

**DIFFERENTIATING THE PRIMARY
REINFORCING AND
REINFORCEMENT-ENHANCING EFFECTS OF
VARENICLINE IN A TWO-LEVER, RAT
SELF-ADMINISTRATION PARADIGM**

by

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University of Pittsburgh, 2013

Nicotine (NIC) has two reinforcement-related effects that may contribute to dependence: 1) NIC acts as a primary reinforcer, and 2) NIC, non-associatively, enhances reinforcement from concurrently available nonpharmacological stimuli. Varenicline (VAR), a partial agonist at nicotinic receptors, is one of the most effective smoking cessation pharmacotherapies. Previous findings from our laboratory show that VAR mimics the reinforcement-enhancing effects of NIC. The present study sought to determine the role of these effects in a self-administration paradigm, in which rats were able to lever press for intravenous VAR and/or a moderately reinforcing visual stimulus (VS). For five groups of rats (i.e., the 2-lever groups), responding on one lever was reinforced with VAR (0.01, 0.06 or 0.1 mg/kg/infusion), NIC (0.06 mg/kg/infusion) or saline (SAL), while responding on a separate lever was reinforced with the VS. Three additional groups were reinforced for pressing a single, active lever and received contingent VAR paired with the VS, the VS only (while receiving noncontingent [i.e., yoked] VAR) or VAR only (all doses 0.1 mg/kg/infusion). Responding was maintained on an FR1 for five sessions, an FR2 for six sessions and an FR5 for fifteen sessions. Compared to responding for the VS in the 2-lever SAL group, responding on the active lever was significantly higher in the contingent VAR + VS group but was not significantly higher in the noncontingent VAR + VS group. Responding for VAR only was not significantly different than responding for SAL only in the 2-lever SAL group. Across the 2-lever VAR groups, responding for the VS did not significantly differ from responding for the VS in the 2-lever SAL group. Certain findings of this study support the notion that VAR has reinforcement-enhancing properties, in that the contingent VAR + VS group demonstrated a significant increase in responding for the VS, compared to saline controls. Although the noncontingent VAR + VS group failed to reach

significance, active-lever responding levels were elevated. Furthermore, VAR did not demonstrate primary reinforcing properties. Interestingly, it could be that rats did not respond sufficiently for VAR in the 2-lever groups to engender a reinforcement-enhancement effect.

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I. INTRODUCTION

Tobacco use is currently the leading cause of preventable disease and premature death in the United States [CDC, 2009]; however, quit rates remain low [Haas et al., 2004]. Of the over 4000 chemicals in a cigarette, nicotine is the putative, primary addictive component [USDHHS, 1988]. Research focused around the underlying processes of nicotine is extensive, but there is still much that is unknown [Dani and Harris, 2005]. Although nicotine acts as a primary reinforcer (i.e., behaviors leading to nicotine delivery are strengthened), these reinforcing properties are relatively weak, in comparison to other highly addictive drugs of abuse. An additional process that may contribute to nicotine use is the ability of nicotine to enhance the reinforcing value of nonpharmacological stimuli (i.e., reinforcement-enhancement) [Donny et al., 2003].

Varenicline (Chantix[®]) is the most effective smoking cessation pharmacotherapy available [Jorenby et al., 2006]. There are a number of other smoking cessation aids on the market, including nicotine-replacement therapy (patch, lozenge, etc.) and sustained-release bupropion (Zyban[®]), but quit success rates are relatively low among these other pharmacotherapies [Cahill et al., 2008, Stead et al., 2008, Hughes et al., 2007]. Previous research demonstrates that varenicline reduced craving and symptoms of withdrawal significantly more than bupropion in a clinical trial [Gonzales et al., 2006] and was more effective than nicotine-replacement therapy during a cessation period [Aubin et al., 2008]. Those participants in the latter experiment, who were unable to remain abstinent but continued to take varenicline, reported a reduction in hedonic value when smoking, compared to participants who also did not remain abstinent but received nicotine-replacement therapy. Furthermore, the abuse liability of varenicline is very low, with the highest therapeutic dose (3mg) demonstrating an abuse liability similar to placebo [McColl et al., 2008].

Data suggest that the therapeutic efficacy of varenicline is due to its ability to substitute for and/or block the reward value of nicotine [Rollema et al., 2007b]. These actions seem to be a result of the partial agonist characteristics of varenicline on $\alpha 4\beta 2$ nicotinic acetylcholine receptors

(nAChRs) in mid-brain reward systems [Foulds, 2006, Mihalak et al., 2006, Rollema et al., 2007b]. Low to moderate doses of varenicline mimic the action of nicotine at nAChRs, while higher doses inhibit the effects of nicotine. One study investigated this hypothesis by looking at brain-stimulation reward (BSR) threshold in rats [Spiller et al., 2009]. Nicotine and low doses of varenicline reduced BSR threshold, while higher varenicline doses increased BSR thresholds. Combining nicotine with varenicline attenuated nicotine-enhanced BSR. Mecamylamine, an antagonist at non- $\alpha 7$ nAChRs, and dihydro- β -erythroidine, an antagonist at $\alpha 4$ nAChRs, blocked the enhancement of BSR seen with varenicline, but this effect was not blocked by methyllycaconitine, an antagonist at $\alpha 7$ nAChRs. In summary, reward function (i.e., BSR threshold) increased when lower doses of varenicline were administered in isolation and decreased when higher doses of varenicline were administered, in combination with nicotine. The results from this study also demonstrated that $\alpha 4$ -containing nAChRs mediated the decrease in BSR threshold of varenicline, whereas $\alpha 7$ nAChRs did not.

Data from our laboratory also support the partial agonist properties of varenicline, in regards to reward function. Two studies assessed the effects of subcutaneous-administered varenicline, alone and in combination with nicotine, while rats pressed a lever for a moderately reinforcing visual stimulus (VS) [Levin et al., 2012]. In the first of the two studies, rats were assigned to one of eight groups [saline, nicotine (0.4 mg/kg), varenicline (0.1, 0.3 or 1.0 mg/kg) or nicotine (0.4 mg/kg) + varenicline (0.1, 0.3 or 1.0 mg/kg)] and received drug before each daily 1-hour session for 14 days. In the second of the two studies, a broader dose range of varenicline was utilized. Rats were assigned to one of six groups [saline, nicotine (0.4 mg/kg) or varenicline (0.01, 0.1, 1.0 or 3.0 mg/kg)] and received 14 sessions of drug exposure. Across both studies, varenicline demonstrated a dose-dependent effect, via VS presentations earned. The 0.1 and 1.0 mg/kg varenicline groups exhibited the highest number of VS presentations, showing similar levels of enhanced responding for the VS as nicotine-treated animals, while the 0.01 and 3.0 mg/kg varenicline groups exhibited levels of responding for the VS that were not significantly different from saline-treated animals. In the nicotine + varenicline groups, varenicline dose-dependently attenuated the reinforcement-enhancing effects of nicotine, with the highest dose (1.0 mg/kg) exhibiting the greatest antagonist effects. The results of these studies are consistent with the partial agonist characteristics of varenicline and support the assertion that the therapeutic efficacy of varenicline may be related to its ability to partially replace the reinforcement-enhancing effects of nicotine at lower doses and inhibit the enhancing effects of nicotine at higher doses.

To date, a limited number of studies have addressed whether varenicline is a primary reinforcer

(i.e., supports operant responding in isolation) [Rollema et al., 2007a, Paterson et al., 2010]. The few studies that have looked at varenicline self-administration found that rats will self-administer the drug but to a lesser degree than rats self-administering nicotine, which corroborates the partial agonist properties of the drug. These studies, however, paired varenicline infusions with a cue light(s) and therefore did not accurately capture the primary reinforcing value of varenicline. Unpublished data from our laboratory also demonstrate a significant increase in varenicline self-administration, compared to rats responding for saline. All drug infusions, however, were paired with the VS, and this confound does not allow for a thorough interpretation of the reinforcing mechanisms underlying varenicline. In other words, animals responded on the same lever for both the drug and VS, and increased responding could therefore be the result of primary reinforcement, reinforcement-enhancement or an amalgam of the two effects. In the current study, we consequently isolated the potential primary reinforcing effects from the potential reinforcement-enhancing effects. This enabled us to adequately address the extent to which primary reinforcement plays a role in varenicline self-administration. In doing so, we utilized a self-administration paradigm but dissociated responding for varenicline and the VS, via separate levers. This allowed rats to concurrently respond for either varenicline or the VS. Utilizing a 2-lever self-administration paradigm separates the two reinforcers animals can respond for and therefore differentiates the reinforcing value of both stimuli [Palmatier et al., 2006].

II. METHODS

A. GENERAL METHOD

1. Subjects

Male Sprague-Dawley rats (Harlan Farms, Indianapolis, IN), weighing between 200-225g upon arrival, were used for the study. Rats were singly housed in hanging mesh wire cages in a colony room that was temperature-controlled between 68 and 70°F. Animals were on a reverse 12-hr light/dark cycle with lights off from 7am-7pm. There was unlimited access to water. Unrestricted chow was provided for the first week after arrival, and the animals were handled and weighed on a daily basis. Following this period, food was restricted to 15g/day for one week while animals continued to be weighed and underwent habituation and training in the operant chambers. After training was complete, diets were restricted to 20g/day [Donny et al., 1995].

2. Apparatus

Experimental testing took place in 15 operant conditioning chambers. The chamber dimensions were 25cm X 31cm X 28cm, for the width, length and height, respectively. There were two retractable levers on one wall, located approximately 2cm from the floor of the chamber. A food pellet dispenser and trough was located between the two levers and was also approximately 2cm from the floor of the chamber. There was a houselight on the same wall, approximately 1cm from the ceiling of the chamber. A white stimulus light was located above both levers but was only activated above the assigned active or VS lever as part of the moderately reinforcing visual stimulus (VS) [Palmatier et al., 2007]. Additional details for the VS are described below in Procedures. Each chamber was also equipped with a syringe pump, which was connected to Tygon[®] tubing, which in turn was connected to the rats catheter and delivered intravenous infusions of the assigned

drug solution.

3. Drugs

Nicotine hydrogen tartrate salt (Sigma, St. Louis, MO) and varenicline tartrate (Pfizer Inc.) were dissolved in 0.9% saline. The dose of nicotine used for self-administration was 0.06 mg/kg/infusion (reported as free base) and was chosen because it reliably supports self-administration [Donny et al., 2003]. The doses of varenicline used were 0.01, 0.06 and 0.1 mg/kg/infusion (reported as free base) and were chosen based on previous varenicline self-administration studies [Rollema et al., 2007a, Paterson et al., 2010]. All solutions were adjusted to a 7.0 (± 0.2) pH level with NaOH and sterilized by being passed through a 0.22 μ m filter.

4. Procedures

The following procedures follow the standard methodology used in our laboratory with a few minor training adjustments [Palmatier et al., 2006].

a. Habituation Animals were placed into the operant chambers unleashed for a 20 min habituation period before training began. During this period, levers were retracted. Houselights were on while rats were in the chamber but were illuminated red to limit visibility.

b. Magazine Training Following habituation to the chambers, rats went through two days of magazine training, in which the association between the sound of the food dispenser and the delivery of food was established. Animals were placed in the chamber for approximately 1 hour, and food pellets were delivered approximately every minute (i.e., 45-75 sec) for a total of 60 pellets. The levers were retracted, and the red houselight remained on throughout the session.

c. Autoshaping There were two autoshaping programs over the course of 4 successive days (2 days of the first program followed by 2 days of the second program). The first program consisted of a 1-hour session in which rats received randomized 15-sec extensions of each lever, followed immediately by delivery of 1 food pellet. Pressing the lever during the 15-sec extension resulted in the delivery of 2 pellets. During the second program, rats received randomized extensions of each lever, and the rats needed to press the lever to end the trial and receive a food pellet. Rats

that received <30 pellets were handshaped to press both levers at the end of the first day. All rats then completed a second day of this autoshaping program to ensure those rats that handshaped were able to receive ≥ 30 pellets within the 1-hour session. During both autoshaping programs, the houselights were illuminated red.

d. Surgery and Recovery During surgery, the rats were anesthetized with isoflurane and implanted with jugular catheters. An analgesic agent (Bupivacaine) was applied to the incision sites before suturing. After surgery, rats recovered for a minimum of 5 days in their home cages. The cannulae were flushed once daily with 0.1ml of sterile saline containing heparin (30 U/ml), Timentin (66.67 mg/ml) and streptokinase (8333 U/ml) to maintain catheter patency and prevent infection. When surgeries began, all animals were placed on restricted feeding schedules of 20g per day, and this feeding schedule was maintained for the remainder of the study. See the Catheter Construction, Catheter Maintenance and Antibiotic Treatment and Testing Catheters sections below for further details.

e. Experimental Drug Phase After a minimum of 5 days of recovery from surgery, animals began the experimental drug phase. Rats were assigned to one of eight groups ($n=11$), and the groups were as follows: 2-lever saline or VS (2L SAL), 2-lever nicotine or VS (2L NIC), 2-lever 0.01 mg/kg varenicline or VS (2L .01 VAR), 2-lever 0.06 mg/kg varenicline or VS (2L .06 VAR), 2-lever 0.1 mg/kg varenicline or VS (2L 0.1 VAR), 0.1 mg/kg varenicline + VS (VAR+VS), yoked 0.1 mg/kg varenicline + VS (YOKED VAR+VS) and 0.1 mg/kg varenicline only (VAR only). For all 2-lever groups, the left lever was the infusion lever, and the right lever was the VS lever. For the remaining three groups, the left lever was the active lever (i.e., responses on this lever had a consequence), and the right lever was the inactive lever (i.e., responses on this lever had no consequence). Responses on the active lever in the VAR+VS group resulted in animals receiving an infusion + the VS. Responses on the active lever in the YOKED VAR+VS and VAR only groups resulted in an infusion only. Animals in the YOKED VAR+VS group received infusions but based off of a paired animal in the VAR+VS group, i.e., when an animal in the VAR+VS group received an infusion for lever presses, its paired animal also received an infusion. The VS is established in our laboratory as maintaining modest rates of responding in rats, and when nicotine is present, this effect is strongly enhanced [Donny et al., 2003]. The VS consisted of illuminating the stimulus light located above the assigned VS lever for 1 sec and offset of the houselight for 1 min. During

this 1-min period, VS lever pressing had no consequence (i.e., a timeout period). There was also a timeout period after each infusion earned on the infusion lever. Responses on the inactive lever had no consequence throughout the session. Groups were exposed to an FR1 schedule of reinforcement for 5 1-hour sessions, an FR2 for 6 1-hour sessions and an FR5 for 15 1-hour sessions.

5. Catheter Construction

Catheters were constructed of a 16cm piece of silastic tubing (0.012 in ID x 0.025 in OD, Dow Corning) and a threaded pedestal bolt (C313G- L20-3UP, Plastics One). The bolt was connected to a 16cm piece of silastic tubing by placing the silastic in Fisherbrand[®] Citrisolv[®] Solvent and Clearing Agent for 30-60 sec and then passing it over 10mm of the bolt. The tubing was then immediately and thoroughly rinsed with distilled water to remove any excess solvent and clearing agent. This was done by flushing 2ml of distilled water through the lumen and rinsing the tubing in 2 separate beakers of distilled water before placing the tubing in a large bath of distilled water for several minutes. A 5mm piece of large silastic tubing (0.04 in ID x 0.085 in OD, Dow Corning) was then placed over the joint and secured with liquid silicon to anchor the bolt and silastic together. A 2 x 2 cm piece of surgical mesh was placed at the base of the bolt and secured with dental cement. At the base of the pedestal, aquarium silicon sealant was placed around the joint between the silastic tubing and the stainless steel tubing. This was done to seal the silastic to the pedestal and strengthen the wall of the silastic tubing where it left the pedestal. Two silicon knobs were added to the catheter and used as anchors during the surgical procedures. Knobs were placed 4cm and 6cm from the tip of the silastic. All catheters were tested by filling the catheter with distilled water and then, using a 21G needle and syringe attached to the bolt, placing slight to moderate pressure on the syringe while plugging the silastic shut. If there were any leaks in the catheter or if the silastic bulged out, the catheter was remade. Placing a large amount of pressure will cause the silastic to bulge (i.e., creating a weak spot even if one was not there before testing), therefore, caution was taken not to damage the silastic. The silastic tip was cut down to 38mm (from end of heat shrink to tip) with a blunt (not beveled) tip. All catheters were sterilized for a minimum of 1 week prior to implantation. The night before surgery, catheters were placed in the fume hood for aeration. Catheters have a dead volume of about 16:1.

6. Catheter Maintenance and Antibiotic Treatment

Animals were treated with both heparin and streptokinase in order to help maintain catheter patency and with Timentin to help fight off post-surgical infections. In each case, the flushing volume was 0.1ml per rat, IV. The appropriate solution varied as a function of the stage of the experiment and occurred as follows:

During Surgery: 1U/ml heparin

First 4 days post-surgery: Flushed animals once per day with solution containing: (per ml) 66.67mg Timentin, 30U of heparin and 8,333U of streptokinase

For remainder of experiment: Flushed animals once per day with solution containing: heparin/Timentin solution

For slow catheter (used as needed): 30U heparin + 8,333U Streptokinase/ml

7. Testing Catheters

A catheter patency test was conducted after completion of the self-administration phase. For testing patency, blood draw was initially attempted. Catheters that failed to give blood were infused with a solution of chloral hydrate. Animals that did not display ataxia were noted as failing the patency test. A total of 12 animals failed the patency test and were excluded from data analysis.

8. Data Analyses

Number of VS presentations and/or infusions earned was the primary dependent variable. Data were analyzed using analyses of variance (ANOVA). The schedules of reinforcement were split into 3 analyses, due to the different number of days on each schedule. A two-way, repeated measures ANOVA was conducted for each phase, as well as the last 5 sessions of the FR5 phase, to take into account the linear effect of Session and Group. Planned pairwise comparisons focused on the effects of varenicline or nicotine, relative to saline. Alpha was set at 0.05.

III. RESULTS

Mean VS presentations for each group across each experimental drug phase are displayed in Figure 1, while mean infusions earned for each group across each phase are displayed in Figure 2. The statistical analyses are expanded upon below.

A. FR1 EXPERIMENTAL DRUG PHASE (5 SESSIONS)

Group differences began to emerge during this phase of the experiment. Statistical analyses revealed that the number of VS presentations earned failed to significantly increase linearly with session in the first 5 sessions under an FR1 ($p=0.79$), but there was a significant interaction between group and the linear change over sessions (i.e., a session X group interaction, $F_{6,62}=3.43$, $p<0.01$), indicating group differences were not stable throughout this phase of the experiment. Relative to the saline group, VS presentations increased linearly in the 2L NIC ($p<0.05$) and VAR + VS ($p<0.05$) groups. The VAR only group was not included in this analysis because these rats were only able to respond for infusions.

Infusions earned increased linearly with session during this phase ($F_{1,62}=87.1$, $p<0.001$), while there was not a significant session X group interaction ($p=0.13$). Relative to the saline group, infusions increased linearly in all groups [2L NIC ($p<0.01$), 2L .01 VAR ($p<0.001$), 2L .06 VAR ($p<0.001$), 2L 0.1 VAR ($p<0.001$), VAR+VS ($p<0.01$) and VAR only ($p<0.001$)]. The YOKED VAR + VS was not included in this analysis because these rats were not given the opportunity to respond for infusions but instead received them based off of a randomly assigned rat in the VAR + VS group.

B. FR2 EXPERIMENTAL DRUG PHASE (6 SESSIONS)

During this phase of the experiment, the number of VS presentations earned increased linearly with session ($F_{1,62}=22.53$, $p<0.001$), but there was not a significant interaction between group and the linear change over sessions ($p=0.53$), indicating stable differences among the groups during this phase. Relative to the saline group, VS presentations increased linearly in all groups [2L NIC ($p<0.01$), 2L .01 VAR ($p<0.01$), 2L .06 VAR ($p<0.01$), 2L 0.1 VAR ($p<0.001$), VAR+VS ($p<0.01$) and YOKED VAR + VS ($p<0.05$)].

Infusions did not increase linearly with session ($p=0.12$), but there was a significant session X group interaction ($F_{6,61}=25.88$, $p<0.01$). Relative to the saline group, infusions only increased linearly in the VAR only group ($p<0.05$).

C. FR5 EXPERIMENTAL DRUG PHASE (15 SESSIONS)

The number of VS presentations earned during the FR5 experimental phase increased linearly with session ($F_{1,61}=34.93$, $p<0.001$), and there was a significant interaction between group and the linear change over sessions ($F_{6,61}=5.97$, $p<0.001$), indicating group differences were not stable throughout the entirety of this phase. Pairwise comparisons revealed that the 2L NIC ($p<0.001$) and VAR + VS ($p<0.01$) groups demonstrated significantly higher VS presentations earned, relative to the 2L SAL group, while all other groups failed to reach significance. The last 5 sessions of this phase were also analyzed in isolation, due to stable responding. The number of VS presentations here increased linearly with session ($F_{1,61}=11.61$, $p<0.001$), but there was not a significant interaction between group and the linear change over sessions ($p=0.75$), indicating group differences were stable by this point in time. Pairwise comparisons, again, revealed that the 2L NIC ($p<0.001$) and VAR + VS ($p<0.001$) groups demonstrated significantly higher VS presentations earned, relative to the 2L SAL group, while all other groups failed to reach significance (see Figure 3).

Infusions also increased linearly with session ($F_{1,62}=19.9$, $p<0.001$), and there was a significant session X group interaction ($F_{6,62}=10.63$, $p<0.001$). Pairwise comparisons revealed that the 2L NIC ($p<0.001$) and VAR + VS ($p<0.001$) groups demonstrated significantly higher VS presentations earned, relative to the 2L SAL group. All other groups again failed to reach significance. During the last 5 sessions of this schedule, infusions increased linearly with session ($F_{1,62}=5.72$, $p<0.05$), but

there was not a significant interaction between group and the linear change over sessions ($p=0.08$), indicating group differences were stable by this point in time. Pairwise comparisons revealed that the 2L NIC ($p<0.001$) and VAR + VS ($p<0.001$) groups demonstrated significantly higher VS presentations earned, relative to the 2L SAL group, while all other groups again failed to reach significance (see Figure 4).

The only groups that demonstrated significantly higher responding for drug and the VS, compared to saline, were the 2L NIC and VAR + VS groups. Although animals in the YOKED VAR + VS group received the same number of infusions as their pair in the VAR + VS group, they still failed to reach significance for VS presentations earned ($p=0.17$), which was also the case for the last 5 sessions of this schedule ($p=0.09$). Despite this, the means of the two groups were still different across the entirety of this schedule (2L SAL VS mean=7.32, SEM= ± 1.83 ; YOKED VAR + VS active mean=11.24, SEM= ± 2.02) and during the last 5 sessions (2L SAL VS mean=7.98, SEM= ± 1.67 ; YOKED VAR + VS active mean=12.4, SEM= ± 1.85), suggesting this analysis may have been significant with a larger n (one animal was lost in the YOKED VAR + VS group, due to failed patency).

IV. DISCUSSION

The primary aim of this study was to assess two potential actions of varenicline in an appropriate model that can distinguish them. The findings of this study show that responding was significantly higher in the VAR + VS group than responding for the VS in the 2L SAL group, supporting the notion that varenicline has reinforcement-enhancing properties. This effect, however, was not seen in the YOKED VAR + VS group. Furthermore, varenicline did not demonstrate primary reinforcing properties, in that responding for varenicline was not significantly different than responding for saline. Similarly, rats did not respond sufficiently for the drug in the 2L VAR groups, which could have led to the lack of reinforcement-enhancement in these groups; responding for the VS across the 2L VAR groups did not significantly differ from responding for the VS in the 2L SAL group. It was hypothesized that varenicline would demonstrate a reinforcement-enhancement effect across all varenicline groups that had concurrent access to the VS and would also demonstrate modest primary reinforcing effects. These hypotheses were, in part, supported. Varenicline engendered reinforcement-enhancement in the VAR + VS group but no other varenicline groups. Additionally, varenicline did not exhibit primary reinforcement.

An intriguing outcome of this data is that the reinforcement-enhancement effect was not witnessed in 2L VAR groups but was witnessed in the VAR + VS group. A possible explanation for this is that the primary reinforcing value of varenicline is not adequate enough to engender responding in rats, and, in turn, receive enough drug to provoke increased responding for the VS. As seen in Figures 2 and 4, animals in the 2L VAR groups were receiving an approximate mean of one infusion per session during the FR5 phase of the experiment. The data therefore suggest that this is an inadequate amount of drug to engender enhancement. As for the VAR + VS group, initial moderate responding for the VS may have, in turn, exposed the rats to the adequate amount of varenicline necessary in order to engender reinforcement-enhancement for the VS (i.e., rats in this group received enough of the drug to generate an enhancing effect).

These results also suggest that varenicline does not have primary reinforcing value. This is evident in both the 2L VAR groups and the VAR only group. As previously mentioned, rats in the 2L VAR groups seem to have not responded enough on the infusion lever, in order to allow for a reinforcement-effect of the VS to occur. Response rates for varenicline in the VAR only group were equally as low and not significantly different than animals responding for saline. These data are important in demonstrating that although varenicline has been reported as being reinforcing, it seems as though this is not due to primary reinforcement of the drug.

A possible explanation for what drove increased self-administration in the Rollema et al. [Rollema et al., 2007a] and Paterson et al. [Paterson et al., 2010] experiments could have been that there was a cue paired with varenicline. As was in our aforementioned unpublished data, these experiments did not allow for an infusion of varenicline in isolation. Although these cues varied from the VS used in our experiments, this does not mean they did not have reinforcing value and/or facilitated learning and therefore contributed to an increase in response rates. Truly differentiating a mildly reinforcing nonpharmacological cue and a varenicline infusion, which was done in the current experiment, helps to better understand the mechanisms driving this increase in behavior.

A limitation of these studies is the use of nicotine-naïve rats, as opposed to rats that are chronically exposed to nicotine. Prior research suggests that repeated exposure to nicotine leads to neuronal adaptations, including desensitization and up-regulation of nAChRs [Quick and Lester, 2002]. It is also thought that chronic nicotine administration leads to tolerance of the drugs pharmacological effects [Benowitz, 2008]. Varenicline is a pharmacotherapy that is targeted toward a population who is chronically exposed to nicotine and was designed to aid smoking cessation for this group. It is therefore important to utilize a similar paradigm in a preclinical model (i.e., a model that incorporates the neuroadaptations that occur with chronic nicotine exposure).

Despite the known adverse health outcomes of smoking, prevalence remains high [CDC, 2009]. This may be, in part, due to the reinforcement-enhancing properties of nicotine. If this is the case, it is crucial to better understand the mechanisms underlying these properties of nicotine and the role of smoking cessation pharmacotherapies in replacing or reducing these effects. This approach may lead to ways in which researchers can improve current smoking cessation aids, such as varenicline, and/or develop novel pharmacotherapies.

V. FUNDING

The National Institutes of Health (DA-10464 and DA-24801) supported this work, and Pfizer, Inc. generously donated varenicline.

VI. DECLARATION OF INTERESTS

Pfizer, Inc. generously donated varenicline.

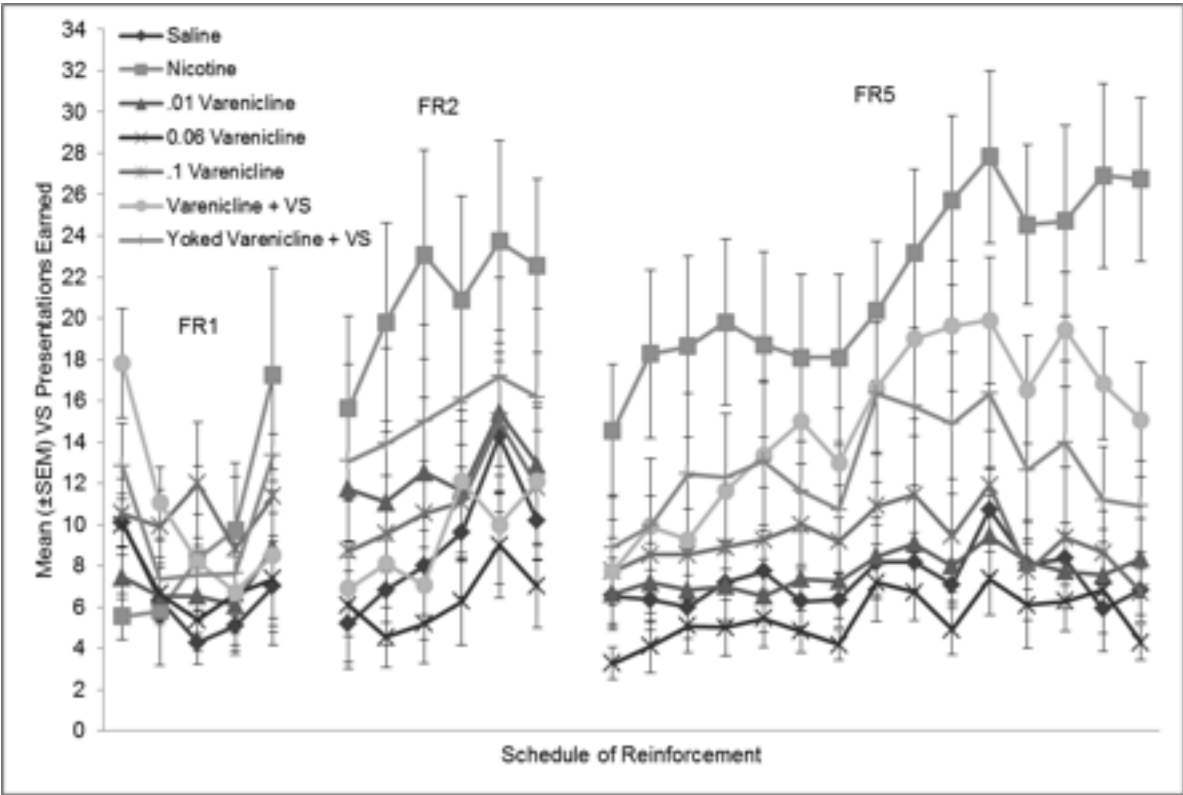


Figure 1: Mean VS presentations earned over testing sessions for each schedule of reinforcement. All groups are represented in the figure legend.

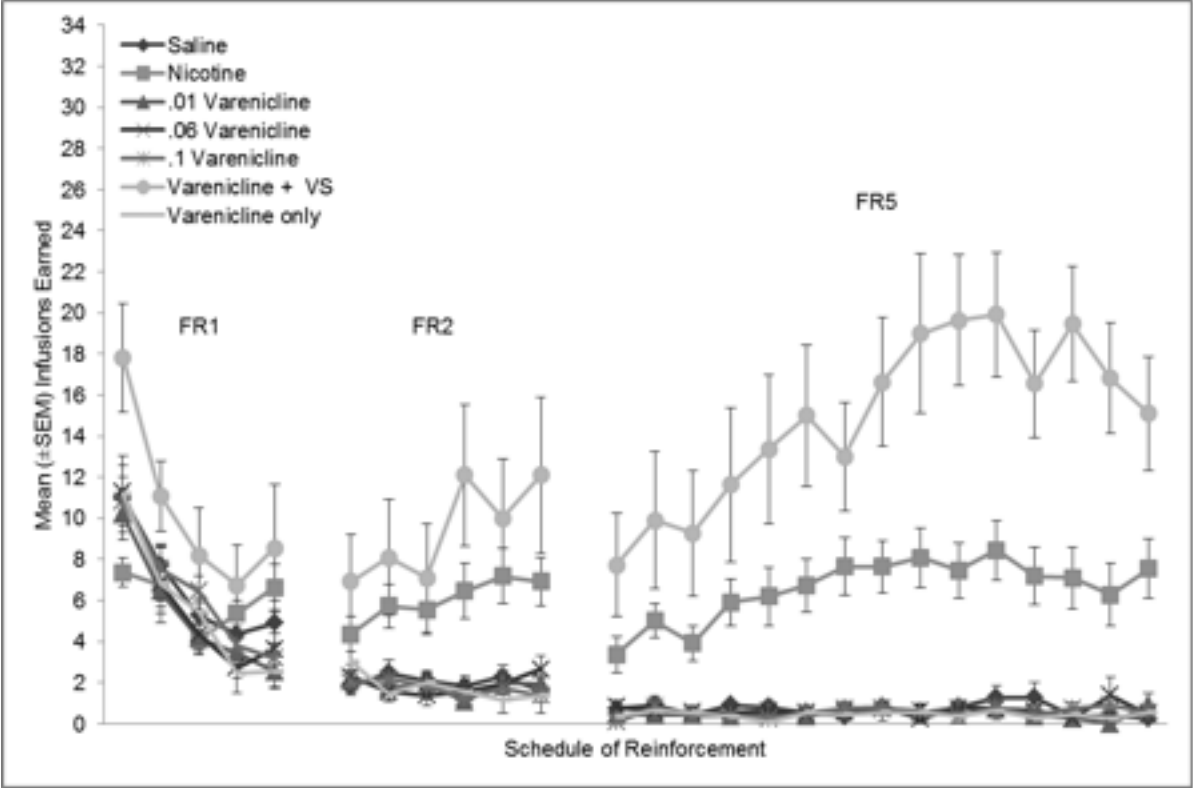


Figure 2: Mean infusions earned over testing sessions for each schedule of reinforcement. All groups are represented in the figure legend.

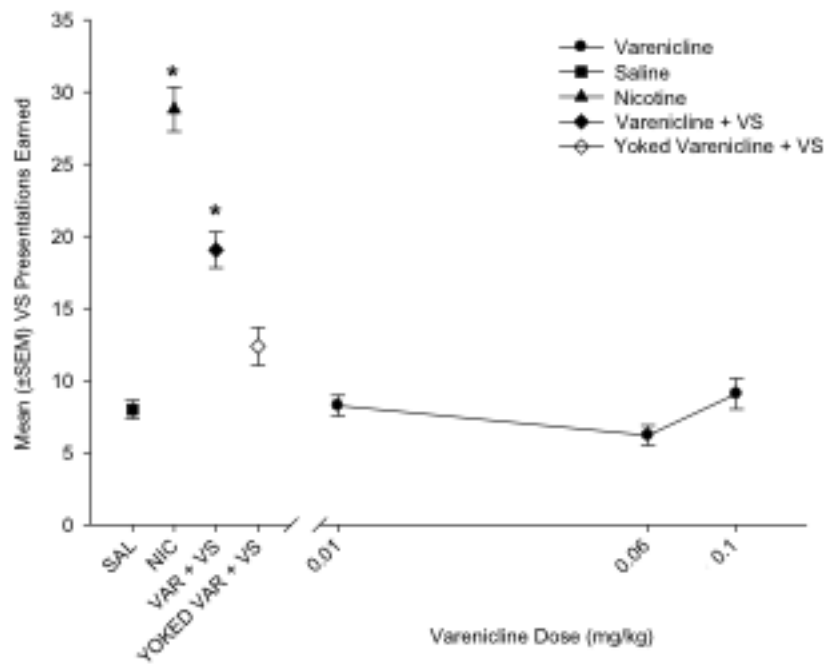


Figure 3: Mean number of VS presentations earned over last 5 sessions of FR5. *indicates significant difference from saline ($p < 0.05$). The 2L NIC and VAR + VS groups showed increased responding for the moderately reinforcing stimulus, while all other groups failed to reach significance.

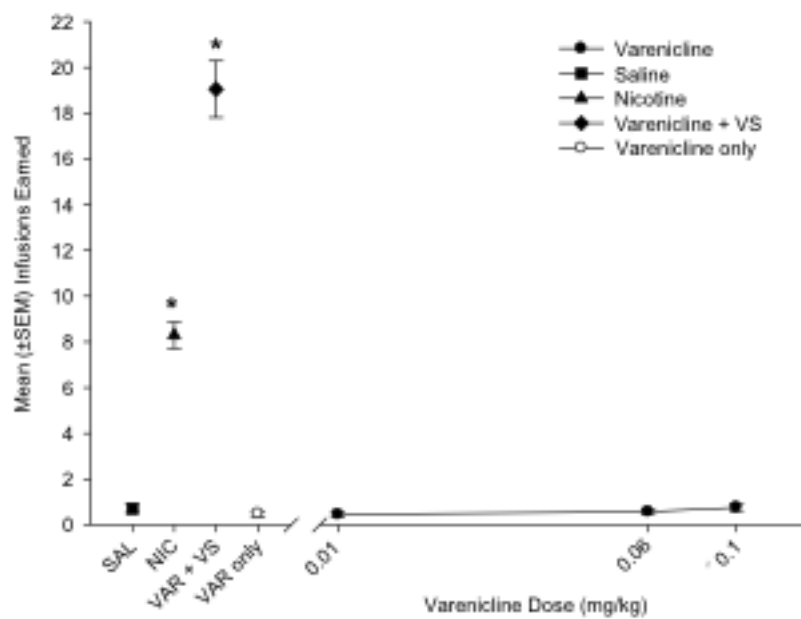


Figure 4: Mean number of infusions earned over last 5 sessions of FR5. *indicates significant difference from saline ($p < 0.05$). The 2L NIC and VAR + VS groups showed increased responding for infusions, while all other groups failed to reach significance.

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