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The Effects of Nicotine on a Probability-Discounting Task

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Abstract

Impulsivity has been implicated in many different neuropsychiatric disorders, such as substance use disorders, Alzheimer's Disease, and schizophrenia. In previous research, the effects of nicotine on attention and delay discounting have been well established. However, delay discounting represents just one aspect of impulsivity, and the other aspects have not been as well studied. The probability-discounting task is frequently used to measure the risky behavior aspect of impulsivity in animal models. We have found that exposure to nicotine, abstinence, and re-exposure to nicotine results in riskier behavior in rats. To test if this behavior is mediated by the α4β2 nicotinic acetylcholine receptors, we then administered an α4β2 nicotinic acetylcholine receptor antagonist, dihydro-β-ethyroidine, and found that the observed behavior on the probability-discounting task is not mediated by the α4β2 nicotinic acetylcholine receptors.

Introduction

Impulsivity has been implicated in drug addiction many times in humans and in animals (Kelsey and Niarula 2013, Weafer and de Wit 2013). Nicotine addiction frequently is comorbid with other addictions and behaviors that could be described as impulsive disorders or that have aspects of impulsive disorders (Potter and Newhouse 2008). However, the research on the effects of nicotine on impulsivity and on the actions of nicotinic acetylcholine receptors (nAChRs) has been contradictory. Moreover, little research has examined the effects of periods of nicotine exposure and abstinence on impulsivity. This research has clinical implications for understanding how impulsivity may be altered when individuals begin to try to stop smoking. The goals of these experiments were (1) to address this gap in the literature and (2) to asses the role of the α4β2 nicotinic acetylcholine receptors in impulsive behavior.

Impulsivity

Impulsive behavior is clinically defined as actions that are poorly conceived, prematurely expressed, unduly risky, or inappropriate to the situation and that often result in undesirable outcomes (Ohmura et al. 2012). From that broad definition, impulsivity can be subdivided into four categories: reflection impulsivity, impulsive action, impulsive choice, and risky behavior. Each aspect of impulsivity can be measured in a number of ways, contributing to the discrepancies seen in the literature. Impulsive choice can also be further subdivided into delay-discounting and probability-discounting, which are measured with their respective tasks. In a delay-discounting task, impulsivity is defined as a greater tendency to value or choose smaller, more immediate rewards over larger, more delayed rewards, even when it is advantageous to choose the larger, delayed reward. In other terms, impulsivity is a lack of delayed gratification. In contrast, the probability-discounting task, while still being a measure of impulsive choice, is

more of a measure of risky behavior (Mendez et al. 2013). Risky behavior is defined as placing oneself in an unsafe situation that can lead to dangerous consequences (Ohmura et al. 2012). With this definition in mind, in a probability-discounting task, choosing the larger, riskier reward is interpreted as impulsive behavior.

Impulsivity appears to be mediated by the medial prefrontal cortex (mPFC), and the various aspects of impulsivity are dissociable. The prelimbic mPFC has been implicated in mediating risky decision making (St Onge and Floresco 2009). The orbitofrontal cortex is involved in delay-discounting behavior and the dorsal anterior cingulated cortex has been implicated in mediating effort-based decision making (Congdon et al 2013).

Actions of nicotine in the brain: roles of nicotinic receptor subtypes

Nicotine binds to nicotinic acetylcholine receptors in the brain. These receptors are ionotropic and are made of pentameric combinations of α (α2-α7) and β (β2-β4) subunits which are coupled to sodium channels. In the human central nervous system, the α4β2 and α7 subtypes are the most widely distributed (Nashmi and Lester 2006). The cholinergic system in the brain is primarily modulatory, and so α4β2 nAChRs are located presynaptically. Receptors that are located presynaptically generally moderate how another neurotransmitter is released. α4β2 nAChRs are expressed in the parietal cortex, cingulate cortex, subiculum, substantia nigra, superior colliculus, medial geniculate nucleus, lateral-dorsal tegmental nucleus, and in lower levels in the nucleus accumbens (Nashmi and Lester 2006). In addition, α4 and β2 mRNAs are transcribed in every dopaminergic and GABAergic neuron in the ventral tegmental area. β2 containing receptors are dense in the limbic system, suggesting that these nicotinic acetylcholine receptors are responsible for the reward-like behavior and relieving negative affect responsible for maintaining addiction (Anderson and Brunzell 2012). Activation of β2 containing nAChRs

causes the reinforcement of reward-like behavior and inactivation of the β2 containing receptors decreases anxiety and fear-like behavior. These β2 receptors also have a high binding affinity for nicotine and endogenous acetylcholine (Anderson and Brunzell 2012). In fact, nicotine has an efficacy equivalent to that of acetylcholine on the α4β2 nAChRs (Anderson and Brunzell 2012).

Nicotinic acetylcholine receptors are unique in that they can experience multiple states of activation. An agonist can activate a nAChR in the traditional sense, but prolonged exposure to an agonist can cause a conformational change in the receptor that decreases its responsivity to be activated. This is known as a desensitizing effect and is similar to nAChR blockade by an antagonist (Levin 2013). Desensitization can occur after prolonged and repeated exposure to an agonist or after prolonged exposure to subactivating concentrations (Wageman et al. 2013). The desensitization by subactivating concentrations can help to explain the actions of nicotine at low doses. Acetylcholine does activate and then desensitize the nAChRs as well, but it is degraded so quickly that the receptors are able to recover fairly rapidly. Nicotine is metabolized much slower than acetylcholine, so the receptor desensitization is prolonged. Nicotinic acetylcholine receptors can also exist in a state of "smoldering activation," where some nicotinic acetylcholine receptors are desensitized and some are activated (Campling et al. 2013). This effect occurs in vivo because nicotine and other agonists can remain in the tissue for hours after drug administration. The smoldering activation depends very much on agonist concentrations. In the day of the average smoker, the levels of nicotine that are present vary greatly. When waking up in the morning after a night of not smoking, the free brain level of nicotine can be as low as 25nM, but can be as high as 465nM in the afternoon after a day of smoking (Rose et al. 2010). The variable concentrations of nicotine could be responsible for the various behavioral effects.

Anderson and Brunzell (2012) showed that low doses of nicotine reduce anxiety, medium doses of nicotine support reward-like behavior, and high doses of nicotine increase anxiety. The dual role of the β2 containing nicotinic acetylcholine receptors can be attributed to smoldering activation. The inactivation of the β2 containing receptors is a result of desensitization and causes the decreased anxiety. The activation causes the reward-like behavior, and both of these effects are present simultaneously in smokers (Anderson and Brunzell 2012). At physiological, sustained concentrations of nicotine seen in active smokers, the majority of α4β2 nAChRs are saturated, but the α7 nAChRs should not be affected (Campling et al. 2013). Because nAChRs are especially prone to being desensitized, it can be difficult to predict if an agonist is exerting its effect because of activation or desensitization. It may be such that, if the main effects of nicotine or other nAChR agonists are because of receptor desensitization and not activation, then the effects may rely on the binding affinity of the ligands, not efficacy.

Nicotine administration causes changes in nicotinic acetylcholine receptor expression. In contrast to how most neurotransmitter systems behave, chronic nicotine treatment causes a dose-dependent upregulation of α4β2 receptor density in the brains of mice and rats (Kassiou et al. 2001). The receptor upregulation is not permanent, and smokers who had abstained for 2 months had normal α4β2 nAChR density. The α4β2 upregulation is seen mostly in the thalamus and cerebellum (but see also Colombo et al. 2013).

The thalamus has the highest density of nicotinic acetylcholine receptors in the brain, and has been implicated in mediating many of the cognitive benefits of nicotine (Levin 2013). The nucleus accumbens has been implicated in impulsivity. Specifically, bilateral excitotoxic lesion of the nucleus accumbens core increased impulsive choice on a delay-discounting task (Dalley et al. 2008). The same effect was not seen by lesioning the nucleus accumbens shell. Interestingly,

the same study also showed that by lesioning both the nucleus accumbens core and nucleus accumbens shell, preference for a larger, delayed reward increased. This supports the finding that choice of the larger, costly reward in both the DDT and PDT was inversely related to α4β2 receptor binding in the nucleus accumbens shell (Mendez et al. 2013). Decreased α4β2 receptor binding in the nucleus accumbens shell should increase preference for the large reward in a delay-discounting task. With this evidence, it is possible that stimulation of the α4β2 nAChRs in the nucleus accumbens through nicotine administration mediates impulsivity. The anterior cingulate cortex and the orbitofrontal cortex have also been implicated in decision-making. Lesioning the anterior cingulate cortex decreased preference for a large reward/high effort over a small reward/low effort option, suggesting that this area is sensitive to effort, not discounting (St Onge and Floresco 2009). Lesions in the orbitofrontal cortex altered behavior in the DDT, suggesting that the orbitofrontal cortex plays a role in delay reinforcement (Dalley et al. 2008). Many studies have shown that several different brain regions are responsible for the varied facets of decision-making, but the thalamus, nucleus accumbens core, and orbitofrontal cortex are the areas most involved in delay- and probability-discounting.

Nicotine and Cognition

The cognitive benefits of nicotine have been well documented (Kumari et al., 2003; Levin et al., 2000; Lawrence et al., 2002). However, nicotinic acetylcholine receptor blockade or desensitization has also been reported to enhance cognition (Levin, 2013). The majority of the evidence for nicotinic acetylcholine receptor involvement in cognition comes from evidence that an agonist such as nicotine and receptor activation is responsible for cognitive improvements. Nicotine and nAChRs' role in cognition is important for understanding addiction and for understanding disease pathology where attentional impairments are seen.

The ascending cholinergic system originating in the basal forebrain and projecting to the cortex is very important in mediating sustained attention (Lawrence et al. 2002). Microdialysis studies can measure the amount of acetylcholine released during tasks that require sustained attention, and they have found that there is a large and sustained increase in acetylcholine efflux in animals actively performing an attentionally demanding task (Dalley et al. 2001). The increases in acetylcholine release were seen specifically in the medial prefrontal cortex and the orbitofrontal cortex, which implicates cortical acetylcholine in attentional processes. Other studies have also implicated release of acetylcholine in the frontal and parietal cortices (Sarter, Parikh, and Howe 2009). The amount of acetylcholine release measured on different tasks suggests that the levels of ACh release are indicative of the demands on attentional performance. Relatedly, α4β2 agonists have been shown to enhance cue detection by increasing the amplitude of signal-evoked cholinergic transients (Sarter, Parikh, and Howe 2009). The prefrontal cortex has also been implicated in cue-detection, so the ACh efflux in that area during attentional demands lends support to this theory. The ACh efflux in the prefrontal cortex is accompanied by prefrontal glutamate release that is mediated by thalamic glutamatergic afferents. When the α4β2 nAChRs located in the thalamus are activated they stimulate glutamate release in the prefrontal cortex, which has also been implicated in cue detection and attentional performance (Grupe et al. 2013). Nicotine administration also causes increased activity in the parietal cortex and attentional improvements (Lawrence et al. 2002). Nicotine-induced performance enhancements have been seen in a rapid visual information-processing task, which requires sustained attention and working memory, and also lends support to the cholinergic system's involvement in cue detection (Lawrence et al. 2002).

The top-down executive functions of attention are responsible for weighing rewards against each other (i.e. reward discounting) and suppression of preemptive responses, both of which are aspects of impulsivity (Sarter and Paolone 2011). Attentional processes are also involved in modulation of working memory. The cholinergic system has been implicated in attention, memory, and impulsivity. It appears that the cholinergic system controls attentional processes, which in turn affects impulsivity.

Multiple studies have shown various aspects of acetylcholine's involvement in memory. The interest here, as well as for acetylcholine's involvement in attention, is in treatment potential for disorders involving impulsivity. Attention relates impulsivity and working memory, and so what affects impulsivity will also have an effect on working memory, and vice versa. Infusions of high doses of α4β2 or α7 nAChR antagonists directly into the hippocampus caused working memory impairments, suggesting the hippocampal nAChRs are important for memory (Felix and Levin 1997). While most nAChRs are located presynaptically, there is evidence that some are located postsynaptically and when activated, depolarize neurons, increase their firing rate, and contribute to long-term potentiation (Colombo et al. 2013). Long-term potentiation is a crucial process for learning and memory. Similar to the effects of nicotine on attention, memory can also be improved by nicotine administration and is accompanied by increased activity in the frontal cortical regions of the brain (Kumari, Grey, and Ffyche 2003).

In support of cholinergic involvement in attention and memory, one of the hallmarks of Alzheimer's Disease is a significant reduction in cortical nicotinic cholinergic receptor binding relative to subjects of the same age (Newhouse et al. 2001). The memory impairments in animals that have received lesions to their cholinergic projections to the hippocampus to model Alzheimer's disease can be reversed by nicotine administration (Yamazaki, Hamaue, and

Sumikawa 2002). The cholinergic lesions disrupt the induction of long-term potentiation (LTP), a crucial working memory process. Nicotine administration can reverse these deficits by enhancing the NMDA receptor response on hippocampal neurons expressing nAChRs. This particular study demonstrates the role of nicotine and nAChRs in attention, but also points out that in the absence of endogenous acetylcholine release, the actions of nicotine were as a result of nAChR activation, not desensitization.

Decreased cholinergic system activity has also been implicated in attention-deficit/hyperactivity disorder (ADHD). In addition to attentional impairments, ADHD patients exhibit various aspects of impulsivity. ADHD patients typically lack behavioral inhibition, which is the ability to delay or refrain from responding due to environmental cues (Potter and Newhouse 2008). This effect may be the result of a combination of decreased cue detection and heightened impulsivity, however, it has been suggested that the deficits in sustained attention are secondary to the deficits in inhibition in the case of ADHD (Potter and Newhouse 2008). Even so, there is very high comorbidity between smokers and ADHD patients. Adolescents with ADHD are both more likely to become smokers and more likely to start smoking at a younger age than controls (Riggs et al. 1999). The severity of impulsivity symptoms are a better predictor of lifetime smoking behavior than the inattentive symptoms, but, considering nicotine's beneficial effect on attention, it is also possible that ADHD patients smoke to self-medicate and address the cognitive dysfunction not treated by most mainstream ADHD therapies (Potter and Newhouse 2008, Wilens and Decker 2007). Similar to patients affected by ADHD, it is thought that schizophrenic patients smoke to correct cognitive deficits as well. The negative symptoms and cognitive deficits of schizophrenia are the best predictor of functional outcome for the patient, and nicotine administration can help relieve some of those cognitive issues.

Interestingly, nicotine administration actually decreases impulsivity and increases behavioral inhibition in an animal model of schizophrenia (Scott and Taylor 2014).

However, nicotine's cognitive benefits only come after tolerance to nicotine's adverse physiological effects. Hahn and Stolerman showed that nicotine produced 8 weeks of stable enhanced attention after a week of poor performance. Initial administration of nicotine can induce nausea because the area postrema in the brain stem recognizes nicotine as a poison. The adverse side effects produced by nicotine are known generally as dysphoria, but can include, tension, anxiety, nausea, and nervousness. Nicotine's adverse side effects are evident in other studies, even if they are not explicitly stated. In a study done by Kirshenbaum et al. (2011), the behavioral effects of nicotine were only seen after repeated administration, not initial exposure. Initial administration of nicotine proved to be too disruptive and tolerance to the dysphoria was needed. These results have been seen in drug-naïve animals and non-smoking humans (Semenova et al. 2007, Foulds et al. 1997).

Nicotine and Impulsivity

As mentioned above, nicotine has been implicated in impulsivity in human and animal models. Nicotine has been shown to increase impulsivity in humans with both acute and routine systemic administration. The effects of systemic nicotine depend on where it binds in the brain and at what concentration. We have hypothesized that activation of the $\alpha4\beta2$ nAChRs in particular are responsible for mediating impulsive behavior in the probability-discounting task. Ohmura et al. (2012) showed that administration of an $\alpha4\beta2$ antagonist in the absence of nicotine suppressed impulsive choice on a 3-choice serial reaction time task, but an $\alpha7$ nAChR antagonist did not.

Most studies have shown that nicotine increases impulsive choice in a delay-discounting task. Kolokotroni et al. (2011) used a delayed reward task to look at impulsive choice and a symmetrically reinforced go/no-go task to look at behavioral inhibition. In this study, impulsivity was defined as being made up of impulsive choice behaviors and failure of inhibitory control, referred to as behavioral disinhibition. In this study, nicotine increased impulsive choice, defined as preference for the smaller, immediate reward, and dose-dependently increased behavioral disinhibition. Administration of a non-competitive, non-selective nicotinic acetylcholine receptor antagonist, mecamylamine, blocked both of these results when administered with nicotine. Nicotine administration in patients with ADHD or schizophrenia decreased behavioral inhibition, suggesting that abnormalities in this aspect of impulsivity are part of these diseases (Kolokotroni et al. 2011). Another study done by Kirshenbaum et al. (2011) corroborates the results in the Kolokotroni et al. (2011) study by finding that repeated, but not initial, administration of nicotine increased behavioral disinhibition in rats performing a conjunctive variable-interval differential-reinforcement-of-low-rate task and a stop-signal task. Mecamylamine also blocked the increased behavioral disinhibition when administered with nicotine in this task. Dallery and Locey (2005) used a delay-discounting task to show that acute and chronic nicotine injections both increased impulsive choice in rats with sustained, but reversible, effects. This experimental design is different from most delay-discounting tasks in that an indifference delay was determined for each rat and each test session started with the indifference delay and increased from there. An indifference delay is the delay at which the animal has no preference for either reward. They found that acute nicotine injections dose-dependently increased preference for the small, immediate reward and that chronic nicotine administration increased impulsive choice, regardless of dose. The increased impulsive choice

seen with chronic nicotine persisted for 30 test sessions after nicotine administration had stopped.

At odds with these results is a study done by Yonezaki, Fadel, and Burk (in preparation). They found that exposure, abstinence, and re-exposure to nicotine increased preference for the large, delayed reward in rats, a trend that is generally thought of as decreased impulsivity. There are a few ways to interpret this result. One is that the nicotine increased preference for the large, delayed reward by relieving responses to negative consequences. The nicotine made the rats less sensitive to the consequences of their choice. Another possible explanation for this result is that nicotine administration leads to perservative behavior. This theory is supported by a study done by Mendez et al. (2013) who looked at nicotine administration on ascending and descending probability-discount tasks. On the descending probability-discounting task, the rats that received nicotine were more likely to choose the large reward. The results from the ascending probability-discounting task, however, were the opposite. Nicotine exposure increased preference for the smaller, certain reward. On an ascending probability-discounting task, all the rats begin choosing the small reward because it is the better choice, so it is possible that nicotine is affecting the rat's ability to be flexible in changing task parameters. The perservative behavior refers to the fact that the rats keep choosing the same reward because that is the reward they started choosing at the beginning of the task.

Semenova et al. (2007) investigated the effects of low and high doses of nicotine in two different strains of rats with different baseline levels of cognition. In the baseline 5-choice serial reaction time task, Wistar rats performed better than Sprague Dawley rats. In the Wistar rats, the only significant effect of nicotine was increased impulsivity at the highest dose of nicotine (0.14mg/kg). The Sprague Dawley rats showed more attentional benefits at the highest dose of

nicotine with more correct responses, but no change in response latency was observed. These results suggest that in an organism with below baseline levels of attentional processing, nicotine can improve attention, but an organism with baseline attention only exhibits increased impulsivity.

The relationship between nicotine and impulsivity on a probability-discounting task is not as strong as the relationship between nicotine and impulsivity on a delay-discounting task. Ohmura et al. (2005) looked at nicotine intake in smokers and measured their performance on 4 discounting tasks (delay and probability discounting of monetary gains and losses). The only significant result was that higher doses of nicotine were associated with a greater tendency to discount delayed rewards. This also suggests that discounting rewards and losses are processed differently in the brain. In a different probability-discounting paradigm, Mitchell et al. (2011) conducted a study where there was a small, safe reward and a large, risky reward that was sometimes accompanied by a foot shock. As the test session progressed, the probability of the rat receiving the foot shock with the large reward went up. This study found that nicotine caused a significant decrease in the choice of the large, risky reward. However, this study did not rule out the possibility that this behavioral result was the product of any of the adverse effects of nicotine. The nicotine could have induced nausea, decreased appetite, or increased tension in the animal, and any of the effects could have contributed to their result. In the presented study, water was used as a reward (Yonezaki et al. in preparation).

We have hypothesized that nicotine administration will increase preference for the large, risky reward on the probability-discounting task. Based on the presented evidence, we also hypothesized that blocking the $\alpha 4\beta 2$ nicotinic acetylcholine receptors with DHβE before

nicotine administration will block the behavioral effects of nicotine on the probability-discounting task.

Materials and Methods

Subjects, Experiment 1

12 male FBNF1 rats were housed in pairs in a temperature- and humidity-controlled room with a 14/10 hour light/dark cycle. Food pellets were available to the subjects in their home cage throughout the experiment. Tap water was available to the subjects for 30 minutes after each test session in their home cages. The subjects were tested once a day, 7 days a week for the duration of the experiment. The experimental procedures were approved by the Institutional Animal Care and Use Committee at the College of William & Mary.

Apparatus

The rats were trained in one of four test chambers, and were tested in the same chamber every day. Each chamber was in a sound-attenuating box. One side of the chamber contained two ports equipped with photocells to detect nose pokes and a dipper with a cup that could be raised to allow water access. One dipper cup contained 0.01mL of water and the other contained 0.06 mL of water. The large reward was located in the right port of two chambers and in the left port of the other two chambers. A panel light was located above each water port and above the lever. A house light was located on the opposite side of the chamber and remained illuminated during all behavioral testing sessions. Behavioral testing programs were controlled by a computer using MED-PC IV software.

Behavioral training prior to drug administration

Rats were shaped to perform the probability-discounting task prior to nicotine exposure. The first stage of behavioral training was water port training, where all lights in the box were

illuminated and both rewards were available with a nose poke into the water port. Criterion for moving onto the next stage of training was entering the water port at least 80 times per test session. In the next stage of testing, appropriate port entries were guided by the panel lights above the ports. During this training stage, a trial was initiated with a lever press.

In the final task, the trial began with a lever extension. A press on the lever initiated a trial. There were three blocks of trials within each session, with 24 trials in each block. The first 12 trials in each block were forced trials in which only the left or the right panel light was illuminated and a nose poke in the port under that light led raising of the dipper 100% of the time in the small reward port, or, in the large reward port, the dipper was raised the proportionate amount of times for the probability that would be tested in that block. There were 6 forced trials for each port, but the order of the forced trials was random. After completing the 12 forced trials, the block consisted of 12 free choice trials, in which both panel lights were illuminated. If the rat did not nose poke in either port during the 10s in which the panel lights were illuminated, that trial was scored as an omission. After an omission, the panel lights turn off and a 60s intertrial interval (ITI) was initiated. A nose poke in the small reward port resulted in the small reward 100% of the time. In subsequent trial blocks, the probability of the rat receiving the large reward after a nose poke in the large reward port declined. In the first block, the large reward was available 100% of the time. In the second block, the large reward was available 33% of the time, and then it was available 17% of the time in the last block of trials. After either dipper was raised, a 60s ITI occurred.

Nicotine administration, Experiment 1

Nicotine bitartrate salt was dissolved in saline, with pH adjusted to 6.8-7.4. All doses were administered using intraperitoneal (IP) injections made at a volume of 1.0mL/kg. On days

1-4, rats received either nicotine (0.4mg/kg) or saline twice per day. In each squad of four animals, there were two nicotine-exposed rats and two saline-exposed rats, with one nicotine- and one saline-exposed animal in chambers with the small reward on the left side of the chamber in each squad. Across the three squads, at least one rat from each condition (nicotine or saline) was trained in each box. The first injection took place directly before placing the animals in the test chambers, and the second injection took place 3-4 hours after behavioral testing. On day 5, all rats received a challenge dose of nicotine (0.1mg/kg) before testing. On days 6 and 7, all rats received saline injections before testing. On day 8, all rats received a challenge nicotine dose (0.1mg/kg) directly before being placed in the testing chamber. On days 9-11, all rats received saline injections before behavioral testing. On day 12, all rats received their final challenge nicotine dose (0.4mg/kg) directly before being placed in the testing chamber.

Subjects, Experiment 2

12 adult male Sprague Dawley rats were housed in pairs in a temperature- and humidity-controlled room on a 14/10 hour light/dark cycle. Food was available ad libitum when the rats were in their home boxes. Water was used as the reward during testing and was available to the rats for 30 minutes immediately after testing. The subjects were tested once a day for the duration of the experiment. The experimental procedures were approved by the Institutional Animal Care and Use Committee at the College of William & Mary.

Drug administration, Experiment 2

The same behavioral training from experiment 1 was used in this experiment and nicotine (0.4 mg/kg) was also prepared in a similar manner to experiment 1. Dihydro-β-erythroidine (DHβE; Sigma), an α4β2 nAChR antagonist, was dissolved in saline and administered in volumes of 1.0 mL/kg. We used a DHβE concentration of 1 mg/kg (Davis and Gould 2006).

Rats received injections on Mondays, Wednesdays, and Fridays, with normal behavioral training occurring on days when there were no injections. Each rat received four injections (saline/vehicle, nicotine/vehicle, saline/DHβE, nicotine/DHβE) in a randomized order with at least two days between each injection. DHβE was injected 15 minutes prior to testing and the nicotine was injected 10 minutes prior to testing.

Behavioral measures and statistical analyses

The probability of selecting the large reward was determined (number of entries into large reward port/total number of port entries) for each block of free choice trials. Behavioral measures were analyzed with ANOVAs, with factors being drug, block, and (in Experiment 1) day. A level of $\alpha = 0.05$ was used for significance. For experiment 1, we also conducted a two-tailed t-test. One-way ANOVAs between blocks for each drug treatment were conducted on the data for experiment 2.

Results

Experiment 1

A drug (saline versus nicotine) X block X day ANOVA was conducted to test whether nicotine affected the probability of entering the large reward port. There were no significant effects or interactions involving day, so the data were averaged across the 12 days of the experiment and a drug X block ANOVA was conducted. We found significance in the between groups interactions. In this experiment, drug (saline versus nicotine) is a between subjects factor and block is a within subjects factor. The main effect of block ($F(2,20) = 82.95$, $p < .05$) shows that there was a significant difference in the probability of entering the large reward port between the two groups of rats (saline pre-exposed and nicotine pre-exposed) across blocks. The percentage by group interaction was also significant ($F(2,20) = 4.876$, $p < .05$). A follow-up

two-tailed t-test was conducted and showed decreasing p values for each block of the test session, approaching significance at block 3 (block 1: $p = 1.00$, block 2: $p = 0.253$, block 3: $p = 0.065$).

Experiment 2

To assess the effects of nicotine alone, we conducted an ANOVA involving drug (saline/vehicle versus nicotine/vehicle) and block as factors. The interaction between saline/vehicle and nicotine/vehicle reaches significance ($p = .019$), which corroborates our result from project 1 (see Figure 2). We then assessed the affects of DHβE alone in a drug (saline/vehicle versus saline/DHβE) X block AVOVA (see Figure 3). There was no significant interaction between these two groups, but the one-way ANOVA between blocks for saline/DHβE did not reach significance ($p = 0.092$). In comparison, the one-way ANOVA between blocks for saline/vehicle did reach significance ($p = 0.004$). To assess whether blocking the α4β2 nicotinic acetylcholine receptors impacted the effects of nicotine, we conducted a drug (nicotine/vehicle versus nicotine/DHβE) X block AVOVA. In these analyses, drug and block are both within subjects factors. It does not appear that the behavior seen in the probability-discounting task is mediated by the α4β2 nAChRs. Examination of group means indicated that nicotine exposure increased the likelihood of entering the large reward port compared to saline administration, as the probability of receiving the large reward decreased. DHβE alone did not affect performance, as a drug (saline/vehicle versus saline/DHβE) X block ANOVA did not yield any significant effects. Similarly, DHβE did not impact the effects of nicotine on probability discounting, as there were no significant effects involving drug in a drug (nicotine/vehicle versus nicotine/DHβE) X block ANOVA (see Figure 4). One-way ANOVA

analyses between blocks for each nicotine/vehicle and nicotine/DHβE drug conditions did not reach significance ($p = 0.807$ and $p = 0.866$, respectively).

Discussion

The differences in the probability of entering the large reward port seen between the nicotine and saline pre-exposed groups in experiment 1 can be interpreted as an increase in risky behavior, or an increase in that aspect of impulsivity. The rats that were pre-exposed to nicotine chose the larger reward more than the saline pre-exposed rats as the reward became less advantageous. Our findings from experiment 2 suggest that this behavior is not mediated by the α4β2 nicotinic acetylcholine receptors.

Looking at the graphical results from experiment 1 (see Figure 1), it is important to note that the saline and nicotine pre-exposed rats exhibited similar percentages of entering the large reward port in the first block when the large reward was available 100% of the time. Both groups of rats almost always entered the large reward port in block 1, suggesting that both groups of rats are capable of discriminating between the large reward and the small reward. Also, this result indicates that the nicotine administration did not affect motivation for the reward.

In Experiment 2, saline/vehicle-exposed rats demonstrated the expected decrease in preference for the larger reward as the probability of receiving that reward declined. Following nicotine/vehicle administration, on the other hand, rats did not significantly alter the probability of entering the large reward port even when the probability of receiving the reward declined. In block 1, nicotine/vehicle caused a lower percentage of large reward choice, possibly due to nicotine-induced dysphoria. There was no significant difference in the performance of the rats between saline/vehicle and saline/DHβE, which tells us that blockade of α4β2 nAChRs does not

affect performance on this task. Blocking the α4β2 nAChRs in the presence of nicotine did not alter the effects of nicotine on percentage of choosing the large reward. However, this result could be a product of the timing of DHβE administration. We decided to administer the DHβE 5 minutes before nicotine, and there has been no consensus on when to administer DHβE. DHβE has been administered 25 minutes before nicotine and 30 minutes before behavioral testing or it has been administered in conjunction with nicotine (Davis and Gould 2006, Reuben and Clarke 2000).

It is encouraging to see that even one dose of nicotine in experiment 2 was enough to create a trend similar to what we saw with pre-exposure to nicotine in experiment 1. In both experiments, as the probability of receiving the large reward declined, the rats who were exposed to saline chose the large reward significantly less with each subsequent block. The rats who were exposed to nicotine did not exhibit this exact behavior in either experiment. Looking at results from both experiments, nicotine appears to stabilize choice of the large reward, even as the probability of receiving the reward declines. The behavioral differences seen in block 1 of experiment 1 and experiment 2 can be explained by the dysphoria caused by initial nicotine administration (Semenova et al. 2007, Foulds et al. 1997). The animals in experiment 1 had previous exposure to nicotine, so when they were initially put into the test boxes, they may not have been as susceptible to the dysphoria nicotine can cause upon initial administration. The animals in experiment 2 required more time before they were able to perform at peak levels during each behavioral test session because they had no tolerance to nicotine.

It is still unclear whether the effect seen in both experiment 1 and experiment 2 is due to a nicotine-induced increase in risky behavior or due to a nicotine-induced increase in perservative behavior. Mendez et al. (2013) showed that nicotine increased perservative

behavior in descending and ascending probability tasks. Based on the Ohmura et al. (2005) results, the relationship between nicotine and probability discounting is not nearly as strong as the relationship between nicotine and delay discounting. In experiment 2, the lack of relationship between the α4β2 nicotinic acetylcholine receptors and behavior on the probability-discounting task suggests that risky behavior might be mediated by another receptor subtype. It is possible that this behavior could be mediated by the α7 nicotinic acetylcholine receptors, which are the second most widespread nicotinic acetylcholine receptor subtype in the brain (Nashmi and Lester 2006). The α7 nAChRs have a much lower affinity for nicotine than the α4β2 receptors, which could explain why the relationship between nicotine and risky behavior on the probability-discounting task is not as robust as the relationship between nicotine and delay discounting. Very little research looking specifically at perservative behavior or lack of behavioral adaptability following nicotine exposure has been reported, so that would be an interesting future direction.

Considering the high incidence of smoking with other impulsive and compulsive conditions such as drug addiction, it is important to investigate the relationship between nicotine and impulsivity (Weafer and de Wit 2013). It might be that those who start smoking have a higher baseline of risky behavior to begin with, but our results from experiment 1 show that nicotine administration increases the probability of choosing the riskier reward in rats that have been pre-exposed to nicotine. Impulsivity is a component of the proposed endophenotype for substance use disorders, so greater impulsivity could be a risk factor for substance use disorders, as well as a possible consequence (Robbins et al. 2012). If the heightened impulsivity is the result of differential expression or activity of nicotinic acetylcholine receptors, then related genes could be investigated to better understand the genetic underpinnings of substance use disorders.

It is also important to investigate the relationship of impulsivity, attention, and nicotinic acetylcholine receptors in diseases such as Alzheimer's and schizophrenia, however the experiments presented here were conducted on wild-type rats, and not animal models of these diseases.

Figure Legends

Figure 1

The graph is a visual representation of the significant between-groups interaction. The two groups exhibit the same behavior, namely a decrease in how often they choose the large, riskier reward, but the saline pre-exposed group exhibited that behavior to a larger degree. The significance is in the difference in the behavioral changes between the groups. It is also important to note that both groups behaved exactly the same during the first block of 100% probability. The nicotine injections did not affect motor activity or desire for water. All changes were as a result of the changes in probability, not adverse effects to nicotine.

Figure 2

This figure shows the effects of nicotine administration compared to saline. The interaction between the two groups is significant. The behavior in block 1 can be explained by the dysphoria caused by initial nicotine exposure. The one-way ANOVA for saline/vehicle demonstrates that the behavior for that drug condition was significantly different from block to block during the test session. The one-way ANOVA for nicotine/vehicle was not significant, and it is clear that the behavior was not significantly different from block to block during the test session.

Figure 3

This figure shows that DHβE administration alone did not significantly alter the behavior of the rats. The interaction between the saline/vehicle condition and the saline/DHβE condition was not significant. While the one-way ANOVA for saline/vehicle was significant, the one-way ANOVA for saline/DHβE was not, as indicated by the two lines not being a perfect overlap. The difference in block 3 was not statistically significant.

Figure 4

As seen in this graph, administering DHβE and nicotine did not impact the behavioral effects of nicotine. The two drug conditions exhibit almost exactly the same behavior. There is no statistical significance in the interaction between the nicotine/vehicle and nicotine/DHβE groups, and neither one-way ANOVA showed significance. DHβE administration did not inhibit the effects of nicotine, so the behavior seen is not medicated by the α4β2 nicotinic acetylcholine receptors.

Figure 1

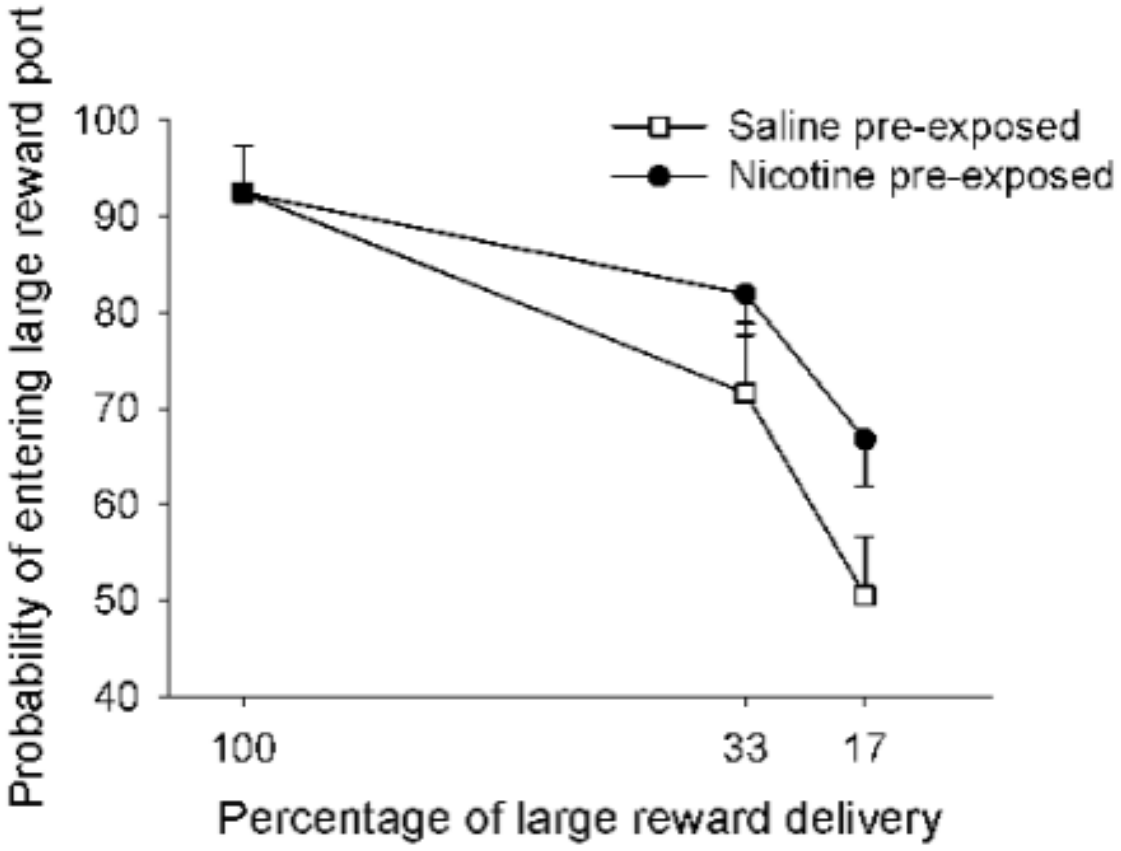

Probability of entering large reward port
100
90
80
70
60
50
40
Saline pre-exposed
Nicotine pre-exposed
100
33
17
Percentage of large reward delivery

Figure 2

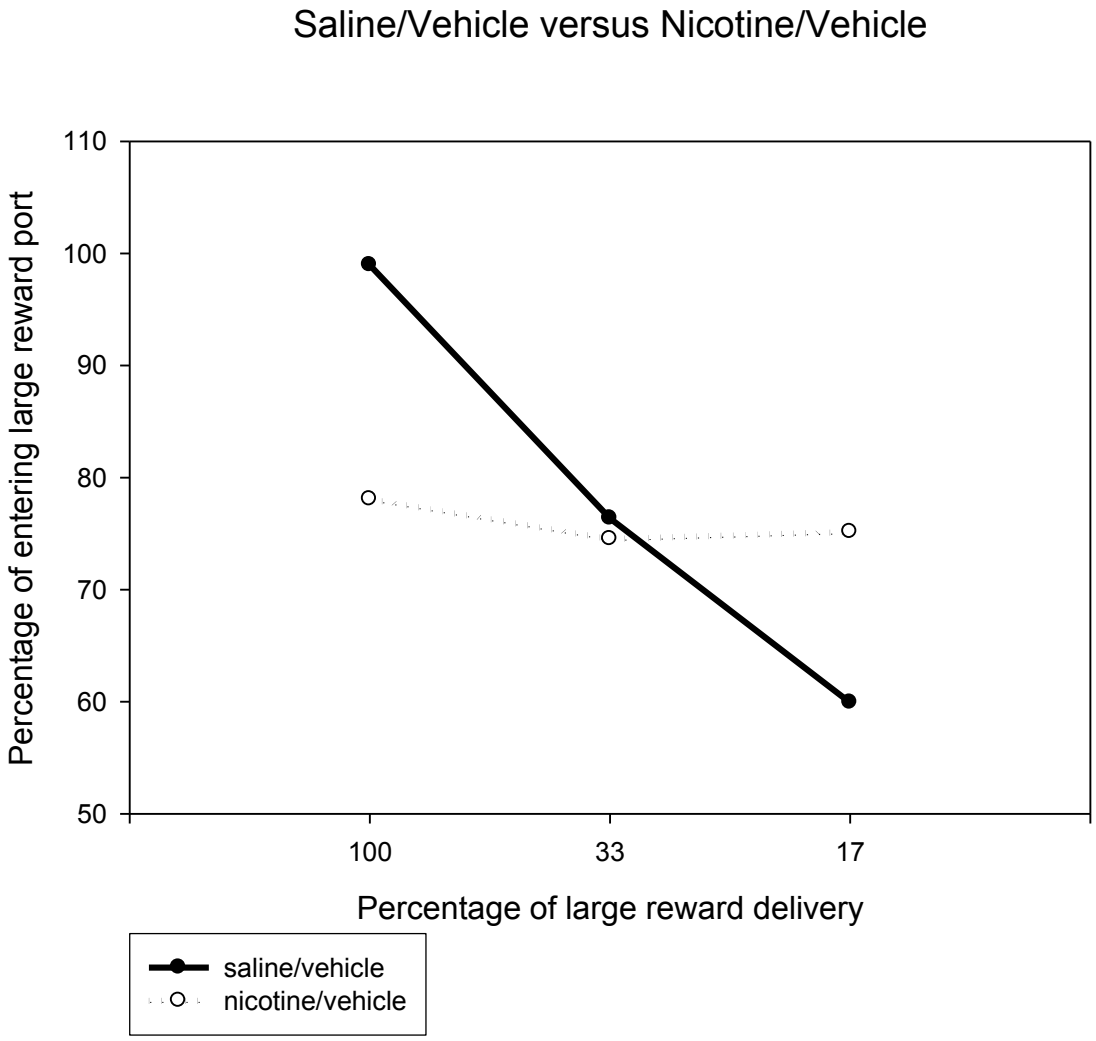

Saline/Vehicle versus Nicotine/Vehicle
Percentage of entering large reward port
110
100
90
80
70
60
50
100
33
17
Percentage of large reward delivery
saline/vehicle
nicotine/vehicle

Figure 3

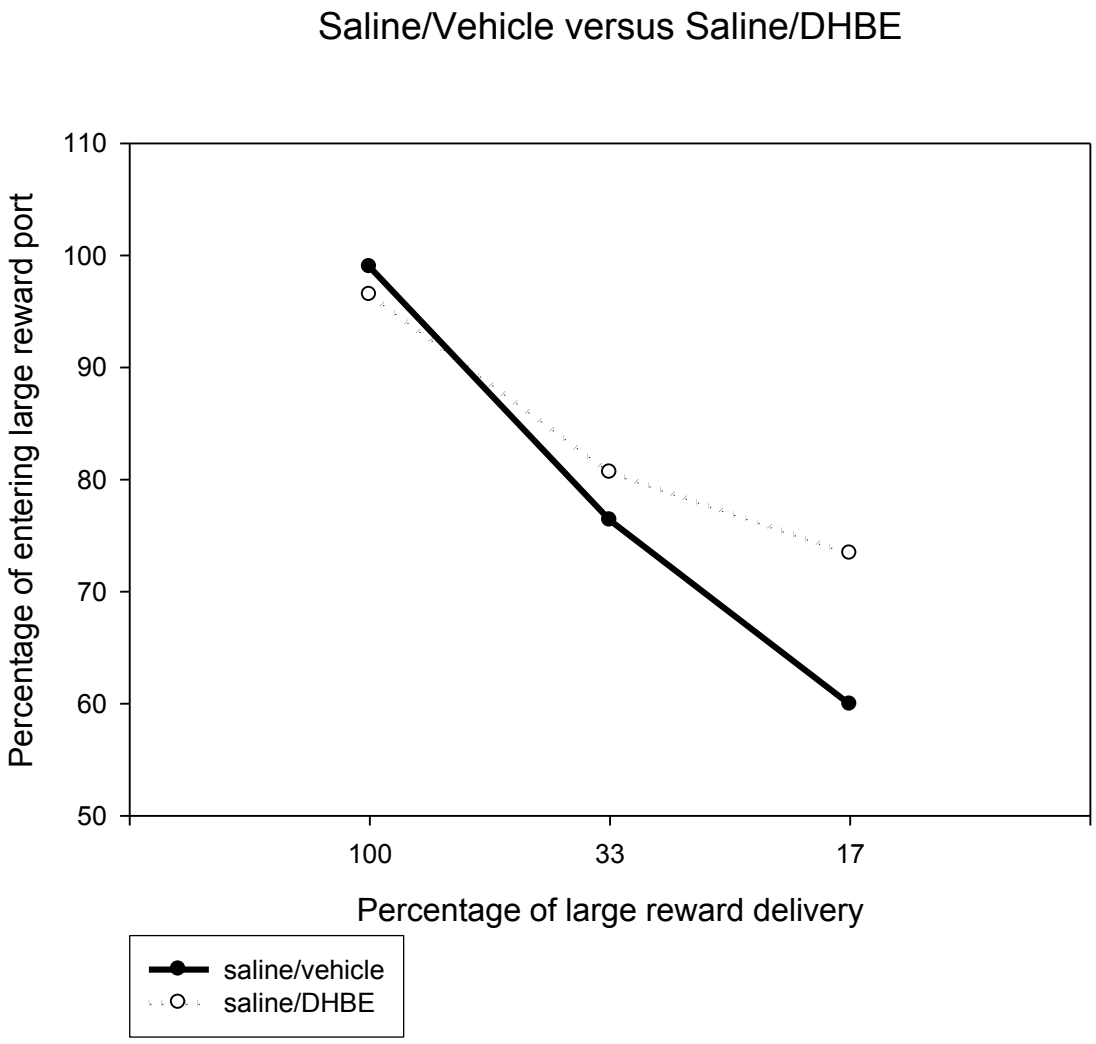
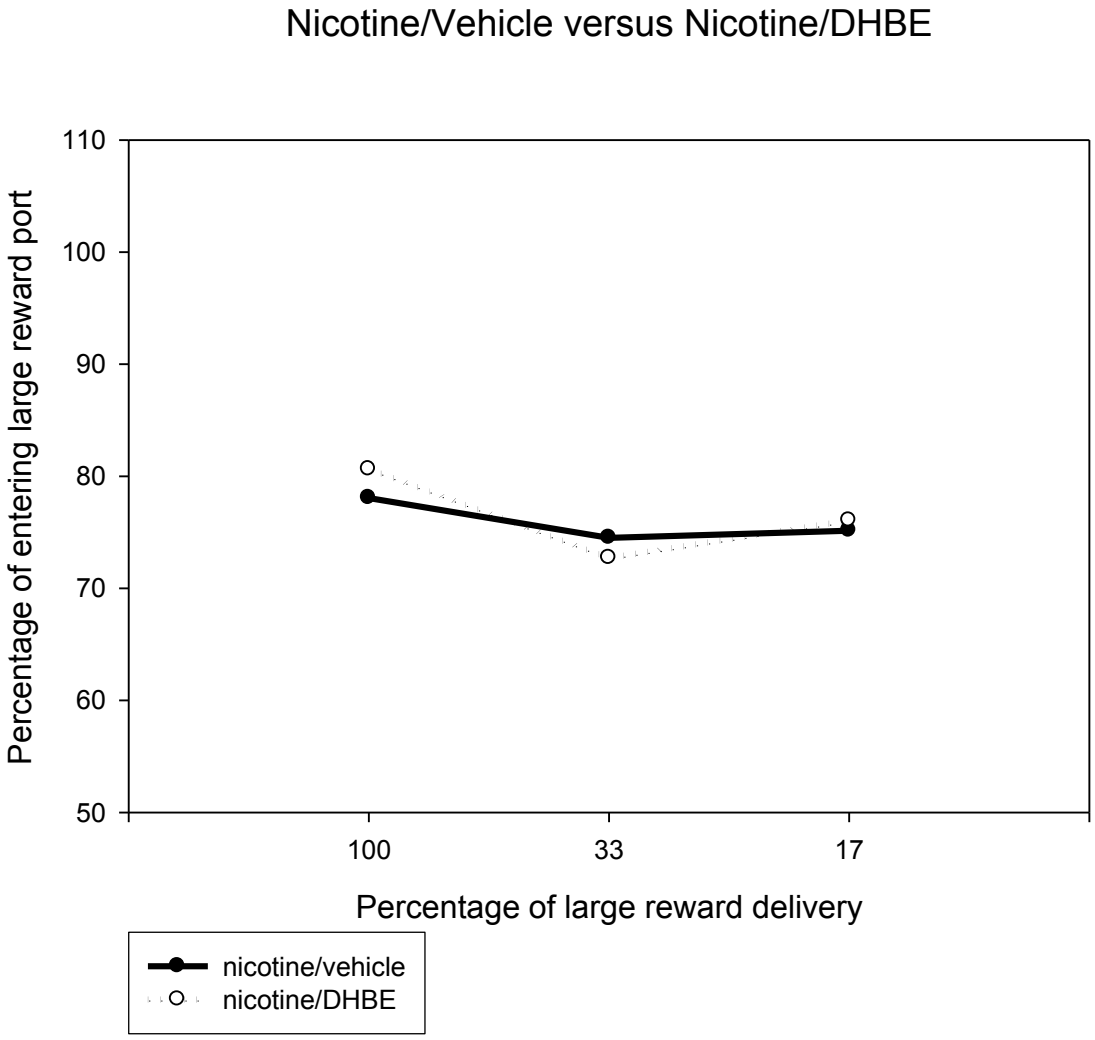

Saline/Vehicle versus Saline/DHBE
Percentage of entering large reward port
110
100
90
80
70
60
50
100
33
17
Percentage of large reward delivery
saline/vehicle
saline/DHBE

Figure 4


Nicotine/Vehicle versus Nicotine/DHBE
Percentage of entering large reward port
110
100
90
80
70
60
50
100
33
17
Percentage of large reward delivery
nicotine/vehicle
nicotine/DHBE

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