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Inhibitory Learning is Modulated by Nicotinic Acetylcholine Receptors

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Abstract

Prior research has established that stimulating nicotinic acetylcholine receptors can facilitate learning and memory. However, most studies have focused on learning to emit a particular behavior, while little is known about the effects of nicotine on learning to withhold a behavioral response. The present study consisted of a dose response analysis of the effects of nicotine on negative occasion setting, a form of learned inhibition. In this paradigm, rats received one type of training trial in which presentation of a tone by itself was followed immediately by food reward. During the other type of trials, the tone was preceded by presentation of a light and no food was delivered after the tone. Rats gradually learned to approach the cup in anticipation of receiving food reward during presentations of the tone alone, but withheld that behavior when the tone was preceded by the light. Nicotine (0.35mg/kg) facilitated negative occasion setting by reducing the number of sessions needed to learn the discrimination between trial types and by reducing the rate of responding on non-reinforced trials. Nicotine also increased the orienting response to the light, suggesting that nicotine may have affected the ability to withhold food cup behavior on non-reinforced trials by increasing attention to the light. In contrast to the effects of nicotine, rats treated with mecamylamine (0.125, 0.5, or 2 mg/kg) needed more training sessions to discriminate between reinforced and non-reinforced trials compared to saline-treated rats. The findings indicate that nicotinic acetylcholine receptors may be active during negative occasion setting and that nicotine can potentiate learned inhibition.

Keywords

inhibition; mecamylamine; attention; nicotine

1. Introduction

A substantial body of research has examined how nicotine, and more generally, stimulation of nicotinic acetylcholine receptors (nAChRs), influences learning and memory (Kenney & Gould, 2008; Levin, 2002). The vast majority of prior research has focused on the

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involvement of nAChRs in learning to emit a certain behavioral response (Felix & Levin, 1997; Ohno et al., 1993), while relatively few experiments have investigated the influence of nAChRs on learning to *omit* a behavior. Yet, a growing number of studies have demonstrated that nicotine can improve the ability to withhold a response (Blondel et al., 2000) and alleviate deficits in inhibitory behavior and impulsivity associated with disorders such as Attention-Deficit/Hyperactivity Disorder (ADHD) and schizophrenia (Migo et al., 2006; Potter & Newhouse, 2004, 2008; Potter et al., 2012). Nevertheless, the behavioral and neurobiological mechanisms that mediate the effects of nicotine on inhibition remain unclear. Moreover, the few studies that have considered the effects of nicotine on inhibition have focused on how it modulates the performance of previously learned tasks (i.e., the expression, or performance of inhibition). Even fewer studies have investigated the effects of nicotine on *learning* to inhibit behavior.

We recently used a negative occasion setting paradigm to test the effects of nicotine on the ability of rats to learn to withhold a behavior based on the presence of a cue in the environment (e.g., a 'stop' signal), an essential aspect of adaptive behavior. Negative occasion setting typically involves a serial feature negative discrimination in which rats are trained to distinguish between two different trial types. During reinforced trials, a target stimulus (e.g., a tone) is presented and immediately followed by food reward. On non-reinforced trials, a feature stimulus (e.g., a light) is presented prior to the tone and indicates the absence of reward following presentation of the tone. Rats learn to approach the food cup during presentation of the tone on reinforced trials but not when the tone is preceded by the light. In other words, rats learn to inhibit responding when the feature precedes the target (Bueno & Holland, 2008; Bouton & Nelson, 1994; Holland, 1984; Holland & Morell, 1996). The feature is thought to modulate the association between the target and the food, resulting in a learned inhibitory response that relies on encoding the meaning of the feature to correctly discriminate between trial types (Holland, 1984). One view of negative occasion setting maintains that on reinforced trials, an excitatory relationship is formed between the tone and food, but on non-reinforced trials, an inhibitory association between the tone and food is gated by the feature stimulus (Bouton & Nelson 1994, 1998; Bouton, 1997; but see Polack et al., 2011).

We previously found that administration of 0.35mg/kg of nicotine enhanced negative occasion setting by facilitating discrimination between trial types and reducing responding during presentation of the tone on non-reinforced trials (MacLeod et al., 2006, 2010). The present study expanded on these prior findings in two important ways: One goal was to determine the effective dose range for nicotine (Experiment 1); the second goal was to determine if the enhancing effects of nicotine on negative occasion setting are due to increased stimulation of nAChRs that are already active during the task. Thus, in Experiment 2, we tested whether stimulation of nAChRs was necessary to learn the feature negative discrimination by treating rats with mecamylamine, a broad spectrum nAChR antagonist. The resulting data provide insight into how nicotine impacts inhibitory behavior, which has implications for understanding and treating several common forms of mental illness that co-occur with substance abuse.

2. Materials and Methods

2.1 Subjects

Male Long Evans rats (9 per group in Experiment 1; 12 per group in Experiment 2) were obtained from Harlan Laboratories (Indianapolis, IN) at 7–8 weeks of age. Rats were housed individually and allowed at least seven days to acclimate to the colony room prior to beginning food restriction and behavioral testing. Water and food (2014 Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Laboratories) were available ad libitum during this acclimation period. During the week prior to behavioral training, rats were handled daily and body weight was gradually reduced to 85% of free-feeding weight. Food restriction was maintained for all rats until completion of behavioral training, with supplemental rat chow given to each rat after the daily session to maintain the target weight. The colony room was maintained on a 14:10 light:dark cycle and monitored and cared for in compliance with the Association for Assessment and Accreditation of Laboratory Animal Care guidelines and the Dartmouth College Institutional Animal Care and Use Committee.

2.2 Drug Preparation and Treatment

In Experiment 1, (–) nicotine hydrogen tartrate (Sigma Aldrich Co., St. Louis, MO, USA) was prepared fresh daily by dissolving in sterile 1X phosphate buffered saline (1X PBS; vehicle) and adjusted to pH 7.0 with 2 N NaOH. Nicotine (or vehicle) was administered subcutaneously at a volume of 2.0 ml/kg, resulting in the equivalent of 0.2, 0.35, or 0.5 mg/kg free-base nicotine. These doses of nicotine were chosen based on our prior work (MacLeod et al., 2006, 2010) and other previous studies that have examined the effects of nicotine on associative learning (e.g., Olausson et al., 2004; Rochford et al., 1996). In Experiment 2, mecamylamine hydrochloride was likewise dissolved in 1X PBS and administered subcutaneously at a volume of 2.0 ml/kg, resulting in doses of 0.125, 0.5, or 2.0 mg/kg. Mecamylamine is a broad-spectrum non-competitive nicotinic acetylcholine receptor antagonist, acting on all the major subtypes of neuronal nAChRs while having little effect on non-nAChRs (Papke et al., 2001). Rats were weighed before each conditioning session and placed in a plastic transporter used to transport subjects to and from the colony room. Nicotine, mecamylamine, or vehicle was administered 10 min before each conditioning session. Following the injection, rats remained in the transporter before being placed in the behavioral chambers.

2.3 Behavioral Apparatus

Behavioral procedures were carried out in standard conditioning chambers (Med Associates, St. Albans, VT). The chambers (24 × 30.5 × 29 cm) consisted of aluminum front and back walls, clear acrylic sides and top, and grid floors. Each chamber was outfitted with a dimly illuminated food cup, recessed in the center of the front wall, a 2.8-W white panel light (which served as the visual stimulus) located 5 cm above the opening to the food cup, and a speaker located 15 cm above and to the right of the food cup, used to present the 1,500 Hz, 78 dB auditory stimulus. Delivery of two 45-mg food pellets (Bioserv, Frenchtown, NJ) served as the unconditioned stimulus. Each chamber was equipped with a pair of infrared photocells located across the entrance to the food cup to monitor entries into the cup and connected to a PC-clone computer. Three additional pairs of photobeam sensors were

mounted in the chamber and used to detect rearing behavior. The sensors were placed 15 cm above the grid floor (i.e., just above the height of the panel light) and were evenly spaced along the wall so that a rearing response produced anywhere in the chamber would be detected by one of the sensors. Each chamber was enclosed in a sound-attenuating cubicle ($62 \times 56 \times 56$ cm) with an exhaust fan to provide airflow and background noise (~ 68 dB) and a red house-light (mounted on the ceiling) to provide background illumination. The cubicles also contained surveillance cameras used to monitor the rats during behavioral training.

2.4 Behavioral Procedure

Each day, rats were placed in plastic transporters and moved from the colony room to the conditioning chambers. One day prior to behavioral training rats were trained to eat from the food cup during a single 64-min session in which two food pellets were randomly delivered 16 times (average intertrial interval (ITI) of 4 min; ranging from 2.5 to 5.5 min). Behavioral training was carried out as described by Holland et al. (1999) and consisted of daily 68-minute sessions with 4 reinforced and 12 non-reinforced trials with an average ITI of 4 min, ranging from 2.5 to 5.5 min (the 1:3 ratio of reinforced to non-reinforced trials was used because it results in faster acquisition compared to a 1:1 ratio). During reinforced trials the tone was presented for 5-sec and followed immediately by the delivery of two food pellets. On non-reinforced trials, the panel light was presented for 5-sec light, followed by a 5-sec empty period, and then a 5-sec presentation of the tone, after which no food was delivered. The two trial types occurred randomly during each session and the presentation order was varied daily.

2.5 Analysis of food cup behavior

In prior studies we found that stimulating nAChRs resulted in more rapid acquisition of the serial feature negative discrimination (MacLeod et al., 2006, 2010). Thus, the primary variable of interest was the number of sessions that were required until rats exhibited successful discrimination between reinforced and non-reinforced presentations of the tone. To assess this, the amount of time that the photobeam in front of the food cup was broken during presentation of the tone was recorded during each trial. The amount of time spent in the food cup was averaged across rats in each group for each trial type. The difference in responding between trial types was obtained by subtracting the time spent in the food cup during the tone on non-reinforced trials from time spent in the food cup during the tone on reinforced trials. Z-scores were calculated by dividing that result by the standard error of the mean (SEM) of the difference in responding across all rats in a group. Successful discrimination between the two trial types was defined as a greater amount of time spent in the food cup during presentation of the tone on reinforced trials than during non-reinforced trials (a Z-score of at least 2.325; $p < 0.01$) as described previously (Meyer & Bucci, 2014).

In addition, group differences in conditioned responding were assessed by conducting logarithmic curve estimations across training sessions for each subject since prior studies have shown that nicotine specifically altered slope of the learning curve on non-reinforced trials (MacLeod et al., 2006, 2010). Thus, learning rates were estimated for each subject and each trial type using linear regression to fit the data over the logarithm of trials. The logarithm was used because it provided the best fit to the individual data over trials. The

regression equation was: $\text{responding} = B_0 + B_1 \cdot \log(\text{trial})$, where the beta coefficient B_1 served as the estimate of learning rate (MacLeod et al., 2006, 2010; MacLeod & Bucci, 2010). The beta coefficients were analyzed with an analysis of variance (ANOVA) using Group as the between subjects variable and Trial type (reinforced, non-reinforced) as the within-subjects variable (alpha level of 0.05). Orthogonal contrasts were used to decompose any significant main effects and interactions.

Finally, to test for potential drug-induced changes in baseline food cup behavior, the amount of time spent with the head in the food cup during the 5-sec period prior to the onset of the conditioned stimulus (CS) was recorded. Group differences in pre-CS behavior were assessed using an independent measures ANOVA (alpha level of 0.05).

2.6 Analysis of rearing behavior

Orienting behavior (rearing on the hind legs with both forepaws off the ground; Holland, 1977) was used as an indicator of attention directed to the feature stimulus (i.e., the light; Gallagher et al., 1990; Kaye & Pearce, 1984; Lang et al., 1997). During presentation of the light on each non-reinforced trial, breaks in the three pairs of photobeams mounted on the walls of the chamber were monitored by the computer. The amount of time that the beams were broken was summed for each trial because previous studies indicate that it is unlikely that a rearing response will simultaneously break more than one photobeam (Keene & Bucci, 2007). A one-way ANOVA and Tukey's HSD test were used to assess group differences in the mean amount of time spent rearing during presentation of the light. An alpha level of 0.05 was used for the analysis.

3. Results

3.1 Experiment 1

The amount of time spent in the food cup during presentation of the tone on reinforced and non-reinforced trials in saline-treated and nicotine-treated rats is shown in Figure 1. Saline-treated rats required 10 training sessions until they began discriminating between reinforced and non-reinforced trials ($\text{SEM} = 3.02, p < 0.005$). In comparison, rats treated with either 0.2 or 0.35 mg/kg of nicotine discriminated between the trial types sooner (8 sessions, $\text{SEM} = 2.89, p < 0.005$ for the 0.2mg/kg group; 7 sessions, $\text{SEM} = 3.58, p < 0.001$ for the 0.35mg/kg group). Rats treated with the highest dose of nicotine (0.5 mg/kg) discriminated between trial types on the 10th session ($\text{SEM} = 3.34, p < 0.001$) like the saline-treated control group.

Beta coefficients were calculated to assess group differences in the learning rates on reinforced and non-reinforced trials (Table 1). Analysis of the beta coefficients revealed a main effect of Trial type [$F(1, 32) = 188.5, p < 0.001$], a marginally-significant main effect of Group ($p = 0.06$) and a significant Group X Trial type interaction [$F(3, 32) = 4.2, p < 0.02$]. Decomposing the interaction revealed that the mean beta coefficient for rats in the 0.35 mg/kg nicotine group was significantly different from that of other groups on the non-reinforced trials as shown in Figure 2 (p 's < 0.04). In other words, the slope of the learning curve on non-reinforced trials was significantly more negative for rats in the 0.35 mg/kg nicotine group, as illustrated in Figure 2. No other pairwise comparisons of beta coefficients

for the non-reinforced trials reached statistical significance. In addition, there were no group differences in the beta values for the reinforced trials ($p > 0.3$).

To test for drug-induced changes in baseline food cup behavior, we analyzed the amount of time spent in the food cup just prior to the onset of any CSs (i.e., pre-CS behavior). Food cup behavior was uniformly low in all groups (saline-treated group, 0.62 ± 0.12 sec; 0.2 mg/kg nicotine, 0.43 ± 0.08 sec; 0.35 mg/kg nicotine, 0.66 ± 0.08 sec, 0.5 mg/kg nicotine, 0.38 ± 0.08 sec). There were no group differences in pre-CS behavior ($p > 0.1$).

To address whether the discrimination was influenced by attention to the inhibitory cue, the time spent rearing during presentation of the light on non-reinforced trials was measured in all groups. As shown in Figure 3, rats treated with 0.35 mg/kg of nicotine reared during presentation of the light more than the rats in the other groups. This was confirmed by an ANOVA that revealed a significant effect of Group [$F(3, 32) = 15.5$, $p < 0.001$]. Post hoc analyses indicated that the 0.35 mg/kg group differed from all other groups (p 's < 0.001) but that none of the other pair-wise groups differences were significant (p 's > 0.7).

3.2 Experiment 2

The amount of time spent in the food cup during presentation of the tone on reinforced and non-reinforced trials in saline-treated and mecamylamine-treated rats is shown in Figure 4. Saline-treated rats required 8 training sessions until they began discriminating between reinforced and non-reinforced trials ($SEM = 2.45$, $p < 0.01$). In contrast, rats treated with mecamylamine required more sessions until they began discrimination between the trial types. Specifically, rats treated with either 0.125 or 0.5 mg/kg of mecamylamine did not discriminate between the trial types until the 10th session ($SEMs = 4.01$ and 2.47 , $p < 0.001$ and $p < 0.01$, respectively). Rats treated with the highest dose of mecamylamine (2 mg/kg) did not discriminate between the trials until the 12th session ($SEM = 3.77$, $p < 0.001$). Analysis of the beta coefficients (Table 2) revealed a main effect of Trial type [$F(1,44) = 50.2$, $p < 0.001$] but no significant main effect of Group ($p > 0.8$) and no significant Group X Trial type interaction ($p > 0.7$), indicating that mecamylamine did not alter the slopes of the learning curves for reinforced or non-reinforced trials, as illustrated in Figure 5.

Food cup behavior during the pre-CS period was low in all groups (saline-treated group, 0.94 ± 0.16 sec; 0.125 mg/kg mecamylamine, 0.85 ± 0.20 sec; 0.5 mg/kg mecamylamine, 0.88 ± 0.17 sec; 2 mg/kg mecamylamine, 1.2 ± 0.24 sec). There were no group differences in pre-CS responding ($p > 0.7$).

To determine if the delay in discrimination caused by mecamylamine was influenced by attention to the inhibitory cue, the time spent rearing during presentation of the light on non-reinforced trials was measured in all groups. As shown in Figure 6, rearing was comparable in all groups, as confirmed by an ANOVA that failed to reveal an effect of Group [$F(3,44)=0.9$, $p > 0.4$].

4. Discussion

One goal of the present study was to determine the effective dose range of nicotine on negative occasion setting. In Experiment 1, we found that nicotine treatment produced a dose-dependent reduction in the number of daily training sessions that were needed to successfully discriminate between reinforced and non-reinforced presentations of the tone. Indeed, saline-treated rats required 10 daily training sessions to successfully discriminate between reinforced and non-reinforced presentations of the tone during the negative occasion setting task. By comparison, rats treated with 0.35 mg/kg of nicotine (free base equivalent) required only 7 sessions to learn the discrimination and rats treated with 0.2 mg/kg of nicotine successfully discriminated in 8 sessions. Rats that received the highest dose of nicotine, 0.5 mg/kg, learned the discrimination in the same number of sessions as saline-treated rats (10 sessions). An enhancing effect of nicotine on negative occasion setting was further demonstrated by the finding that 0.35 mg/kg specifically reduced responding during presentation of the tone on non-reinforced trials, as evidenced by a significantly more negative slope of the learning curve. These data replicate our prior findings (MacLeod et al., 2006, 2010) and delineate the effective doses at which nicotine modulates negative occasion setting.

In addition, nicotine enhanced the amount of orienting behavior directed to the feature stimulus, i.e., the light. Indeed, consistent with our prior study (MacLeod et al., 2010), rats treated with 0.35 mg/kg spent more time rearing on the hind legs during presentation of the light on non-reinforced trials compared to saline-treated rats. Orienting behavior is often interpreted as an attentional response (Pavlov, 1927; Sokolov, 1963; Gallagher et al., 1990; Kaye & Pearce, 1984; Lang et al., 1997) and thus nicotine may have enhanced inhibition of responding to the tone on non-reinforced trials by increasing attention to the feature stimulus. Specifically, it has been suggested that the negative feature may “gate” the inhibitory properties of the target stimulus (the tone), thus allowing the tone to obtain both excitatory and inhibitory properties (Bouton & Nelson, 1994; Holland, 1984; but see Polack et al., 2011). Presentation of the light may thus activate the inhibitory association of the tone with food, resulting in less food cup behavior during the tone on trials in which the light precedes the tone. An increase in attention to the light may enhance the gating effect of the feature such that it is more likely to control responding to the target. Consistent with this attentional interpretation of the effect of nicotine on negative occasion setting, it has previously been shown that nicotine enhances performance in the five-choice serial reaction time task by improving the ability to attend to and detect visual stimuli (Blondel et al., 2000; Young et al., 2013). Similarly, nAChR stimulation improves sustained attention (Stolerman et al., 2000; McGaughy et al., 1998; Tsutsui-Kumura et al., 2010). This notion is also consistent with previous findings that nAChRs play a key role in sensory gating, a commonly studied model of sensory inhibition (Luntz-Leybman et al., 1992). Nicotine enhanced pre-pulse inhibition (Semenova et al., 2003, 2004; Acri et al., 1994) and has also been shown to enhance latent inhibition, indicating that nicotinic receptors can modulate the ability to decrease attention to uninformative stimuli (Rochford et al., 1996).

Although it was beyond the scope of the present study to test which specific brain regions and circuits were affected by nicotine, a wealth of prior research allows for some useful

speculation. For example, Davis et al. (2007) have demonstrated that the enhancing effects of nicotine on contextual fear memory are mediated by the hippocampus. Moreover, studies that have tested the role of nAChRs in hippocampal-dependent forms of inhibition, such as pre-pulse inhibition and sensory gating, have demonstrated that antagonism of nAChRs impairs performance (Erhardt et al., 2004; Shepard et al., 2003). Consistent with this, negative occasion setting is also impaired by lesions of the hippocampus (Holland et al., 1999). Given the high density of nAChRs in hippocampus (Tribollet et al., 2004), it seems likely that the nicotine may affect negative occasion setting by acting on hippocampal nAChRs. However, future studies are needed to directly test this.

A second goal of the current study was to determine if nAChRs are normally active during negative occasion setting. In Experiment 2, treatment with mecamylamine had the opposite effect as nicotine in that it increased the number of sessions that were needed before rats learned the discrimination. One interpretation of these data is that nAChRs are normally active during this form of inhibitory learning and are required for negative occasion setting. This is consistent with studies in humans demonstrating that mecamylamine impaired performance in a stop-signal reaction time task that is procedurally very similar to the negative occasion setting paradigm used here (Potter et al., 2012). However, it remains to be determined whether nAChRs are required for inhibition per se, or if the results of Experiment 2 reflect a general impairment in learning (e.g., a working memory deficit).

The present study utilized the negative occasion setting paradigm specifically because it shares procedural similarities with the stop-signal task that has been used to study the effects of nicotine on behavioral inhibition in humans (Potter & Newhouse, 2004, 2008; Potter et al., 2012). Nicotine affects stop-signal reaction time specifically by reducing the amount of time needed to inhibit behavior when signaled by a 'stop' stimulus (Potter & Newhouse, 2004, 2008; Potter et al., 2012). Similarly, nicotine enhanced negative occasion setting here by reducing responding to the tone when it had been preceded by the light. However, further studies could use a variant of this procedure to more specifically examine the contribution of nAChRs to inhibition. For example, a specific effect of nAChR blockade on inhibition could be tested by assessing the effects of nicotinic receptor compounds in a procedure that is otherwise identical to the one used here, except that the light and the tone are presented simultaneously and instead of serially on the non-reinforced trials. This procedure is commonly referred to as a 'conditioned inhibition' paradigm and also involves learning to inhibit responding on non-reinforced trials, albeit through different behavioral mechanisms than the negative occasion setting paradigm (Bouton, 2007). Specifically, the feature acquires a direct inhibitory association with the unconditioned stimulus (Rescorla & Holland, 1977). This results in a classically testable form of inhibition, in which the feature can delay excitatory conditioning of a novel cue (retardation) and suppress responding to another excitatory CS when introduced into a compound with this CS (summation). Note that while it is true that prior studies have examined nicotine in the context of occasion setting or conditioned inhibition (Bevins et al., 2006; Murray et al., 2011; Palmatier & Bevins, 2008), those studies have asked a fundamentally different question than the one address here. Indeed, in the studies by Bevins and colleagues, nicotine itself was used *as the stimulus* (i.e., nicotine itself was the occasion setter or the conditioned inhibitor). In contrast,

the focus of the present line of work is to determine how nicotine *modulates other stimuli* that serve as occasion setters or conditioned inhibitors.

Another implication of the findings from Experiment 2 is that the enhancing effects of nicotine on negative occasion setting observed in Experiment 1 may be due to potentiation of nAChRs that are normally active during the task. However, it is important to note that the effects of nicotine and mecamylamine on negative occasion setting were not simple mirror images of each another. For example, the nicotine-induced reduction in the number of training sessions needed to learn the discrimination was accompanied by an increase in orienting to the feature stimulus. In contrast, mecamylamine-treated rats required more sessions to discriminate between reinforced and non-reinforced trials, but did not exhibit a difference in orienting behavior compared to control rats. This could be the result of a floor effect, since the levels of rearing to the light are normally quite low as evidenced by the saline-treated groups in Experiments 1 and 2. Alternatively, this result may indicate that the enhancing effect of nicotine on negative occasion setting may not simply reflect a potentiation of normal activity at nAChRs during learning. Consistent with this idea, the effect of nicotine on the number of sessions required to learn the discrimination was associated with an effect on conditioned responding during non-reinforced trials, whereas mecamylamine did not affect conditioning on those trials. A similar pattern of results has been demonstrated in other forms of learning. For instance, while systemic nicotine administration has been shown to enhance contextual fear memory, blocking nAChRs with an antagonist is without affect (Gould & Wehner, 1999).

Thus, although the finding that mecamylamine delayed acquisition of the conditioned discrimination suggests that nAChRs are normally active during this task, the enhancing effects of nicotine may be at least partially due to other mechanisms. For example, nAChRs are located on dopaminergic neurons and their stimulation results in increased dopaminergic tone and enhancement of dopamine-related functions (Rapier et al., 1990). In particular, acetylcholine modulates striatal and mesolimbic dopamine systems (Wainer & Mesulam, 1990; Garzon et al., 1999) and nicotine affects the release of dopamine in these pathways (Rapier et al., 1990). Thus, nicotine may affect inhibition by interacting with dopaminergic systems that regulate reward and motivational processes (Viggiano et al., 2002). Similarly, nicotine has also been shown to stimulate the release of norepinephrine (Sterley et al., 2014; Westphalen et al., 2009; Zhao et al., 2007), which among other functions, modulates attention by regulating activity of prefrontal cortical neurons (Newman et al., 2008; McGaughy et al., 2008). We have previously shown that negative occasion setting is dependent on the prefrontal cortex (MacLeod & Bucci, 2010) and thus nicotine may act to enhance negative occasion setting by altering attentional functions mediated by prefrontal norepinephrine levels.

In summary, the results of Experiments 1 and 2 add to a small but growing literature regarding the role of cholinergic systems in inhibitory behavior. Indeed, prior studies have demonstrated that manipulation of acetylcholine receptors or selective cholinergic lesions interfere with motor inhibition (Ivliev, 1999) and impulsive choice behavior (Dalley et al., 2004). Nicotine administration also alleviates deficits in so-called behavioral inhibition in persons with ADHD (Potter & Newhouse, 2004, 2008). The present findings expand this

line of research by demonstrating that nicotine also enhances the ability to *learn* an inhibitory response, a result that has important implications for understanding nicotine use and abuse in normal as well as clinical populations. In addition, the data suggest that one way nicotine may affect inhibitory processes is by altering attention to stimuli that signal the need to withhold a behavioral response. This is supported by studies indicating that nicotine improves intra- and extra-dimensional shifts in attention (Allison & Shoaib, 2013) and improves performance in various choice reaction time tasks (Young et al., 2013; Tsutsui-Kimura et al., 2010).

Conclusions

The present data provide new evidence that nicotine can improve learning that involves withholding a behavioral response. These findings complement the extensive literature on nicotinic modulation of excitatory conditioning and other forms of learning that are instead centered on the *emission* of a response. The enhancing effects of nicotine on negative occasion setting may be attributable to an increase in attention to the feature stimulus (or ‘stop signal’), although future studies are needed to examine the effects of nicotine on inhibitory associations per se. Regardless, the findings inform our understanding of how nicotine may alleviate inhibitory deficits as demonstrated in persons with ADHD (Potter & Newhouse, 2004, 2008). Moreover, the enhancing effects of nicotine on inhibitory behavior provide insight into the nature of co-occurring mental illness and nicotine abuse (Kumari & Postma, 2005), particularly in conditions associated with endogenous alterations in nAChR function, such as schizophrenia (Ripoll et al., 2004; Martin et al., 2004; Schwarcz et al., 2001).

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Highlights

- Negative occasion setting, a form of inhibitory learning, was enhanced by nicotine
- Nicotine may modulate the ability to withhold a behavioral response by altering attention
- Blockade of nicotinic acetylcholine receptors impaired negative occasion setting

Figure 1.

Responding during presentation of the tone on reinforced and non-reinforced trials in Experiment 1. Nicotine had a dose-dependent effect on the number of sessions needed to learn the discrimination. Data are means $\pm$ SEM. Asterisks indicate the session at which rats started to exhibit significant discrimination between the trial types based on the Z-score analysis ($p < 0.01$). Abbreviations: R=reinforced trials, NR=non-reinforced trials.

Figure 2A

Figure 2B

Figure 2.

Responding during reinforced (A) and non-reinforced (B) trials in Experiment 1. Nicotine (0.35mg/kg) decreased responding to the tone when it was preceded by the light as evidenced by a more negative slope of the learning curve on non-reinforced trials. Data are means $\pm$ SEM. * $p < 0.05$.

Figure 3.

Orienting behavior (rearing) exhibited during presentation of the light on non-reinforced trials in Experiment 1. Nicotine (0.35mg/kg) significantly increased the amount of time spent rearing when the light was presented. Data are means $\pm$ SEM. * $p < 0.001$.

Figure 4.

Responding during presentation of the tone on reinforced and non-reinforced trials in Experiment 2. Mecamylamine increased the number of sessions needed to learn the discrimination. Data are means $\pm$ SEM. Asterisks indicate the session at which rats started to exhibit significant discrimination between the trial types based on the Z-score analysis ($p < 0.01$). Abbreviations: R=reinforced trials, NR=non-reinforced trials.

Figure 5A

Figure 5B

Figure 5.

Responding during reinforced (A) and non-reinforced (B) trials in Experiment 2.

Mecamylamine had no effect on the rate of learning on either reinforced or non-reinforced trials. Data are means $\pm$ SEM.

Figure 6.

Orienting behavior (rearing) exhibited during presentation of the light on non-reinforced trials in Experiment 2. Mecamylamine had no effect on the amount of time spent rearing when the light was presented. Data are means $\pm$ SEM.

Table 1

Beta values (slope) for nicotine-treated rats in Experiment 1

	<u>Reinforced Trials</u>	<u>Non-reinforced trials</u>
Saline	0.83 ± 0.19	0.08 ± 0.16
0.2 mg/kg	1.17 ± 0.18	0.59 ± 0.17
0.35 mg/kg	0.64 ± 0.14	$-0.31 \pm 0.10^*$
0.5 mg/kg (* $p < 0.04$)	0.91 ± 0.26	0.43 ± 0.26

Table 2

Beta values (slope) for mecamylamine-treated rats in Experiment 2

	<u>Reinforced Trials</u>	<u>Non-reinforced trials</u>
Saline	0.76 ± 0.24	0.24 ± 0.28
0.2 mg/kg	1.14 ± 0.19	0.75 ± 0.16
0.35 mg/kg	0.92 ± 0.18	0.52 ± 0.17
0.5 mg/kg	0.76 ± 0.44	0.42 ± 0.16