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Effects of nicotine on attention: role of orexin-1 receptors

Stacy Pitcairn

Effects of nicotine on attention: role of orexin-1 receptors

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by

Stacy Pitcairn

Accepted for ____Honors_____

_____Joshua A. Burk_____
Type in the name, Director

___Robin C. Looft-Wilson_____
Type in the name

__Paul D. Kieffaber_____
Type in the name

Williamsburg, VA
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Abstract

The orexin (also called hypocretin) system projects to a wide array of brain regions and is activated by drugs of abuse. The orexin system is best known for its function in wakefulness and arousal, but recent research has suggested that orexins play a vital role in attentional processing. The orexin-1 receptor has been implicated in crucial mechanisms of attention, specifically the transmission of acetylcholine to the cortex. Nicotine is a commonly administered psychoactive drug that has been shown to have cognitive-enhancing effects. Nicotine appears to target the orexin system, suggesting a potential role of the orexin system in mediating the cognitive enhancing effects seen with nicotine. However, the role of orexinergic function in the attentional-promoting effects of nicotine has not been fully described. The present study was designed with two goals in mind: 1) to identify a nicotine dose that improves rat performance in a sustained-attention task and 2) to examine the effect of chronic nicotine administration in conjunction with a selective orexin-1 antagonist, SB-334867, in rats in a sustained-attention task. The results of these experiments suggest that acute nicotine administration impairs attentional functioning, and that chronic nicotine administration slightly improves performance in the sustained-attentional task. Administration of chronic nicotine with SB-334867 blocks the beneficial effect of chronic nicotine in the sustained-attention task. Although these data are preliminary, the findings of this study could provide a novel target that mimics nicotine-induced cognitive activation and give insight into the behavioral and attentional effects of nicotinic activation and orexin-1 antagonism.

Introduction

Orexinergic mechanisms of attention

The orexin system is a vast neuropeptide system that is known for its role in regulating homeostatic mechanisms (Boss et al. 2015). Perhaps it most well-known for its function in promoting wakefulness and arousal, however, the orexin system is also implicated in reward processing, motivation, and addiction (Boss et al. 2015). Because attentional processing requires a general state of arousal, the orexin system has also been suggested to be involved in attentional processing (Fadel and Burk 2009). Attention is a vital cognitive function that consists of detection and selection of stimuli in the environment and the suppression of irrelevant stimuli (Poorthius et al. 2013). Orexin releasing neurons are located in the lateral hypothalamus and contiguous perifornical area (LH/PFA) and have projections to other brain regions, including to areas involved in attention such as the basal forebrain (BF) and the cortex (Fadel and Burk 2009). The transmission of acetylcholine to the cortex is a key neural mechanism that supports attentional processing (Klinkenberg et al. 2011, Himmelheber et al. 2000). Orexin releasing neurons have been shown to promote attention by stimulating the basal forebrain to release acetylcholine to the cortex (Fadel and Burk 2009, Fadel and Frederick-Duus 2008).

The orexin system is composed of two orexin peptides (orexin-A and orexin-B) and two G-protein-coupled receptors (the orexin-1 and the orexin-2 receptor). Orexin B has preferential binding for the orexin-2 receptor, whereas orexin A has high affinity for the orexin-1 and orexin-2 receptors (Sakurai et al. 1998). The orexin-1 receptor in particular has been implicated in attention, as it has been shown to contribute to orexin A-induced increases in cortical acetylcholine release (Fadel and Frederick-Duus 2008. Dong et al. 2006). Administration of orexin-A to the basal forebrain has been shown to enhance performance in rats during a

sustained attention task possibly via enhancement of cortical cholinergic inputs (Zajo, Fadel and Burk 2016). Further support for this conclusion comes from experiments showing that application of orexin peptides can increase cortical acetylcholine release (Li et al. 2016, Boschen, Fadel and Burk 2009). All of this evidence together suggests that the orexin system and the transmission of acetylcholine has a role in attentional functioning.

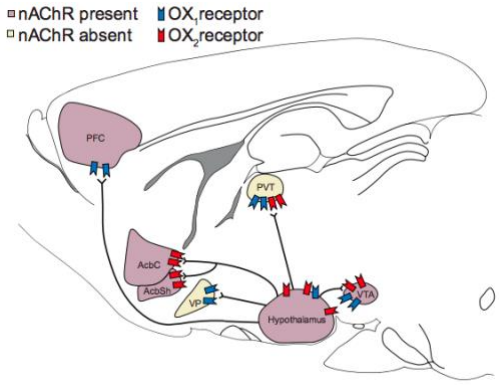
Nicotine-activated mechanisms of attention

Nicotine is a naturally-occurring psychoactive compound that is most commonly found in tobacco products. Nicotine acts as a cholinergic receptor agonist and has been shown to increase acetylcholine transmission to nicotinic acetylcholine receptors (nAChRs) in the cortex (Poorthius et al. 2013). Despite some of the well-known deleterious effects of tobacco products, nicotine has been shown to enhance cognitive functions related to executive functioning, attention, memory, response inhibition, contextual learning, reward-related learning and reward processing, emotion, anxiety and decision-making, (Zaarindast and Khakpai 2019, Wood et al. 2016, Nees 2015, Swan and Lessov-Schlaggar, 2007). Ricciardi et al. (2013). Allison et al. 2013 showed that acute nicotine improved performance in an attentional set shifting task in rodents (Allison et al. 2013). Nicotine has also been the studied as a therapeutic target for cognitive enhancement in diseases including schizophrenia and Alzheimer's Disease (Featherstone et al. 2015, Lombardo et al 2015). More recent studies have suggested improved performance in an attentional set-shifting task in rats, with an even greater improvement seen in rodents that had ketamine-induced cognitive deficits consistent with a rodent model of schizophrenia (Wood et al. 2016). However, the findings of nicotine's effect on attention have not always shown enhancement of the attentional function. A review by Matta et al. 2008 suggests that the effects

of nicotine on cognitive function in rats are complex, and many studies have found no effect and some have observed nicotine-induced impairment on cognitive tasks (Matta et al. 2008).

Potential interactions between nicotinic and orexinergic system

There is a growing body of evidence that suggests interaction between the orexinergic system and the behavioral and cognitive effects of nicotine. Recently, researchers have noted the shared anatomical areas between nAChRs and orexin-1 receptors. The following figure from Khoo et al. 2019 demonstrates that orexin receptors and nAChRs are present in varying densities in brain regions such as the hypothalamus, the ventral tegmental area, and the prefrontal cortex (Khoo et al. 2019). However, in the context of attention, the most relevant brain area shown would be the prefrontal cortex, which contains both nAChRs and orexin-1 receptors. The prefrontal cortex has been shown consistently to be involved in human attentional functioning, including sustained attention (Fortenbaugh et al. 2017).



Khoo, S. Y. S., McNally, G. P., & Clemens, K. J. (2019). The orexin system and nicotine addiction: preclinical insights. In *Neuroscience of Nicotine* (pp. 509-517). Academic Press.

Additionally, orexin neurons are targets of acute or chronic nicotine administration, and the LH/PFA appears to be particularly sensitive to nicotine. (Pasumarthi and Fadel, 2009). Specifically, studies have also shown that acute nicotine administration activates orexin neurons and nicotine upregulates expression of orexins and orexin receptors in the rat brain (Pasumarthi,

Reznikov and Fadel 2006, Kane et al. 2000). Recent research has suggested that blocking nicotine receptors leads to decreased activation of orexin neurons, measured by fos-immunoreactivity (Simmons et al. 2016). These results suggest that (i) acute nicotine increases orexin activity in part through nAChRs on orexin neurons, (ii) chronic nicotine desensitizes and upregulates nAChRs and (iii) nicotinic receptor blockade leads to decreased orexinergic activity in nicotine-exposed rats (Simmons et al. 2016). With all of this evidence taken together, it appears as though nicotine and the orexinergic system have various, although undefined, degrees of interaction. Both systems also rely heavily upon cholinergic transmission, possibly having a role as a mechanism of attentional processing in the orexinergic projections to the PFC and BF.

Despite these findings, recent studies regarding orexins and nicotine have been primarily focused on the role of orexin in addiction and withdrawal behaviors. The role of orexinergic function in the arousing and attentional-promoting effects of nicotine has not yet been described. Further investigation into nicotinic acetylcholine receptors and their potential interaction with the orexin system could give insight into the mechanism of action for nicotine-induced cognitive enhancement. The goal of the present experiment is 1) to identify a nicotine dose that shows improved attentional performance compared to saline and 2) to examine the effect of chronic nicotine administration in conjunction with a selective-1 antagonist, SB-334867, in rats in a sustained-attention task.

Attentional measures

Due to the presence of attentional deficits in many neurodegenerative and neuropsychiatric conditions, the methods of measuring attention are crucial in preclinical studies. Types of attention commonly measured are sustained, spatial, divided, and alternating attention.

(Yantis & Johnson 1990). In rodents, two common paradigms for rodent models are the five-choice serial reaction task and the sustained-attention task. This experiment utilizes the sustained-reaction task due to its advantages. The sustained-reaction task is advantageous because it is non-spatial (Zajo et al. 2016). Additionally, the sustained-attention task includes a non-signal trial, so there is a behavioral measurement even when no signal is presented (Zajo et al 2016). Many drugs with psychoactive characteristics produce multiple, simultaneous effects on different cognitive processes, such as attention, motivation, understanding of task rules, motor control, or other psychological processes. The attention paradigm used should confidently characterize responses as the result of attentional deficits. One analysis of the construct validity of this task tested whether manipulations that are known to disrupt attention also disrupt performance in the task, as well as tested whether known alterations of motivation would produce a response pattern distinct from "attention-related" manipulations (Echevarria et al. 2005). These findings supported the use of this task as a measure of attention in rat subjects (Echevarria et al. 2005). The different measures used in this task, such as rate of hits, misses, correct rejections, and omissions, as well as different signal durations, blocks, presence of the distracter, lead to specific patterns of responses that can elucidate a "pure" attentional deficit or the influence of other psychological processes.

Experiment 1 Goal

The goal of the first study was to identify a dose of nicotine that shows improved attentional performance compared to saline.

Materials and methods

Subjects

A total of 14 Sprague-Dawley rats were utilized throughout the duration of the experiment. The rats were group-housed in a temperature and humidity-controlled environment with a 14:10-h light/dark cycle. All behavioral testing took place at least six days per week between the hours of 8AM and 4PM. Animals were water restricted and food was available ad libitum for the duration of the experiment. On days where behavioral testing occurred, rats were given access to water for ten minutes following their time in the task. The rats were allowed 20 minutes of water access on days where no behavioral testing occurred. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the College of William & Mary.

Apparatus

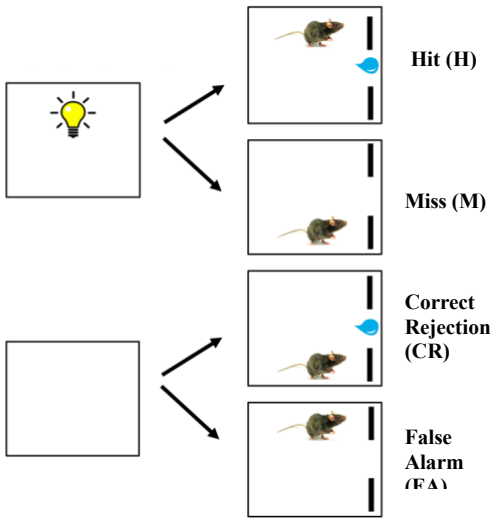
The rats were trained in one of 12 operant boxes (Med Associates Inc.,) each housed within a sound-attenuating box and controlled by MedPC IV software. Each box contained two retractable levers on each side, a water port with a water delivery dipper (0.01 ml) located between the two levers, and three panel lights. One panel light was positioned directly above each retractable lever, and the third panel light was located above the water port. A house light was located on the other side of the box and remained illuminated throughout the testing session.

Animal training prior to drug administration

Rats trained in three phases to reach performance criteria in the sustained-attention task. During the first phase of training, the retractable levers were extended into the chamber at all

times. The water dipper was raised (2.0 s access to 0.01 ml tap water) as reinforcement for each lever press. To minimize a lever bias, five consecutive presses on a lever resulted in the discontinuation of water access until the other lever was pressed. Rats were required to meet a criterion of 120 reinforcers per session for three sessions in order to move to the next training stage.

During the second stage, the rats were trained to discriminate between signals (1 s illumination of the panel light) and nonsignals (no illumination of the panel light). After a signal or no signal, the retractable levers were extended into the chamber. Half of the rats were given water access for pressing the left lever after a signal, which was recorded as a hit. If the rat pressed the right lever after a signal trial, the trial was recorded as a miss. Following no signal presentation, a press on the right lever was recorded as a correct rejection and water access was provided. A press to the left lever was recorded as a false alarm.



Gill et al. (2000), adapted by Pitcairn for current study

The rules of the task were reversed for half of the rats. Incorrect choices were followed by a correction trial that was identical to the previous trial in which only the correct lever was extended for 90 s. If the errors occurred on signal trials, the panel light remained illuminated for

the duration of the lever extension during the forced choice trial. For all trials, if no lever press was made within 3 s after lever extension, the trial was scored as an omission. The intertrial interval (ITI) for this stage of training was 12 s. Criterion for completion of this stage of training was set at 70% hits and 70% correct rejections for three consecutive sessions.

During the final version of the task, three signal durations were used: 250, 80, and 25 ms and correction trials were not included. The presentation of these signal durations were randomly varied in order to increase attentional demands relative to a single signal (Kolega 1987). In addition, the ITI was reduced to 9 ± 3 s. The changes to the signal duration and the ITI were designed to increase attentional demands (Parasuraman et al. 1987; Kolega et al. 1990). Each testing session lasted between 25 and 35 minutes with a total of 126 trials each session (63 signal, 63 nonsignal). The animals trained in this task until a criterion of ≤70% hits on the 250-ms signal, ≤70% correct rejections, and ≥20 omissions for three consecutive sessions was met. Animals were considered prepared for drug administration after reaching criterion. Subjects, apparatus, and animal training prior to drug administration were identical for both experiments.

Acute nicotine administration

Once rats met performance criteria, they were considered prepared for drug administration. In order to examine the effect of acute nicotine administration, rats received a single i.p. injection of saline (n=2) or nicotine (0.1mg/kg n=2 ,0.2mg/kg n=3; dose given as salt). These doses were selected based upon the guidelines set forth by Matta et al. 2008. Injections were administered approximately five minutes before placement in the task, with peak brain nicotine levels occurring approximately 15 mins after injection (Matta et al. 2008).

Modified attention task with distracter

Immediately following drug administration procedures, rats were placed in a modified version of the sustained attention task with the distracter. This task is divided into three equal blocks with 42 trials in each block. In the first block of the task, the sustained-attention task functions as normal, which serves as a baseline reading. In the second block of the task, the house light flashes continuously at a duration of 0.5 second on followed by 0.5 second off. This is considered the distracter. It is disorienting, and increases attentional demands. In the third block, the distracter is removed and the normal task resumes. This allows for assessment of how the rat performs with the distracter present, how the rat's attentional performance recovers from the distracter in the third block. and how rats perform differently across groups and drug doses.

Procedures for nicotine preparation

Nicotine hydrogen tartrate salt (Sigma-Aldrich, CA, USA) was dissolved in 0.9% saline and pH was adjusted to physiological range using NaOH solution. Nicotine solutions were kept cool in a fridge and removed when required. Nicotine was administered no more than six days following preparation.

Behavioral measures and statistical analyses

The number of hits (H), misses (M), correct rejections (CR) and false alarms (FA), and omissions were recorded for each testing session. Omissions are recorded when a rat does not press any lever when both of the choice levers are extended. Each session was divided into three blocks to assess the effect of the drug within each session.

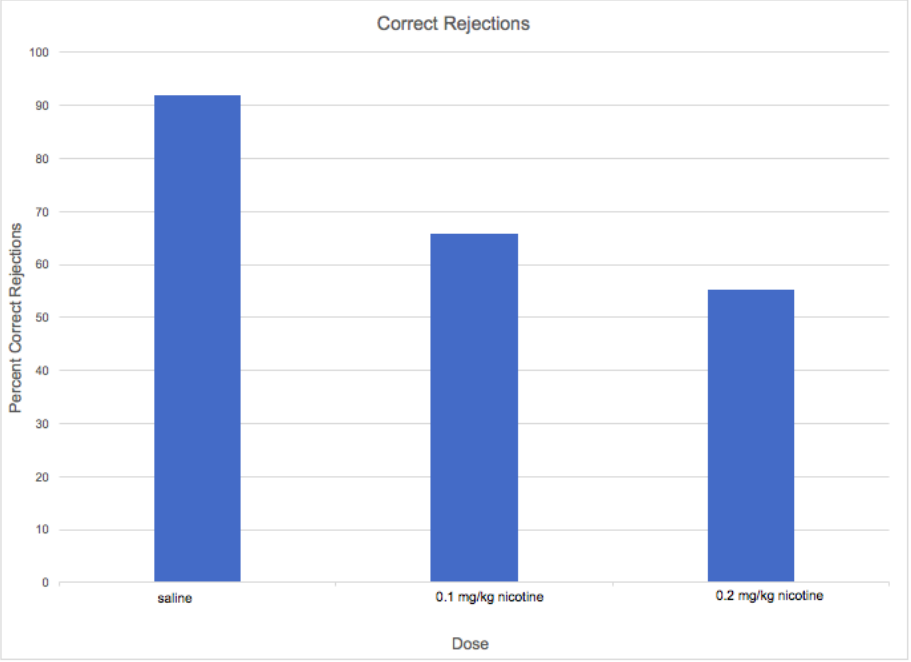
Repeated-measures Analyses of Variance (ANOVAs) were conducted. The factors in each analysis included drug dose (saline, 0.1 mg/kg, 0.2 mg/kg) and block (block 1, block 2,

block 3). For hits, signal duration (250, 80, 25 ms) were included. An alpha level of .05 was used to determine statistical significance in all analyses.

Experiment 1 Results

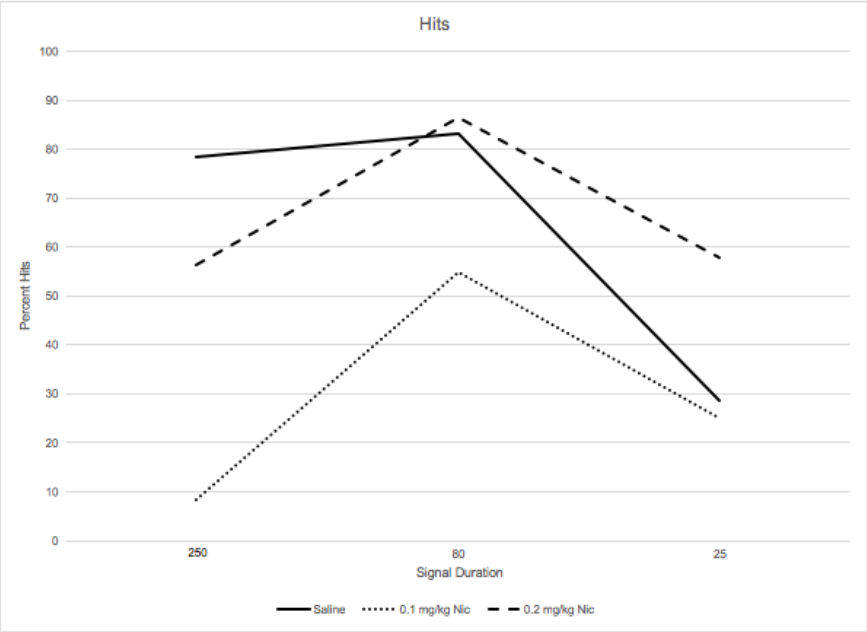
Correct rejections (CR) reflect accuracy on non-signal trials. For correct rejections, a dose X block ANOVA yielded a significant effect of dose ($F(2,13) = 5.314$, $p = 0.021$). The average percent of correct rejections was analyzed per group of nicotine doses (saline, 0.1mg/kg, and 0.2 mg/kg) as shown in Fig. 1. The rats administered saline had the highest percent of correct rejections, indicating the highest accuracy in non-signal trials. The rats administered the 0.1 mg/kg dose had a lower percentage of correct rejections. Rats administered 0.2mg/kg had the lowest percentage of correct rejections, indicating the strongest impairment in performance during non-signal trials.

Figure 1: Acute nicotine administration impairs accuracy in non-signal trials



Hits indicate accuracy on signal trials. The signals used in this experiment were 250, 80, and 25 ms durations. There was a significant signal duration X dose interaction ($F(4, 52) = 4.126$, $p = .01$). The lowest accuracy is seen at the longest signal duration for both nicotine groups (Fig. 2).

Figure 2: Effect of acute nicotine administration on percent hits



Experiment 2 Hypothesis

Based upon the results of the first experiment that showed a deleterious effect of acute nicotine administration, the second experiment was designed to examine chronic nicotine administration. After chronic nicotine administration, rats will be administered a selective orexin-1 antagonist, SB-334867. During five days of chronic nicotine administration, attentional performance is expected to improve. Administration of SB-334867 is expected to block the beneficial effect of chronic nicotine in the sustained-attention task.

Methods: Chronic nicotine and SB-334867 administration

For this experiment, each rat received a single i.p. injection of either nicotine (0.1mg/kg n=5; dose given as salt) or a vehicle (n=2). Nicotine was prepared as previously described, and injections were administered approximately five minutes before placement in the task. Chronic nicotine administration was performed as described in Arnold et al. (2003). The rats were injected once per day for five consecutive days. On day 6, half of the rats in the experimental group received the nicotine injection followed by a single i.p. injection of SB-334867 (3.0 mg/kg) approximately 5 minutes later. This dose of SB-334867 has been documented to show a significant impairment in task performance, particularly during the longest duration signal (Boschen et al. 2009). The other half of rats in the experimental group received nicotine followed by a vehicle. On day 8, the rats that received the SB-334867 on day 6 were instead given a vehicle, and the rats that received the vehicle were given SB-334867. The control group received one injection of saline followed by one injection of vehicle in the same regimen.

Immediately following drug administration procedures, rats were placed in the modified version of the sustained attention task with the distracter. This task is divided into three equal blocks. In the first block of the task, the sustained-attention task functions as it has previously, which serves as a baseline reading. In the second block of the task, the house light flashes continuously. This is considered the distracter, a disorienting stimulus that increases attentional demands. In the third block, the distracter is removed and the normal task resumes. This allows for assessment of how the rat performs with the distracter present, how the rat's attentional performance recovers from the distracter in the third block. and how rats perform differently across groups and drug doses.

Procedures for SB-334867 preparation

The nonpeptide orexin-1 receptor antagonist SB-334867 (Tocris Biosciences, UK) was suspended in a vehicle solution of 1.0 ml saline, 200 mg hydroxypropyl-beta-cyclodextrin, and 125 µl 100% dimethyl sulfoxide. Preparation of SB-334867 occurred immediately prior to the injection.

Behavioral measures and statistical analyses

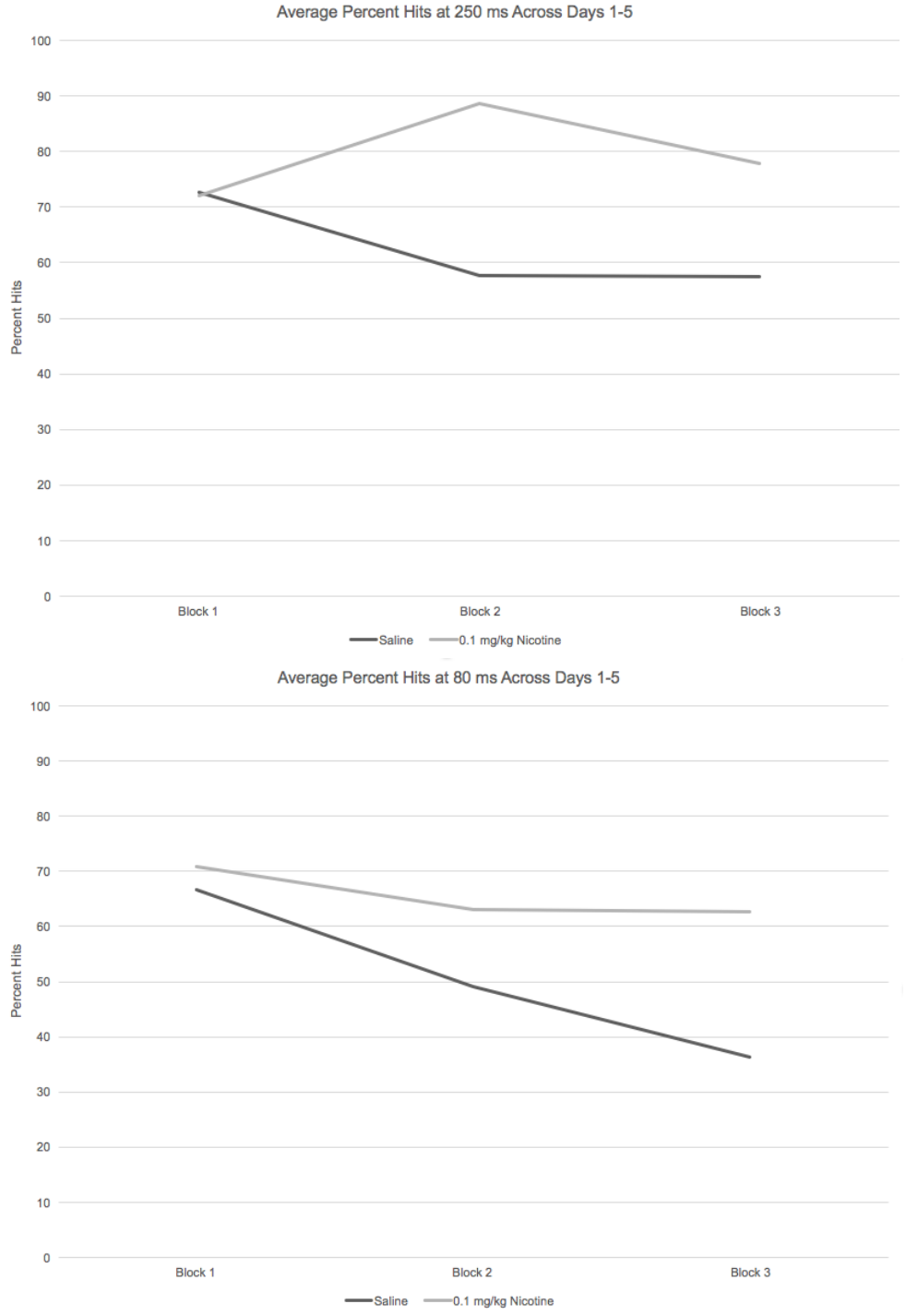
The number of hits (H), misses (M), correct rejections (CR) and false alarms (FA), and omissions were recorded for each testing session. Omissions are recorded when a rat does not press any lever when both of the choice levers are extended. Each session was divided into three blocks to assess the effect of the drug within each session.

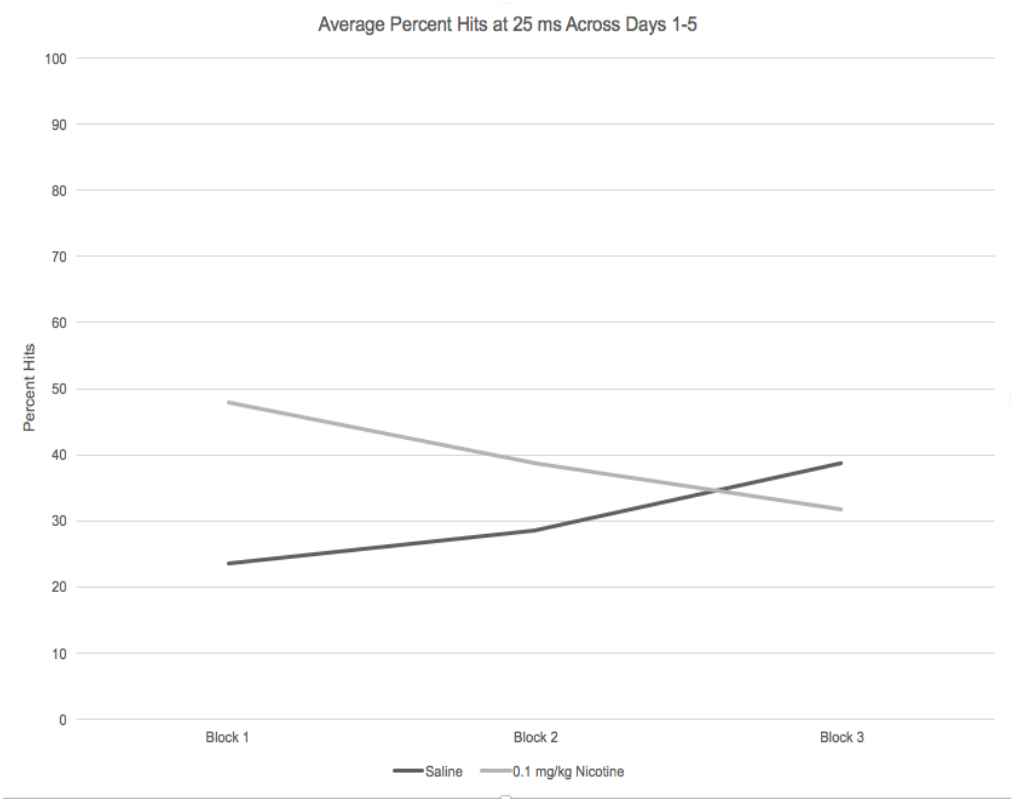
Repeated-measures Analyses of Variance (ANOVAs) were conducted. The factors in each analysis included drug (SB-334867, vehicle) and block (block 1, block 2, block 3). For analyses of hits, signal duration (250, 80, 25 ms) were included. An alpha level of .05 was used to determine statistical significance in all analyses.

Experiment 2 Results

When comparing the average performance between the nicotine and saline groups across five days of chronic administration, the trend shows a slight improvement for the group administered nicotine (Fig. 3). A block X signal duration ANOVA was conducted on the rats treated with nicotine and a significant block X signal duration interaction was observed ($F(4,16) = 3.770$, $p = .024$). However, ANOVA was not conducted between the nicotine and the saline rats due to the small sample size of the saline group, and thus the observations are eported as a trend.

Figure 3: Nicotine groups show trend of improved accuracy compared with saline across five-day chronic nicotine administration at all signal durations

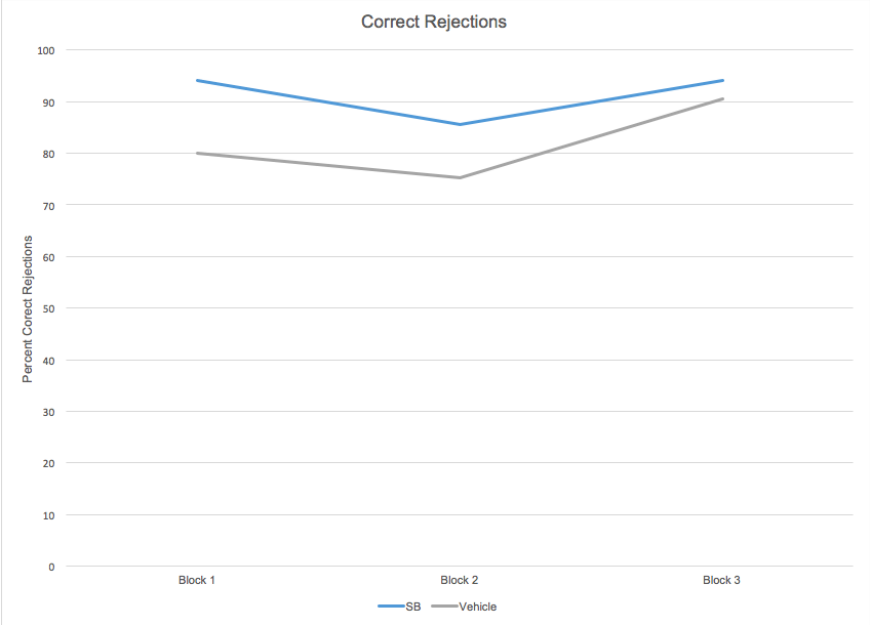




Omissions were also analyzed for the rats administered nicotine. A one-factor ANOVA was conducted to analyze the effect of block and the effect was not significant ($F(2,8) = .862$, $p = .458$).

On the days where SB-334867 was administered, the rats that were administered nicotine performed with higher accuracy in non-signal trials with the treatment of SB-334867 compared to saline (Fig. 4). A drug X block ANOVA was performed in order to assess effects of SB-334867. The effect of the drug was significant ($F(1,4)=10.060$, $p=.034$) and the effect of block was significant ($F(2,8) = 5.998$, $p= .026$). However, the effect of drug X block was not significant ($F(2,8) = .901$, $p = .444$).

Figure 4: SB-334867 treatment improves accuracy in non-signal trials



For hits, the rats performed with higher accuracy when receiving the vehicle rather than the SB-334867 for all signal durations (Fig. 5a, b, c). This is the opposite trend that was observed in the non-signal trials. For hits, a drug X block X signal duration ANOVA yielded a significant effect of drug ($F(1,8) = 17.975$, $p = 0.013$) and signal duration ($F(2,8) = 17.588$, $p = .001$), but it was not significant for drug X block ($F(2,8)= 2.055$, $p = .190$) or drug X signal duration ($F(2,8) = .194$, $p = .827$).

Figure 5a: Percent Hits at 250 ms signal duration

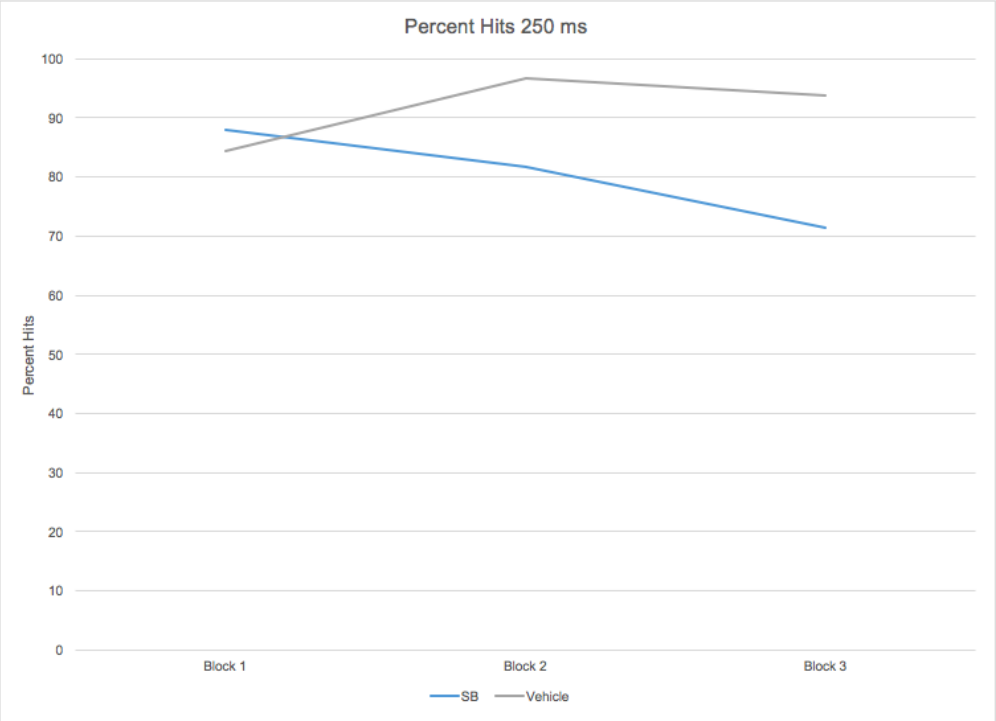


Figure 5b: Percent Hits at 80 ms signal duration

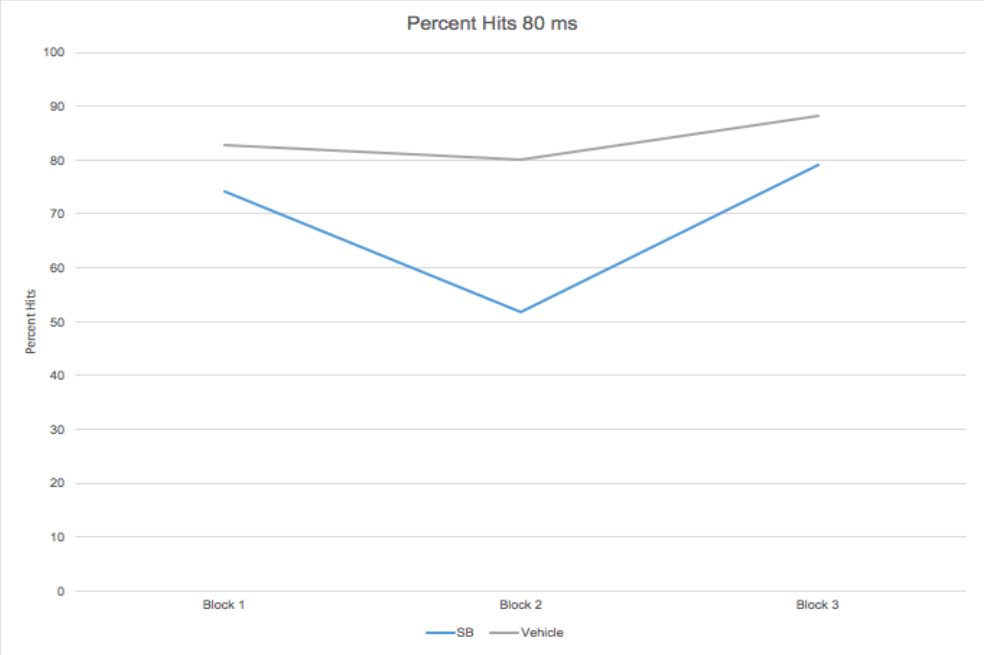
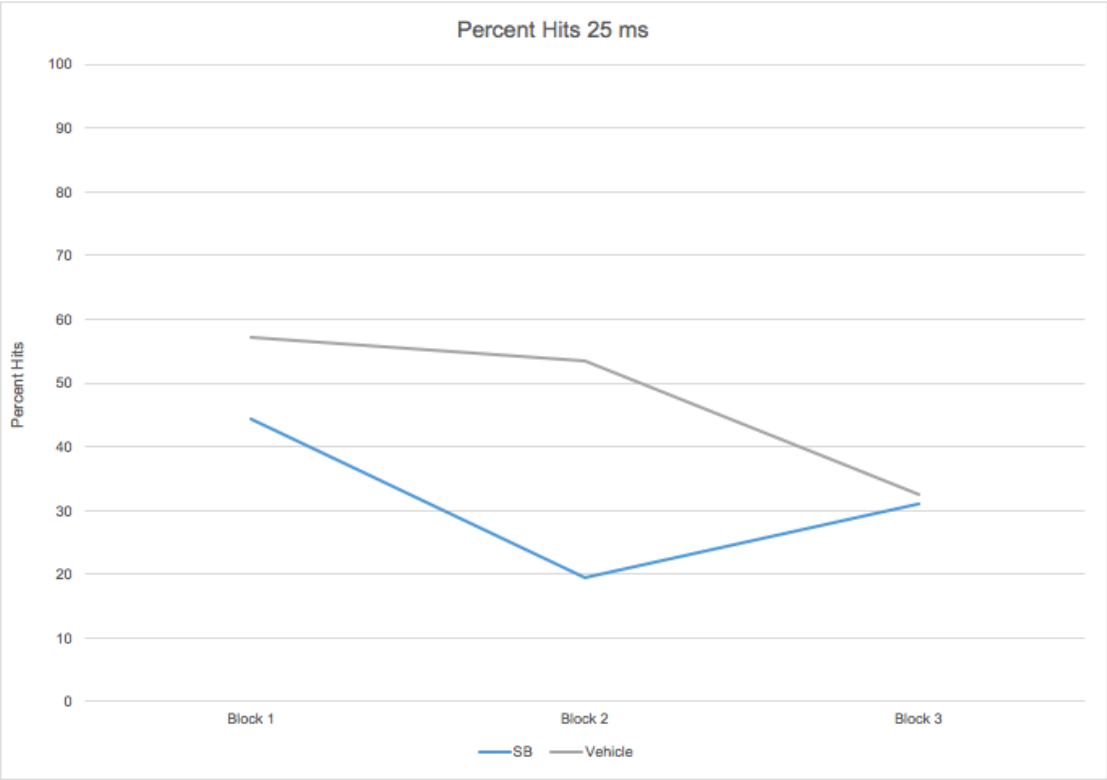


Figure 5c: Percent Hits at 25 ms Signal Duration



Discussion

For the first experiment, the significant effect of dose suggests that acute nicotine administration impairs cognitive functioning in a dose-dependent manner in non-signal trials. When analyzing hits, the lowest accuracy was observed at the longest signal duration. This suggests that the impairment is in processing the rules of the task, rather than an impairment in perception of the signal. This impairment was not seen in the rats that received saline. These results demonstrate that acute nicotine administration impairs cognitive functioning in the sustained-attention task.

In the second experiment, the data from the nicotine group showed a trend of improved performance during the five days of chronic nicotine administration compared to the group that

received a vehicle. The nicotine group also showed no significant effect on omissions, which are recorded when a rat does not press any lever when both of the choice levers are extended. This suggests that the drug treatment did not significantly affect the rats' response rate. With SB-334867 treatment, the opposite trend between correct rejections and hits is likely due to the presence of a side bias, because the same lever that is recorded as a "correct rejection" in non-signal trials is recorded as a "miss" during signal trials. It is possible that during the distracter block the animals mistake the flashing house light for the signal light. This implies that administration of SB-334867 is shifting rat performance and that animals are adopting a simpler cognitive strategy in order to complete the task. Rats are highly trained to reduce a side bias in the training phase, and thus these findings demonstrate the presence of cognitive deficits with treatment of SB-334867. These preliminary data support the hypothesis that chronic nicotine administration slightly improves attentional performance during days 1-5 compared to acute administration and that SB-334867 administration impairs cognitive functioning following nicotine-induced improvement compared to a vehicle.

Although there were significant findings, this study remains limited and further research can assist in elucidating the mechanisms of nicotine-induced cognitive enhancement. Future studies should be conducted using a larger sample size, as well as chronic nicotine at 0.1 mg/kg and 0.2 mg/kg doses rather than a single dose. Additionally, only a single selective antagonist was utilized during the experiment, while other antagonists may have shown stronger effects. For example, Howe et al. 2010 found that nicotine administered alone failed to improve performance of the sustained-attention task, whereas nicotine combined with a selective nAChR antagonist was beneficial. Further studies may also consider administering nicotine into specific brain areas,

such as the LH/PFA, rather than systemic administration, as the LH/PFA are where orexins are localized and appears to be particularly sensitive to nicotine (Pasumarthi and Fadel, 2009).

Nicotine is the most widespread abused drug which interacts with central nAChRs (Zarrindast et al. 2019). The findings of this study could be useful in future clinical applications for those with cognitive and attentional deficits, as seen in schizophrenia and Alzheimer's Disease. In both of these conditions. nicotine has been studied as a therapeutic cognitive-enhancing drug. However, the potential addiction to nicotine and the availability of tobacco products and e-cigarettes are adverse consequences to nicotine therapies. Instead, targeting the orexinergic system may mimic the cognitive-enhancing benefit of nicotine without the harmful effects. Although more research needs to be conducted in order to fully understand the interaction between orexinergic and nicotinic mechanisms of cognitive enhancement, this study gives insight into the behavioral and attentional effects of nicotinic activation and orexin-1 antagonism.

Acknowledgments

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