and a within-session effect of probability block were achieved (Cardinal et al. 2000b; Simon et al. 2007; Winstanley et al. 2006). Following stable responding, animals were treated every other day with systemic administration of one of four doses of a cholinergic drug no more than 30 minutes prior to testing, with treatments administered in a counterbalanced order across animals. On the days between treatment days animals were run in the task with no pharmacological manipulation.

Delay Discounting Task

In the delay discounting task, rats were again given the option between a small immediate, guaranteed reward and a large discounted reward. However, in the delay discounting task, the large rewards were guaranteed on each trial, but delayed in their delivery. Sessions were 60 minutes long and consisted of five blocks of 12 trials each. Each 60-second trial began with illumination of the food trough and house lights. In this task, a press on one lever (either left or right, counterbalanced across subjects) resulted in 1 food pellet delivered immediately, and a press on the other lever resulted in delivery of 3 food pellets after a delay. The duration of the delay varied with each block (0s, 4s, 8s, 16s, 32s) on a schedule that has been shown to produce a robust discounting curve (Evenden and Ryan 1996b; Simon et al. 2009; Winstanley et al. 2006). Following stable responding, animals were again treated every other day with systemic administration of one of four doses of a cholinergic drug no more than 30 minutes prior to testing, with treatments administered in a counterbalanced order. On the days between treatment days animals were run in the task with no pharmacological manipulation.

Instrumental Responding Task

Instrumental responding for single pellet food rewards was first assessed using fixed ratio schedules (FR1, 3, 10, 20, 40, one schedule/day). Test sessions in the fixed ratio task were 30 minutes in length and occurred once a day. After testing with the fixed ratio schedules, instrumental responding was assessed in one session using a progressive ratio schedule of reinforcement, on which the number of lever presses required to earn a reward increased with each successive reward earned (1, 4, 10, 20, 35, …) (Barr and

Phillips 1999; Cetin et al. 2004; Kheramin et al. 2005). These sessions varied in length, ending only after an hour with no reward delivery had passed (the breakpoint).

Open Field Test

To obtain a baseline measure of exploratory behavior when introduced to a novel environment, rats were placed in locomotor activity chambers for a 1 hour test session, during which data on horizontal activity and time spent in center region were collected to assess locomotor and exploratory behavior, respectively. Locomotor activity was tested under red light and white noise conditions.

Radioligand Binding Protocol

A receptor binding experiment examined nAChR levels in brain regions associated with impulsive and risky decision making, including structures within the striatum and prefrontal cortex. Sixteen rats were trained and tested in both the probability and risk discounting task. Rats were tested in one of the two tasks until 5 consecutive sessions of stable responding were reached, then switched over to the other task again until 5 sessions of stable responding were achieved. Immediately following completion of the two discounting tasks, rats were run in the instrumental responding task and Open Field Test described above. Rats were then deeply anesthetized with inhaled isoflurane and sacrificed for brain harvesting.

Collected brains were immediately frozen at -30^{o} C in isopentane for 60 seconds, and stored at -80^{o} C. Twenty micrometer thick coronal brain sections were taken on a cryostat at -18^{o} C from cortical (orbitofrontal, medial prefrontal, infralimbic, prelimbic,

cingulate, and agranular insular cortex) and limbic (nucleus accumbens shell and core, basolateral amygdala, and ventral and dorsal hippocampus) structures in the forebrain, as well as structures within the midbrain (ventral tegmental area,), and mounted onto Superfrost-plus microscope slides (VWR, USA). Slides were kept on ice during cutting, then desiccated overnight at 4^{o} C and temporarily stored at -80^{o} C. Using receptor autoradiography, nAChR subtype levels were identified using ^{125}I radioligands. Epibetadine was used to identify α4β2 nAChR subtype, while α-bungarotoxin was used to identify the α7 nAChR subtype. Tissue sections were pre-incubated in fresh Tris-HCl buffer solution (50 mM Tris-HCl base, 120 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, and 1 mM $MgCl_2$, adjusted to pH 7.4 with 10 N HCl) for 5 minutes. For total binding, sections were incubated at room temperature with predetermined radioligand for a set amount of time, within the Tris-HCl buffer. Adjacent sections designated for nonspecific binding were processed in the presence of 100 μM of nicotine hydrogen tartrate, which was added to pre-incubation and incubation buffers. Sections were incubated at room temperature for 60 minutes and washed in ice-cold incubation buffer twice for 5 minutes each, followed by ice-cold ddH_2O for 30 seconds, dried under constant airflow for 1 hour at room temperature and exposed to film (Kodak Biomax MR Film) along with [^{125}I]-standards of known radioactivity.

After film exposure for several days (2 days for epibetadine, 3 days for α-bungarotoxin) the films were developed in D19 Kodak developer for 4 minutes, rinsed in water, and fixed in Kodak Rapid Fixer for 5 minutes. The slides were then exposed to vapor fixation and stained in Cresyl Violet solution to assist the analysis. Images from

autoradiograms were quantitatively analyzed using a PC-based image analysis system (InterFocus Imaging Ltd., UK). A calibration curve of radioactivity nCi/mg tissue versus optical density was generated using the [^{125}I]-standard for receptor ligand binding.

Statistical Analysis

Raw data files were exported from Graphic State software and were compiled using a custom macro written for Microsoft Excel (Dr. Jonathan Lifshitz, Spinal Cord and Brain Injury Research Center, University of Kentucky). Statistical analyses were conducted in SPSS 16.0. Response omission and intertrial interval (ITI) activity during treatment days were assessed using repeated measures ANOVA. Stable behavior was determined using a two-way repeated measures ANOVA (Session x Probability/Delay), across 5 consecutive days, and was defined by a main effect of trial block in the absence of a main effect or interaction involving day. Primary and post-hoc analyses of the probabilistic and delay discounting tasks on drug treatment days were conducted using two-way repeated measures ANOVA (Dose X Probability/Delay). Stable behavior was also determined for intertreatment days using a two-way repeated measures ANOVA (Day x Probability/Delay), across each of the 4 days that followed the treatment days, and was defined by the absence of a main effect of day or interaction between day and block. Relationships between baseline behavioral performance and receptor binding data were determined using two-tailed Pearson's bi-variate correlations. In all cases, *p* values less than .05 were considered significant.

EXPERIMENT 1. ROLE OF NICOTINIC AND MUSCARINIC RECEPTORS IN PROBABALISTIC DECISION MAKING

Experiment 1.1. Effects of the Acetylcholinesterase Inhibitor Donepezil on Performance in the Probability Discounting Task

To begin to understand the role cholinergic systems may play in risky behavior, the effects of the centrally acting reversible acetylcholinesterase inhibitor donepezil on performance in the probability discounting task were assessed. This task characterizes the ability of varying food reward probabilities to promote discounting of a large reward, and is considered to assess risk-taking behavior (Mobini et al. 2002). Based on the general cognitive enhancing effects reported following increases in acetylcholine (Cutuli et al. 2008; Furey et al. 2000; Furey et al. 2008; Ricciardi et al. 2009), it was predicted that donepezil would improve decision making by decreasing disadvantageous risky responding in the probability discounting task.

Treatment

Following stable responding in the probability discounting task, animals were treated every other day with systemic administration of one of four doses of donepezil (0, 0.3, 1.0, 3.0 mg/kg, i.p.) 30 minutes prior to testing in the task. All drugs were purchased from Sigma Aldrich, except for donepezil, which was purchased from A&A Pharmachem. All drugs were dissolved in 0.9% saline, and administered at a volume of 1 ml/kg.

Results

No significant effects of donepezil treatment on response omissions were observed during test days in the probabilistic discounting task (Table 1). Conversely, a significant effect of donepezil treatment on ITI locomotor activity, during test days in the probabilistic discounting task, was observed ($F_{(3,42)} = 4.74$, $p < .01$, Table 1). Post-hoc analyses revealed no significant effects when comparing ITI activity following treatment with only the low or middle dose of donepezil to that following treatment with saline; however, a significant decrease in activity was observed when comparing activity following saline treatment to that following treatment with the high dose of donepezil ($p < .01$).

Table 1. Effects of Cholinergic Drugs on Response Omissions and Locomotor Activity During Testing in the Probability Discounting Task.

	Dose	Donepezil Experiment 1.1	Nicotine Experiment 1.2	Oxotremorine Experiment 1.3	Mecamylamine Experiment 1.4	Atropine Experiment 1.5
Mean Percent Choice Trials Omitted	Veh	0.3%	0.3%	0.4%	2.0%	4.5%
	Low	4.4%	0.1%	10.8% *	1.2%	22.4% *
	Med	0.0%	0.0%	4.3%	1.6%	41.6% *
	Hi	0.7%	6.1% *	24.9% *	2.1%	49.3% *
Mean ITI Locomotor Activity (beam breaks)	Veh	2068	2382	1983	1618	1395
	Low	1858	2621	1499	1463	1383
	Med	1877	2939 *	1480 *	1637	1230
	Hi	1481 *	1949	987 *	1490	1319

* = significantly different from Veh

Analysis of 5 days of baseline responding prior to testing using a two-way repeated measures ANOVA showed no significant main effect of day ($F_{(4,56)} = 1.13$, $p = .35$) or interaction between day and block ($F_{(16,224)}=.89$, $p = .58$), but a significant effect of probability ($F_{(4,56)} = 171.66$, $p < .01$). This suggests that animals were responding stably before treatment began and that they discounted the large reward as a function of the probability of its delivery. The effects of all doses of donepezil on the performance of rats in the probabilistic decision making task were assessed using a two-factor repeated measures ANOVA. A significant main effect of dose ($F_{(3,39)} = 2.91$, $p < .05$) was observed in our initial analysis, although there was no significant interaction between dose and block ($F_{(12,156)} = 1.70$, $p = .07$). Post-hoc analysis using two-factor ANOVA compared each drug dose with saline control conditions and revealed no significant effects, although treatment with the high dose of donepezil caused a near significant increase in preference for the large risky reward ($p = .08$), when compared to choice preference following treatment with saline.

Although there were no significant effects found when comparing each dose individually with saline conditions, interesting trends in performance were observed following treatment with each dose of donepezil. A small shift in preference toward the smaller, safer reward was observed following treatment with either the low or middle dose of donepezil; conversely, a moderate shift in preference toward the larger, risky reward was observed following treatment with the high dose of donepezil (Figure 1(A)). These trends, along with an overall main effect of drug dose, supported additional analyses of the data obtained. Baseline performance on the probability discounting task

allows for the characterization of the rats' "natural" propensity for risk-taking. To examine how donepezil administration interacted with baseline levels of risk taking, animals from this experiment were split into "risk-taking" and "risk-averse" groups based on whether they fell above or below the median percentage of choices (averaged across blocks) of the large, risky reward during the 5 days of stable responding immediately prior to testing with the drug.

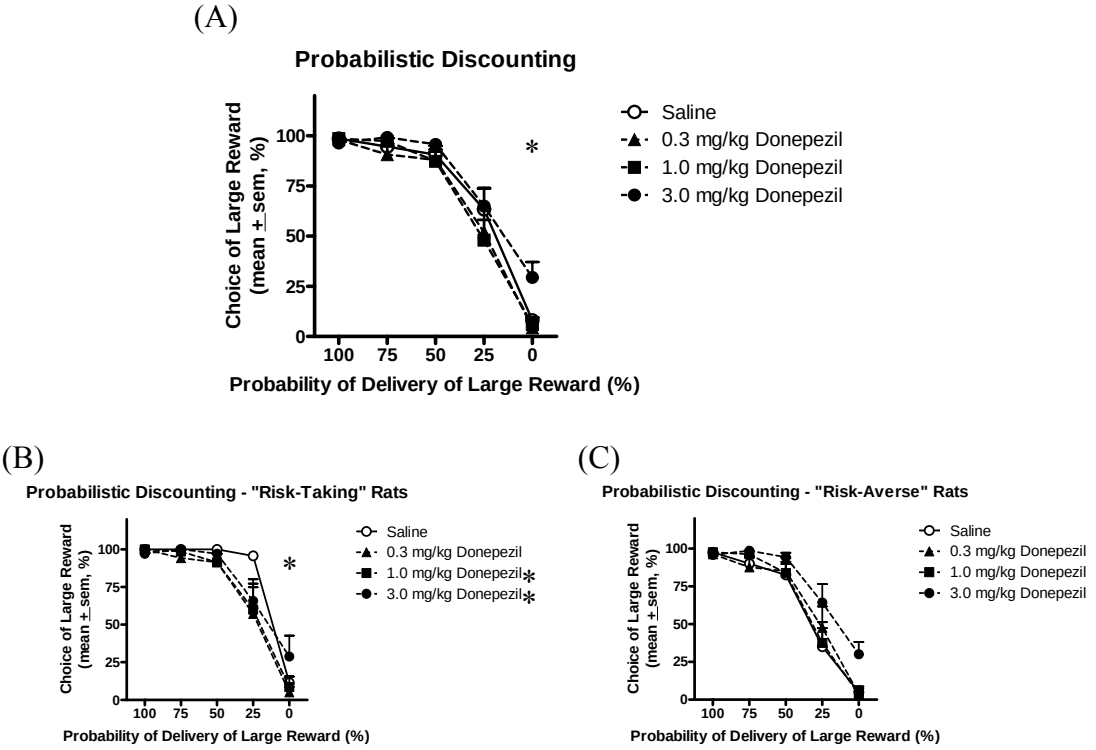


Figure 1. Effects of donepezil treatment on responding in the probability discounting task. (A) Treatment with donepezil significantly affected performance in the probability discounting task, when testing all rats. However, post-hoc analyses revealed no between group differences. (B) Treatment with all doses of donepezil significantly shifted the performance of risk-taking rats in the probability discounting task. Post-hoc analyses revealed that the middle and high doses of donepezil (black triangles, squares, and circles, respectively) significantly shifted preference towards the small reward when compared to performance following treatment with saline. (white circles) (C) No significant shift in performance was observed in risk-averse rats following treatment with donepezil, when compared to performance following treatment with saline. * $p < .05$.

The effects of all doses of donepezil on the performance of risk-taking rats in the probabilistic decision making task were assessed using a two-factor repeated measures ANOVA. In risk taking rats, a significant main effect of dose ($F_{(3,18)} = 3.76$, $p < .05$) and a significant interaction between dose and block ($F_{(12,72)} = 2.62$, $p < .01$) were observed. Individual post-hoc comparisons with a two-factor ANOVA revealed that following treatment with the middle and high doses of donepezil prior to testing, risk-taking rats significantly shifted their preference towards the smaller, safer reward across the five blocks, when compared to their performance after saline treatment ($ps < .01$). Although treatment with the low dose of donepezil caused a similar shift in preference to that seen with the higher doses in risk-taking rats, this effect was not quite significant ($p = .06$). Thus, donepezil appeared to decrease risk-taking in rats with high baseline levels of risk-taking (Figure 1(B)).

A two-factor repeated measures ANOVA was again used to assess the effects of donepezil on the performance of risk-averse rats in the probabilistic decision making task. There was no significant main effect of dose ($F_{(3,18)} = 2.95$, $p = .06$) or interaction between dose and block ($F_{(12,72)} = 1.17$, $p = .32$). Risk-averse rats did show a modest shift toward preference for the larger risky reward, when comparing choice preference following treatment with the highest dose of donepezil to that following treatment with saline. Nonetheless, analysis suggests that donepezil does not appear to significantly alter risk-taking in rats with low baseline levels of risk-taking (Figure 1(C)).

The most apparent difference between the risk-taking and risk-averse rats was that risk taking rats chose the larger, risky reward much more often during the 4th

session block. Selection of the large reward is considered a "risky" decision because delivery of the large reward is not guaranteed (except during block 1). Furthermore, although selection of the large reward is risky, these choices do not necessarily become less advantageous, or "poor" decisions, until the 4th session block (25% probability), when choice of the large reward yields less total food delivery across the 10 choice trials (5 pellets), than selection of the small guaranteed reward (10 pellets). Because donepezil decreased risky decision making in risk-taking rats, these data suggest that increases in acetylcholine availability can improve cost-benefit decision making. The observed results in risk-taking rats support our prediction that increases in acetylcholine can decrease risky decision making, as determined by the probability discounting task. These results also suggest that the observed effect may be dependent on differences in baseline risk behavior, which in turn, may be dependent on intrinsic differences in cholinergic function (the relationship between such intrinsic differences in acetylcholine systems and decision making was investigated in Experiment 3).

Experiment 1.2. Effects of the Nicotinic Receptor Agonist Nicotine on Performance in the Probability Discounting Task

Changes in nicotinic acetylcholine receptor (nAChR) function may underlie deficits in cost-benefit decision making, which are characteristic of several psychopathological conditions including ADHD, Parkinson's disease, and addiction (Clark and Robbins 2002; Coyle et al. 1983; Kalivas and Volkow 2005; Thompson et al. 2007). The purpose of this experiment was to investigate the effects of acute

administration of several doses of the non-selective nAChR agonist nicotine on probabilistic decision making. Research has shown increases in cognitive function following acute nicotine administration (Cutuli et al. 2008; Furey et al. 2000; Furey et al. 2008; Ricciardi et al. 2009). However, both acute and chronic nicotine have been shown to cause increases in impulsive decision making (Dallery and Locey 2005). Based on the observed decrease in risky behavior following treatment with donepezil, it was predicted that rats with high baseline levels of risk-taking would shift their preference toward the smaller, safer reward following acute treatment with nicotine.

Treatment

This experiment used the same design and statistical analysis as Experiment 1.1; however, the effects of the nAChR agonist nicotine (0, 0.1, 0.3, 1.0 mg/kg, s.c.) were investigated instead. In addition, the large reward consisted of 4 pellets and the probability of its delivery was decreased across the 5 blocks at a schedule of 50, 25, 12.5, and 0 percent. This change was made in an attempt to shift the baseline response curve to be more centered in the parametric space.

Results

Significant effects of nicotine treatment on response omissions and ITI locomotor activity, during test days in the probabilistic discounting task, were observed (Table 1). A significant effect of nicotine dose was observed when comparing response omissions during treatment days ($F_{(3,42)} = 6.83$, $p < .01$). Post-hoc analyses revealed no significant effects when comparing response omissions following treatment with only the low or middle dose of nicotine to that following treatment with saline, but omissions

significantly increased with the high dose of nicotine ($p < .05$). A significant effect of nicotine dose was also observed when comparing ITI locomotor activity during treatment days ($F_{(3,30)} = 7.16$, $p < .01$). Post-hoc analyses revealed no significant effects when comparing ITI activity following treatment with only the low or high dose of nicotine to that following treatment with saline; however, results do show that ITI activity significantly increased when comparing activity following treatment with the middle dose of nicotine to that following treatment with saline ($p < .05$).

Analysis of 5 days of baseline responding prior to testing using a two-way repeated measures ANOVA showed no significant main effect of day ($F_{(4,56)} = 1.40$, $p = .25$) or interaction between day and block ($F_{(16,224)} = .83$, $p = .66$), but a significant effect of probability ($F_{(4,56)} = 255.85$, $p < .01$), indicating that animals were responding stably before treatment began. The effects of all doses of nicotine on the performance of rats in the probabilistic decision making task were assessed using a two-factor repeated measures ANOVA. A significant main effect of dose ($F_{(3,36)} = 13.04$, $p < .001$) and a significant interaction between dose and block ($F_{(12,144)} = 4.64$, $p < .001$) were observed. Individual post-hoc comparisons with a two-factor ANOVA revealed that treatment with the highest dose of nicotine significantly increased preference for the large risky reward when compared to saline ($p < .01$). Post-hoc analyses revealed no significant effects when comparing performance on the task following treatment with the low and middle dose of nicotine with performance after saline treatment. Thus, nicotine appeared to increase risk-taking in rats (Figure 2(A)).

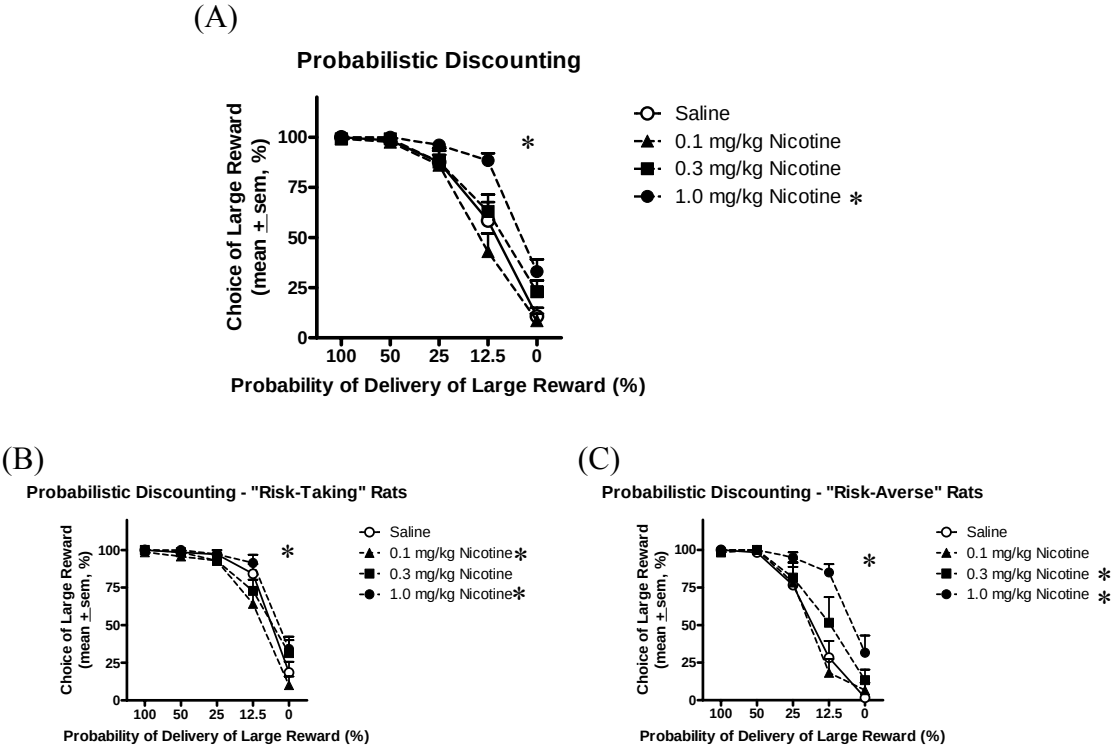


Figure 2. Effects of nicotine treatment on responding in the probability discounting task. (A) Analysis of treatment with all doses of nicotine showed a significant shift in performance in the probability discounting task. Post-hoc analyses revealed that the high dose of nicotine (black circles) significantly shifted preference towards the large reward when compared to performance following treatment with saline. (white circles). (B) Risk-taking rats treated with all doses of nicotine significantly shifted their preference in the probability discounting task. Post-hoc analyses revealed that the low dose of nicotine (black triangles) significantly shifted preference towards the small, safe reward, while treatment with the high dose of nicotine (black circles) significantly shifted preference towards the large, risky reward, when compared to performance following treatment with saline. (white circles) (C) Risk-averse rats treated with all doses of nicotine also significantly shifted their performance in the probability discounting task. Post-hoc analyses revealed that both the middle (black squares) and high dose of nicotine (black circles) resulted in a significant increase in preference towards the large, risky reward, when compared to performance following treatment with saline. (white circles). * $p < .05$.

As with the donepezil experiment (Experiment 1.1), animals were split into risk-taking and risk-averse groups based on whether they fell above or below the median

percent choice (averaged across blocks) of the large, risky reward during the 5 days of stable responding immediately prior to testing. The effects of all doses of nicotine on the performance of risk-taking rats in the probabilistic decision making task were assessed using a two-factor repeated measures ANOVA. A significant main effect of dose ($F_{(3,18)} = 5.45$, $p < .01$) and a significant interaction between dose and block ($F_{(12,72)} = 1.93$, $p < .05$) were observed. Individual post-hoc comparisons using a two-way repeated measures ANOVA revealed a significant increase in preference for the large risky reward, when comparing performance following treatment with the highest dose of nicotine to performance following saline treatment ($p < .01$), in risk-taking rats. Conversely, post-hoc analyses also revealed a significant decrease in preference for the large risky reward, when comparing performance on the task following treatment with the low dose of nicotine to performance after saline treatment ($p < .05$). No post-hoc effects were observed when comparing performance on the task following treatment with the middle dose of nicotine with performance after saline treatment. Thus, nicotine appeared to both increase and decrease risk-taking in rats with high baseline levels of risk-taking, depending on the dose (Figure 2(B)).

The effects of all doses of nicotine on the performance of risk-averse rats in the probabilistic decision making task were also assessed using a two-factor repeated measures ANOVA. A significant main effect of dose ($F_{(3,15)} = 12.21$, $p < .001$) and a significant interaction between dose and block ($F_{(12,60)} = 4.58$, $p < .001$) were observed. Individual post-hoc analyses using a two-factor ANOVA resulted in a significant increase in preference for the large risky reward ($ps < .05$), when comparing rats

performance following treatment with both the middle and high dose of nicotine to performance following saline treatment, in risk-averse rats. Post-hoc analyses revealed no significant changes in choice preference when comparing performance following treatment with the low dose of nicotine to performance after saline treatment. Thus, nicotine appeared to increase risk-taking in rats with low baseline levels of risk-taking (Figure 2(C)).

Findings suggest that nAChRs are involved in probabilistic decision making. The results observed in risk-taking rats following treatment with the low dose of nicotine support our predictions (based on Experiment 1.1) that increases in nAChR activity would decrease risk-taking. Interestingly, treatment with the high dose of nicotine caused increases in risky behavior in both risk-taking and risk-averse rats. Together with the findings of Dallery and Locey, these data suggest that acute treatment with nicotine in rats will cause shifts in choice preference when testing in the probability and delay discounting tasks. What remains unclear are the mechanisms through which acute treatment with nicotine affects discounting in these tasks. Activation of nAChRs within the mesolimbic system has been shown to increase reward salience and reinforcement, as well as incentive motivation (Picciotto et al. 2008; Zhang and Sulzer 2004). These motivational increases may explain why nicotine treated rats were willing to respond for larger amounts of food reward, even when that reward was accompanied by a high risk of loss.

Experiment 1.3. Effects of the Muscarinic Receptor Agonist Oxotremorine on Performance in the Probability Discounting Task

Although muscarinic acetylcholine receptors (mAChRs) have been implicated in various forms of executive function, whether or not mAChR are involved in cost-benefit decision making is unknown (Friedman 2004; Gasbarri et al. 1997; Levin et al. 1997a). The purpose of this experiment was to investigate the effects of acute administration of several doses of the non-selective mAChR agonist oxotremorine on probabilistic decision making. Although general muscarinic activation within the prefrontal cortex is associated with enhanced cognition (Chen et al. 2004; Chouinard et al. 1995; De Rosa and Hasselmo 2000), the effects of mAChR activation within the mesolimbic system on cost-benefit decision making are unclear. There is some evidence that the mAChRs subtypes M^1, M^3, M^4, and M^5 are implicated in the evaluation of rewards; for example, activation of the M^1 subtype has been shown to increase at the end of a meal, suggesting a possible role in signaling hedonic satiation and inhibition of reward seeking and taking behaviors (Levey et al. 1995; Pratt and Kelley 2004; Pratt et al. 2007). Based on the observed decrease in risky behavior following treatment with donepezil, it was predicted that rats with high baseline levels of risk-taking would shift their preference toward the smaller, safer reward following acute treatment with oxotremorine.

Treatment

This experiment used the same experimental design and statistical analysis as Experiment 1.2; however, the effects of the non-selective mAChR agonist oxotremorine (0, 0.03, 0.1, 0.3 mg/kg, i.p.) were investigated instead.

Results

Significant effects of oxotremorine on response omissions and ITI locomotor activity during test days in the probabilistic discounting task were observed (Table 1). A significant effect of oxotremorine dose was observed when comparing response omissions during treatment days ($F_{(3,45)} = 10.49$, $p < .01$). Post-hoc analyses revealed a near significant effect when comparing response omissions following treatment with middle dose of oxotremorine to that following treatment with saline ($p = .06$), and response omissions significantly increased following treatment with the low and high doses of oxotremorine ($p < .05$). A significant effect of oxotremorine dose was observed when comparing ITI locomotor activity during treatment days ($F_{(3,42)} = 5.17$, $p < .01$). Post-hoc analyses revealed no significant effects when comparing ITI activity following treatment with only the low dose of oxotremorine to that following treatment with saline; however, results do show that ITI activity significantly decreased when comparing activity following treatment with the either the middle or high dose of oxotremorine to that following treatment with saline ($ps < .05$).

Analysis of 5 days of baseline responding prior to testing using a two-way repeated measures ANOVA showed no significant main effect of day ($F_{(4,52)} = .38$, $p = .82$) or interaction between day and block ($F_{(16,208)} = .92$, $p = .55$), but a significant effect of probability ($F_{(4,52)} = 12.48$, $p < .01$). This suggests that animals were responding stably before treatment began. Treatment with the high dose of oxotremorine greatly increased response omissions in the task (8 rats omitting responses in at least 7 of the 10 choice trials for at least 1 block with the high dose, versus 2 rats with the low and

medium dose), resulting in insufficient data for analysis. Thus, data from the high dose were eliminated in subsequent analyses. Analysis of data from saline, as well as the low and middle doses of oxotremorine, using a two-factor repeated measures ANOVA revealed no significant main effect of dose ($F_{(2,22)} = .91$, $p = .42$) or interaction between dose and block ($F_{(8,88)} = 1.58$, $p = .14$). Thus, oxotremorine did not appear to affect risk-taking when analyzing data from all rats (Figure 3(A)).

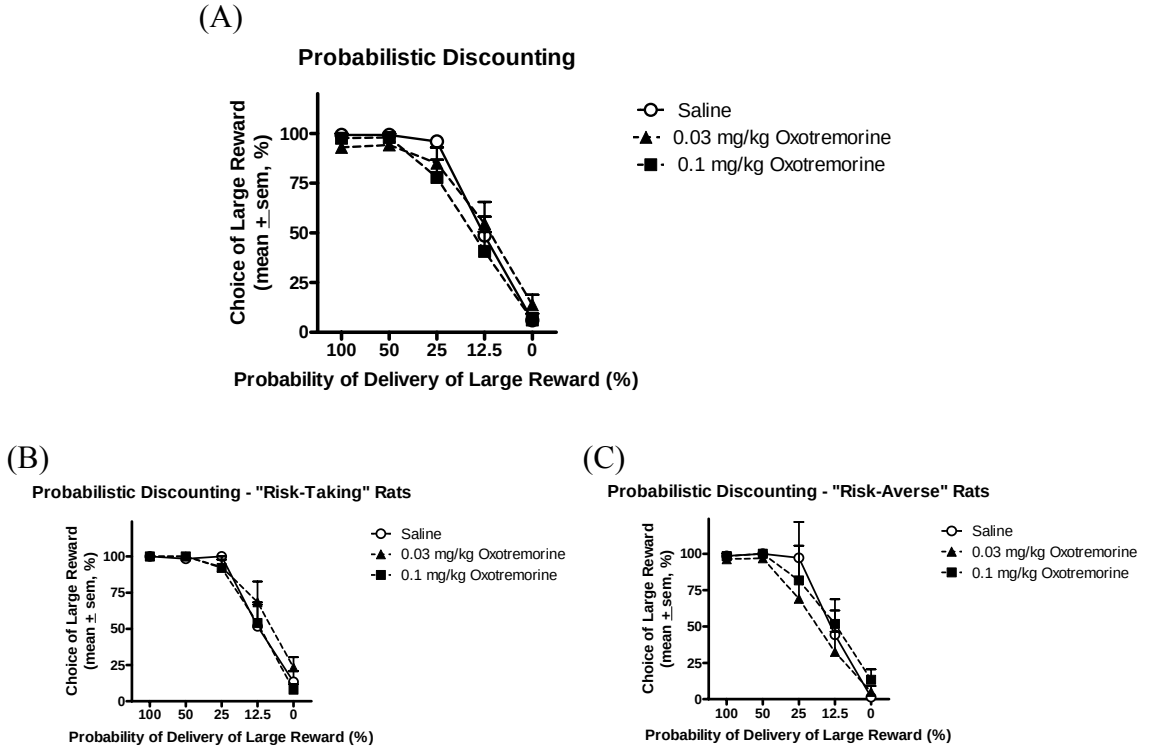


Figure 3. Effects of oxotremorine treatment on responding in the probability discounting task. (A) Treatment with oxotremorine resulted in no significant shifts in performance in the probability discounting task, when compared to preference following treatment with saline. Effects of the highest doses are not reported due an increase in response omissions. (B) Treatment with oxotremorine showed no significant shift in risk-taking rats in performance on the probability discounting task, when compared to performance following treatment with saline. (C) Similar to risk-taking rats, risk averse rats showed no significant shift in performance following treatment with oxotremorine, when comparing to performance following treatment with saline.

As in previous experiments, animals were split into risk-taking and risk-averse groups based on whether they fell above or below the median percent of choice for the large, risky reward, totaled across blocks. The effects of all doses of oxotremorine on the performance of risk-taking and risk-averse rats in the probabilistic decision making task were assessed using a two-factor repeated measures ANOVA. Similar to the results observed when comparing all rats, in risk-taking rats, results indicated no main effect of dose ($F_{(2,8)} = 1.54$, $p = .27$) or interaction between dose and block ($F_{(8,32)} = .90$, $p = .53$), on performance in the task. Thus, oxotremorine did not appear to affect risk-taking in rats with high baseline levels of risk-taking (Figure 3(B)). Furthermore, no main effect of dose ($F_{(2,12)} = .75$, $p = .49$) or interaction between dose and block ($F_{(8,48)} = 1.13$, $p = .36$) on performance was observed in risk-averse rats. Thus, oxotremorine did not appear to affect risk-taking in rats with low baseline levels of risk-taking (Figure 3(C)). Available data from this experiment did not support our predicted results that activation of mAChRs would decrease risky decision making, as assessed by performance in the probability discounting task.

Experiment 1.4. Effects of the Nicotinic Receptor Antagonist Mecamylamine on Performance in the Probability Discounting Task

In addition to testing the effects of nicotinic and muscarinic AChR agonists on probabilistic discounting, this dissertation project also examined the effects of nicotinic and muscarinic AChR antagonists on probabilistic discounting. Experiment 1.4 used systemic administration of the non-selective nAChR antagonist mecamylamine, to

determine the effects of nAChR blockade on performance in the probability discounting task. Based on data from Experiment 1.2, suggesting that increased activation of nAChR increases risk-taking behavior when testing all rats, it was predicted that blockade of nicotinic cholinergic receptors would decrease risk-taking behavior in all rats. This would be evident as a decrease in preference for the large risky reward on the probabilistic decision making task following acute treatment with the nAChR antagonist mecamylamine.

Treatment

This experiment used the same design and statistical analysis as Experiment 1.3; however, the effects of the nAChR antagonist mecamylamine (0, 0.5, 1.0, 2.0 mg/kg, s.c.), on performance in the probability discounting task, were investigated instead.

Results

No significant effects of mecamylamine treatment on response omissions and locomotor activity during test days in the probabilistic discounting task were observed (Table 1). Analysis of 5 days of baseline responding prior to testing using a two-way repeated measures ANOVA showed no significant main effect of day ($F_{(4,56)} = 1.29$, $p = .29$) or interaction between day and block ($F_{(16,224)} = .86$, $p = .62$), but a significant effect of probability ($F_{(4,56)} = 476$, $p < .01$), indicating that animals were responding stably before treatment began. The effects of all doses of mecamylamine on the performance of rats in the probabilistic decision making task were assessed using a two-factor repeated measures ANOVA. No significant main effect of dose ($F_{(3,36)} = 2.02$, $p = .13$) or interaction between dose and block ($F_{(12,144)} = 1.68$, $p = .08$) were observed. However,

rats did show a modest shift toward preference for the larger risky reward, when comparing choice preference following treatment with the highest dose of mecamylamine to that following treatment with saline (Figure 4(A)).

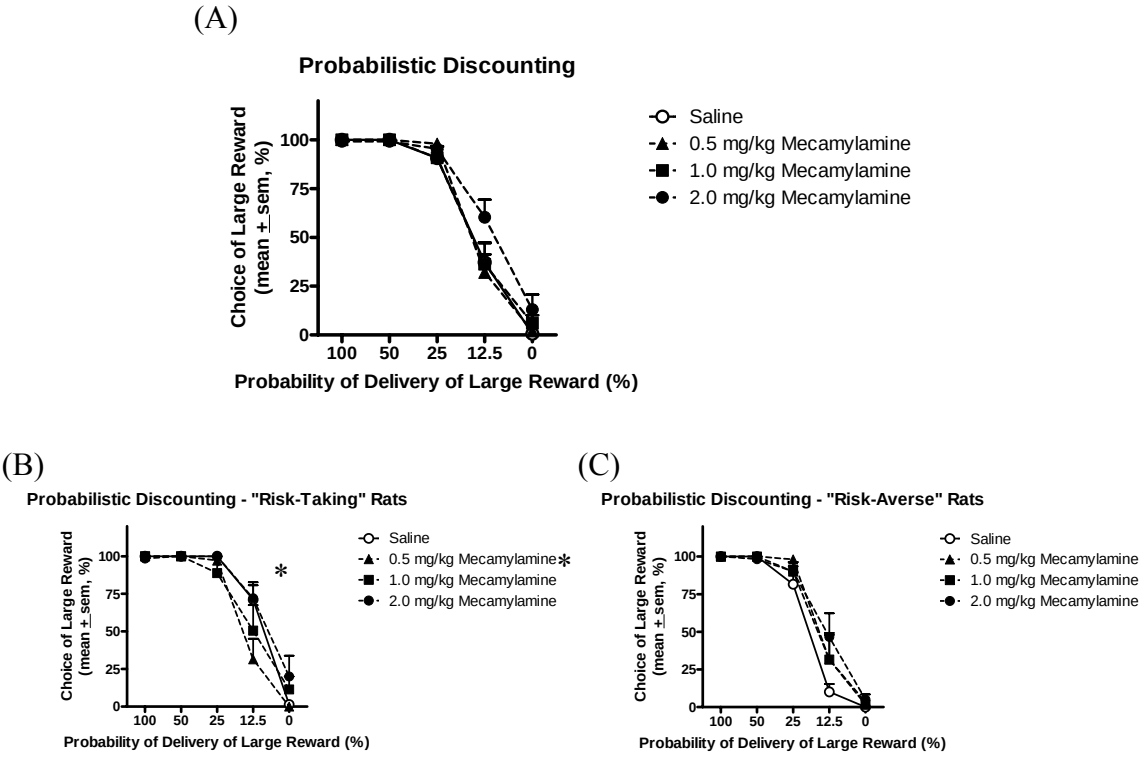


Figure 4. Effects of mecamylamine treatment on responding in the probability discounting task. (A) Treatment with all doses of mecamylamine resulted in no significant shift in preference for reward, when compared to performance following treatment with saline. (B) Treatment with all doses of mecamylamine significantly shifted the performance of risk-taking rats in the probability discounting task. Post-hoc analyses revealed that the 0.5 dose of mecamylamine (black triangles) significantly shifted preference towards the small, safe reward when compared to performance following treatment with saline. (white circles) (C) Risk-averse rats did not significantly shift their performance in the probability discounting task following treatment with mecamylamine, when compared to performance following treatment with saline. * $p < .05$.

Animals were again split into risk-taking and risk-averse groups based on whether they fell above or below the median percent choice (averaged across blocks) of

the large, risky reward, during the 5 days of stable responding immediately prior to testing. The effects of all doses of mecamylamine on the performance of risk-taking rats in the probabilistic decision making task were assessed using a two-factor repeated measures ANOVA. In risk taking rats, no significant main effect of dose ($F_{(3,18)} = 2.70$, $p = .08$), but a significant between dose and block interaction ($F_{(12,72)} = 2.21$, $p < .05$) was observed. Individual post-hoc comparisons with a two-factor ANOVA revealed that following treatment with the low dose of mecamylamine, risk-taking rats significantly shifted their preference towards the smaller, safer reward across the five blocks, when compared to their performance after saline treatment ($p < .05$). Thus, mecamylamine appeared to decrease risk-taking in rats with high baseline levels of risk-taking (Figure 4(B)).

No main effect of dose ($F_{(3,15)} = 2.36$, $p = .11$) or interaction effect ($F_{(12,60)} = 1.35$, $p = .21$) was observed in risk-averse rats, again when comparing all doses of mecamylamine. However, rats did show a modest shift toward preference for the larger risky reward, when comparing choice preference following treatment with the highest dose of mecamylamine to that following treatment with saline (Figure 4(C)).

The results of this experiment do not support our predictions (based on Experiment 1.2) that decreases in nAChR activity cause a decrease in risky-choice in all rats. However, treatment with the low dose did cause a significant decrease when analyzing data from only risk-taking rats, providing further support that nAChRs are involved in probabilistic decision making. These data suggest that rats with high baseline levels of risky decision making will show more discounting of large food rewards that

are risky following acute treatment with mecamylamine. Activation of nAChRs in the ventral tegmental area has been shown to increase reward salience and reinforcement, as well as incentive motivation (Picciotto et al. 2008; Zhang and Sulzer 2004). These reported nAChR activity-dependent increases in reward salience and reinforcement may explain why blockade of nAChRs decreased motivation for the larger food reward options, particularly when accompanied by a high risk of reward loss.

Experiment 1.5. Effects of the Muscarinic Receptor Antagonist Atropine on Performance in the Probability Discounting Task

This experiment used systemic administration of the non-selective mAChR antagonist atropine, to determine its effects on performance on the probability discounting task. Because high levels of mAChR activation caused problems with instrumental performance in the task, and lower levels of activation did not appear to affect risk-taking, it was predicted that blockade of mAChR would not strongly affect performance in the probabilistic task and that this would be evident in both whole-group and split analyses. Blockade of mAChR could also interfere with task performance in a non-specific manner. This would be evident as an increase in the number of omitted responses, as seen with oxotremorine.

Treatment

Experiment 1.5 used the same experimental design and statistical analysis as Experiment 1.4 described above. However, the effects of the mAChR antagonist atropine (0, 0.3, 1, 3.0 mg/kg, i.p.) were investigated instead.

Results

A significant effect of atropine dose was observed when comparing response omissions during treatment days in the probabilistic discounting task ($F_{(3,45)} = 17.55$, $p < .01$, Table 1). Post-hoc analyses revealed significant effects when comparing response omissions following treatment with each individual dose of atropine to that following treatment with saline ($ps < .01$). Similar to results seen with the high dose of nicotine, the effect of the low dose of atropine on omissions was primarily carried by a few rats, allowing for group data analysis. No significant effect of atropine treatment, on ITI locomotor activity, during treatment days in the probabilistic discounting task, was observed (Table 1).

Analysis of 5 days of baseline responding prior to testing using a two-way repeated measures ANOVA showed no significant main effect of day ($F_{(4,56)} = 2.09$, $p = .10$) or interaction between day and block ($F_{(16,224)} = 1.34$, $p = .18$), but a significant effect of probability ($F_{(4,56)} = 311.21$, $p < .01$). This suggests that animals were responding stably before treatment began. Treatment with the middle and high dose of atropine greatly increased response omissions in the task (following treatment with the low dose, 4 rats omitted responses in at least 7 of the 10 choice trials available in a block, for at least 1 block, versus 10 and 9 rats with the medium and high dose, respectively), resulting in insufficient data for reliable analysis. Thus, data from the middle and high dose were eliminated in subsequent analyses. Analysis of data from saline and the low dose of atropine, using a two-factor repeated measures ANOVA, revealed no significant main effect of dose ($F_{(1,9)} = .83$, $p = .39$), but a significant

interaction between dose and block ($F_{(4,36)} = .10$, $p < .001$). Thus, atropine appeared affect performance across the probability discounting task when testing all rats, resulting in poor decision making (Figure 5).

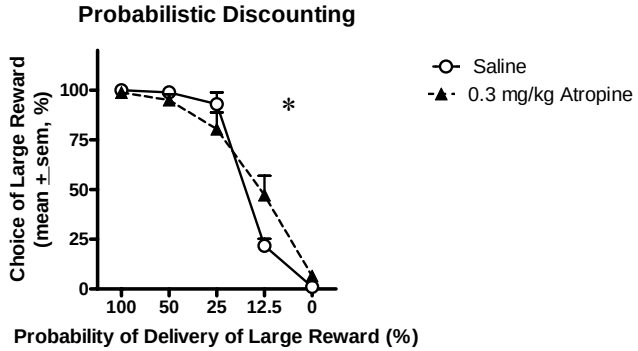


Figure 5. Effects of atropine treatment on responding in the probability discounting task. Treatment with the low dose (black triangles) resulted in a significant shift in performance across the task, when compared to performance following treatment with saline (white circles). Effects of higher doses are not reported due an increase in response omissions. * $p < .05$.

As mentioned, treatment with all doses of atropine increased response omissions, preventing reliable whole group analysis in all rats with either the middle or high dose of atropine. Additionally due to a lack of sufficient data, split group analyses for the low dose could not be reported with confidence. Available data from this experiment do not support our predicted results that blockade of mAChRs would not significantly affect risky decision making, as assessed by the probability discounting task.

Experiment 1 Summary and Discussion

Results from Experiment 1 provide evidence suggesting that both nicotinic and muscarinic AChRs are involved in probabilistic cost-benefit decision making (Table on page 65). Activation of nAChRs following treatment with the high dose of nicotine increased preference for the large risky reward in all rats (although interestingly, both activation of nAChRs following treatment with the low dose of nicotine and blockade of nAChRs following treatment with the low dose of mecamylamine decreased preference for the large risky reward in rats that had high baseline levels of risky decision making). The role of mAChRs in probabilistic discounting was difficult to determine due to side effects that increased response omissions in the task. Results from the limited data obtained suggest that activation of mAChRs may affect probabilistic discounting; however, mAChR blockade may impair decision making when risk of reward loss is involved, resulting in disadvantageous decision making across the probability discounting task. Experiments examining the effects of lower doses of mAChR agonists and antagonists that do not increase response omissions in the probability discounting task may address the question of whether or not mAChR are involved in probabilistic decision making.

The effects of acute nicotine on probability discounting were the most prominent effects observed in Experiment 1. Increases in risky decision making following activation of nAChRs with the high dose of nicotine were observed in all rats, as well as in both risk-taking and risk-averse subgroups of rats. These increases in risky decision making observed following acute treatment with the high dose of nicotine do not support

our prediction that increases in nAChR activity would decrease risk-taking, suggesting instead that acute nAChR activation causes increases in risky decision making. Conversely, the finding observed with the low dose of nicotine in risk taking rats (decreased risky decision making) do support our predicted results, as well as research showing that nicotine treatment can enhance cognitive processes such as increases in attention and memory (Cutuli et al. 2008; Furey et al. 2000; Furey et al. 2008; Ricciardi et al. 2009). Furthermore, the findings observed following treatment with the low dose of nicotine suggest that the effects of nicotine on probabilistic decision making appear to depend on both nicotine dose and on baseline performance in the task.

The mechanisms underlying the observed increase in risky decision making following treatment with the high dose of nicotine are unclear. As discussed, activation of nAChRs in the ventral tegmental area has been shown to increase reward salience and reinforcement, as well as incentive motivation (Picciotto et al. 2008; Zhang and Sulzer 2004). These motivational increases may explain why rats treated with the high dose of nicotine were willing to continue to respond for larger amounts of food reward, even when that reward was accompanied by an increasing risk of loss. Further research investigating the increases in risky decision making observed in the probability discounting task following treatment with nicotine may help identify the neurobiological mechanisms underlying this effect.

EXPERIMENT 2. ROLE OF NICOTINIC AND MUSCARINIC RECEPTORS IN IMPULSIVE DECISION MAKING

Experiment 2.1. Effects of the Acetylcholinesterase Inhibitor Donepezil on Performance in the Delay Discounting Task

Experiment 2 used a design similar to that of Experiment 1; however, this experiment assessed performance on a "delay discounting" task and not in the probability discounting task. The delay discounting task characterizes the ability of delays in delivery to promote discounting of a larger reward and increase selection of a smaller immediate reward. This task is considered to assess impulsive behavior and the ability (or inability) to delay gratification (Evenden and Ryan 1996a). The first experiment in this set used systemic administration of the acetylcholinesterase inhibitor donepezil to determine its effects on performance in the delay discounting task. Based on data from our laboratory and research suggesting that increased acetylcholine levels increase prefrontal cortical-based cognition (Cutuli et al. 2008; Furey et al. 2000; Furey et al. 2008; Ricciardi et al. 2009), it was predicted that increased synaptic levels of acetylcholine would improve decision making as assessed by the delay discounting task. Specifically, it was predicted that donepezil treatment would decrease impulsive responding in high-impulsive rats, causing a shift in preference towards the larger, delayed reward.

Treatment

In this experiment, the effects of the acetylcholinesterase inhibitor donepezil (0, 0.3, 1.0, 3.0 mg/kg, i.p.), on performance in the delay discounting task, were investigated.

Results

No significant effects of donepezil treatment on response omissions, during test days in the delay discounting task, were observed (Table 2). Conversely, a significant effect of donepezil treatment on ITI locomotor activity, during test days in the delay discounting task, was observed ($F_{(3,45)} = 6.34$, $p < .01$, Table 2). Post-hoc analyses revealed no significant effects when comparing ITI activity following treatment with only the low or middle dose of donepezil to that following treatment with saline; however, a significant decrease in activity was observed when comparing activity following saline treatment to that following treatment with the high dose of donepezil ($p < .05$).

Analysis of 5 days of baseline responding prior to testing using a two-way repeated measures ANOVA showed no significant main effect of day ($F_{(4,60)} = .94$, $p = .45$) or interaction between day and block ($F_{(16,240)} = .94$, $p = .52$), but a significant effect of delay ($F_{(4,60)} = 78.72$, $P < .01$). This suggests that animals were responding stably before treatment began and that they discounted the large reward as a function of the delay to its delivery.

Table 2. Effects of Cholinergic Drugs on Response Omissions and Locomotor Activity During Testing in the Delay Discounting Task.

	Dose	Donepezil Experiment 2.1	Nicotine Experiment 2.2	Oxotremorine Experiment 2.3	Mecamylamine Experiment 2.4	Atropine Experiment 2.5
Mean Percent Choice Trials Omitted	Veh	0.3%	0.1%	0.1%	0.5%	3.2%
	Low	0.3%	0.3%	0.4%	0.0%	3.3%
	Med	0.0%	0.1%	0.3%	0.0%	3.2%
	Hi	6.8%	0.1%	0.5%	0.3%	7.2% *
Mean ITI Locomotor Activity (beam breaks) breaks)	Veh	2491	2883	3136	2277	2372
	Low	2533	3006	3175	2325	2348
	Med	2558	3729	3278	2219	2311
	Hi	1804 *	3780 *	2748 *	2209	1815 *

* = significantly different from Veh

The effects of all doses of donepezil on the performance of rats in the delay discounting task were assessed using a two-factor repeated measures ANOVA. No significant main effect of dose ($F_{(3,42)} = .26$, $p = .86$) or interaction between dose and block ($F_{(12,168)} = 1.33$, $p = .20$) were observed. Thus, donepezil does not appear to affect performance in the delay discounting task, when testing all rats (Figure 6(A)).

To examine how donepezil administration interacted with baseline levels of impulsivity, animals from this experiment were split into non-impulsive and impulsive groups based on whether they fell above or below the median percentage of choices (averaged across blocks) of the large, delayed reward, during the 5 days of stable responding immediately prior to testing. The effects of all doses of donepezil on the performance of non-impulsive rats in the delay discounting task were assessed using a two-factor repeated measures ANOVA. In non-impulsive rats, no significant main effect

of dose ($F_{(3,18)}$ = .86, p = .48) or interaction between dose and block ($F_{(12,72)}$ = .95, p = .50) was observed. Thus, donepezil does not appear to affect impulsive choice in rats with low baseline levels of impulsivity (Figure 6(B)).

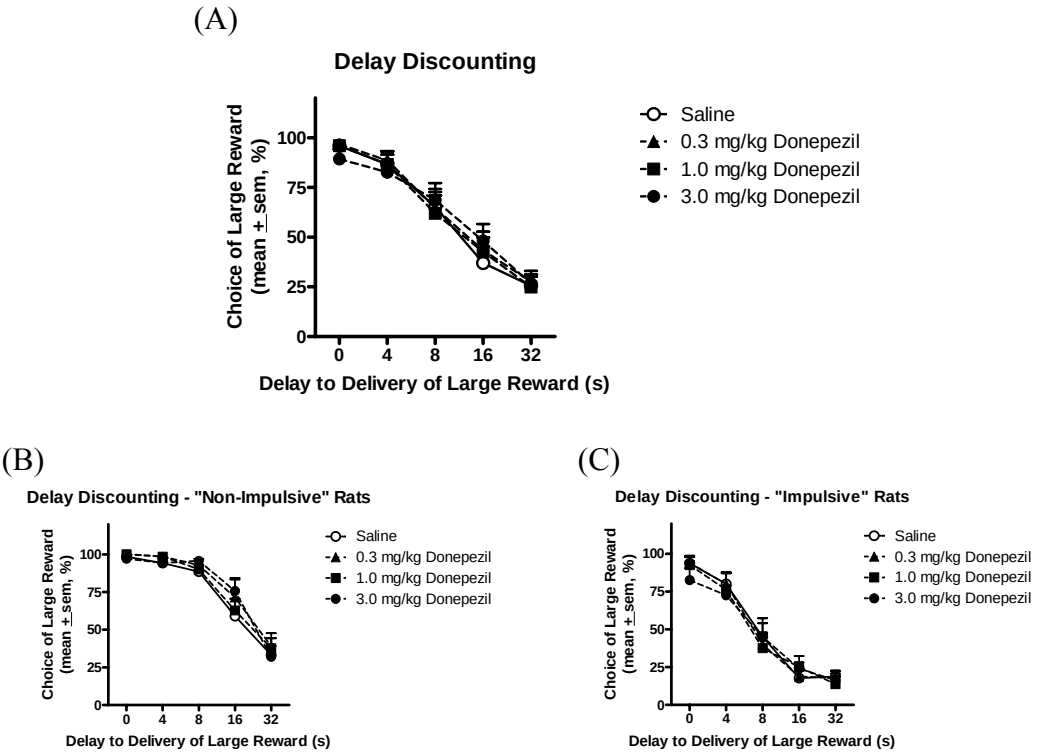


Figure 6. Effects of donepezil treatment on responding in the delay discounting task. (A) Treatment with all doses of donepezil did not significantly affect performance in the delay discounting task, when compared to performance following treatment with saline. (B) Treatment with all doses of donepezil did not significantly shift the performance of non-impulsive rats in the delay discounting task when compared to performance following treatment with saline. (C) No significant shift in performance was observed in risk-averse rats when treated with donepezil and comparing to performance following treatment with saline.

A two-factor repeated measures ANOVA was again used to assess the effects of all doses of donepezil on the performance of impulsive rats in the delay discounting task. There was no significant main effect of dose ($F_{(3,21)}$ = .24, p = .87) or interaction

between dose and block ($F_{(12,84)}$ = .88, p = .57). Thus, donepezil did not appear to significantly alter impulsive choice in rats with high baseline levels of impulsivity (Figure 6(C)). Results from this study do not support our predictions (based on Experiment 1.1) that increases in AChR activity would decrease impulsive choice, as no effects were observed in either the full or split group analyses. Together with the findings of Experiment 1.1, these findings suggest that increases in synaptic levels of acetylcholine following treatment with donepezil affect risky decision making, but not impulsive decision making.

Experiment 2.2. Effects of the Nicotinic Receptor Agonist Nicotine on Performance in the Delay Discounting Task

The second experiment in this set used systemic administration of the non-selective nAChR agonist nicotine, to determine its effects on performance in the delay discounting task and impulsive decision making. Data from Experiment 1.2 suggest that the effect of nicotine treatment on preference for a larger reward that is disadvantageous due to risk of reward omission is dependent on both nicotine dose and baseline performance. While treatment with a high dose of nicotine increased risky choice in all rats, treatment with a low dose of nicotine decreased risky choice in rats with high baseline levels of risky decision making. Based on the data from Experiment 1, as well as research from Dallery et al. showing increases in impulsive choice following acute nicotine treatment, it was predicted that activation of nAChRs with a low dose of nicotine would decrease disadvantageous, impulsive decision making in the delay

discounting task, while treatment with a high dose would increase impulsive decision making. Decreased impulsive choice would be evident by a shift in preference toward the larger delayed reward, while increases in impulsive choice would be evident by a shift in preference toward the smaller, immediate reward following acute treatment with the nAChR agonist nicotine.

Treatment

Experiment 2.2 used the same experimental design and statistical analysis as Experiment 2.1 described above, except that the effects of the nAChR agonist nicotine (0, 0.1, 0.3, 1.0 mg/kg, s.c.) on delay discounting were investigated instead.

Results

No significant effect of nicotine treatment on response omissions, during test days in the delay discounting task, was observed (Table 2). Conversely, a significant effect of nicotine treatment on ITI locomotor activity, during test days in the delay discounting task, was observed ($F_{(3,45)} = 2.91$, $p < .05$, Table 2). Post-hoc analyses revealed no significant effects when comparing ITI activity following treatment with only the low dose of nicotine to that following treatment with saline; however, significant increases in activity were observed when comparing activity following saline treatment to that following treatment with either the middle or high dose of nicotine (*p*s < .05).

Analysis of 5 days of baseline responding prior to testing using a two-way repeated measures ANOVA showed no significant main effect of day ($F_{(4,56)} = 1.78$, $p = .15$) or interaction between day and block ($F_{(16,224)} = 1.67$, $p = .05$), but a significant

effect of block ($F_{(4,56)} = 76.89$, $p < .01$), suggesting that responding was stable prior to nicotine treatment. The effects of all doses of nicotine on the performance of rats in the delay discounting task were assessed using a two-factor repeated measures ANOVA. No significant main effects of dose ($F_{(3,45)} = 1.85$, $p = .15$) or interaction between dose and block ($F_{(12,180)} = .99$, $p = .46$) were observed. Thus, nicotine does not appear to affect impulsive choice when testing all rats (Figure 7(A)).

As with the donepezil experiment (Experiment 2.1), animals were split into non-impulsive and impulsive groups based on whether they fell above or below the median percent choice (averaged across blocks) of the large, delayed reward, during the 5 days of stable responding immediately prior to testing. The effects of all doses of nicotine on the performance of non-impulsive rats in the delay discounting task were assessed using a two-factor repeated measures ANOVA. No significant main effect of dose ($F_{(3,21)} = .53$, $p = .67$) or interaction between dose and block ($F_{(12,84)} = .93$, $p = .52$) were observed. Thus, nicotine does not appear to affect impulsivity in rats with low baseline levels of impulsive choice (Figure 7(B)).

The effects of all doses of nicotine on the performance of impulsive rats in the delay discounting task were also assessed using a two-factor repeated measures ANOVA. A significant main effect of dose ($F_{(3,21)} = 3.32$, $p < .05$), but no significant interaction between dose and block ($F_{(12,84)} = .70$, $p = .75$) was observed. Post-hoc comparisons of performance following treatment with each dose of nicotine to performance following saline treatment revealed no significant shifts in choice preference in the delay discounting task, although treatment with the high dose did result

in a modest increase in preference for the large delayed reward (p = .06), when compared to saline controls. Thus, nicotine appeared to modestly decrease impulsive choice in rats with high baseline levels of impulsive choice (Figure 7(C)).

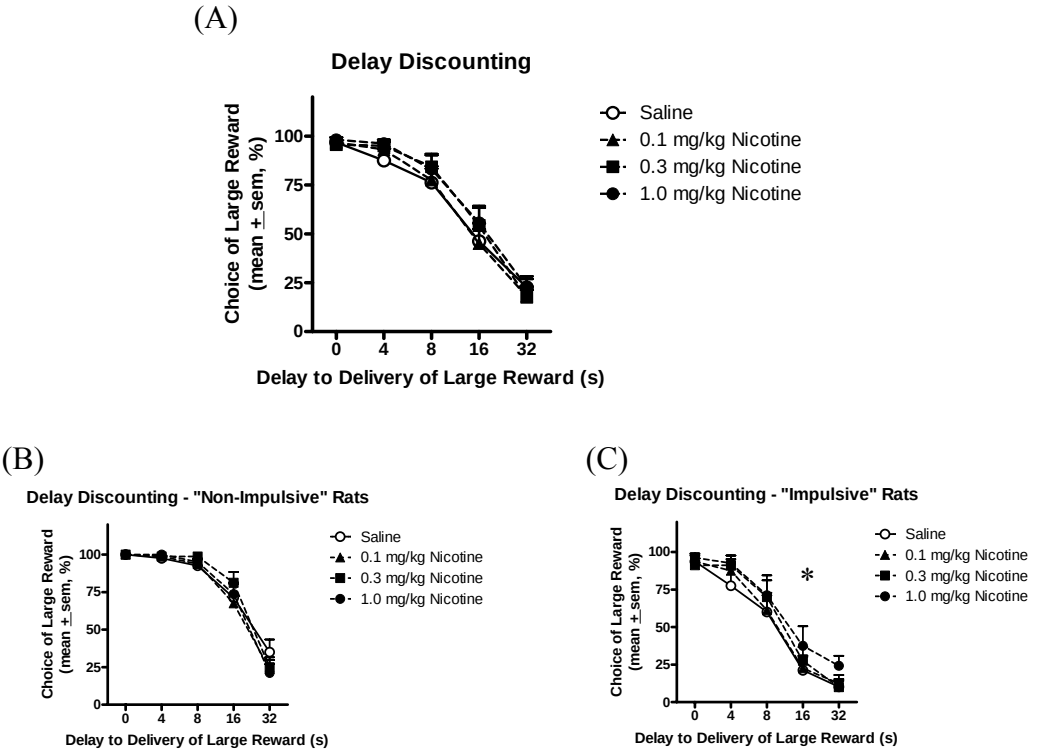


Figure 7. Effects of nicotine treatment on responding in the delay discounting task. (A) Analysis of treatment with all doses of nicotine suggests no significant shifts in performance on the delay discounting task, when compared to performance following treatment with saline. (B) Treatment with all doses of nicotine did not significantly shift the performance of non-impulsive rats in the delay discounting task when comparing to performance following treatment with saline. (C) Treatment with all doses of nicotine significantly shifted the performance of impulsive rats in the delay discounting task, when compared to saline controls. Post-hoc analyses revealed no significant between group differences, when comparing performance in the delay discounting task following treatment with nicotine to performance following treatment with saline. * $p < .05$.

Activation of nAChRs did not affect performance in the delay discounting task when analyzing performance in all or non-impulsive rats; however, nicotine did appear to decrease impulsive choice in impulsive rats. It should be noted, however, that the significance in these results was limited to the omnibus ANOVA and were not obtained with between-group analyses. Although results from this study did not clearly support our predictions (based on Experiment 1.2 and Dallery and Locey 2005) that activation of nAChRs with a low dose of nicotine would decrease impulsive decision-making, while treatment with a high dose would increase impulsive decision making, they do suggest that nAChRs are involved in impulsive decision making.

The observed findings are contrary to the increases in impulsive choice found by Dallery et al.; however, this may be due to differences between the fixed delay discounting task used in these experiments and the adjusting delay procedure used by Dallery et al. In our fixed delay task, impulsive choice is measured as a percentage of preference for immediate versus delayed rewards, while in the adjusted delay procedure impulsive choice is represented by an indifference point that indexes the value of a delayed reward by adjusting the delay to large reward. Similar opposing effects in delay discounting have also been observed in different experiments, following treatment with acute amphetamine (Cardinal et al. 2000a; de Wit et al. 2002; Stanis et al. 2008; Wade et al. 2000).

Research has shown increases in cognitive function following acute nicotine administration (Cutuli et al. 2008; Experiment 1.2; Furey et al. 2000; Furey et al. 2008; Ricciardi et al. 2009). Enhanced cognitive processes may explain why rats that had high

baseline levels of impulsive choice were willing to wait for a larger, more advantageous food reward following nicotine treatment. As mentioned, nAChR are also involved in reward motivation. Given that nicotine increased preference for the larger reward in both the probabilistic and delay discounting task, an alternative explanation for both sets of findings is that the effects in the delay discounting task may result from acute increases in motivation for the larger reward, even when temporal costs are increased over time. Notably, research investigating the effects of amphetamine on impulsive choice, using the same fixed delay design used in this experiment, has shown results similar to that seen here with nicotine (Setlow et al. 2009; Slezak and Anderson 2009). Like amphetamine, nicotine can cause increases in mesolimbic dopamine release, suggesting that increases in mesolimbic dopamine levels may underlie these drug effects on cost-benefit decision making through increases in motivation for rewards and reward related cues. Further research investigating the increases in preference for the larger reward observed in the delay discounting (and probability discounting) task following treatment with nicotine may help identify the neurobiological mechanisms underlying this effect.

Experiment 2.3. Effects of the Muscarinic Receptor Agonist Oxotremorine on Performance in the Delay Discounting Task

The third experiment in this set used systemic administration of the non-selective mAChR agonist oxotremorine, to determine its effects on performance in the delay discounting task and impulsive decision making. Due to motoric impairments that resulted in a large number of trial omissions in the task following treatment with the high

dose of oxotremorine in Experiment 1.3, a lower dose replaced the highest dose used in the probability discounting task. Based on limited data from Experiment 1.3, it was predicted that activation of mAChRs following systemic administration of oxotremorine would not affect performance in the delay discounting task when assessed in either full or impulsive-split analyses.

Treatment

Experiment 2.3 used the same experimental design and statistical analysis as Experiment 2.2 described above, however, in this experiment the effects of the mAChR agonist oxotremorine (0, 0.003, 0.01, 0.03 mg/kg, i.p.) were investigated instead.

Results

No significant effect of oxotremorine treatment on response omissions, during test days in the delay discounting task, was observed (Table 2). Conversely, a significant effect of oxotremorine treatment on ITI locomotor activity, during test days in the delay discounting task, was observed ($F_{(3,45)} = 4.07$, $p < .05$, Table 2). Post-hoc analyses revealed no significant effects when comparing ITI activity following treatment with only the low or middle dose of oxotremorine to that following treatment with saline, and a near significant decrease in ITI activity, when comparing activity following treatment with the high dose of oxotremorine to that following treatment with saline ($p = .07$).

Analysis of 5 days of baseline responding prior to testing using a two-way repeated measures ANOVA showed no significant main effect of day ($F_{(4,56)} = 1.70$, $p = .16$) or interaction between dose and block ($F_{(16,224)} = 1.37$, $p = .16$), but a significant effect of probability ($F_{(4,56)} = 73.80$, $p < .01$). This suggests that animals were responding

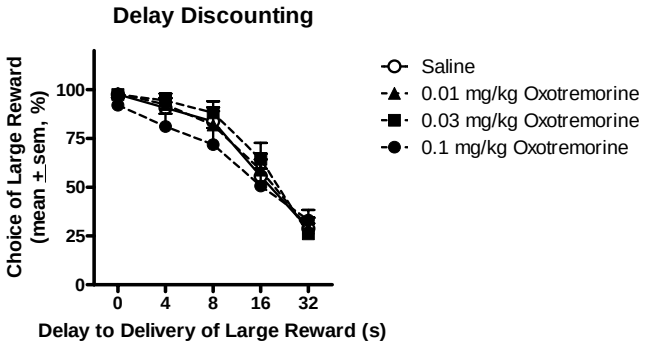
stably before treatment began. Analysis of data from all doses of oxotremorine, using a two-factor repeated measures ANOVA, revealed no significant main effect of dose ($F_{(3,45)} = 2.37$, $p = .08$) or interaction between dose and block ($F_{(12,180)} = 1.38$, $p = .18$). Thus, oxotremorine does not appear to affect impulsive choice, when testing all rats (Figure 8(A)).

As in previous experiments, animals were split into non-impulsive and impulsive groups based on whether they fell above or below the median percent of choice for the large, delayed reward, totaled across blocks. The effects of all doses of oxotremorine on the performance of non-impulsive and impulsive rats, in the delay discounting task, were assessed using a two-factor repeated measures ANOVA. Results indicate no main effect of dose ($F_{(3,21)} = .82$, $p = .50$), but a significant interaction between dose and block ($F_{(12,84)} = 2.26$, $p < .05$), on performance in the delay discounting task with non-impulsive rats. Post-hoc comparisons of performance following treatment with each dose of oxotremorine to performance following saline treatment resulted in no significant shifts in choice preference. Nonetheless, oxotremorine did appear to increase impulsive choice in rats with low baseline levels of impulsive choice (Figure 8(B)).

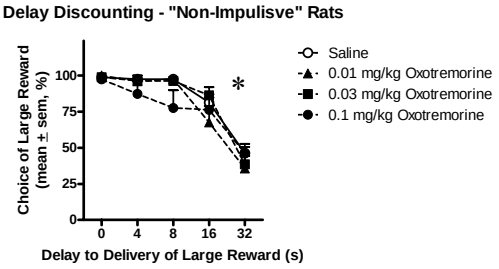
A significant main effect of dose ($F_{(3,21)} = 3.71$, $p < .05$), but no significant interaction effect ($F_{(12,84)} = 1.26$, $p = .26$) on performance was observed in impulsive rats, again when comparing all doses of oxotremorine. Although post-hoc comparisons of performance following treatment with each dose of oxotremorine to performance following saline treatment resulted in no significant shifts in choice preference, treatment with both the low and middle dose did cause moderate increases in preference

for the large delayed reward, when compared to choice preference following saline treatment (*ps* = .08). Results suggest that oxotremorine may modestly decrease impulsive choice in rats with high baseline levels of impulsive choice (Figure 8(C)).

(A)



(B)



(C)

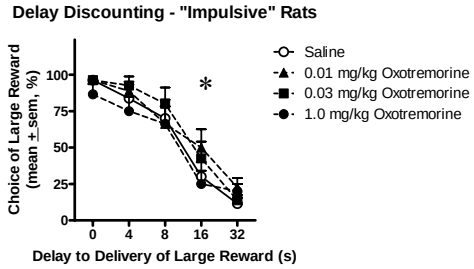


Figure 8. Effects of oxotremorine treatment on responding in the delay discounting task. (A) Analysis of treatment with all doses of oxotremorine suggests no significant shift in performance on the delay discounting task, when compared to performance following treatment with saline. (B) Treatment with all doses of oxotremorine resulted in a significant interaction effect, represented by a shift in performance across the delay discounting task in non-impulsive rats. Post-hoc analyses reveal no significant effects when comparing performance following treatment of each dose of oxotremorine to performance after saline treatment. (C) Impulsive rats showed a significant main effect of dose represented by a shift in performance, as a result of dose of oxotremorine. Similar to non-impulsive rats, no main effects were found when comparing performance following each dose to performance following saline treatment. * $p < .05$.

Results from this experiment do not support our predicted results that oxotremorine would not affect impulsive decision making. Although activation of mAChRs did not significantly affect performance in the delay discounting task when assessing all rats, significant shifts in reward preference were observed in the split group analyses (in both impulsive and non-impulsive rats). Similar to results seen with donepezil in the probability discounting task, the effect of oxotremorine was dependent on baseline impulsive choice, in that the direction of the shift in reward preference was different for the impulsive and non-impulsive group. These group differences may, in turn, be dependent on intrinsic differences in cholinergic function (see Experiment 3). Results from this experiment suggest that mAChR are involved in the discounting of delayed rewards.

Experiment 2.4. Effects of the Nicotinic Receptor Antagonist Mecamylamine on Performance in the Delay Discounting Task

The fourth experiment in this set used systemic administration of the non-selective nAChR antagonist mecamylamine, to determine its effects on impulsive decision making. Based on results from Experiment 2.2 showing that nAChR activation modestly increases choice of the large delayed food reward in rats with low baseline levels of impulsive choice, it was predicted that blockade of nAChRs would decrease preference for the large delayed reward, and increase impulsive choice. This would be evident by a decrease in preference for the large delayed reward.

Treatment

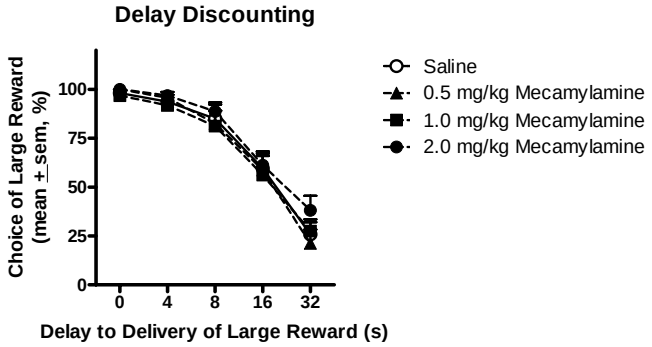
Experiment 2.4 used the same experimental design and statistical analysis as Experiment 2.3 described above. However, the effects of the nAChR antagonist mecamylamine (0, 0.5, 1, 2 mg/kg, s.c.) on performance in the delay discounting task were investigated instead.

Results

No significant effects of mecamylamine treatment on response omissions and locomotor activity, during test days in the delay discounting task, were observed (Table 2). Analysis of 5 days of baseline responding prior to testing using a two-way repeated measures ANOVA showed no significant main effect of day ($F_{(4,60)} = 1.37$, $p = .26$) or interaction between day and block ($F_{(16,240)} = .97$, $p = .49$), but a significant effect of probability ($F_{(4,60)} = 91.46$, $p < .01$), indicating that animals were responding stably before treatment began.

The effects of all doses of mecamylamine on the performance of rats in the delay discounting task were assessed using a two-factor repeated measures ANOVA. No significant main effects of dose ($F_{(3,45)} = 2.68$, $p = .06$) or interaction between dose and block ($F_{(12,180)} = .94$, $p = .51$) were observed. However, treatment with the highest dose of mecamylamine did appear to cause a slight increase in preference for the large delayed reward. Results suggest mecamylamine does not significantly affect impulsive choice, when testing all rats (Figure 9(A)).

(A)



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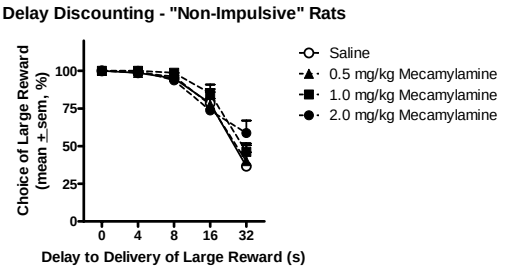


(C)

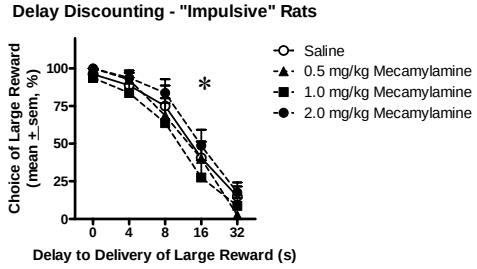


Figure 9. Effects of mecamylamine treatment on responding in the delay discounting task. (A) Analysis of treatment with all doses of mecamylamine suggests no significant shift in performance on the delay discounting task, when compared to performance following treatment with saline. (B) Treated with all doses of mecamylamine did not significantly shift the performance of non-impulsive rats in the delay discounting task, when compared to performance following treatment with saline. (C) Treatment with all doses of mecamylamine significantly shifted performance in a dose dependent manner, in the delay discounting task, when compared to performance following treatment with saline. Post-com comparisons show no significant difference in choice preference, when comparing performance following each individual dose of mecamylamine, to that following saline. * $p < .05$

Animals were again split into non-impulsive and impulsive groups based on whether they fell above or below the median percent choice (averaged across blocks) of the large, delayed reward, during the 5 days of stable responding immediately prior to testing. The effects of all doses of mecamylamine on the performance of non-impulsive rats in the delay discounting task were assessed using a two-factor repeated measures

ANOVA. No significant main effect of dose ($F_{(3,21)} = 1.02$, $p = .40$) or interaction between dose and block ($F_{(12,84)} = 1.67$, $p = .09$) were observed. Interestingly, similar to results seen with all rats, treatment with the highest dose of mecamylamine did cause a slight increase in preference for the large delayed reward. Nonetheless, statistical analysis suggests that mecamylamine does not appear to affect impulsive choice in rats with low baseline levels of impulsive choice (Figure 9(B)).

The effects of all doses of mecamylamine on the performance of impulsive rats in the delay discounting task were also assessed using a two-factor repeated measures ANOVA. A significant main effect of dose ($F_{(3,21)} = 5.47$, $p < .01$), but no significant interaction between dose and block ($F_{(12,84)} = .91$, $p = .54$) were observed. Post-hoc comparisons of performance following treatment with each dose of mecamylamine to performance following saline treatment resulted in no significant shift in choice preference. Nonetheless, results suggest that mecamylamine can affect impulsive choice in rats with high baseline levels of impulsivity (Figure 9(C)).

Results from this experiment do not support our predictions that blockade of nAChR would decrease preference for the large delayed reward. Together with the findings of Experiment 1.4, these data suggest that acute treatment with mecamylamine causes decreased preference for large, risky food rewards in risk-taking rats in the probability discounting task. Furthermore, while mecamylamine clearly affected performance in the delay discounting task, direction of the effect on performance is not clear. Pharmacological activation and blockade of nAChRs appear to modulate cost-

benefit decision making; however, more research is needed to fully identify the role of nAChRs.

Experiment 2.5. Effects of the Muscarinic Receptor Antagonist Atropine on Performance in the Delay Discounting Task

The fifth experiment in this set used systemic administration of the non-selective mAChR antagonist atropine to determine its effects on impulsive decision making. Due to problems in Experiment 1.5 with task performance in the probability discounting task following treatment with the middle and high dose of atropine, two lower doses of atropine replaced the two higher doses used in Experiment 1.5. Based on the lack of effect on risk-taking following treatment with the lowest dose of atropine, it was predicted that blockade of mAChR would not affect performance in the delay discounting task when assessing rats jointly or as impulsive-split groups. Blockade of mAChR may also interfere with behavioral participation in the task via motoric or cognitive impairments. These effects would be evident as an increase in response omissions, as seen with atropine in the probability discounting task.

Treatment

Experiment 2.5 used the same experimental design and statistical analysis as in Experiment 2.4 described above. However, the effects of the mAChR antagonist atropine (0, 0.03, 0.1, 0.3 mg/kg, i.p.) were investigated instead.

Results

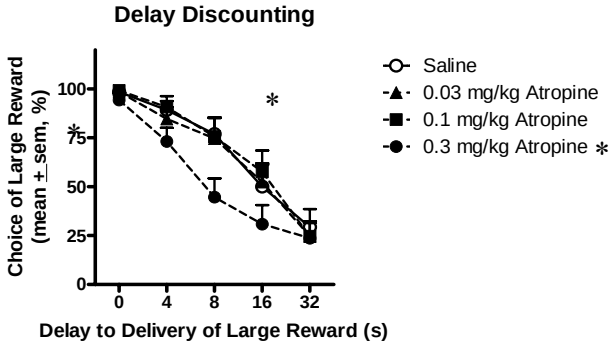
Significant effects of atropine treatment on response omissions and ITI locomotor activity during test days in the delay discounting task were observed (Table 2). A significant effect of atropine dose was observed when comparing response omissions during treatment days ($F_{(3,45)} = 8.89$, $p < .01$). Post-hoc analyses revealed no significant effects when comparing response omissions following treatment with only the low or middle dose of atropine to that following treatment with saline; however, results do show that response omissions significantly increased when comparing omissions following treatment with the high dose of atropine to that following treatment with saline ($p < .01$). Effects of the high dose of atropine on response omissions were carried by only a few rats (3 of 16 rats omitted 7 or more trials within a block), allowing for group data analysis. A significant effect of atropine dose was observed when comparing ITI locomotor activity during treatment days ($F_{(3,45)} = 4.03$, $p < .05$). Post-hoc analyses revealed no significant effects when comparing ITI activity following treatment with only the low or middle dose of atropine to that following treatment with saline; however, results do show that ITI activity significantly decrease when comparing activity following treatment with the high dose of atropine to that following treatment with saline ($p < .01$).

Analysis of 5 days of baseline responding prior to testing using a two-way repeated measures ANOVA showed no significant main effect of day ($F_{(4,56)} = .89$, $p = .48$) or interaction between day and block ($F_{(16,224)} = .68$, $p = .81$), but a significant effect of probability ($F_{(4,56)} = 66.71$, $p < .01$). This suggests that animals were responding

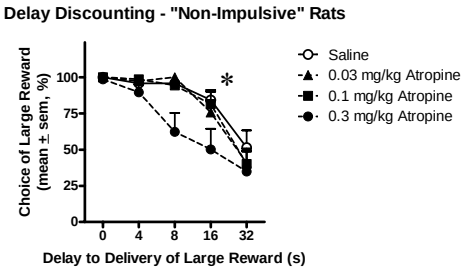
stably before treatment began. Analysis of data from all doses of atropine, using a two-factor repeated measures ANOVA, revealed a significant main effect of dose ($F_{(3,36)}$ = 6.81, $p < .01$) and interaction between dose and block ($F_{(12,144)} = 2.74$, $p < .01$). Post-hoc analysis using a two-way repeated measure ANOVA reveal that although treatment with the low or middle dose of atropine does not appear to significantly affect choice preference in the delay discounting task, treatment with the high dose of atropine results in a significant decrease in preference for the large delayed reward, when compared to choice preference following treatment with saline ($p < .05$). Thus, treatment with the mAChR antagonist atropine results in an increase in impulsive choice across all rats (Figure 10(A)).

As in previous experiments, animals were split into impulsive and non-impulsive groups based on whether they fell above or below the median percent of choice for the large, delayed reward, totaled across blocks. A two-factor repeated measures ANOVA was used to assess the effects of all doses of atropine on the performance of rats with low baseline levels of impulsive choice in the delay discounting task. There was a significant main effect of dose ($F_{(3,18)} = 3.15$, $p = .05$) and interaction between dose and block ($F_{(12,72)} = 1.98$, $p < .05$) observed (Figure 10(B)). Post-hoc analyses revealed no significant effect on choice preference following treatment with each dose of atropine; however, treatment with the highest dose of atropine did produce a near significant decrease in preference for the large delayed reward ($p = .06$).

(A)



(B)



(C)

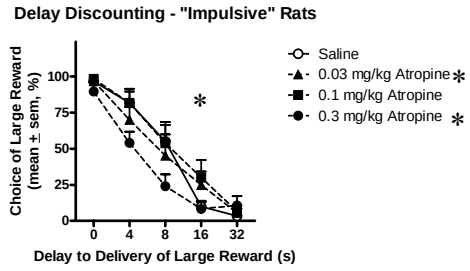


Figure 10. Effects of atropine treatment on responding in the delay discounting task. (A) Analysis of treatment with all doses of atropine, in all rats, suggests a significant shift in performance on the delay discounting task when compared to performance following treatment with saline. Post-hoc analyses revealed that treatment with the high dose (black circles) significantly decreased preference for the large delayed reward when compared to treatment with saline (white circles). (B) Analysis of treatment with all doses of atropine, in impulsive, suggests a significant shift in performance on the delay discounting task when compared to performance following treatment with saline. Post-hoc analyses revealed no significant effect of each individual dose on choice preference, when compared to treatment with saline (white circles). (C) Analysis of treatment with all doses of atropine, in all rats, suggests a significant shift in performance on the delay discounting task when compared to performance following treatment with saline. Post-hoc analyses revealed that treatment with the low and high dose (black triangles and circles, respectively) significantly decreased preference for the large delayed reward when compared to treatment with saline (white circles). * $p < .05$

The effects of all doses of atropine on the performance of non-impulsive rats in the delay discounting task were assessed using a two-factor repeated measures ANOVA. In rats with high baseline levels of impulsive choice, a significant main effect of dose

($F_{(3,18)} = 4.29$, $p < .05$) and interaction between dose and block ($F_{(12,72)} = 3.08$, $p < .01$) was observed. Post-hoc analyses revealed a near significant decrease in preference for the large delayed reward when comparing performance following treatment with the middle dose of atropine to that of saline ($p = .07$). Interestingly, treatment with the low dose of atropine revealed a significant interaction between dose and block ($p < .05$), showing a decrease in choice for the large delayed reward for the first 3 blocks, then increasing choice for the large delayed reward for blocks 4 and 5. Furthermore, treatment with the high dose of atropine resulted in a significant decrease in preference for the large delayed reward ($p < .05$). The results suggest that treatment with atropine significantly increases impulsive choice in rats with high baseline levels of impulsivity (Figure 10(C)). Results from this experiment do not support our predicted results that blockade of mAChRs would not affect performance in the delay discounting task in all rat or split group analysis, and suggest that blockade of mAChRs significantly increases impulsive choice.

Experiment 2.6. Effects of the Nicotinic Receptor Agonist Nicotine on Response Perseveration in the Probability Discounting Task

During the course of completing Experiment 2, a number of studies investigating probabilistic discounting were conducted at the University of British Columbia, and published by principal investigator Stan Floresco. In these studies, Floresco and colleagues showed that, similar to our effects following treatment with acute nicotine (Experiment 1.2), systemic acute injections of amphetamine (St Onge et al. 2010), as

well as inactivation of the medial prefrontal cortex (St. Onge and Floresco 2009), can cause increases in preference for a large risky reward in a probability discounting task in which the probability of large reward delivery descends across a test session (as in Experiment 1). Interestingly, their studies further demonstrate that when the order of probabilities is reversed (probability of reward delivery ascends across a test session), these same manipulations lead rats to prefer the smaller safe reward relative to controls. The authors suggest that increases in choice for the large risky reward in the probability discounting task with descending probabilities of reward delivery may be due to response perseveration rather than actual increased preference for large risky rewards. In Experiment 1, risky decision making was assessed with decreasing probabilities of delivery of the large reward across a test session. Following treatment with nicotine, rats began the task by selecting the large reward, and then maintained their preference for this initial selection longer than when testing occurred following saline treatment. Therefore, based on the findings of Floresco and colleagues, it may be argued that the maintained preference for the initially chosen large reward observed following acute nicotine injections (Experiment 1.2) may have occurred, at least in part, as a result of drug-induced response perseveration.

To address this issue, the rats used in Experiment 2 were tested in several control experiments. In the first control experiment, the effects of nicotine on probability discounting were tested in the descending probability version of the probability discounting task used in Experiment 1. This experiment was necessary to verify that

similar results as those seen in our initial experiment testing the effects of nicotine on probability discounting (Experiment 1.2) could be replicated with this group of rats.

Following this initial control experiment, the effects of the highest dose of nicotine was tested in a version of the probability discounting task in which the probabilities of delivery of the large reward ascended as the test session progressed. With this order of presentation of reward probabilities, animals will produce an inverted version of the response curve obtained with descending probabilities of large reward delivery. Since the probability of the large reward begins at 0%, rats will initially chose the small guaranteed reward; however, as the task progresses and the probability of delivery of the large reward increases, rats will shift their preference over to the large risky reward (Floresco and Whelan 2009). If nicotine causes perseverative responding, then it should decrease rats' preference for the large reward as the probability of its delivery increases. However, if nicotine causes increased preference for large rewards, then the opposite pattern of behavior should emerge.

A third control experiment was used to further characterize potential nicotine-induced increases in response perseveration. In this experiment we used a within-session shift task, in which the large reward shifts within a single test session from being delivered (100% probability of delivery) for the first two blocks, to not being delivered at all (0% probability) in blocks 3, 4, and 5. If nicotine induces response perseveration, then following treatment with nicotine, animals should continue to choose the large reward significantly more during blocks 3, 4 and 5, when compared to performance following saline treatment. This simpler version of the task provides a more stringent

test of response perseveration in the absence of any requirements for calculating changes in reward probabilities.

As discussed, activation of nAChRs in the ventral tegmental area has been shown to increase reward salience and reinforcement, as well as incentive motivation (Picciotto et al. 2008; Zhang and Sulzer 2004). These reported nAChR activity-dependent increases in reward salience and reinforcement may explain why acute treatment with nicotine increased preference for the larger, albeit risky reward, in the probability discounting task. Based on these findings, it was predicted that nicotine treatment would increase preference for the large risky reward in both the descending and ascending probability discounting task, as well as the within-session shift task.

Treatment

Following completion of Experiment 2.5, rats from this experiment were trained in the probability discounting task with descending probabilities of large reward delivery (100, 50, 25, 12.5, 0%) until stable responding was achieved. Rats were then treated with nicotine using the same within subject design and discounting task used in Experiment 1; however, in this experiment only the highest dose of nicotine was used, limiting treatment to only 2 days. After completion of the descending large reward delivery probability version of the task used in Experiment 1, rats were trained and tested similarly in the probability discounting task with ascending probabilities of large reward delivery (0, 12.5, 25, 50, 100%), using the same protocol. Finally, upon completion of the ascending probability task, rats were again tested using the same protocol with a

within-session shift test, in which the delivery of the large reward switched from guaranteed to not delivered (100, 100, 0, 0, 0%).

Results

Analysis of 5 days of baseline responding prior to testing in the descending probability discounting task using a two-way repeated measures ANOVA showed no significant main effect of day ($F_{(4,60)} = 1.63$, $p = 1.78$) or interaction between day and block ($F_{(16,240)} = .68$, $p = .81$), but a significant effect of probability ($F_{(4,60)} = 296.15$, $p < .01$). This suggests that animals were responding stably before treatment began. Analysis of data using a two-factor repeated measures ANOVA revealed a significant main effect of dose ($F_{(1,15)} = 19.83$, $p < .05$) and interaction between dose and block ($F_{(4,60)} = 5.74$, $p < .01$). As in Experiment 1.2 the high dose of nicotine appeared to affect risky choice by increasing preference for the initially chosen large risky reward, when testing all rats with descending probabilities of delivery of the large risky reward (Figure 11(A)).

Analysis of 5 days of baseline responding prior to testing in the ascending probability discounting task using a two-way repeated measures ANOVA, showed a significant man effect of day ($F_{(4,60)} = 5.52$, $p < .01$) and block ($F_{(4,60)} = 330.16$, $p < .01$), as well as an interaction between day and block ($F_{(16,240)} = 2.31$, $p < .01$). Analysis of data in the ascending probability discounting task revealed a significant main effect of dose ($F_{(1,15)} = 11.07$, $p < .01$) and interaction between dose and block ($F_{(4,60)} = 5.49$, $p < .01$). Interestingly, when testing with ascending probabilities of large risky reward delivery, the high dose of nicotine appeared to affect risky choice not by increasing preference for the larger, risky reward (as we saw with the descending probabilities), but

by increasing preference for the smaller guaranteed reward that was initially preferred (Figure 11(B)). Finally, analysis of data in the within-session perseveration test revealed a significant main effect of dose ($F_{(1,13)} = 23.75$, $p < .01$) and interaction between dose and block ($F_{(4,52)} = 22.13$, $p < .01$). The high dose of nicotine appeared to maintain preference for the larger, initially chosen reward when compared to treatment with saline (as seen with the descending probabilities), by increasing preference for the large reward option during blocks when it was not delivered (Figure 11(C)).

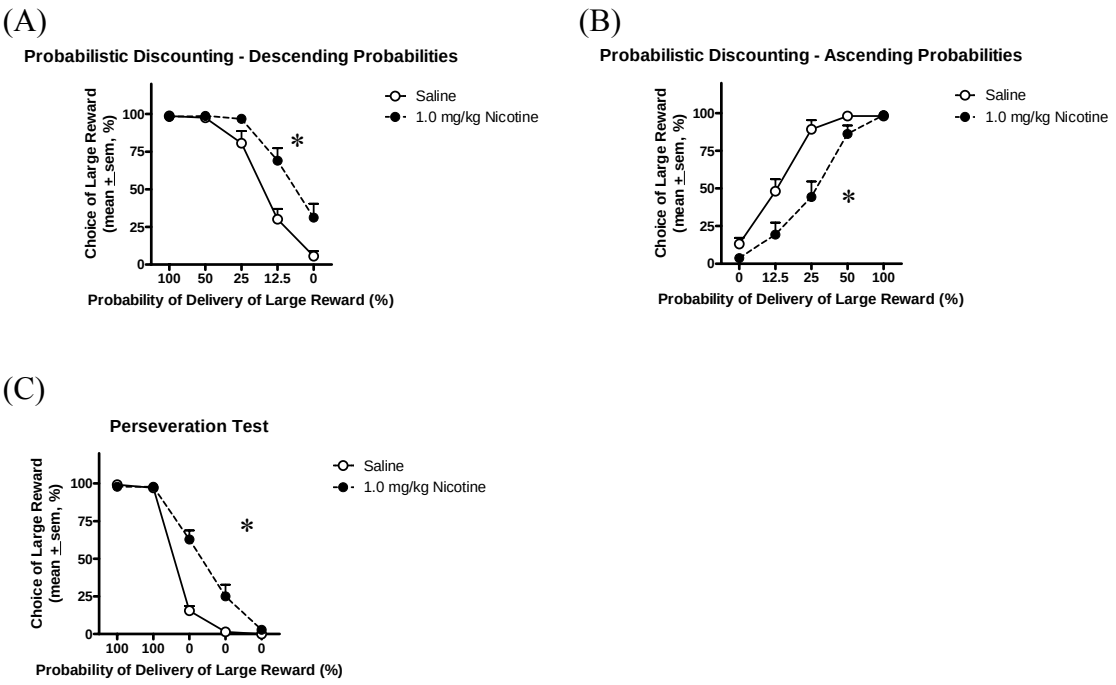


Figure 6. Effects of nicotine on within-session response perseveration. (A) Treatment with nicotine (black circles) resulted in a significant shift in performance in rats when compared to performance following treatment with saline (white circles), in the probability discounting task with descending probabilities of delivery of the large reward. (B) Treatment with nicotine (black circles) resulted in a significant shift in performance in rats when compared to performance following treatment with saline (white circles), in the probability discounting task with ascending probabilities of delivery of the large reward. (C) Treatment with nicotine (black circles) resulted in a significant shift in performance in rats when compared to performance following treatment with saline (white circles), in a within-session perseveration test. * $p < .05$.

Results from this experiment do not support our predicted results that treatment with nicotine would cause an increase in choice of the large risky reward. The experiment instead showed that nicotine treatment increased preference for the initially selected reward in all tasks, regardless of whether that reward was the small safe reward (ascending probabilities) or the large risky reward (descending probabilities, within-session shift). These findings suggest that the increases in choice of the large delayed reward following treatment with the high dose of nicotine in Experiment 1.2 and 2.6 are not simply due to increases in the salience and/or reinforcement value of reward, but may be due, at least in part, to increases in perseverative responding. The results of this experiment identify a need to consider issues of behavioral perseveration in tasks assessing the effects of nicotine on the discounting of probabilistic rewards.

Experiment 2 Summary and Discussion

Results from Experiment 2 suggest that both nicotinic and muscarinic AChRs are involved in cost-benefit decision making involving delays (Table 3). Activation of nAChRs appeared to increased preference for the large delayed reward in rats with high baseline levels of impulsive choice; however, this directionality was not statistically confirmed, as a lack of between group effects was observed. Results from this experiment also demonstrate that blockade of nAChRs by all doses of mecamylamine caused significant shifts in preference for delayed rewards in rats with high baseline levels of impulsive choice; however, a lack of significant effects by any individual dose made the directionality of these effects difficult to interpret. Interestingly, a recent study

with humans showed that oral administration of mecamylamine caused decreases in tolerance for delays when testing patients with ADHD (high baseline levels of impulsive choice) in the Choice Delay Task, resulting in even higher levels of impulsivity (Potter et al. 2009). Again, the data suggests that the effects of nicotinic receptor agonists and antagonists on discounting of delayed rewards may depend on both dose used and baseline levels of impulsive choice.

Table 3. Summary of Cholinergic Behavioral Pharmacology Experiments

	Donepezil (AChE-)	Nicotine (nAChR+)	Oxotremorine (mAChR+)	Mecamylamine (nAChR-)	Atropine (mAChR-)
Effects on Risky Choice (% choice of large reward in probability discounting task)	Affected behavior in all rats, direction unclear. Decreased in risk-taking rats	Increased in all rats	No effects	Decreased in risk-taking rats	Decreased then increased in all rats
Effects on Impulsive Choice (% choice of small reward in delay discounting task)	No Effects	Affected behavior in impulsive rats, direction unclear	Affected behavior in impulsive and non-impulsive rats, direction unclear.	Affected behavior in impulsive rats, direction unclear	Increased in all rats

Investigation of the role of mAChRs in delay discounting suggests that these receptors are also involved in impulsive decision making. The effects of mAChR activation by oxotremorine were limited to our analysis of all treatment doses in split group analysis. Although it appears that oxotremorine increases impulsive choice in non-

impulsive rats and decreases it in impulsive rats, because the effects by individual doses were not seen, the directionality of any effects of oxotremorine was difficult to determine. Interestingly, the most prominent effect on delay discounting was observed following blockade of mAChR by atropine. Results show that acute injections of the high dose of atropine decreased impulsive choice in all rats. Prior to these experiments, the role of muscarinic receptors in delay discounting had not been investigated. These findings are very different from those observed following nAChR activation and may occur as a result of differences in the structure and function of the cholinergic receptor type. While activation of ionotropic nAChRs has been shown to have an excitatory effect on cellular processes, metabotropic mAChR have been shown to be both excitatory and inhibitory. This suggests that mAChR may play a modulatory role in cost-benefit decision making. The complex organization of mAChR subtypes complicates the interpretation of our data and highlights the need for more research investigating the biological mechanisms of each mAChR subtype and how they can affect cost-benefit decision making.

The effects of acute systemic injections of nicotine on response perseveration within the probability discounting task were also examined in Experiment 2. In a version of the probabilistic discounting task with ascending probabilities of reward delivery, nicotine caused rats to choose the smaller safer reward significantly more than with saline. Similarly, when testing choice preference in a within-session shift task, rats continued to respond on the lever that initially yielded a larger reward, even when such responding yielded no reward. The results from this control experiment suggest that our

initially observed effects of nicotine on probabilistic discounting (increased preference for the large, risky reward), may be due to nicotine-induced increases in response perseveration. The findings from our control experiments also rule out several alternative explanations for our initially observed effects of nicotine on probabilistic discounting. First, because nicotine treatment shifted behavior both toward (descending probabilities) and away from (ascending probabilities) choice of the large reward, nicotine did not likely alter choice behavior as a result of changes in reward detection or preference. The observed changes both toward and away from the large reward, even in the middle block of trails when rewards are equal on the two levers, also suggest that our results cannot be attributed to changes in preference for variable over fixed reward delivery.

The findings observed in the probability discounting task following treatment with nicotine are similar to those seen following temporary inactivation of the PFC. In a recent study by St. Onge et al (2009), inactivation of mPFC resulted in an increase in preference for a large risky reward when testing with descending probabilities of reward delivery, while a decrease was observed when testing with ascending probabilities. These data replicate the findings of several studies showing that inactivation of PFC can promote perseverative behaviors (Baran et al. 2010; Del'Guidice et al. 2009). The authors of this study suggest these results may occur as a result of general disruptions in response flexibility represented as a failure to change responding in the face of changing reward values. Interestingly, a recently published study by St. Onge et al (2010) replicated the effect observed in probability discounting following inactivation of mPFC

using systemic administration of amphetamine. Because amphetamine is known to cause significant increases in dopamine levels within the corticomesolimbic system, these data suggests that increases in dopamine can also cause increases in response perseveration within the probabilistic discounting task.

The effects observed in impulsive rats in the delay discounting task following treatment with nicotine, although not statistically significant, were in the direction of an increase in preference for the initially selected large reward. What remains unclear, however, is whether this increase in preference for this large delayed reward occurred as a result of improved cognitive performance or as a result of increased perseveration, as the large reward lever is the lever that was initially preferred. As mentioned above, only one other study has investigated the effects of nicotine in rats on performance in a delay discounting task (Dallery and Locey 2005). Contrary to our findings, in this experiment, animals treated with nicotine showed a decreased preference for the large delayed reward (increased impulsive choice) in a version of the delay discounting task that adjusts the delay to the large reward based on preference during the previous block of testing (Mazur 1987). Interestingly, in this adjusted delay experimental design, choice preference for the large delayed reward during the first block was, on average, below 50%. It is possible, then, that increases in choice of the small immediate reward following treatment with a high dose of nicotine in this version of the delay discounting task, as observed by Dallery and Locey (2005), were partially due to increases in perseverative responding for the reward option initially selected small, immediate reward lever. However, the design of the adjusted delay design used by Dallery and

Locey presents less of an opportunity for initial lever preferences than the set delay design used in Experiment 2, and therefore may be less susceptible to the development of response perseveration. Following this line of thought, if nicotine treatment does cause some degree of increased impulsive choice, in addition to the response perseveration inducing effects observed in Experiment 2.6, then this may explain why perseverative effects were not as prominent when testing in the delay discounting task in Experiment 2.2, than they were in the probability discounting task in Experiment 1.2. In other words, In Experiment 2.2, nicotine-induced increases in impulsive choice (decreased preference for the large delayed reward) may have washed out any extended responding for the initially selected larger reward, resulting from response perseveration. That is, any increases in impulsive choice (preference for the small immediate reward) caused by nicotine may not have been observable as a result of perseveration on the large reward lever. Indeed, the mechanisms underlying the increases in impulsive choice observed by Dallery and Locey following treatment with a low dose of nicotine remain unclear.

Although it is reasonable to conclude that some type of perseverative behavior is occurring as a result of nicotine administration, this explanation cannot be generalized to all forms of cost-benefit decision making, as results from a recent experiment in our lab testing the effect of nicotine on a risk discounting task (in which the probability of a shock associated with a large reward increases across blocks), show no perseveration-inducing effects of nicotine (Mitchell et al – in preparation). Additionally, research testing the effects of nicotine on reversal learning has produced mixed results; while

some studies have reported deficits in response flexibility following nicotine treatment, others have shown no effect (Brown et al. 2010; Loh et al. 1993).

Future control studies, similar to those conducted in Experiment 2.6 for probability discounting, may help address the issue of perseverative responding in the delay discounting task following treatment with acute nicotine. If the delays used in Experiment 2 were presented in a descending order, with the large delay associated with the large reward in the first block, then we could expect that rats acutely treated with nicotine would initially prefer the small reward. Therefore, if the small increase in preference for the large delayed reward observed following nicotine treatment in Experiment 2.2 was due to an increase in perseverative responding, we would expect rats tested with descending delays to continue to choose the initially preferred small reward even as the delay to the large reward decreases; however, if increases in preference for the large reward following treatment with nicotine are due to decreases in impulsive choice then we would then expect nicotine treated rats to chose the large delayed reward more than saline controls, during the test session.

It should be noted that increases in perseverative responding in the delay discounting task following acute nicotine treatment may also be linked to deficits in the temporal organization of behavior. Experiments examining the effects of nicotine on a version of the delay discounting task in which the delay to the large reward remains constant, may address the issue of nicotine-induced disruptions of long term monitoring of delays in the task. That is, changes in choice preference when testing the effects of nicotine in a version of the delay discounting task with constant delays, would suggest

that nicotine may impair the ability to appropriately guide behavior based on temporal information, rather than affect choice for rewards with different temporal costs. The pattern of results following nicotine administration is consistent with an interpretation of perseverative responding. However, the cause of this possibly perseverative behavior is not clear. One possibility is that increases in the salience of reward-related cues following treatment with nicotine results in increased preference for the initially selected reward. Acute treatment with nicotine has indeed been shown to increase preference for reward-related cues (Cohen et al. 2004; Olausson et al. 2004; Wang et al. 2007). The observed effects in the discounting tasks may therefore result from nicotine induced effects on attention and motivation for rewards and reward-related cues. That is, significant nicotine induced increases in the salience of rewards and reward-related cues, following treatment with nicotine, may account for nicotine-induced increases in motivation and preference for an initially more rewarding reward or reward-related cue, such that responding for those rewards and cues is maintained even when they become disadvantageous as the task progresses. Importantly, this initially selected reward may vary and will be dependent on the design of the given task. Research investigating the role of key structures within the mesolimbic system on cost-benefit decision making will be necessary to further characterize the role of nicotine in cost-benefit decision making.

Results from Experiments 1 and 2 suggest that both nicotinic and muscarinic AChRs can affect cost-benefit decision making when delay of reward or risk of reward loss is involved. Experiment 3 used a different approach to further characterize a possible role of nAChRs in cost-benefit decision making, by examining correlations

between baseline levels of cost-benefit decision making and intrinsic nAChR subtype levels. This was accomplished by using receptor binding techniques in drug naive rats, to examine the relationship between intrinsic α4β2 and α7 nAChR subtypes levels and baseline performance on both the probabilistic and delay discounting task.

EXPERIMENT 3. RELATIONSHIP BETWEEN BASELINE NICOTINIC RECEPTOR LEVELS AND COST-BENEFIT DECISION MAKING

The cholinergic system has been repeatedly shown to play a role in component processes involved in cost-benefit decision making, including executive function and reward motivation (Cutuli et al. 2008; Furey et al. 2008; Picciotto et al. 2008; Ricciardi et al. 2009; Zhang and Sulzer 2004). Recent findings from Experiment 2.6 further suggest that the cholinergic system is also involved in response flexibility, when testing cost-benefit decision making in the probability discounting task. As mentioned above, populations known to have deficits in cost-benefit decision making include patients with psychopathologies such as schizophrenia, Alzheimer's disease, and addiction, among others (Clark and Robbins 2002; Coyle et al. 1983; Euteneuer et al. 2009; Kalivas and Volkow 2005; Thompson et al. 2007; Weiler et al. 2009; Yip et al. 2009). Patients with these conditions have characteristically impulsive and/or risky behaviors, which negatively impact many aspects of their lives (Clark and Robbins 2002; Coyle et al. 1983; Euteneuer et al. 2009; Kalivas and Volkow 2005; Thompson et al. 2007; Weiler et al. 2009; Yip et al. 2009).

Research using rodents with genetically "knocked-out" nAChR, as well as direct infusions of nicotinic agonists or antagonist into the prefrontal cortex and mesolimbic system of rodents, provides evidence for the involvement of nAChR in processes involved in cost-benefit decision making (Collins et al. 1989; George et al. 2000; Levin et al. 2009; Levin et al. 1997b; Marks et al. 1987; Reavill and Stolerman 1990). The

α4β2 and α7 nAChR subtypes in particular are highly expressed in the brain, and genetic deletion of these nAChR subtypes has been shown to cause behavioral impairments in tasks that measure component processes involved in cost-benefit decision making, such as conditioned place preference (preference for contexts associated with drug rewards) and 5 choice serial reaction task (attention for food reward associated stimuli) (Curzon et al. 2006; Hoyle et al. 2006; Walters et al. 2006). Similarly, direct infusions of nAChR agonists directly into the prefrontal cortex enhance cognitive processes implicated in cost-benefit decision making, such as working and reference memory for food rewards, while infusions of cholinergic antagonists in these structures impair these same functions (Levin et al. 2009; Levin et al. 1997b). These findings are complemented by human post-mortem studies and studies using radiolabeled ligand binding to identify AChR density levels in patients with schizophrenia and Alzheimer's disease. Patients with these disorders show decreased levels of the α4β2 nAChR subtype in both the striatum and the cortex when compared to controls (Breese et al. 2000; Durany et al. 2000; Flynn and Mash 1986; Warpman and Nordberg 1995). Similarly, individuals diagnosed with schizophrenia have shown decreased levels of the α7 nAChR subtype within the prefrontal cortex (Guan et al. 1999).

Receptor binding studies investigating cholinergic receptor levels in drug users have also identified changes in nAChR levels in structures implicated in cost-benefit decision making. Neuroimaging and post-mortem studies in nicotine addicts have reported increases in nAChR densities. Specifically, current smokers showed long-term increases in baseline α4β2 nAChR levels within the frontal cortex and striatum, when

compared to ex- and non-smoking controls (Breese et al. 1997; Court et al. 1998; Mukhin et al. 2008). Research with animals supports these findings, reporting increases in cortical and striatal autoradiographic binding of α4β2 nAChRs in rodents chronically exposed to nicotine (Collins et al. 1989; Marks et al. 1987).

Only a handful of studies have used receptor binding techniques to investigate how baseline differences in nAChR may be related to baseline differences in cognitive function (Le Foll et al. 2009; Woodruff-Pak et al. 2008), and no studies to date have examined whether differences in baseline nAChR levels are related to differences in performance on tasks assessing cost-benefit decision making. To address relationships between nAChR expression and cost-benefit decision making, we used receptor autoradiography to correlate nAChR binding in brain areas implicated in cost-benefit decision making processes, with behavioral performance in the probability and delay discounting tasks. Results from these experiments were expected to speak to the possibility of using cholinergic α4β2 and α7 receptors as biomarkers and/or potential targets for the treatment of psychopathological conditions in which cost-benefit decision making is impaired.

Experiment 3.1. Relationship Between α4β2 Nicotinic Receptors and Performance on the Probabilistic and Delay Discounting Tasks

In the brain, the α4β2 nAChR subtype predominates in density and distribution and is implicated in executive and motivational processes (Granon et al. 2003; Le Foll et al. 2009; Perry et al. 2002; Picciotto et al. 1998; Woodruff-Pak et al. 2008). Receptor

binding studies have shown decreases in levels of α4β2 in prefrontal cortical areas of patients with schizophrenia and Alzheimer's disease (Breese et al. 2000; Durany et al. 2000; Flynn and Mash 1986; Warpman and Nordberg 1995). Additionally, receptor binding studies in nicotine dependent subjects have identified higher α4β2 nAChR density levels within the mesolimbic system (Breese et al. 1997; Court et al. 1998; Mukhin et al. 2008). These conditions have been associated with impaired cost-benefit decision making, including impulsive and risky behaviors (Clark and Robbins 2002; Coyle et al. 1983; Euteneuer et al. 2009; Kalivas and Volkow 2005; Thompson et al. 2007; Weiler et al. 2009; Yip et al. 2009). Based on this research, along with the results observed in Experiment 1 and 2, we aimed to identify correlations between α4β2 nAChR subtype density levels and behavioral performance on the probability and delay discounting task.

Results from Experiment 1.2 suggest that activation of nAChRs with a high dose of nicotine increases choice of the large risky reward in the probabilistic discounting task, while treatment with a low dose of nicotine decreases risky decision making in rats with high baseline levels of risky choice. Results from Experiment 2.6 further suggest that the effects observed in the probabilistic discounting task occurred as a result of increases in response perseveration, possibly due to enhanced motivational salience of the initially-selected reward. Because of the increase in response perseveration in the probabilistic discounting task following treatment with a high dose of nicotine, we predicted that higher levels of α4β2 nAChR levels would be associated with greater

preference for the large risky reward in the probability discounting task (increased risky choice).

Based on the observed decrease in preference for the large risky reward following treatment with a low dose of nicotine, we further predicted that low levels of α4β2 nAChRs would be associated with decreased preference for the large risky reward (decreased risky choice), resulting in a positive linear relationship between choice of the large risky reward in the probability discounting task (risky choice) and α4β2 nAChR levels. Perseverative, habitual responding may be occurring as a result of increases in dopaminergic firing within the mesolimbic system (Ellinwood and Kilbey 1975; Faure et al. 2010; Hsieh et al. 2010; Loh and Beck 1989). Based on this understanding, as well as research suggesting that nicotine induces dopamine release in the mesolimbic system (Mifsud et al. 1989; Rada et al. 2001; Wang et al. 2007), we predicted that increases in α4β2 nAChR levels correlated with increases in preference for the initially more rewarding choice (large reward lever), would be observed in structures within the mesolimbic system.

Although results from Experiment 2.2 showed a significant main effect of nicotine administration in the impulsive subgroup of rats in the delay discounting task, no significant between group post-hoc effects were observed. Nonetheless, nicotine did appear to cause a slight increase in responding for the large reward, in impulsive rats. It remains unclear whether this increase was due to increases in response perseveration, improved cognitive performance, or some other effect of nicotine. Nonetheless, the

findings from both Experiments 1 and 2 suggest that increased activation of α4β2 nAChRs may be associated with increased preference for the large delayed reward.

Similar to our predicted results with probability discounting, we predicted a positive linear relationship between α4β2 nAChR levels and delay discounting, with increased preference for the large delayed reward (decreased impulsive choice) associated with higher levels of α4β2 nAChR. Whether the increases in preference for the large delayed reward in Experiment 2.2 occurred as a result of improved cognition or increased perseverative responding remains unclear. Because of this ambiguity, brain regions in which we would expect to see correlations between nAChR levels and decision making were difficult to predict; however, identified locations resulting from this research may help elucidate the mechanisms underlying increases in preference for the large, delayed reward following acute nicotine administration. For example, higher α4β2 nAChR levels within cortical regions associated with greater preference for the large delayed reward could suggest that the increase in preference for the large delayed reward following nicotine administration occurred as a result of improved cognitive function. Conversely, higher α4β2 nAChR density levels within mesolimbic system that are associated with greater preference for the larger delayed reward could suggest that the increase in preference for the large delayed reward occurred as a result of nicotine-induced preservative responding.

Treatment

Rats were trained in both discounting tasks, as well as a number other behavioral tasks (instrumental responding on various schedules of reinforcement, open field test)

and sacrificed as described in the methods section above, and brains were sliced and prepared for receptor autoradiography. Heteromeric α4β2-containing nAChR were identified using ^{125}I-epibatidine binding. For total binding, sections were incubated at room temperature with 0.4 nM [^{125}I]-epibetadine for 1 hour, within the Tris-HCl buffer. Epibetadine-incubated slides were then exposed to the imaging film for 2 days to allow receptor level density analysis.

Results

Bi-variate correlations were observed between performance in the decision making tasks and other behavioral measures (Table 4). A positive linear correlation was observed between baseline performance in the two discounting tasks ($r = .57$, $p < .05$), such that as preference for the large risky reward increased, so did preference for the large delayed reward (Figure 12(A)). A positive linear correlation was also found between choice preference in the probability discounting task and performance in the Open Field Test ($r = .50$, $p < .05$), such that as choice preference for the larger risky reward increased, so did the total time the rat spent in the open, center region of the activity chamber (Figure 12(B)). Furthermore, there was a negative linear correlation observed between choice preference in the delay discounting task and performance in a fixed ratio 1 instrumental responding task ($r = -.50$, $p < .05$). In this correlation, preference for the larger delayed reward increased in the delay discounting task as the total number of lever presses decreased in the instrumental responding task (Figure 12(C)).

Table 4. Correlation matrix displaying linear relationships between behavioral measures.

	% choice of large risky reward	% choice of large delayed reward	Fixed Ratio 1	Fixed Ratio 3	Fixed Ratio 10	Fixed Ratio 20	Fixed Ratio 40	Prog Ratio	Horiz Activity	Time in Center
% choice of large risky reward	X	**r = .57 p < .05***	r = -.06 p = .81	r = -.31 p = .25	r = -.12 p = .65	r = -.35 p = .19	r = -.28 p = .30	r = -.21 p = .44	r = -.02 p = .95	**r = .50 p < .05***
% choice of large delayed reward	X	X	**r = -.50 p < .05***	r = -.39 p = .14	r = -.24 p = .37	r = -.27 p = .31	r = -.19 p = .48	r = .03 p = .92	r = -.11 p = .70	r = .04 p = .89
Fixed Ratio1	X	X	X	r = .35 p = .18	r = .28 p = .30	r = .07 p = .81	r = .14 p = .61	r = -.21 p = .43	r = .35 p = .19	r = -.16 p = .55
Fixed Ratio 3	X	X	X	X	**r = .69 p < .01***	**r = .56 p < .05***	**r = .53 p < .05***	r = .02 p = .95	r = -.12 p = .66	r = -.12 p = .65
Fixed Ratio 10	X	X	X	X	X	**r = .80 p < .01***	**r = .85 p < .01***	r = .41 p = .11	r = -.30 p = .25	r = .09 p = .75
Fixed Ratio 20	X	X	X	X	X	X	**r = .96 p < .01***	**r = .73 p < .01***	r = -.30 p = .26	r = -.07 p = .79
Fixed Ratio 40	X	X	X	X	X	X	X	**r = .73 p < .01***	r = -.28 p = .29	r = -.14 p = .68
Prog Ratio	X	X	X	X	X	X	X	X	r = -.20 p = .47	r = -.23 p = .38
Horiz Activity	X	X	X	X	X	X	X	X	X	r = -.17 p = .53
Time in Center	X	X	X	X	X	X	X	X	X	X

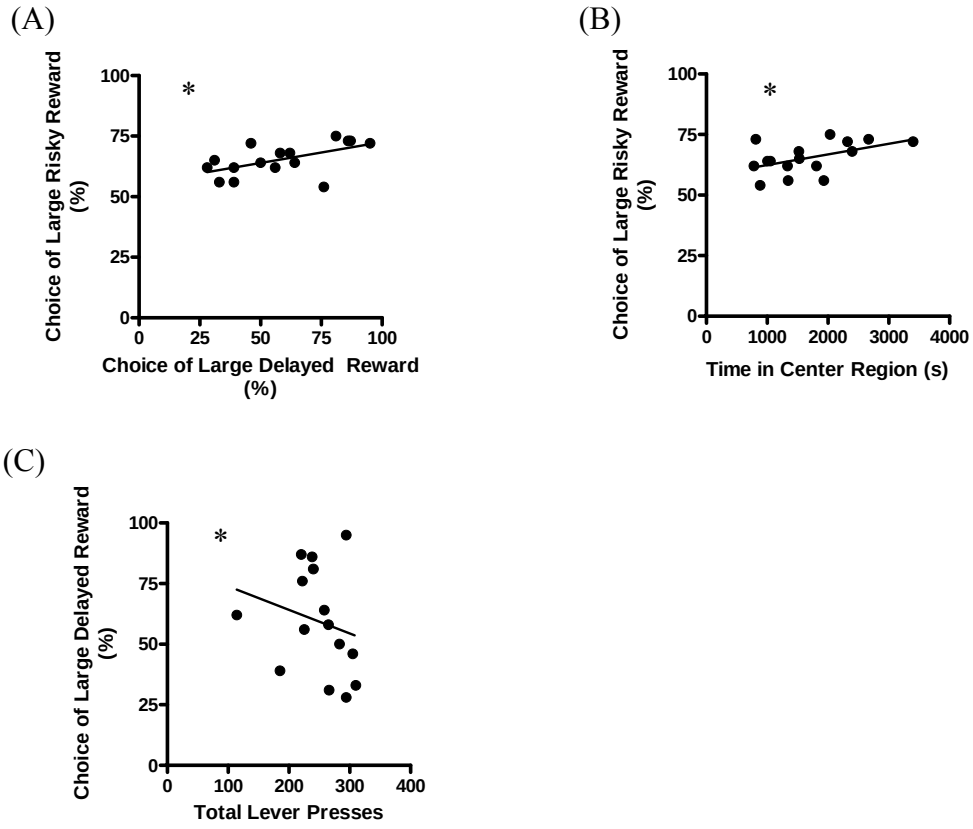


Figure 12. Linear associations between behavioral measures. (A) Baseline performance in the probability discounting task was positively correlated with baseline performance in the delay discounting task, such that as preference for the large risky reward increased, so did preference for the large delayed reward. (B) Baseline performance in the probability discounting task was also positively correlated with baseline performance in the activity test, such that as preference for the risky delayed reward increased, so did time spent in the open center region of the activity chamber. (C) Baseline performance in the delay discounting task was negatively correlated with baseline performance in the fixed ratio 1 instrumental responding task, such that, as preference for the large delayed reward increased, total number of bar presses during a 30 minute FR1 instrumental responding test. * $p < .05$.

Receptor density levels were assessed in cortical and limbic structures in the forebrain, as well as structures within the midbrain (Figure 13). Pearson's bi-variate correlations were found between nAChRs in the brain and performance in both decision making tasks, as well as instrumental responding and open field behavioral measures. A

negative linear correlation was observed between performance in the probability discounting task and α4β2 nAChR density levels in the nucleus accumbens shell (r = -.59, p < .05), such that as preference for the large risky reward increased, α4β2 nAChR density levels in the nucleus accumbens shell decreased (Figure 14).

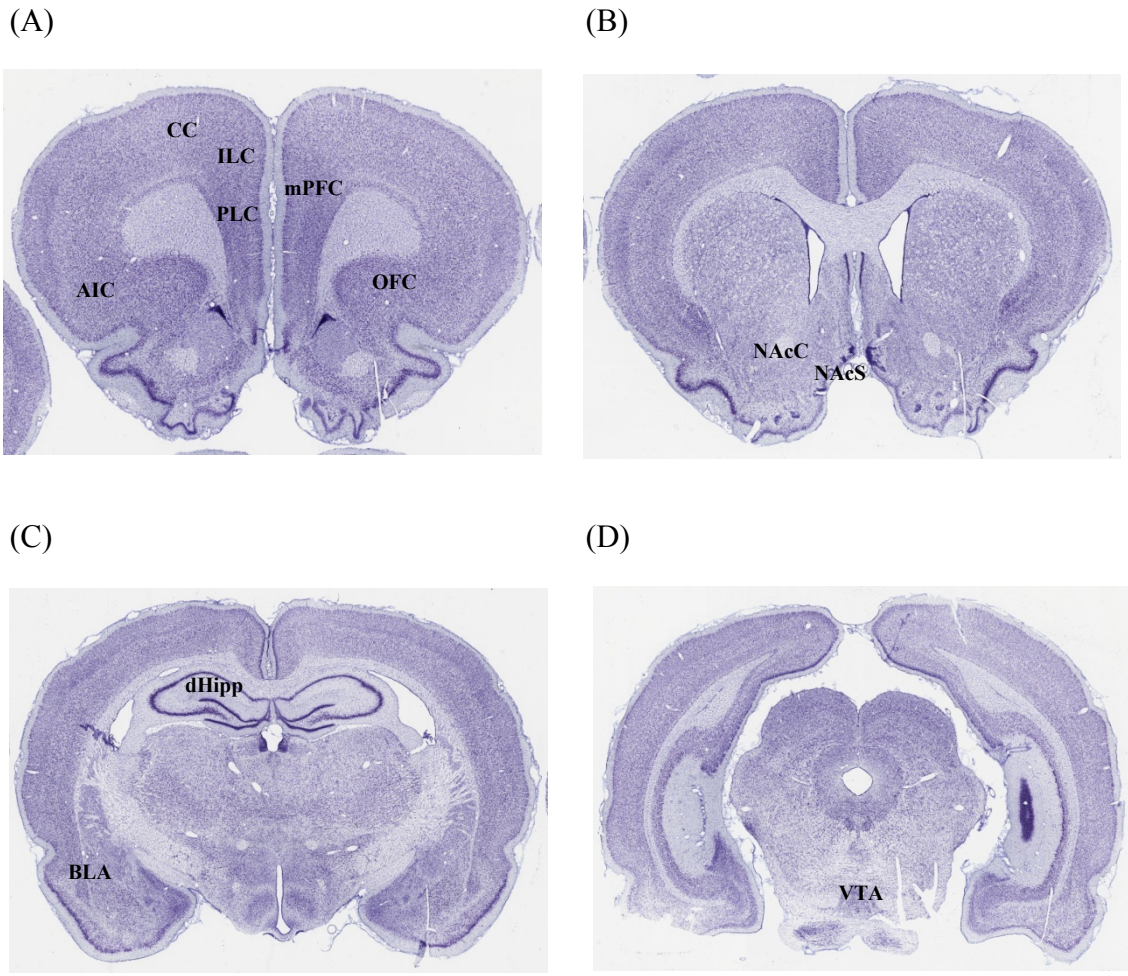


Figure 13. Cerebral structures in which nAChR subtype levels were determined. Twenty micrometer thick coronal brain sections were taken from (A) cortical (orbitofrontal OFC, medial prefrontal mPFC, infralimbic ILC, prelimbic PLC, cingulated CC, and agranular insular AIC cortex) and (B) & (C) limbic (nucleus accumbens shell NAcS and core NAcC, basolateral amygdala BLA, and dorsal hippocampus dHIPP) structures in the forebrain, as well as structures within the (D) midbrain (ventral tegmental area VTA). (Jones 2007)

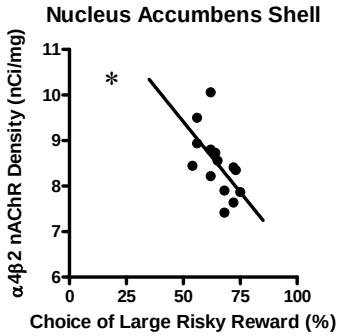


Figure 14. Linear association between risky decision making and α4β2 nAChR density levels. Baseline performance in the probability discounting task was negatively correlated with baseline α4β2 nAChR density levels. As preference for the large risky reward increased, α4β2 nAChR density levels in the nucleus accumbens shell decreased. * $p < .05$.

Similar to results seen with the probability discounting task, a negative linear correlation was observed between performance in the delay discounting task and nAChR density levels in the nucleus accumbens shell ($r = -.61$, $p < .05$), in that greater preference for the large delayed reward was associated with lower α4β2 nAChR density levels in this region (Figure 15(A)). However, performance in the delay discounting task also had a similar correlation with α4β2 nAChR density levels in the dorsal hippocampus ($r = -.60$, $p < .05$), such that greater preference for the large delayed reward was associated with lower α4β2 nAChR density levels in this region (Figure 15(B)).

Pearson's bi-variate correlations were also used to investigate the relationships between baseline α4β2 nAChR density levels in specific brain regions and performance in several behavioral tasks (Table 5). A positive linear correlation was found between

performance in the instrumental responding task on a fixed ratio 1 reinforcement schedule and nAChR levels in the orbitofrontal, medial prefrontal, and prelimbic cortex, as well as ventral and dorsal hippocampus (*ps* < .05), such that as responding in this version of the instrumental task increased, so did α4β2 nAChR density levels in these sites. Additionally, a similar relationship was found between performance in the progressive ratio version of the instrumental responding task and α4β2 nAChR density levels in the ventral hippocampus ($p < .05$). No correlations were observed between performance in the fixed ratio 3, 10, 20, and 40 versions of the instrumental responding task and α4β2 nAChR density levels in the brain regions assessed.

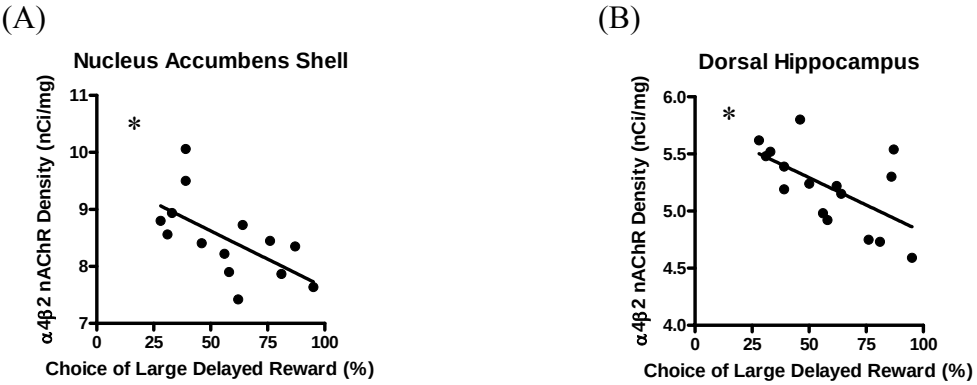


Figure 15. Linear association between impulsive decision making and α4β2 nAChR density levels. (A) Baseline performance in delay discounting task was negatively correlated with baseline α4β2 nAChR density levels. As preference for the large delayed reward increased, α4β2 nAChR density levels in the nucleus accumbens shell decreased. (B) Baseline performance in delay discounting task was also negatively correlated with baseline α4β2 nAChR density levels. As preference for the large delayed reward increased, α4β2 nAChR density levels in the hippocampus also decreased.

Table 5. Correlation matrix displaying linear relationships between α4β2 nAChR density levels and performance in behavioral tasks.

	% choice of large risky reward	% choice of large delayed reward	Fixed Ratio1	Progressive Ratio	Horizontal Activity
OFC	r = -.22 p = .44	r = -.35 p = .20	**r = .59** **p < .05***	r = -.40 p = .14	**r = .62** **p < .05***
mPFC	r = -.31 p = .26	r = -.27 p = .33	**r = .57** **p < .05***	r = -.14 p = .63	**r = .63** **p < .05***
CC	r = .22 p = .41	r = .19 p = .49	r = .16 p = .56	r = -.39 p = .13	r = .43 p = .09
PLC	r = -.26 p = .34	r = -.30 p = .28	**r = .56** **p < .05***	r = -.26 p = .35	r = .36 p = .19
ILC	r = -.18 p = .54	r = -.37 p = .19	r = .44 p = .11	r = -.14 p = .64	r = .06 p = .83
AIC	r = .09 p = .75	r = .03 p = .91	r = .07 p = .80	r = -.43 p = .10	r = .15 p = .58
vHipp	r = -.03 p = .92	r = -.25 p = .36	**r = .57** **p < .05***	**r = -.54** **p < .05***	r = .44 p = .09
dHipp	r = -.03 p = .91	**r = -.60** **p < .05***	**r = .53** **p < .05***	r = -.40 p = .13	r = .23 p = .39
NAcC	r = .21 p = .45	r = .10 p = .73	r = .44 p = .11	r = -.36 p = .18	**r = .65** **p < .01***
NAcS	**r = -.59** **p < .05***	**r = -.61** **p < .05***	r = .37 p = .19	r = -.03 p = .92	**r = .59** **p < .05***
BLA	r = -.10 p = .75	r = -.54 p = .07	r = .10 p = .76	r = -.35 p = .27	r = .07 p = .83
VTA	r = .21 p = .45	r = -.08 p = .78	r = -.05 p = .85	r = -.50 p = .06	r = -.15 p = .59

Density levels of α4β2 nAChRs were also linearly correlated with horizontal activity in the Open Field Test, such that as horizontal photobeam breaks increased, so did α4β2 nAChR density levels in the orbitofrontal and medial prefrontal cortex (*ps* < .05). Additionally, a similar positive correlation was found between horizontal photobeam breaks and α4β2 nAChR density levels in the nucleus accumbens core and shell (*ps* < .05). No correlations were found between time spent in the center region of the activity chamber and α4β2 nAChR density levels in the brain.

Several correlations were observed between α4β2 receptor binding in specific brain regions and performance on the decision making tasks. In particular, a negative linear correlation was observed between α4β2 nAChR levels in the nucleus accumbens shell and preference for the large risky reward in the probability discounting task, such that as α4β2 nAChR levels in the nucleus accumbens shell increased, preference for the large risky reward decreased (decreased risky choice). These findings do not support our predicted results (based on our behavioral pharmacological experiments) that higher levels of α4β2 nAChR would be associated with greater preference for the large risky reward (increased risky choice). A negative linear correlation was also observed between the delay discounting task and α4β2 nAChR levels in the nucleus accumbens shell and dorsal hippocampus. As α4β2 nAChR density levels in the nucleus accumbens shell and dorsal hippocampus increased, preference for the large delayed reward decreased (increased impulsive choice). These finding do not support our predicted results (based on our behavioral pharmacological experiments) that higher levels of α4β2 nAChR would be associated with greater preference for the large delayed reward (decreased

impulsive choice). Although the role of α4β2 nAChR in cost benefit decision making remains unclear, the findings from this experiment do suggest that α4β2 nAChR are indeed involved in cost-benefit decision making. Furthermore, the negative correlation between high levels of α4β2 nAChR with greater preference for the large reward in both tasks suggests shared neurobiological mechanisms.

Experiment 3.2. Relationship Between α7 Nicotinic Receptors and Performance on the Probabilistic and Delay Discounting Tasks

As mentioned above, these experiments focused on nAChR subtypes with roles well established in executive function and reward motivation. The α7 nAChR subtype comprises about 10% of all nAChRs in the mammalian brain and is also implicated in executive function and incentive motivation (Curzon et al. 2006; Hoyle et al. 2006; Keller et al. 2005; Young et al. 2004; Zanetti et al. 2006); however, research on the role of the α7 nAChR subtype in executive function and incentive motivation is more limited than that on α4β2 (Berger et al. 1998; Chang and Berg 1999; Gray et al. 1996; Hefft et al. 1999; McGehee et al. 1995; Messi et al. 1997). Of note, receptor binding studies have shown decreases in α7 nAChR levels in prefrontal cortical areas of patients with schizophrenia and Alzheimer's disease (Breese et al. 2000; Durany et al. 2000; Flynn and Mash 1986; Warpman and Nordberg 1995).

As discussed in Experiment 3.1, results from our behavioral pharmacological experiments (Experiment 1.2 and 2.2) suggest that activation of nAChRs following treatment with a high dose of nicotine can increase choice of the large risky or delayed

reward in the probabilistic or delay discounting tasks, respectively. Therefore, we predicted that higher levels of α7 nAChR levels would be associated with greater preference for the large risky reward in the probability discounting task (increased risky choice) and the large delayed reward in the delay discounting task (decreased impulsive choice). As discussed, the perseverative, habitual responding observed in the probabilistic discounting task may be occurring as a result of increases in dopaminergic firing within the mesolimbic system (Ellinwood and Kilbey 1975; Faure et al. 2010; Hsieh et al. 2010; Loh and Beck 1989). Based on this understanding, as well as research suggesting nicotine-dependent increases in dopamine levels (Mifsud et al. 1989; Rada et al. 2001; Wang et al. 2007), we predicted that the relationship between probabilistic discounting and nAChR density levels would be linear and that increases in nAChR levels would be seen in structures found within the mesolimbic system. Whether the increases in preference for the large delayed reward in the delay discounting task occurred as a result of improved cognition or increased perseverative responding remains unclear; therefore, areas in which nAChR levels may be correlated with this task are difficult to determine. Identified locations resulting from this research may help elucidate the mechanisms underlying our observed increase in preference for the large, delayed or risky reward following acute nicotine administration.

Treatment

Using receptor autoradiography, homomeric α7 nAChRs were identified using ^{125}I-labeled α-bungarotoxin. For total binding, sections were incubated at room

temperature with 5 nM [^{125}I]-α-bungarotoxin for 2 hours, within the Tris-HCl buffer. Film was exposure to α-bungarotoxin slides for 3 days.

Results

Pearson's bi-variate correlations were used to investigate the relationships between baseline α7 nAChR density levels in specific brain regions and baseline performance in several behavioral tasks (Table 6). A negative linear correlation was observed between performance in the delay discounting task and nAChR density levels in the basolateral amygdala (r = -.54, p < .05), in that greater preference for the large delayed reward was associated with lower α7 nAChR density levels in this region (Figure 16). Performance in the probability discounting task was not associated with α7 nAChR density levels in any brain regions analyzed.

Similar to results seen with α4β2 nAChR, a positive linear correlation was found between performance in the instrumental responding task with a fixed ratio 1 reinforcement schedule and α7 nAChR levels in several areas within the brain, including the orbitofrontal and agranular insular cortex, as well as the nucleus accumbens core and basolateral amygdala (ps < .05), such that as responding in this version of the instrumental task increased, so did α7 nAChR density levels in these sites. No linear correlations were observed between performance in the fixed ratio 3, 10, 20, and 40 versions of the instrumental responding task and α7 nAChR density levels in the brain regions that were assessed. Additionally, a negative linear relationship was found between performance in the progressive ratio version of the instrumental responding task and α7 nAChR density levels in the orbitofrontal and agranular insular cortex (p < .05).

Table 6. Correlation matrix displaying linear relationships between α7 nAChR density levels and performance in behavioral tasks.

	% choice of large risky reward	% choice of large delayed reward	Fixed Ratio1	Progressive Ratio	Horizontal Activity
OFC	r = .37 p = .18	r = -.38 p = .16	**r = .62** **p < .05 ***	**r = -.61** **p < .05 ***	r = .08 p = .77
mPFC	r = .18 p = .50	r = -.36 p = .19	r = .30 p = .28	r = -.22 p = .43	r = -.11 p = .69
CC	r = .25 p = .37	r = .11 p = .69	r = .02 p = .96	r = -.27 p = .34	r = -.19 p = .51
PLC	r = .14 p = .62	r = -.40 p = .14	r = .42 p = .12	r = -.23 p	r = -.04 p = .88
ILC	r = .02 p = .93	r = .42 p = .12	r = .24 p = .39	r = -.06 p = .84	r = -.17 p = .54
AIC	r = .26 p = .35	r = .25 p = .37	**r = .54** **p < .05 ***	**r = -.54** **p < .05 ***	r = .15 p = .60
vHipp	R = -.06 p = .82	r = -.03 p = .90	r = .11 p = .68	r = **.18** p = .50	r = -.14 p = .61
dHipp	R = -.06 p = .83	r = -.22 p = .42	r = .33 p = .21	r = -.11 p = .68	r = .31 p = .24
NAcC	r = .16 p = .67	r = .31 p = .39	**r = .64** **p < .05 ***	r = .29 p = .41	r = .28 p = .44
NAcS	R = -.32 p = .25	r = -.28 p = .31	r = .11 p = .71	r = -.04 p = .90	r = -.04 p = .88
BLA	R = -.41 p = .13	**r = -.54** **p < .05 ***	**r = .53** **p < .05 ***	r = -.27 p = .33	**r = .61** **p < .05 ***
VTA	R = -.12 p = .68	r = -.05 p = .85	r = -.15 p = .59	r = .05 p = .85	r = -.26 p = .35

Density levels of α7 nAChRs were also positively correlated with horizontal activity in the Open Field Test, such that as horizontal photobeam breaks increased, so did α7 nAChR density levels in the basolateral amygdala (*ps* < .05). No correlations were found between time spent in the open center region of the activity chamber and α7 nAChR density levels in the brain.

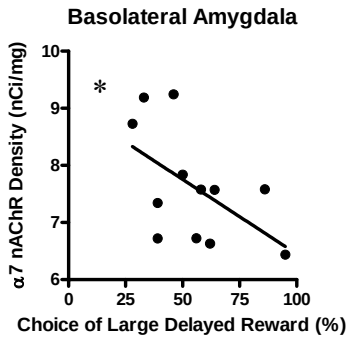


Figure 16. Linear association between impulsive decision making and α7 nAChR density levels. Baseline performance in the delay discounting task was negatively correlated with baseline α7 nAChR density levels. As preference for the large delayed reward increased, α7 nAChR density levels in the basolateral amygdala decreased. * p < .05.

Results suggest that α7 nAChR density levels are not associated with choice preference in the probability discounting task. In the delay discounting task, results show that as α7 nAChR density levels in the basolateral amygdala increased, choice of the large delayed reward decreased (increased impulsive choice), resulting in a negative linear relationship. Similar to Experiment 3.1, findings from the delay discounting task did not support our prediction (based on our behavioral pharmacological experiments)

that higher levels of α7 nAChR levels would be associated with greater preference for the large delayed reward (decreased impulsive choice). Although the role of α7 nAChR in cost benefit decision making remains unclear, the findings from this experiment suggest that α7 nAChR are indeed involved in cost-benefit decision making.

Experiment 3 Summary and Discussion

In Experiment 3, the relationships between performances in several behavioral tasks were assessed. Among the various behavioral measures there were several significant linear correlations; of particular interest, however, was a positive linear correlation between the probability and delay discounting tasks, showing that greater preference for the large risky reward was associated with greater preference for the large delayed reward. Correlations observed between probability and delay discounting may suggest similar underlying cognitive and neuronal processes involved in action-outcome contingencies (Green and Myerson 1996; Mazur 1997; Sozou 1998).

This finding is not entirely surprising since it has been suggested by some studies that probability and delay discounting may share similar underlying processes (Green and Myerson 1996; Mazur 1997; Sozou 1998). For example, choosing an uncertain reward for 5 trials, and receiving it only after the 5^{th} trial can be somewhat equivalent to a very long delay (Cardinal 2006). In addition, the reward type is identical and the reward magnitudes are very similar in both discounting tasks, resulting in some likely contextual overlap between the two tasks. Nonetheless, dissociable processes, such as differences in eventual predictability of rewards and absolute reward magnitude, may be

observed between discounting of delayed rewards and discounting of uncertain rewards (Green and Myerson 2004; Ho et al. 1999; Mitchell 2003; Simon et al. 2009), and several previous studies from our laboratory have shown no relationship between performance in the two tasks (Mendez et al. 2010; Simon et al. 2009). More research is needed to identify shared and separate target mechanisms for the treatment of impaired risky and impulsive behaviors.

A correlation was also found between performance in the probabilistic discounting task and locomotor activity in the Open Field Test, such that animals that were more likely to choose the large risky reward were also more likely to spend more time in the open center region of the activity chamber. The association between increases in choice of the large risky reward and time spent in the center of the chamber during the activity test is not surprising, as time spent in a open field has often been used as a measure of anxiety and risky behavior (Mikics et al. 2005). Finally, a correlation was found between the delay discounting task and instrumental responding for food reward, such that greater preference for the large delayed reward was associated with less total lever pressing during a 30 minute test session in the fixed ratio 1 version of the instrumental responding task. This relationship is consistent with the hypothesis that increased responding on low fixed reinforcement schedules reflects greater sensitivity to immediate reinforcement (Johansen et al. 2009; Sagvolden et al. 1992a; Sagvolden et al. 1992b).

Results from Experiments 1 and 2 suggest that pharmacological manipulation of both nicotinic and muscarinic AChRs can affect cost-benefit decision making when

delay of reward or risk of reward loss is involved. ACh levels within the nucleus accumbens, hippocampus, and amygdala have all been associated with changes in processes implicated in cost benefit decision making, such as motivation for reward seeking and taking, and processes involved in the learning and memory concerning reward and reward associated cues (Crespo et al. 2006; Schroeder and Packard 2004; Smith et al. 2004a; Smith et al. 2004b). Results from Experiment 3 associate differences in nAChR levels within all of these structures with differences in cost-benefit decision making. Data from Experiment 3.1 suggest that higher α4β2 nAChR levels in the nucleus accumbens shell are associated with decreased preference for the large risky reward in the probability discounting task (decreased risky decision making) and decreased preference for the large delayed reward in the delay discounting task (increased impulsive decision making). Furthermore, it was found that increases in α4β2 nAChR levels in the dorsal hippocampus were associated with decreased preference for the larger delayed reward in the delay discounting task.

Although some of the specific brain areas in which we observed relationships between nAChR levels and performance in the discounting tasks were unexpected, research has been conducted that may elucidate why these areas may be implicated. The association between lower levels of α4β2 nAChR levels in the ventral hippocampus and higher breakpoints when responding for food on the progressive ratio task is similar to that seen in a recent study that implicates the hippocampus in addictive behaviors. Using squirrel monkeys, Le Foll et al (2009) found that lower levels of α4β2 nAChR within the hippocampus were associated with higher breakpoints when responding for intravenous

nicotine on a progressive ratio schedule. These findings may help explain the observed association found between α4β2 nAChR levels in the dorsal hippocampus and choice preference in the delay discounting task. That is, because rats with lower levels of α4β2 nAChR within the hippocampus were more likely to work and wait for a future reward in the progressive ratio task (represented by higher breakpoints), then it may also be expected that rats with lower levels of α4β2 nAChRs within the hippocampus would also be more likely to wait for larger, albeit delayed, reward in the delay discounting task.

Results from Experiment 3.2 show that although α7 nAChR density levels are not associated with choice preference in the probability discounting task, α7 receptor levels are associated with choice preference in the delay discounting task. Activation of α7 nAChRs on the terminals of dopaminergic neurons projecting from the VTA to the ventral striatum is implicated in dopamine release within the nucleus accumbens; however, no significant correlations between α7 levels in nucleus accumbens and impulsive or risky decision making were observed. Interestingly, increases in α7 nAChR density levels in the basolateral amygdala were (BLA) were associated with decreased preference for the large delayed reward (increased impulsive choice). Destruction of the BLA has been shown to increase impulsive choice (Winstanely Theobald, Cardinal, Robbins 2004), an effect that may result from impairments in utilizing representations of the incentive value of delayed rewards to guide behavior. Therefore, high levels of α7 nAChR levels within the BLA may be causing effects similar to those observed with BLA inactivation – i.e. causing the value of large reward to not be maintained across delays, and in turn promoting impulsive choice. Finally, the fact that there were fewer

associations between α7 nAChR and performance in cost-benefit decision making tasks relative to α4β2 nAChR may reflect the relatively lower levels of expression of α7 nAChRs in the brain.

Activation of nAChR in the nucleus accumbens has been shown to induce increases in processes that promote responding for large rewards, including attention to, learning of, and/or motivation for, rewards and reward-related cues (Mifsud et al. 1989; Rada et al. 2001; Wang et al. 2007). Therefore, the observed associations between increases in α4β2 nAChR level in the nucleus accumbens shell and decreases in preference for the large reward in both the delay and probability discounting task in Experiment 3.1 were a bit surprising. One explanation for this discrepancy may lie in the fundamental difference between the effects of nAChR activation resulting from endogenous receptor activation (such as that occurring in our untreated rats in Experiment 3) and receptor activation resulting from exogenous drug administration (such as that occurring in our nicotine-treated rats in Experiments 1 and 2). While higher levels of endogenous nAChR activation may lead to decreased preference for a larger reward, activation of nAChR with a high dose of nicotine may cause greater preference for larger rewards.

One explanation for the observed relationship between higher levels of endogenous nAChRs and lower preference for large rewards may stem from a recent study in which the authors propose that ACh within the nucleus accumbens shell is involved in avoidance behaviors, possibly through mediation of GABAergic output (Hoebel 2007). In this study, microdialysis during several avoidance behaviors shows

that increases in aversive behavior correlate with increases in acetylcholine release in the shell (such as that which occurs at the end of a meal). These findings suggest that endogenous acetylcholine activation of nAChRs within the shell may be causing increases in avoidance (of delays to reward or risk of loss of reward) in rats with higher levels of α4β2 nAChR in the nucleus accumbens shell.

The effects of systemic injections of nicotine in Experiment 1 and 2 were most prominent in the probability discounting task in rats that had low choice preference for the large risky reward, and were limited in the delay discounting task to rats that had low choice preference for the large delayed rewards. When further considering the results of Experiment 3, as well as research showing that activation of nAChR in the nucleus accumbens are involved in processes that promote responding for large rewards, including attention to, learning of, and/or motivation for, rewards and reward-related cues (Mifsud et al. 1989; Olausson et al. 2006; Rada et al. 2001; Wang et al. 2007), it may be argued that rats with low baseline preference for the large risky or delayed rewards in Experiments 1 and 2 were also the rats that had the highest baseline levels of nicotinic receptors within the nucleus accumbens shell. Therefore, it is possible that in Experiments 1 and 2, any observed increases in preference for the large risky or delayed reward occurred in rats with low baseline preference for this reward because they had a higher number of available nAChR onto which systemically injected nicotine could bind, and subsequently exert a large effect (increased motivation for rewards and reward related cues).

As suggested in Experiment 2, increases in perseverative responding following systemic injections of nicotine may be due to increases in attention and motivation directed toward an initially presented larger reward, to the degree that appropriate consideration of subsequent changes in reward-cost contingencies are impaired. Given that AChR have been highly implicated in behavioral and biological component processes involved in cost-benefit decision making, the lack of associations between nAChR subtypes levels in cortical structures implicated in cost-benefit decision making and performance in the discounting tasks was surprising. Interestingly, however, it has been repeatedly shown that several processes relevant to cost benefit decision making (learning, motivation) can be affected by cholinergic and dopaminergic interactions (Bertorelli and Consolo 1990; Imperato et al. 1994; Puttfarcken et al. 2000; Weiner et al. 1990; Zanetti et al. 2006). This suggests that nAChR activation may not directly affect cortical-dependent processes involved in cost-benefit decision making, including decision making and conflict resolution, but instead may act indirectly through other neurotransmitter systems.

Research suggests that the effects of cholinergic and dopaminergic interactions within the mesolimbic system on cost-benefit decision making may be modulated by the feed-forward, complementary organization of cholinergic and dopaminergic neurons (Balfour 2004; David et al. 2006; Mifsud et al. 1989; Rada et al. 2001; Wang et al. 2007). It may therefore be the case that nicotine's effects on cost-benefit decision making are indirect and mediated by nAChR-dependent increases in dopamine release.

More research is needed to examine how cholinergic interactions with other neurotransmitters (particularly dopamine) can affect cost-benefit decision making.

SUMMARY, DISCUSSION, AND CONCLUSIONS

Results Summary

Current research suggests a role for both nicotinic and muscarinic AChRs in psychological processes necessary for cost-benefit decision making, including executive functions involving the PFC and incentive motivation involving the mesolimbic system (Cutuli et al. 2008; Furey et al. 2008; Picciotto et al. 2008; Ricciardi et al. 2009). To date, however, there has only been one study that has used animals to investigate the causal role of cholinergic systems in cost-benefit decision making (Dallery and Locey 2005). The present study suggests that the cholinergic system is indeed involved in cost-benefit decision making. Specifically, results suggest that increases in acetylcholine levels resulting from inhibition of acetylcholinesterase decrease risky behavior in risk-taking rats (Experiment 1.1). Results also suggest that activation of nAChRs by nicotine increases risky decision making (Experiment 1.2), whereas blockade of nAChRs causes a decrease in risky decision making (Experiment 1.4). Investigation of the role of mAChRs in probabilistic discounting was limited due to an increase in response omissions. Limited data, however, do show that activation of mAChRs does not appear to affect risky decision making (Experiment 1.3), while blockade of mAChRs can cause poor decision making across the probability discounting task (Experiment 1.5). Regarding the role of cholinergic receptors in impulsive decision making, results suggest that inhibition of acetylcholinesterase does not appear to affect impulsive choice (Experiment 2.1). Results from this task do suggest, however, that activation of nAChRs

decreases impulsive choice in impulsive rats (Experiment 2.2). Blockade of nAChRs also affected impulsive choice in impulsive rats, although the directionality of these effects was difficult to interpret (Experiments 2.4). Blockade of mAChR appeared to decrease impulsive choice in both impulsive and non-impulsive rats (Experiment 2.5), while activation of these receptors with oxotremorine caused effects that appeared to depend on baseline levels of impulsive choice (Experiment 2.3). Finally, analysis of radioligand binding densities for specified nAChR subtypes suggests that α4β2 and α7 nAChR may be related to risky and/or impulsive choice as determined by the probabilistic and delay discounting task, and that nAChR in selected subcortical structures (NAcc, Hipp, BLA) may be directly involved in cost-benefit decision making (Experiment 3).

General Discussion and Conclusions

The overall goal of this dissertation was to determine the role that cholinergic receptors play in cost-benefit decision making. Results from Experiment 1 identify a number of effects on performance in the probability discounting task following cholinergic manipulations; however, the most prominent effect observed was a nicotine-induced increases in risky decision making. The results from control studies suggest that these effects may be due, at least in part, to increases in perseverative responding. In Experiment 2, several effects on performance in the delay discounting task were observed following cholinergic receptor manipulation, with the most prominent effect observed being an increase in impulsive decision making following treatment with the

mAChR antagonist atropine. These findings suggest that cholinergic receptors are indeed involved in cost-benefit decision making and that the mechanisms underlying different types of cost-benefit decision making (impulsive, risky) are dissociable.

Results from this dissertation also suggest that intrinsic differences in nAChR subtype levels within mesolimbic structures (NAcc, Hipp, BLA) can predict baseline levels of impulsive and risky decision making. Negative correlations were observed between nAChR subtype levels in the nucleus accumbens shell and preference for the large delayed or risky reward, suggesting that shared mechanisms may underlie the observed increase in preference for the large reward in the two discounting tasks. Contrary to what was expected based on Experiment 1 and 2 (in which increases in activation of nAChR was associated with greater preference for the large risky or delayed reward), findings from Experiment 3 showed that increases in nAChR levels predict decreased preference for the large risky or delayed reward. However, when considering that the most prominent effects of nicotine were seen in rats with low baseline preference for the large reward regardless of risk (animals presumed to have high baseline levels of nAChR), one may conclude that high baseline levels of nAChR serve to predispose animals to larger increases in preference for large rewards following high levels of exogenous nAChR activation (perhaps as a result of the overall greater number of nAChRs that can respond to the agonist).

Given the feed-forward organization of ACh and dopamine interactions within the mesolimbic system, and the role of dopamine in encoding rewards and reward-related cues, one would predict that increases in cholinergic receptor activation would

increase attention towards and motivation for reward associated stimuli. Similar effects are indeed observed following treatment with the dopamine increasing drug amphetamine (St Onge et al. 2010). These shared effects may be due to their shared neurobiological functions and feed-forward organization. Similarly, the apparent increase in preference for the larger delayed reward when testing the effects of nicotine in the delay discounting task (albeit small, and limited to the impulsive subgroup of rats) are similar to those reported in a recent study in which acute amphetamine increased preference for the large delayed reward in the delay discounting task (Setlow et al. 2009). Again, these findings support the idea that acetylcholine and dopamine work in a feed-forward manner that can amplify attention and motivation for rewards and reward related cues.

Notably, no correlations between nAChR subtypes in structures within the PFC and choice preference in the delay or probability discounting task were observed. Correlations between mAChR subtypes within these key structures on cost-benefit decision making are unknown. Therefore, although the PFC is strongly implicated in cost-benefit decision making, the role of ACh within the PFC may be limited to activation of mAChRs. Future research should continue to identify nicotinic and muscarinic cholinergic mechanisms involved in cost-benefit decision making. Such studies could expand our findings by examining the effects of administration of cholinergic drugs that bind to specific nicotinic and muscarinic receptor subtypes on performance in cost-benefit decision making tasks. These proposed studies could be even more informative if administration of these drugs were limited to the specific brain

regions that Experiment 3 identified as being associated with individual differences in cost-benefit decision making (NAcc, Hipp, BLA). Finally, this research could be even more particularly useful when conducted with animals that model psychopathological conditions in which there is known to be impaired cost-benefit decision making. This dissertation project offers, for the first time, insight into the role of acetylcholine in risky and impulsive decision making, possibly providing data for the advancement of, intervention and care for individuals suffering from impaired cost-benefit decision making.

REFERENCES

Balfour DJ (2004) The neurobiology of tobacco dependence: a preclinical perspective on the role of dopamine projections to the nucleus. Nicotine Tobacco Research 6: 899-912

Baran S, Armstrong C, Niren D, Conrad C (2010) Prefrontal cortex lesions and sex differences in fear extinction and perseveration. Learning and Memory 17: 267-268

Barr A, Phillips A (1999) Withdrawal following repeated exposure to d-amphetamine decreases responding for a sucrose solution as measured by a progressive ratio schedule of reinforcement. Psychopharmacology 141: 99-106

Beer JS, Knight RT, D'Esposito M (2006) Controlling the integration of emotion and cognition. Psychological Science 17: 448-453

Berger F, Gage FH, Vijayaraghavan S (1998) Nicotinic receptor-induced apoptotic cell death of hippocampal progenitor cells. Journal of Neuroscience 18: 6871-6881

Bertorelli R, Consolo S (1990) D1 and D2 dopaminergic regulation of acetylcholine release from striata of freely moving rats. Journal of Neurochemistry 54: 2145-2148

Bickel W, Odum A, Madden G (1999) Impulsivity and cigarette smoking: delay discounting in current, never, and ex-smokers. Psychopharmacology 146: 447-454

Breese C, Lee M, Adams C, Sullivan B, Logel J, Gillen K, Marks M, Collins A, Leonard S (2000) Abnormal regulation of high affinity nicotinic receptors in subjects with schizophrenia. Neuropsychopharmacology 23: 351-364

Breese CR, Marks MJ, Logel J, Adams CE, Sullivan B, Collins AC, Leonard S (1997) Effect of smoking history on [3H]nicotine binding in human postmortem brain. Journal of Pharmacological Experimental Therapeutics 282: 7-13

Brown DC, Nichols JA, Thomas F, Dinh L, Atzori M (2010) Nicotinic modulation of auditory attentional shift in the rat. Behavioural Brain Research 210: 273-279

Butcher (1994) Cholinergic neurons and networks. In: Paxinos G (ed) The Rat Nervous System. Academic Press, San Diego, pp 1003-1016

Cardinal R (2006) Neural systems implicated in delay and probabilistic reinforcement. Neural Networks 19: 1277-1301

Cardinal RN, Robbins TW, Everitt BJ (2000a) The effects of d-amphetamine, chlordiazepoxide, a-flupenthixol and behavioural manipulations on choice of signalled and unsignalled delayed reinforcement in rats. Psychopharmacology 152: 362-375

Cardinal RN, Robbins TW, Everitt BJ (2000b) The effects of d-amphetamine, chlordiazepoxide, α-flupenthixol and behavioural manipulations on choice of signalled and unsignalled delayed reinforcement in rats. psychopharmacology 152: 362-375

Cetin T, Freudenberg F, Fuchtemeier M, Koch M (2004) Dopamine in the orbitofrontal cortex regulates operant responding under a progressive ratio of reinforcement in rats. Neuroscience Letters 370: 114-117

Chang KT, Berg DK (1999) Nicotinic acetylcholine receptors containing alpha 7 subunits are required for reliable synaptic transmission in situ. J. Neurosci. 19: 3701-3710

Chen K, Baxter M, Rodefer J (2004) Central blockade of muscarinic cholinergic receptors disrupts affective and attentional set-shifting. European Journal of Neuroscience 20: 1081-1088

Chouinard ML, Gallagher M, Yasuda RP, Wolfe BB, McKinney M (1995) Hippocampal muscarinic receptor function in spatial learning-impaired aged rat. Neurobiology of Aging 16: 955-963

Clark L, Robbins TW (2002) Decision-making deficits in drug addiction. Trends in Cognitive Sciences 6: 361-363

Cohen C, Perrault G, Griebel G, Soubrie P (2004) Nicotine-associated cues maintain nicotine-seeking behavior in rats several weeks after nicotine withdrawal: reversal by the cannabinoid (CB1) receptor antagonist, rimonabant (SR141716). Neuropsychopharmacology 30: 145-155

Collins A, Marks M, Pauly J (1989) Differential effect of chronic nicotine treatment on nicotinic receptor numbers in various brain regions of mice. Journal of Substance Abuse 1: 273-286

Court JA, Lloyd S, Thomas N, Piggott MA, Marshall EF, Morris CM, Lamb H, Perry RH, Johnson M, Perry EK (1998) Dopamine and nicotinic receptor binding and the levels of dopamine and homovanillic acid in human brain related to tobacco use. Neuroscience 87: 63-78

Coyle JT, Price DL, DeLong MR (1983) Alzheimer's disease: a disorder of cortical cholinergic innervation. Science 219: 1184-1190

Crespo JA, Sturm K, Saria A, Zernig G (2006) Activation of muscarinic and nicotinic acetylcholine receptors in the nucleus accumbens core is necessary for the acquisition of drug reinforcement. J. Neurosci. 26: 6004-6010

Critchley HD, Rolls ET (1996) Hunger and satiety modify the responses of olfactory and visual neurons in the primate orbitofrontal cortex. J Neurophysiol 75: 1673-1686

Curzon P, Anderson DJ, Nikkel AL, Fox GB, Gopalakrishnan M, Decker MW, Bitner RS (2006) Antisense knockdown of the rat [alpha]7 nicotinic acetylcholine receptor produces spatial memory impairment. Neuroscience Letters 410: 15-19

Cutuli D, Foti F, Mandolesi L, Bartolo PD, Gelfo F, Federic F, Petrosini L (2008) Cognitive performance of healthy young rats following chronic donepezil administration. Psychopharmacology 197: 661-673

Dallery J, Locey ML (2005) Effects of acute and chronic nicotine on impulsive choice on rats. Behavioural Pharmacology 16: 15-23

David V, Besson M, Changeux J, Granon S, Cazala P (2006) Reinforcing effects of nicotine microinjections into the ventral tegmental area of mice: dependence on

cholinergic nicotinic and dopaminergic D1 receptors. Neuropharmacology 50: 1030-1040

De Rosa E, Hasselmo ME (2000) Muscarinic cholinergic neuromodulation reduces proactive interference between stored odor memories during associative learning in rats. Behavioral Neuroscience 114: 32-41

de Wit H, Enggasser JL, Richards JB (2002) Acute administration of d-amphetamine decreases impulsivity in healthy volunteers. Neuropsychopharmacology 27: 813-825

Decker M, Bannon A, Curzon P, Gunther K, Brioni J, Holladay M (1997) ABT-089 [2-methyl-3(2-(S)-pyrrolidinylmethoxy) pyridine dihydrochloride]. II. A novel cholinergic channel modulator with effects on cognitive performance in rats and monkeys. Journal of Pharmacological Experimental Therapeutics 283: 247-258

Del'Guidice T, Nivet E, Escoffier G, Baril N, Caverni J-P, Roman FS (2009) Perseveration related to frontal lesion in mice using the olfactory H-maze. Behavioural Brain Research 205: 226-233

Dellu F, Mayo W, Cherkaoui J, Le Moal M, Simon H (1991) Learning disturbances following excitotoxic lesion of cholinergic pedunculo-pontine nucleus in the rat. Brain Research 544: 126-132

Diaz del Guante MA, Cruz-Morales SE, Prado-Alcalá RA (1991) Time-dependent effects of cholinergic blockade of the striatum on memory. 122: 79-82

Durany N, Zochling R, Boissl K, Paulus W, Ransmayr G, Tatschnerf T, Danielczyk W, Jellingerg K, Deckertb J, Riedererb P (2000) Human post-mortem striatal α4β2 nicotinic acetylcholine receptor density in schizophrenia and Parkinson's syndrome. Neuroscience Letters 287: 109-112

Ellinwood EH, Kilbey MM (1975) Amphetamine stereotypy: the influence of environmental factors and preponent behavioral patterns on its topography and development. Biological Psychiatry 10: 3-16

Elliott R, Newman JL, Longe OA, Deakin JFW (2003) Differential response patterns in the striatum and orbitofrontal cortex to financial reward in humans: a parametric functional magnetic resonance imaging study. Journal of Neuroscience 23: 303-307

Euteneuer F, Schaefer F, Stuermer R, Boucsein W, Timmermann L, Barbe MT, Ebersbach G, Otto J, Kessler J, Kalbe E (2009) Dissociation of decision-making under ambiguity and decision-making under risk in patients with Parkinson's disease: a neuropsychological and psychophysiological study. Neuropsychologia 47: 2882-2890

Evenden J, Ryan C (1996a) The pharmacology of impulsive behavior in rats: the effects of drugs on response choice with varying delays of reinforcement. Psychopharmacology 128: 161-170

Evenden JL, Ryan CN (1996b) The pharmacology of impulsive behaviour in rats: the effects of drugs on response choice with varying delays of reinforcement. Psychopharmacology 128: 161-170

Faure A, Leblanc-Veyrac P, El Massioui N (2010) Dopamine agonists increase perseverative instrumental responses but do not restore habit formation in a rat model of Parkinsonism. Neuroscience 168: 477-486

Field M, Santarcangelo M, Sumnall H, Goudie A, Cole J (2006) Delay discounting and the behavioural economics of cigarette purchases in smokers: the effects of nicotine deprivation. Psychopharmacology V186: 255-263

Floresco S, Whelan J (2009) Perturbations in different forms of cost/benefit decision making induced by repeated amphetamine exposure. Psychopharmacology 205: 189-201

Flynn D, Mash D (1986) Characterization of L-[3H]nicotine binding in human cerebral cortex: comparison between Alzheimer's disease and the normal. Journal of Neurochemistry 47: 1948-1954

Friedman JI (2004) Cholinergic targets for cognitive enhancement in schizophrenia: focus on cholinesterase inhibitors and muscarinic agonists. Psychopharmacology 174: 45-53

Furey ML, Pietrini P, Alexander GE, Schapiro MB, Horwitz B (2000) Cholinergic enhancement improves performance on working memory by modulating the functional activity in distinct brain regions: a positron emission tomography regional cerebral blood flow study in healthy humans. Brain Research Bulletin 51: 213-218

Furey ML, Ricciardi E, Schapiro MB, Rapoport SI, Pietrini P (2008) Cholinergic enhancement eliminates modulation of neural activity by task difficulty in the prefrontal cortex during working memory. Journal of Cognitive Neuroscience 20: 1342-1353

Gasbarri A, Sulli A, Pacitti C, Puglisi-Allergra S, Cabib S, Castellano C, Introini-Collison I, McGaugh JL (1997) Strain-dependent effects of D2 dopaminergic and muscarinic-cholinergic agonists and antagonists on memory consolidation processes in mice. Behavioural Brain Research 86: 97-104

George TP, Verrico CD, Picciotto MR, Roth RH (2000) Nicotinic modulation of mesoprefrontal dopamine neurons: pharmacologic and neuroanatomic characterization. Journal of Pharmacological Experimental Therapeutics 295: 58-66

Good CH, Lupica CR (2009) Properties of distinct ventral tegmental area synapses activated via pedunculopontine or ventral tegmental area stimulation in vitro. the Journal of Physiology 587: 1233-1247

Granon S, Faure P, Changeux J-P (2003) Executive and social behaviors under nicotinic receptor regulation. Proceedings of the National Academy of Sciences of the United States of America 100: 9596-9601

Gray R, Rajan A, Radcliffe K, Yakehiro M, Dani J (1996) Hippocampal synaptic transmission enhanced by low concentrations of nicotine. Nature 383: 713-716

Green L, Myerson J (1996) Exponential versus hyperbolic discounting of delayed outcomes: risk and waiting time. American Zoologist 36: 496-505

Green L, Myerson J (2004) A discounting framework for choice with delayed and probabilistic rewards. Psychological Bulletin 130: 769-792

Guan Z, Zhang X, Blennow K, Nordberg A (1999) Decreased protein level of nicotinic receptor alpha7 subunit in the frontal cortex from schizophrenic brain. Neuroreport 10: 1779-1782

Hefft S, Hulo S, Bertrand D, Muller D (1999) Synaptic transmission at nicotinic acetylcholine receptors in rat hippocampal organotypic cultures and slices. Journal of Physiology 515: 769-776

Ho MY, Mobini S, Chiang TJ, Bradshaw CM, Szabadi E (1999) Theory and method in the quantitative analysis of "impulsive choice" behaviour: implications for psychopharmacology. Psychopharmacology 146: 362-372

Hoffman W, Moore M, Templin R, McFarland B, Hitzemann R, Mitchell S (2006) Neuropsychological functionand delay discounting in methamphetamine-dependent individuals. Psychopharmacology 188: 162-170

Hoyle E, Genn RF, Fernandez C, Stolerman IP (2006) Impaired performance of alpha7 nicotinic receptor knockout mice in the five-choice serial reaction time task. Psychopharmacology 189: 211-223

Hsieh PC, Yeh TL, Lee IH, Huang HC, Chen PS, Yang YK, Chiu NT, Lu RB, Liao M-H (2010) Correlation between errors on the Wisconsin Card Sorting Test and the availability of striatal dopamine transporters in healthy volunteers. Journal of Psychiatry Neuroscience 35: 90-94

Imperato A, Obinu MC, Dazzi L, Gessa GL (1994) Does dopamine exert a tonic inhibitory control on the release of striatal acetylcholine in vivo. European Journal of Pharmacology 251: 271-279

Johansen E, Killeen P, Russell V, Tripp G, Wickens J, Tannock R, Williams J, Sagvolden T (2009) Origins of altered reinforcement effects in ADHD. Behavioral and Brain Functions 5: 7

Jones EG (2007) Whole brain map - rattus norvegicus. 30 micron Nissl stained coronal sections. www.brainmaps.org.

Kadir A, Darreh-Shori T, Almkvist O, Wall A, Grut M, Strandberg B, Ringheim A, Eriksson B, Blomquist G, Langstrom B, Nordberg A (2008) PET imaging of the in vivo brain acetylcholinesterase activity and nicotine binding in galantamine-treated patients with AD. Neurobiology of Aging 29: 1204-1217

Kalivas PW, Volkow ND (2005) The neural basis of addiction: a pathology off motivation and choice. American Journal of Psychiatry 162: 1403-1413

Keller JJ, Keller AB, Bowers BJ, Wehner JM (2005) Performance of [alpha]7 nicotinic receptor null mutants is impaired in appetitive learning measured in a signaled nose poke task. Behavioural Brain Research 162: 143-152

Kheramin S, Body S, Herrera FM, Bradshaw CM, Szabadi E, Deakin JFW, Anderson IM (2005) The effect of orbital prefrontal cortex lesions on performance on a progressive ratio schedule: implications for models of inter-temporal choice. Behavioural Brain Research 156: 145-152

Le Foll B, Chefer SI, Kimes AS, Shumway D, Stein EA, Mukhin AG, Goldberg SR (2009) Baseline expression of alpha4beta2 nicotinic acetylcholine receptors predicts motivation to self-administer nicotine. Biological Psychiatry 65: 714-716

Lemay S, Chouinard S, Blanchet P, Masson H, Soland V, Beuter A, Bedard MA (2004) Lack of efficacy of a nicotine transdermal treatment on motor and cognitive deficits in Parkinson's disease. Progress in Neuro-Psychopharmacology and Biological Psychiatry 28: 31-39

Levey AI, Edmunds SM, Koliatsos V, Wiley RG, Heilman CJ (1995) Expression of m1-m4 muscarinic acetylcholne receptor proteins in rat hppocampus and regulation by cholinergic innervation. The Journal of Neuroscience 15: 4077-4092

Levin E, Kaplan S, Boardman A (1997a) Acute nicotine interaction with nicotinic and muscarinic antagonists: working and reference memory effects in the 16-arm radial maze. Behavioral Pharmacology 8: 236-242

Levin E, Perkinsa A, Brothertona T, Qazia M, Bereza C, Montalvo-Ortiza J, Davisa K, Williams P, Christophera N (2009) Chronic underactivity of medial frontal cortical β2-containing nicotinic receptors increases clozapine-induced working

memory impairment in female rats. Progress in Neuro-Psychopharmacology and Biological Psychiatry 33: 296-302

Levin E, Torry D, Christopher N, Yu X, Einstein G, Schwartz-Bloom R (1997b) Is binding to nicotinic acetylcholine and dopamine receptors related to working memory in rats? Brain Research Bulletin 43: 295-304

Loh EA, Beck CHM (1989) Rats treated chronically with the benzodiazepine, diazepam or with ethanol exhibit reduced variability of behavior. Alcohol 6: 311-316

Loh EA, Smith AM, Roberts DCS (1993) Evaluation of response perseveration of rats in the radial arm maze following reinforcing and nonreinforcing drugs. Pharmacology Biochemistry and Behavior 44: 735-740

Marks M, Stitzel J, Collins A (1987) Influence of kinetics of nicotine administration on tolerance development and receptor levels. Pharmacology Biochemistry and Behavior 27: 505-512

Mazur JE (1987) An adjusting amount procedure for studying delayed reinforcement. In: Commons ML, Mazur JE, Nevin JA, Rachlin H (eds) Quantitative Analysis of Behavior: The Effects of Delay and of Intervening Events on Reinforcement Value. Erlbaum, Hillsdale, NJ, pp 55-73

Mazur JE (1997) Choice of delay, probability, and conditioned reinforcement. Animal Learning and Behavior 25: 131-147

McGehee D, Heath M, Gelber S, Devay P, Role L (1995) Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. Science 269: 1692-1696

Mendez IA, Simon NW, Hart N, Mitchell MR, Nation JR, Wellman PJ, Setlow B (2010) Self-administered cocaine causes long-lasting increases in impulsive choice in a delay discounting task. Behavioral Neuroscience 124: 470-477

Messi ML, Renganathan M, Grigorenko E, Delbono O (1997) Activation of Î±7 nicotinic acetylcholine receptor promotes survival of spinal cord motoneurons. FEBS letters 411: 32-38

Mifsud JC, Hernandez L, Hoebel BG (1989) Nicotine infused into the nucleus accumbens increases synaptic dopamine as measured by in vivo microdialysis. Brain Research 478: 365-367

Mikics É, Barsy B, Barsvári B, Haller J (2005) Behavioral specificity of non-genomic glucocorticoid effects in rats: effects on risk assessment in the elevated plus-maze and the open-field. Hormones and Behavior 48: 152-162

Mitchell SH (1999) Measures of impulsivity in cigarette smokers and nonsmokers. Psychopharmacology 146: 455-464

Mitchell SH (2003) Discounting the value of commodities according to different types of costs. In: Heather N, Vuchinich RE (eds) Choice, Behavioral Economics and Addiction. Oxford, UK: Elsevier, pp 339-357

Mobini S, Body S, Ho MY, Bradshaw C, Szabadi E, Deakin J, Anderson I (2002) Effects of lesions of the orbitofrontal cortex on sensitivity to delayed and probabilistic reinforcement. Psychopharmacology V160: 290-298

Mobini S, Chiang TJ, Ho MY, Bradshaw CM, Szabadi E (2000) [Without Title]. Psychopharmacology 152: 390-397

Mukhin AG, Kimes AS, Chefer SI, Matochik JA, Contoreggi CS, Horti AG, Vaupel DB, Pavlova O, Stein EA (2008) Greater nicotinic acetylcholine receptor density in smokers than in nonsmokers: a PET study with 2-18F-FA-85380. Journal of Nuclear Medicine 49: 1628-1635

O'Doherty JP (2004) Reward representations and reward-related learning in the human brain: insights from neuroimaging. Current Opinions in Neurobiology 14: 769-776

Olausson P, Jentsch J, Taylor J (2004) Nicotine enhances responding with conditioned reinforcement. Psychopharmacology V171: 173-178

Olausson P, Jentsch JD, Tronson N, Neve RL, Nestler EJ, Taylor JR (2006) {Delta}FosB in the nucleus accumbens regulates food-reinforced instrumental behavior and motivation. Journal of Neuroscience 26: 9196-9204

Perry DC, Xiao Y, Nguyen HN, Musachio JL, Dávila-García MI, Kellar KJ (2002) Measuring nicotinic receptors with characteristics of alpha4beta2, alpha3beta2 and alpha3beta4 subtypes in rat tissues by autoradiography. Journal of Neurochemistry 82: 468-481

Picciotto MR, Addy NA, Mineur YS, Brunzell DH (2008) It is not "either/or": activation of desensitization of nicotinic acetylcholine receptors both contribute to behaviors related to nicotine addiction and mood. Progress in Neurobiology 84: 329-342

Picciotto MR, Zoli M, Rimondini R, Lena C, Marubio LM, Pich EM, Fuxe K, Changeux J-P (1998) Acetylcholine receptors containing the [beta]2 subunit are involved in the reinforcing properties of nicotine. Nature 391: 173-177

Potter AS, Newhouse PA, Bucci DJ (2006) Central nicotinic cholinergic systems: a role in the cognitive dysfunction in Attention-Deficit/Hyperactivity Disorder? Behavioural Brain Research 175: 201-211

Potter AS, Ryan KK, Newhouse PA (2009) Effects of acute ultra-low dose mecamylamine on cognition in adult Attention-Deficit/Hyperactivity Disorder (ADHD). Human Psychopharmacology 24: 309-317

Pratt WE, Kelley AE (2004) Nucleus accumbens acetylcholine regulates appetitive learning and motivation for food via activation of muscarinic receptors. Behavioral Neuroscience 118: 730-739

Pratt WE, Spencer RC, Kelley AE (2007) Muscarinic receptor antagonism of the nucleus accumbens core causes avoidance to flavor and spatial cues. Behavioral Neuroscience 121: 1215-1223

Puttfarcken P, Jacobs I, Faltynek C (2000) Characterization of nicotinic acetylcholine receptor-mediated [3H]-dopamine release from rat cortex and striatum. Neuropharmacology 39: 2673-2680

Rada P, Jensen K, Hoebel BG (2001) Effects of nicotine and mecamylamine-induced withdrawal on extracellular dopamine and acetylcholine in the rat nucleus accumbens. Psychopharmacology 157: 105-110

Reavill C, Stolerman I (1990) Locomotor activity in rats after administration of nicotinic agonists intracerebrally. British Journal of Pharmacology 99: 273-378

Reynolds B, Richards J, Horn K, Karraker K (2004) Delay discounting and probability discounting as related to cigarette smoking status in adults. Behavioral Processes 64: 333-344

Ricciardi E, Pietrinia P, Schapiroc M, Rapoportd S, Fureye M (2009) Cholinergic modulation of visual working memory during aging: a parametric PET study. Brain Research Bulletin 79: 322-332

Sagvolden T, Hendley ED, Knardahl S (1992a) Behavior of hypertensive and hyperactive rat strains: hyperactivity is not unitarily determined. Physiology and Behavior 52: 49-57

Sagvolden T, Metzger MA, Schiorbeck HK, Rugland AL, Spinnangr I, Sagvolden G (1992b) The spontaneously hypertensive rat (SHR) as an animal model of childhood hyperactivity (ADHD): changed reactivity to reinforcers and to psychomotor stimulants. Behavioral Neural Biology 58: 103-112

Schroeder JP, Packard M (2004) Facilitation of memory for extinction of drug-induced conditioned reward: role of amygdala and acetylcholine Learning and Memory 11: 641-647

Setlow B, Mendez I, Mitchell M, Simon N (2009) Effects of chronic administration of drugs of abuse on impulsive choice (delay discounting) in animal models. Behavioral Pharmacology 20: 380-389

Simon NW, Gilbert RJ, Mayse JD, Bizon JL, Setlow B (2009) Balancing risk and reward: a rat model of risky decision making. Neuropsychopharmacology 34: 2208-2217

Simon NW, Mendez IA, Setlow B (2007) Cocaine exposure causes long-term increases in impulsive choice. Behavioral Neuroscience 121: 543-549

Slezak J, Anderson K (2009) Effects of variable training, signaled and unsignaled delays, and d-amphetamine on delay-discounting functions. Behavioral Pharmacology 20: 424-436

Smith DE, Rapp PR, McKay HM, Roberts JA, Tuszynski MH (2004a) Memory impairment in aged primates is associated with focal death of cortical neurons and atropy of subcortical neurons. The Journal of Neuroscience 24: 4373-4381

Smith JE, Co C, Yin X, Sizemore GM, Ligouri A, Johnson WE (2004b) Involvement of cholinergic neural system in intravenous cocaine self-administration. Neuroscience Biobehavioral Research 27: 841-850

Sozou PD (1998) On hyperbolic discounting and uncertain hazard rates. Proceedings of the Royal Society of London. Series B: Biological Sciences 265: 2015-2020

Squire LR (1986) Mechanisms of memory. Science 232: 1612-1619

St Onge JR, Chiu YC, Floresco SB (2010) Differential effects of dopaminergic manipulations on risky choice. Psychopharmacology 211: 209-221

St. Onge JR, Floresco SB (2009) Prefrontal cortical contribution to risk-based decision making. Cerebral Cortex 20: 1816-1828

Stanis J, Marquez Avila H, White M, Gulley J (2008) Dissociation between long-lasting behavioral sensitization to amphetamine and impulsive choice in rats performing a delay-discounting task. Psychopharmacology 199: 539-548

Thompson A, Molina B, Pelham W, Gnagy E (2007) Risky driving in adolescents and young adults with Childhood ADHD. Journal of Pediatric Psychology 32: 745-759

Tom SM, Fox CR, Trepel C, Poldrack RA (2007) The neural basis of loss aversion in decision-making under risk. Science 315: 515-518

Wade TR, de Wit H, Richards JB (2000) Effects of dopaminergic drugs on delayed reward as a measure of impulsive behavior in rats. Psychopharmacology 150: 90-101

Walters C, Brown S, Changeux J-P, Martin B, Damaj M (2006) The β2 but not α7 subunit of the nicotinic acetylcholine receptor is required for nicotine-conditioned place preference in mice. Psychopharmacology 184: 339-344

Wang Z, Faith M, Patterson F, Tang K, Kerrin K, Wileyto EP, Detre JA, Lerman C (2007) Neural substrates of abstinence-induced cigarette cravings in chronic smokers. Journal of Neuroscience 27: 14035-14040

Warpman U, Nordberg A (1995) Epibatidine and ABT 418 reveal selective losses of alpha 4 beta 2 nicotinic receptors in Alzheimer brains. Neuroreport 6: 2419-2423

Weiler JA, Bellebaum C, BrÃ¼ne M, Juckel G, Daum I (2009) Impairment of probabilistic reward-based learning in schizophrenia. Neuropsychology 23: 571-580

Weiner DM, Levey AI, Brann MR (1990) Expression of muscarinic and acetylcholine and dopamine receptor mRNAs in rat basal ganglia. Proceedings of the National Academy of Sciences of the United States of America 87: 7050-7054

Weller JA, Levin IP, Shiv B, Bechara A (2007) Neural correlates of adaptive decision making for risky gains and losses. Psychological Science 18: 958-964

Winhusen TM, Somoza EC, Harrer JM, Mezinskis JP, Montgomery MA, Goldsmith RJ, Coleman FS, Bloch DA, Leiderman DB, Singal BM, Berger P, Elkashef A (2005) A placebo-controlled screening trial of tiagabine, sertraline and donepezil as cocaine dependence treatments. Addiction 100: 68-77

Winstanley CA, Theobald DEH, Dalley JW, Cardinal RN, Robbins TW (2006) Double dissociation between serotonergic and dopaminergic modulation of medial prefrontal and orbitofrontal cortex during a test of impulsive choice. Cerebral Cortex 16: 106-114

Wittfoth M, Schardt DM, Fahle M, Herrmann M (2009) How the brain resolves high conflict situations: double conflict involvement of dorsolateral prefrontal cortex. Neuroimage 44: 1201-1209

Woodruff-Pak DS, Lehr MA, LI JG, Liu-Chen LY (2008) Young and older good learners have higher levels of brain nicotinic receptor binding. Neurobiology of Aging 31: 1032-1043

Yip S, Sacco K, George T, Potenza M (2009) Risk/reward decision-making in schizophrenia: a preliminary examination of the influence of tobacco smoking and relationship to Wisconsin Card Sorting Task performance. Schizophrenia Research 110: 156-164

Young JW, Finlayson K, Spratt C, Marston HM, Crawford N, Kelly JS, Sharkey J (2004) Nicotine improves sustained attention in mice: evidence for invovlement of the alpha7 nicotine acetylcholine receptor. Neuropsychopharmacology 29: 891-900

Yu AJ, Dayan P (2005) Uncertainty, neuromodulation, and attention. Neuron 46: 681-692

Zanetti L, D'Exaerde AdK, Zanardi A, Changeux J, Picciotto M, Zoli M (2006) Inhibitin of both a7 and b2 nicotinic acetylcholine receptors in necessary to prevent development of sensitization to cocaine-elicited increases in extracellular dopamine levels in the ventral striatum. Psychopharmacology 187: 181-188

Zhang H, Sulzer D (2004) Frequency-dependent modulation of dopamine release by nicotine. Nature Neuroscience 7: 581-582

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