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The effects of acute, chronic, and withdrawal from chronic nicotine on novel and spatial object recognition in male C57BL/6J mice

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Abstract

Rationale—Spatial and novel object recognition learning is different from learning that uses aversive or appetitive stimuli to shape acquisition because no overt contingencies are needed. While this type of learning occurs on a daily basis, little is known about how nicotine administration affects it.

Objectives—To determine the effects of acute, chronic, and withdrawal from chronic nicotine on two related but distinct incidental learning tasks, novel and spatial object recognition.

Methods—In C57BL/6J mice, the effects of acute (0.045–0.18 mg/kg), chronic (6.3 mg/kg/day), and withdrawal from chronic nicotine on novel and spatial object recognition were examined.

Results—With a 48-h delay between training and testing, acute nicotine enhanced spatial (difference score, saline=3.34 s, nicotine=7.71 s, $p=0.029$) but resulted in a deficit in novel object recognition (difference score, saline=8.76 s, nicotine= 4.48 s, $p=0.033$). Chronic nicotine resulted in a strong trend towards a deficit in spatial object recognition (difference score, saline=4.01 s, nicotine=1.81 s, $p=0.059$) but had no effect on novel object recognition, and withdrawal from chronic nicotine disrupted spatial object recognition (difference score, saline=3.00 s, nicotine=0.17 s, $p=0.004$) but had no effect on novel object recognition.

Conclusions—The effects of nicotine on spatial object recognition shift from enhancement to deficit as administration changes from acute to chronic and withdrawal. These effects were specific for spatial object recognition, which may be due to differing underlying neural substrates involved in these tasks. Understanding how nicotine alters learning has implications for understanding diseases associated with altered cholinergic function.

Keywords

Acetylcholine; Learning and memory; Hippocampus; Addiction; Mice; Rodent; Object-place memory; Nicotine; Object recognition; Spatial memory

Introduction

Nicotine administration is known to alter learning and memory in both rodents and humans (Heishman et al. 2010; Kenney and Gould 2008a; Levin et al. 2006). Using a contextual fear

conditioning task in mice, we have previously found that acute nicotine enhances learning, chronic nicotine is without effect, and withdrawal from chronic nicotine results in learning deficits (Davis et al. 2005; Gould and Higgins 2003; Gould and Wehner 1999). However, it is unknown if these various effects of different nicotine administration protocols generalize to other learning and memory tasks. One type of learning that has received less attention is automatic or incidental learning. This type of learning refers to the formation of memories that occur due to interacting with stimuli in the environment in the absence of intention or motivation to form such memories (Wang and Morris 2010). The automatic and incidental processing of information concerning what and where objects are in the environment is critical to the successful completion of daily activities in humans (Postma et al. 2008). While it is difficult to unequivocally determine what constitutes incidental learning in animals, a behavioral paradigm that models this learning would require rapid encoding and lack of explicit external reward or punishment (Wang and Morris 2010). Object recognition satisfies these requirements as a model of incidental learning.

Object recognition tasks exploit the natural propensity of rodents to spontaneously explore novel objects or spatial configurations over familiar objects or previously experienced spatial configurations (Bevins and Besheer 2006; Dere et al. 2007; Ennaceur 2010; Ennaceur and Delacour 1988). The effects of various nicotine administration protocols on novel or spatial object recognition (NOR and SOR, respectively) is largely unknown. Because there are differences in the neural substrates involved in reinforced versus unreinforced spatial learning (Gaskin et al. 2009; Okada and Okaichi 2010; White et al. 2003), the effects of nicotine on SOR may be different from the effects on nicotine on spatial learning that is motivated by appetitive or aversive stimuli.

Learning and memory tasks that depend upon hippocampal function have been found to be particularly sensitive to both the enhancing and detrimental effects of nicotine (Kenney and Gould 2008a). For example, trace-cued and contextual fear conditioning are both hippocampus-dependent tasks, and both forms of learning are modulated by nicotine administration whereas the closely related, but hippocampus independent, delay-cued fear conditioning task is unaffected by nicotine administration (Davis et al. 2005; Gould et al. 2004; Gould and Higgins 2003; Raybuck and Gould 2009). Similarly, performance on the hippocampus-dependent water maze and radial-arm maze are also modulated by nicotine administration (Bernal et al. 1999; Levin et al. 2006; Scerri et al. 2006; Sharifzadeh et al. 2005). Many of the effects of nicotine on fear conditioning have been found to require the direct action of nicotine in the hippocampus (Davis and Gould 2009; Davis et al. 2007; Raybuck and Gould 2009), further suggesting that hippocampal function is particularly amenable to modulation by nicotine. The hippocampus is critically involved in spatial tasks (Parkinson et al. 1988; Warburton and Brown 2010) and may play a role in novel object recognition along with other brain areas such as the perirhinal cortex (Winters et al. 2008). Thus, we hypothesized that acute nicotine administration would enhance and withdrawal from chronic nicotine would disrupt SOR, while these effects may differ for perirhinal cortex-dependent NOR.

Methods

Subjects

Subjects were male C57BL/6J mice (8–12 weeks old, Jackson Labs, Bar Harbor, ME) group housed 2–4 per cage and kept on a 12:12 light dark cycle (lights on at 07:00). Mice were tested during the light cycle so as to allow comparisons to previous studies (e.g. Besheer and Bevins 2003; Bevins et al. 2001; Gould and Wehner 1999; Kenney and Gould 2008b). Mice had ad libitum access to food and water. All procedures were approved by the Temple

University Institutional Animal Care and Use Committee and performed in accordance with NIH standards.

Apparatus

Object recognition occurred in an open field arena made of opaque gray Plexiglas (50×60×25 cm) and dimly lit from above (27 lx). A camera mounted above the open field arena was used for recording and observation of behavior. Two sets of identical objects were used in the experiments: one set of objects consisted of an inverted 50 ml falcon tube (Fisher Scientific, Pittsburgh, PA) filled with clean mouse bedding, and the other set consisted of a 10-cm high tower made of yellow and green plastic interlocking blocks. All objects were affixed to a piece of gray Plexiglas (7.5×7.5 cm) to prevent mice from moving or tipping over objects. For spatial object recognition, two visual cues were placed on two adjacent walls of the open field arena: a black and white striped pattern (21×19.5 cm) was affixed to the center of the northern wall and a black and gray checkered pattern (26.5×20 cm) was placed at the center of the western wall.

Object recognition procedures

Prior to any behavioral manipulations, mice were handled for 1 min twice a day for 3 days in the room in which training and testing were performed. Mice that received intraperitoneal injections were also handled as if receiving an injection. Following 3 days of handling, mice were pre-exposed to the open field arena for 10 min a day for 2 days. Training occurred 1 day following the second pre-exposure: mice were placed into the open field arena for 10 min and allowed to explore two objects placed in the NE and NW corners that were either identical (NOR) or different (SOR). Objects were placed approximately 5 cm from the walls of the open field arena. At 24 or 48 h following training, mice were re-exposed to the open field arena for 10 min with either one of the previously explored objects replaced with an object the mouse had not previously explored (NOR), or the object that was previously in the NW corner was moved to the SW corner (SOR) (Fig. 1). Mice will preferentially explore a novel object over a previously experienced object or a spatially displaced object over one that has remained stationary. As suggested by Bevins and Besheer (2006), a mouse that was not used in experiments was allowed to explore the arena prior to pre-exposures, training, and testing so that the field would have a familiar smell to the first animal of each day. Objects were counterbalanced across locations and conditions. The open field arena and all objects were thoroughly wiped down with 70% ethanol before and after all behavioral procedures.

During training and testing in NOR and SOR, mice were scored for exploratory behavior via observation on a television monitor using hand-held stopwatches. Exploration was defined as a mouse having its nose directed toward the object within approximately 1 cm (Bevins and Besheer 2006). Climbing or sitting on objects was not scored as object exploration. Mice that did not spend more than a total of 10 s exploring the objects on training or testing were excluded from analysis (less than 15% of all animals). Prior to scoring behavioral experiments, all experimenters were initially tested to ensure that they did not differ in their scoring by more than 10%.

Drugs and administration

Nicotine hydrogen tartrate salt (Sigma, St. Louis, MO; all doses reported as freebase) was dissolved in physiological saline.

Acute nicotine—Mice were administered acute nicotine intraperitoneally at doses ranging from 0.045 to 0.18 mg/kg at a volume of 0.01 ml/g (Gould and Higgins 2003). In most experiments, nicotine or saline was administered 5 min prior to training and testing. In one

experiment (Fig. 3c), nicotine was administered 5 min prior to training or testing only, as indicated. In another experiment (Fig. 3d), nicotine was administered immediately following training and 5 min prior to testing.

Chronic nicotine—Mice were implanted with subcutaneous mini-osmotic pumps (Alzet, model 1002) for the administration of chronic nicotine at a dose of 6.3 mg/kg/day. Pumps were filled with 100 μ l of saline or nicotine and implanted while mice were anesthetized via isoflurane (5% induction, 2–3% maintenance). A small incision was made on the lower back of the mouse, the pump was implanted, and the wound was closed with surgical staples. Mice were trained in NOR or SOR 12 days following pump implantation.

Withdrawal from chronic nicotine—Mice were implanted with subcutaneous mini-osmotic pumps and administered saline or nicotine at a dose of 6.3 mg/kg/day as described above. Pumps were removed 12 days following implantation while mice were anesthetized as described above. Mice were trained in NOR or SOR 24h following pump removal.

Data and statistical analyses

Exploratory behavior during testing was transformed into difference scores and preference ratios for comparison across groups. The difference score indicates the difference in time spent exploring the novel over the familiar (NOR) or the spatially displaced over the stationary object (SOR). The preference ratio was calculated and defined as: preference ratio_{N/D} = $E_{N/D} / (E_{N/D} + E_{F/S})$ where preference ratio_{N/D} = preference ratio of novel/displaced object, $E_{N/D}$ = exploration time of novel/displaced object and $E_{F/S}$ = exploration time of familiar/stationary object.

Data were analyzed using one-way ANOVAs or independent samples *t* tests where appropriate. To determine whether mice demonstrated significant preference for the novel or displaced object, one-sample *t* tests were performed to test if responding was different from chance with a test value of 0 or 0.5 representing chance for difference scores or preference ratios, respectively. Mice more than 2.5 standard deviations from the mean on exploratory measures were considered outliers and removed from statistical analyses (two animals).

Results

Acute nicotine

Previous work suggests that acute doses of nicotine ranging from 0.045 to 0.18 mg/kg are most likely to have an effect in learning and memory tasks in C57BL/6J mice (Gould and Higgins 2003). Thus, the effects of three doses of systemic nicotine (0.045, 0.09, and 0.18 mg/kg) administered at training and testing on NOR and SOR were examined (Fig. 2). With a 24-h delay between training and testing, acute nicotine had no effect on the preference for the novel (Fig. 2a; $F(3,30) < 1$ for both the difference score and preference ratio) or displaced object (Fig. 2b; $F(3,30) = 1.42$, $p = 0.25$ for difference score; $F(3,30) < 1$ for preference ratio). One-sample *t* tests revealed that all treatment groups in the NOR task demonstrated recognition of the novel object ($ps < 0.05$), and all treatment groups in the SOR task demonstrated recognition of the spatially displaced object ($ps < 0.05$) except for the 0.045 mg/kg nicotine-treated group which displayed a strong trend towards spatial object recognition (difference score, $p = 0.065$; preference ratio, $p = 0.093$).

In an effort to make the NOR and SOR tasks more difficult and potentially reveal an effect of nicotine on a more challenging version of the task, the time between training and testing was increased to 48 h (Fig. 3). The findings from the acute dose response on NOR and SOR with a 24-h difference between training and testing suggested that the 0.09 mg/kg dose of

nicotine would be most effective at enhancing this task, as there was a weak trend towards enhancement at this dose. Additionally, previous work has found this dose to be effective in enhancing contextual fear conditioning in mice (Gould and Higgins 2003). With 48 h between training and testing in NOR, 0.09 mg/kg nicotine resulted in a deficit in recognition of the novel object (Fig. 3a) as measured by both the difference score ($t(14)=2.37$, $p=0.033$) and preference ratio ($t(14)=2.28$, $p=0.038$). One-sample t tests revealed that both the saline- and nicotine-treated groups recognized the novel object ($ps<0.05$), suggesting that acute nicotine resulted in a deficit but not complete disruption of novel object recognition. With 48 h between training and testing in SOR, 0.09 mg/kg nicotine resulted in an enhancement in recognition of the spatially displaced object (Fig. 3b) as measured by the difference score ($t(15)=2.41$, $p=0.029$) and preference ratio ($t(15)=2.33$, $p=0.034$) and both treatment groups demonstrated recognition of the spatially displaced object as determined by one-sample t tests ($ps<0.05$). The finding that acute nicotine enhanced SOR at a 48-h delay was also replicated (data not shown, $t(28)=2.41$, $p=0.023$).

To determine if the enhancing effect of acute nicotine on SOR was due to alterations in acquisition or recall of the task, nicotine (0.09 mg/kg) was administered prior to training or testing only of SOR with a 48-h delay. Administration of nicotine at training or testing only had no effect on the recognition of the spatially displaced object (Fig. 3c) as measured by the difference score ($F(2,30)<1$) and the preference ratio ($F(2,30)<1$); all three treatment groups demonstrated recognition of the spatially displaced object as determined by one-sample t tests ($ps<0.05$). This finding is in agreement with previous work demonstrating that nicotine must be administered prior to both training and testing in contextual fear conditioning to enhance learning (Gould and Higgins 2003; Gould and Wehner 1999; Kenney and Gould 2008b).

The enhancing effect of acute nicotine on SOR with a 48-h delay between training and testing could be due to either an alteration in acquisition or consolidation of the memory formed during training. To tease these two stages of memory formation apart, nicotine was administered immediately following training and before testing in SOR with a 48-h delay. Administration of nicotine following training and before testing had no effect on the recognition of the spatially displaced object (Fig. 3d) as measured by the difference score ($t(13)<1$) and the preference ratio ($t(13)<1$); both groups demonstrated recognition of the spatially displaced object as measured by the difference score ($ps<0.05$) and the group administered nicotine demonstrated recognition of the spatially displaced object as measured by the preference ratio ($p<0.05$), whereas the saline-treated group demonstrated a strong trend towards an effect ($p=0.059$). This finding suggests that nicotine specifically alters acquisition of SOR and that the enhancement of this task is not due to a sensitization effect of nicotine being administered for a second time at testing.

In all acute nicotine administration experiments, no difference in overall exploration of the objects was observed during either training or testing, suggesting that the observed effects were not due to changes in locomotor activity or anxiety (Table 1).

Chronic nicotine

Previous work suggests that during chronic nicotine administration, tolerance develops to the effects of acute nicotine on learning and memory (Davis et al. 2005). To determine if tolerance develops to the effects of acute nicotine in NOR and SOR, 6.3 mg/kg/day of nicotine was administered to mice leading up to and during training and testing in object recognition (Fig. 4). This dose of nicotine was chosen because it results in plasma nicotine levels similar to those seen following administration of the 0.09 mg/kg acute dose of nicotine and matches levels seen in smokers (Davis et al. 2005). Chronic nicotine administration had no effect on NOR (Fig. 4a) as measured by the difference score ($t(19)<1$)

or the preference ratio ($t(19) < 1$) and both treatment groups demonstrated recognition of the novel object ($p < 0.05$), suggesting that tolerance developed to the detrimental acute effects of nicotine on this task. In contrast, chronic nicotine administration resulted in a deficit in SOR (Fig. 4b); there was a strong trend towards an effect as measured by the difference score ($t(20) = 2.00$, $p = 0.059$) and a significant effect as measured by the preference ratio ($t(20) = 2.30$, $p = 0.032$). One-sample t tests revealed that both saline- and chronic nicotine-treated mice demonstrated recognition of the spatially displaced object ($p < 0.05$). Thus, chronic nicotine reduced, but did not abolish, recognition of the spatially displaced object. No effect of chronic nicotine administration was observed on overall object exploration during training or testing in either NOR or SOR, suggesting that there were not alterations to overall locomotor activity or anxiety due to nicotine administration (Table 1).

Withdrawal from chronic nicotine

Previous work has found that withdrawal from 12 days of chronic nicotine administration results in deficits in hippocampus-dependent learning tasks that are sensitive to enhancement by acute nicotine administration such as contextual and trace-cued fear conditioning (Davis et al. 2005; Raybuck and Gould 2009). Thus, we hypothesized that withdrawal from chronic nicotine would also result in deficits in spatial object recognition. NOR and SOR were examined 24 h following withdrawal from 6.3 mg/kg/day nicotine (Fig. 5). With a 24-h delay between training and testing in object recognition, withdrawal from chronic nicotine had no effect on either NOR (Fig. 5a) as measured by the difference score ($t(16) < 1$) and preference ratio ($t(16) < 1$) or SOR (Fig. 5c) as measured by the difference score ($t(27) < 1$) and preference ratio ($t(27) < 1$); all groups in both NOR and SOR experiments demonstrated recognition of the novel and spatially displaced objects, respectively ($p < 0.05$). At a 48-h delay between training and testing in object recognition, withdrawal from chronic nicotine administration did not alter NOR (Fig. 5b) as measured by the difference score ($t(18) < 1$) and preference ratio ($t(18) < 1$) and both treatment groups demonstrated recognition of the novel object ($p < 0.05$). In contrast, at a 48-h delay between training and testing, withdrawal resulted in a deficit in SOR (Fig. 5d) as measured by both the difference score ($t(19) = 3.24$, $p = 0.004$) and the preference ratio ($t(19) = 2.54$, $p = 0.020$). One-sample t tests revealed that mice withdrawn from chronic nicotine did not demonstrate recognition of the spatially displaced object as measured by both the difference score ($p = 0.76$) and preference ratio ($p = 0.68$), whereas saline-treated mice did ($p < 0.05$). No effect of withdrawal from chronic nicotine was observed on overall object exploration during training or testing in either NOR or SOR, suggesting that the effects were not due to changes in overall locomotor activity or anxiety (Table 1).

Discussion

Spatial and novel object recognition are differentially sensitive to the effects of acute, chronic, and withdrawal from chronic nicotine administration. Acute nicotine administration enhanced SOR but produced a deficit in NOR. In contrast, chronic nicotine treatment had no effect on NOR, suggesting that tolerance developed for disruptive effects of acute nicotine on NOR, but resulted in a deficit in SOR. Withdrawal from chronic nicotine administration produced a greater deficit in SOR than chronic nicotine but had no effect on NOR. Additionally, the effects of nicotine were only evident when there was a 48-h difference between training and testing with no effect at a 24-h interval. Thus, the effects of nicotine on cognitive and learning processes are not universal but are specific to the type of task, the difficulty of the task, and the nicotine treatment duration.

The enhancement of SOR due to acute nicotine administration is consistent with previous work, suggesting that acute nicotine can enhance learning that has a spatial component to it, such as contextual learning and learning to navigate the Morris water maze (Kenney and

Gould 2008b; Sharifzadeh et al. 2005). The lack of an effect of acute nicotine on NOR with a 24-h delay between training and testing is in agreement with one study that found no effect of nicotine in NOR at a 1-h delay (Sambeth et al. 2007) but at odds with two previous studies in which acute nicotine enhanced NOR at a 24-h delay (Bura et al. 2007; Puma et al. 1999). However, there is an important difference in the overall results of the present study and those studies of both Bura et al. (2007) and Puma et al. (1999). While both the present study and previous work examined the effects of acute nicotine at a 24-h delay between training and testing in NOR, in the present study, all mice demonstrated recognition of the novel object, whereas in the studies of both Bura et al. (2007) and Puma et al. (1999), saline-treated animals did not demonstrate recognition of the novel object, but nicotine-treated animals did. Thus, the present study and previous work examined different phenomenon; in the present study, the ability of nicotine to modulate learning was examined, whereas Bura et al. (2007) and Puma et al. (1999) examined the ability of nicotine to generate learning in a situation that normally did not result in learning. This suggests that when certain experiences are subthreshold for formation or recall of a memory that is associated with NOR, acute nicotine administration may push the memories above threshold for remembering, whereas if the experience is sufficient for memory formation, then nicotine may have no effect or result in a deficit.

The opposing effects of acute nicotine on SOR and NOR with a 48-h delay between training and testing suggest that acute nicotine administration may be shifting how information is processed and consolidated following initial object exposure. With nicotine administration, information processing may be shifted to a strategy that not only favors recognition of a spatial displacement, but does so at the expense of processing specific information about the objects themselves. Given that nicotine is known to enhance contextual memories but not those that involve a discrete cue (Gould and Higgins 2003; Gould and Wehner 1999), it may be the case that learning in the presence of nicotine results in more efficient processing of disparate cues, in some cases to the detriment of processing discrete information. While previous work has found that nicotine does not result in a deficit in conditioning to a discrete cue (Gould and Higgins 2003; Gould and Wehner 1999), the discrete cue in these studies were relatively simple auditory stimuli and not a more complex object as in the present study and therefore may involve different learning processes; this may explain why cued fear conditioning is not disrupted by acute nicotine but NOR is. Finally, because administration of acute nicotine at testing only had no effect on SOR, nicotine is most likely not altering retrieval of the memory but learning of the spatial configuration of the objects, as has been shown for other types of hippocampus-dependent learning (Portugal et al. 2008). Thus, nicotine administration may result in enhancement of one type of learning at the expense of another type.

Chronic nicotine administration resulted in a deficit in SOR, but tolerance developed for the disruptive effect of acute nicotine on NOR. The deficit in SOR due to chronic nicotine administration was unexpected, as previous work in mice has found that tolerance develops to the enhancing effects of acute nicotine in contextual and trace-cued fear conditioning (Davis et al. 2005; Raybuck and Gould 2009). However, the development of deficits in SOR with chronic nicotine treatment when acute nicotine treatment produced enhancement could be viewed as an overshoot of tolerance. That is, if tolerance reflects physiological adjustments that oppose the acute effects of a drug in an attempt to return the system to homeostasis (Raybuck and Gould 2009; Solomon and Corbit 1973), then a shift towards decreased SOR would be expected with chronic treatment. The effects of chronic nicotine on other spatial tasks have varied. In the water maze, Abdulla et al. (1996) found that chronic nicotine enhanced memory retention in young Sprague–Dawley rats and acquisition in older rats, and Bernal et al. (1999) found enhancement of acquisition in C57BL/6 mice due to chronic nicotine administration. Chronic nicotine has also been found to enhance spatial working

memory as measured by the radial-arm maze in Sprague–Dawley rats (Levin et al. 1993; Levin et al. 1990). In agreement with the present study, Scerri et al. (2006) found deficits in the acquisition and retention of the water maze in Sprague–Dawley rats due to chronic nicotine administration. Thus, the effects of chronic nicotine on tasks that contain a spatial component are quite variable and may be particularly sensitive to the specific task, species differences, and experimental parameters such as dose of nicotine. However, increasing evidence suggests that chronic nicotine use in humans is associated with cognitive deficits (Nooyens et al. 2008; Sabia et al. 2008).

In both animals and humans, withdrawal from chronic nicotine is known to result in learning and memory deficits (Domier et al. 2007; Jacobsen et al. 2005; Kenney and Gould 2008a; Mendrek et al. 2006) and in the present study, withdrawal from chronic nicotine administration resulted in a deficit in SOR but had no effect on NOR. The lack of an effect of withdrawal from chronic nicotine administration on NOR agrees with previous work performed in rats in which withdrawal was found to have little effect on the recognition of a novel object (Besheer and Bevins 2003), suggesting that this lack of an effect is not specific to mice. Importantly, withdrawal from chronic nicotine did not affect overall object exploration, suggesting that the observed deficit in SOR was one of learning and not due to changes in locomotor activity or anxiety. Additionally, the effect of withdrawal is unlikely to simply be an extension of the deficits found in SOR due to chronic nicotine administration because chronic nicotine-treated mice still demonstrated recognition of the spatially displaced object, whereas withdrawal resulted in no recognition whatsoever. This finding is in line with previous work in which a task that was enhanced by acute nicotine, contextual fear conditioning, also showed deficits during withdrawal (Davis et al. 2005). Additionally, since NOR was not affected by nicotine withdrawal, the differences in the neural substrates involved in these two related but distinct object recognition tasks may help in identifying specific brain areas that are particularly sensitive to the effects of withdrawal.

The cognitive and somatic effects of nicotine withdrawal tend to be most severe within 24–48 h following withdrawal while typically tapering off around 72–96 h later (Besheer and Bevins 2003; Damaj et al. 2003; Isola et al. 1999; Shoaib and Bizarro 2005; Yamazaki et al. 2006). In the present study, mice were trained in object recognition 24 h following withdrawal and tested either 24 or 48 h after training. The two different intervals between training and recall were used because the difficulty of object recognition tasks is known to increase considerably with the retention interval between training and testing (de Bruin and Pouzet 2006; Ennaceur and Delacour 1988; Frick and Gresack 2003) and thus the longer interval might be more sensitive to disruption. Indeed, the detrimental effects of nicotine withdrawal were only seen with the longer training–testing interval. These findings suggest that task difficulty is an important parameter to consider when examining the effects of nicotine on cognitive performance, though it is also possible that the time interval between withdrawal and testing influences the severity of the deficit.

Judgment of familiarity is central to the performance of object recognition tasks as rodents will only preferably explore a novel object or spatial configuration if they can judge the previously explored object or spatial configuration as being familiar (Ennaceur 2010). A previous study examining the effects of nicotine on environmental familiarization found that acute nicotine interfered with environmental familiarization whereas chronic nicotine was without effect (Bevins et al. 2001). These results are not in conflict with our findings for multiple reasons. First, different species were used. Second, we examined object recognition, whereas Bevins et al. (2001) examined environmental familiarization. Third and most important, we habituated the mice to the environment before administering nicotine. Specifically, in the present study, mice received two pre-exposure sessions to the environment in which the objects were experienced prior to any nicotine administration.

thereby potentially minimizing the environment as a variable, whereas in Bevins et al. (2001), rats were given nicotine upon initial exposure to the environment. Interestingly, this difference could suggest that the effects of nicotine may differ depending on whether nicotine is administered in a familiar or novel environment.

The differences in the response to acute, chronic nicotine and withdrawal from chronic nicotine in SOR and NOR may be due to the fact that different brain regions are involved in these tasks. The hippocampus is a critical neural component for the processing of the spatial and contextual information that is integral to the performance of SOR (Eichenbaum 2004; Smith and Mizumori 2006), whereas the perirhinal cortex is the critical structure involved in processing object-specific information important for NOR and is not vital to the recognition of configural discriminations (Bachevalier and Nemanic 2008; Horne et al. 2010; Winters et al. 2008). Indeed, cholinergic function in the perirhinal cortex is important for synaptic plasticity and the performance of NOR (Warburton et al. 2003; Winters and Bussey 2005). It may be the case that acute nicotine administration disrupts the flow of cholinergic transmission in the perirhinal cortex to produce a deficit in NOR, although no previous studies have examined the effects of nicotine on synaptic plasticity in the perirhinal cortex. In contrast, the effects of nicotine on synaptic plasticity in the hippocampus are well documented (Fujii et al. 1999; Kenney and Gould 2008a). The enhancing effect of acute nicotine and the detrimental effect of withdrawal from chronic nicotine on hippocampus-dependent contextual fear conditioning are both known to depend upon the direct action of nicotine in the hippocampus (Davis and Gould 2009; Davis et al. 2007). The findings from the present study that acute nicotine enhanced and withdrawal from chronic nicotine disrupted SOR are consistent with this previous work and suggest that these effects may be related to changes in hippocampal function. Finally, chronic nicotine has been found to disrupt the dopaminergic and cholinergic modulation of synaptic transmission in the temporo-ammonic pathway of the hippocampus (Nakauchi et al. 2007), a finding that may help explain, in part, the deficit in SOR due to chronic nicotine administration, but requires further examination.

Several factors contribute to the performance of object recognition tasks. Deficits in novel or spatial object recognition could be attributed to memory-related processes such as state dependence of the familiarization process, interference with memory for the field or interference with object memory. Alternatively, deficits or facilitation of object recognition memories could be due to non-memory-related processes such as motor effects or changes in object perception. State dependence for the familiarization to the objects or their spatial locations is unlikely to explain the findings in the present study because the deficit in NOR and the enhancement of SOR due to acute nicotine, and the deficit in SOR due to chronic and withdrawal from chronic nicotine, were observed when animals were in the same drug state at both training and testing. State-dependent theory would predict that deficits would arise when animals were in different drug states at training and testing (Overton 1991), which was not the case in the present study, and prior work suggests that nicotine does not produce robust state-dependent effects (Bevins et al. 2007). Additionally, it is also unlikely that interference with the memory for the field would play a significant role in the observed deficits in NOR and SOR in the present study given that mice were thoroughly familiarized to the field prior object exposure. Non-memory-related processes are also unlikely to explain the data from the present study as there were no differences observed in overall object exploration due to the various drug treatments (Table 1), which suggests there was no alteration in motivation or locomotor activity. Furthermore, if general non-memory related processes, memory for the field, or state-dependent effects were altered due to nicotine administration and these contributed to the performance on NOR and SOR, one would expect similar effects of nicotine on these tasks. Given that the effects of acute, chronic, and withdrawal from chronic nicotine on NOR and SOR tasks were different, it is unlikely that

non-memory related or nonspecific processes that would affect both types of learning significantly contributed to the results. Thus, the most parsimonious explanation for the present data is that the various effects of nicotine on NOR and SOR reflect how nicotine differentially alters the cognitive processing, and therefore the neural substrates that underlie these tasks.

Overall, nicotine administration alters both NOR and SOR. The differences between the effects of nicotine in these tasks are likely due to differences in the underlying neural substrates involved in these tasks and how nicotine alters these substrates. The findings in the present study agree with previous work that made use of appetitive and aversive behavioral paradigms and extend this literature to demonstrate that incidental learning, such as SOR, is sensitive to the effects of acute nicotine and withdrawal from chronic nicotine. This sensitivity, however, is expressed when the task is made more difficult suggesting that nicotine has a greater effect when there is a heavier cognitive load. A better understanding of how nicotinic receptors contribute to learning and memory processes will aid in the development of more efficacious therapeutics for a variety of disorders in which cholinergic function is altered, such as nicotine addiction, Alzheimer's disease, Parkinson's disease, and schizophrenia (Besson et al. 2007; Picciotto and Zoli 2008; Woodruff-Pak and Gould 2002).

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Fig. 1. General experimental procedure outlining the placement of objects in the open field arena for both NOR and SOR during training and 24/48 h later during testing. *Underlined* objects indicate those that are preferentially explored during testing

Fig. 2.

The effects of acute nicotine on NOR and SOR with a 24-h delay between training and testing. **a** The effects of nicotine administered prior to training and testing on NOR as measured by both the difference score and preference ratio ($n=7-10$). **b** The effects of nicotine administered prior to training and testing on SOR as measured by both the difference score and preference ratio ($n=9-12$). White bar represents saline-treated mice and progressively *darker shades of gray* represent mice that received various doses of nicotine as indicated. Data represents mean $\pm$ SEM

Fig. 3.

The effects of acute nicotine on NOR and SOR with a 48-h delay between training and testing. **a** The effects of acute nicotine administered prior to training and testing on NOR as measured by both the difference score and the preference ratio ($n=8$). **b** The effects of acute nicotine administered prior to training and testing on SOR as measured by both the difference score and the preference ratio ($n=8-9$). **c** The effects of nicotine administration at training or testing on SOR as measured by both the difference score and the preference ratio ($n=10-13$). **d** The effects of nicotine administered immediately following training and prior to testing on SOR as measured by both the difference score and the preference ratio ($n=7-8$). * $p<0.05$ compared to saline-treated mice. *White bars* represents saline-treated mice and *light gray bars* represents mice administered 0.09 mg/kg nicotine prior to both training and testing (**a**, **b**), training only (**c**), or following training and before testing (**d**). *Dark gray bar* represents mice administered 0.09 mg/kg nicotine prior to testing only. Data represents mean $\pm$ SEM

Fig. 4.

The effects of chronic nicotine on NOR and SOR with a 48-h delay between training and testing. **a** The effects of chronic nicotine administration on NOR as measured by both the difference score and the preference ratio ($n=10-11$). **b** The effects of chronic nicotine administration on SOR as measured by the preference ratio and the difference score ($n=10-12$). $*p<0.05$, $^{\dagger}p=0.059$ compared to saline-treated mice. *White bars* represent mice treated with chronic saline and *gray bars* represent mice treated with 6.3 mg/kg/day of chronic nicotine. Data represents mean $\pm$ SEM

Fig. 5.

The effects of withdrawal from chronic nicotine on SOR and NOR. **a** The effects of withdrawal from chronic nicotine on NOR with a 24-h delay between training and testing ($n=8-10$). **b** The effects of withdrawal from chronic nicotine on NOR with a 48-h delay between training and testing ($n=10$). **c** The effects of withdrawal from chronic nicotine on SOR with a 24-h delay between training and testing ($n=13-16$). **d** The effects of withdrawal from chronic nicotine on SOR with a 48-h delay between training and testing ($n=10-11$).

* $p<0.05$ compared to saline-treated mice. *White bars* represent mice withdrawn from chronic saline and *gray bars* represent mice withdrawn from 6.3 mg/kg/day of chronic nicotine. Data represents mean $\pm$ SEM

Table 1

Total object exploration time during training and testing in object recognition

Experiment	Group (n)	Training—total object exploration time (s)	Testing—total object exploration time (s)
Acute nicotine			
NOR—24 h	Saline (10)	28.91±3.08	23.72±3.67
	0.045 Nic (7)	30.39±3.32	27.17±2.91
	0.09 Nic (9)	28.04±3.28	25.53±3.30
	0.18 Nic (8)	26.03±2.38	25.76±3.27
NOR—48 h	Saline (8)	30.06±3.30	24.91±2.21
	0.09 Nic (8)	28.98±2.71	23.30±2.21
SOR—24 h	Sal (12)	31.33±3.02	20.31±1.77
	0.045 Nic (10)	31.45±1.96	16.33±1.59
	0.09 Nic (9)	34.98±2.51	19.76±1.86
	0.18 Nic (10)	33.29±2.66	16.65±1.57
SOR—48 h	Sal (9)	29.51 ±2.03	21.23 ±2.44
	0.09 Nic (8)	27.98±3.65	22.25±1.81
Chronic nicotine			
NOR—48 h	Saline (10)	32.51±2.33	21.74±1.77
	Chronic Nic (11)	32.92±3.41	21.23±2.71
SOR—48 h	Saline (10)	34.72±3.70	21.53±2.07
	Chronic Nic (12)	36.21±3.19	19.99±1.91
Nicotine withdrawal			
NOR—24 h	Saline (10)	29.48±2.82	21.43±1.80
	Nic W/D (8)	30.49±2.48	22.82±2.05
NOR—48 h	Saline (10)	23.74±2.74	19.77±1.87
	Nic W/D (10)	24.87±2.09	21.93±2.22
SOR—24 h	Saline (16)	30.56±3.05	20.63±1.47
	Nic W/D (13)	31.11±1.82	18.99±2.07
SOR—48 h	Saline (11)	27.06±2.48	18.43±1.62
	Nic W/D (10)	25.02±1.20	16.97±2.02

Data represent mean ± SEM