A comparison of brain and behavioral effects of varenicline and nicotine in rats

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1. Introduction

Varenicline is a partial agonist at the α4β2 nicotinic acetylcholine receptor [1,2] and is used as an aid to smoking cessation, resulting in smoking cessation rates that are higher than those achieved with nicotine replacement products such as the patch, gum and spray [3,4]. Varenicline was developed based on the hypothesis that a partial agonist would help smokers to quit by blocking the impact of nicotine when smoking again, while on the other hand still providing some stimulation of the reinforcement of nicotine during a lapse [5]. Several clinical studies have shown that varenicline reduces the severity of nicotine withdrawal and attenuates the reinforcement of nicotine during a lapse [5]. Since varenicline and nicotine both act at nicotinic acetylcholine receptors, it is of interest to compare in vivo effects of varenicline and nicotine that contribute to reducing withdrawal symptoms and help to prevent relapse to smoking. Results from preclinical in vitro and in vivo studies are consistent with partial agonist properties of varenicline.

It binds with much higher affinity and significantly lower intrinsic efficacy to α4β2 nAChRs than nicotine [1]. In vivo, varenicline produces lower maximal mesolimbic dopamine release than nicotine, attenuates the nicotine-induced dopamine release to the level seen with varenicline alone, and reduces nicotine self-administration in rats [1]. We conducted studies in rats to learn more about differences in the actions of varenicline and nicotine on the brain using blood oxygenation level dependent (BOLD) contrast. With repeated dosing, nicotine causes sensitization, manifested as stronger peak BOLD activation in the hippocampus (HP), prefrontal cortex (PFC) and ventral tegmental area (VTA), and more prolonged activation in the HP, NAcc, PFC and VTA [6], all part of the greater limbic system which is involved with addiction [7]. Sensitization is thought to be key to nicotine's addictive potential [8,9] and since varenicline has no abuse potential [10], we hypothesized that varenicline would not induce locomotor sensitization. In this report, we examined the effects of the first dose and repeated doses of varenicline.
on brain activation and locomotor activity and contrast the results with those of our prior experiments with nicotine [6].

Finally, since there has been interest in the potential therapeutic use of nicotinic agents e.g. for Attention Deficit Disorder (ADD) [11], this study also evaluated the impact of varenicline on performance in the Morris water maze model. ADD is a well established risk factor for smoking among adolescents [12], and youths with ADD may be using nicotine to self-medicate symptoms of inattention. However, nicotine is too addictive to be used as a therapeutic and based on a previous report [13] we hypothesized that varenicline would improve cognitive functioning.

2. Materials and methods

2.1. Animal care

Subjects were male Sprague-Dawley rats (250–350 g) obtained from Charles River Laboratories (Wilmington, MA). Animals were housed singly in Plexiglas cages and maintained in ambient temperature (22–24 °C) on a 12 h light/12 h dark cycle with lights on at 09:00 h for experiments 1 and 3, and 6:00 h for experiment 2. Food and water were provided ad libitum. The procedures were approved and monitored by the University of Massachusetts Medical School Institutional Animal Care and Use Committee.

2.2. Drug dose and administration

During preliminary studies we found that at doses of 0.4 mg/kg varenicline produced much greater BOLD activation than 0.4 mg/kg nicotine (all drug doses are expressed as the base), that we had used to induce BOLD activation and locomotor sensitization in our prior study using Sprague-Dawley rats [6]. However, when varenicline was administered at a dose of 0.04 mg/kg and nicotine at a dose of 0.4 mg/kg, the BOLD activation was similar. We used the 0.04 mg/kg i.v. dose in the BOLD experiments for comparison with nicotine, since the recommended daily dose of varenicline (2 mg) is about one tenth that of the nicotine obtained from smoking. However, nicotine is too addictive to be used as a therapeutic and may be using nicotine to self-medicate symptoms of inattention.

2.3. Experiment #1: locomotion

2.3.1. Methods

Adult male Sprague-Dawley rats were matched for body weight and randomly assigned to varenicline or saline treatment groups (n=8/group). On day 1 all animals were placed in a non-reflective black open field 121 cm by 121 cm for 30 min to habituate to the environment while the distance traveled was tracked using Observer® 5.0 for Windows software (Noldus Information Technology, Wageningen, Netherlands) and a Canon ZR100 color digital video camera (Canon, NY) mounted 135 cm above the center of the open field. On each of the following 6 days, animals received daily subcutaneous (SC) injections of either varenicline 0.04 mg/kg or an equivalent volume of physiological saline. Immediately following each injection, locomotion was monitored in the open field for 30 min. As a test for locomotor sensitization, activity as measured by percent change in distance traveled over baseline was analyzed using repeated measures analysis of variance (ANOVA) with a within-subjects factor of day and between-subjects factor of drug.

2.3.2. Results

In response to saline on day 1, there was no difference in locomotor activity between the varenicline and saline groups (Fig. 1). Repeated saline injections on days 2–7 had no effect on locomotion. The first dose of varenicline did not produce locomotor activation above that observed for saline, nor was any increase in locomotor activity observed in response to repeated doses of varenicline over six days.

2.4. Experiment #2: spatial learning

2.4.1. Methods

Spatial learning was assessed in a Morris Water Maze (MWM) [14] using a modification of the methods from Epp et al. [15]. The maze consisted of a plastic pool (180 cm diameter; 60 cm high) filled with tap water (26 ± 1 °C) to a depth of 28 cm. The testing environment incorporated salient visual cues that remained constant throughout the study to provide animals with spatial markers. The platform was a clear Plexiglas stand (10 cm diameter, 26 cm high), positioned 2 cm below the water surface and 26 cm from the maze wall in the southwest quadrant. The platform was not visible from the surface of the water.

Animals (n=8/group) were randomly assigned to receive one of the six test solutions (saline, nicotine 0.4 mg/kg, varenicline 0.04 mg/kg, varenicline 0.1 mg/kg, varenicline 0.4 mg/kg, or varenicline 1 mg/kg) via SC injection before the first trial on each day. One day prior to testing (day 0) the animals were habituated to the testing environment by being placed in the maze for 3 min with the platform removed. Spatial learning acquisition consisted of three trials each day over six consecutive days. Entry into the water maze was randomized between one of the four possible start locations (north, east, southeast, and northwest). For each trial the animal had 2 min to navigate to the hidden platform. The elapsed time (latency) between entering the pool and locating the platform was recorded for each rat. Once an animal located the platform it was allowed to remain there for 10 s before being removed. Animals that failed to locate the platform in the allotted 2 min were guided to the platform’s location and allowed a 10-s platform sit. Following each trial, animals were placed in an incubator for a 2 min inter-trial interval. The three consecutive trials on each day were averaged to produce a single value for each day.

2.4.2. Results

By day 3, the saline control animals had mastered the maze and showed only slight improvements in performance times on subsequent days, so Fig. 2 shows data only for the first three days. Analysis of variance (ANOVA) revealed a significant between-group effect on the first day of testing (F(5,39)=2.45, p<0.01), with
animals treated with each dose of varenicline performing significantly better than the saline treated animals. After day 1, differences between varenicline doses and saline did not reach statistical significance. On day 3, the animals treated with varenicline 0.04 mg/kg outperformed those treated with nicotine 0.4 mg/kg (F(1, 13) = 4.66, p < 0.05) and this difference was of borderline significance on day 6 (p = 0.06).

There were no significant differences between nicotine and saline on days 1 and 2, but by day 3 the nicotine-treated animals began to fall behind the saline controls (p = 0.06) and showed significantly worse performance than controls on days 5 (F(1, 13) = 4.66, p < 0.05) and 6 (F(1, 13) = 4.66, p < 0.01).

2.5. Experiment #3: brain activation

2.5.1. Study design
To assess the effect of the first dose of varenicline in naive animals, 6 animals received a dose of varenicline (0.04 mg/kg) administered via the lateral tail vein while the animal was in the scanner. To test the effects of repeated dosing, 8 rats received a dose of varenicline (0.04 mg/kg) subcutaneously on each of 5 consecutive days, while 8 control animals received saline. On the following day, they received a sixth dose (IV) while in the scanner. The IV route was used in the scanner (1) to allow for comparisons with our prior studies of nicotine administered IV, (2) because it ensures that the drug is delivered to the brain rapidly and with less individual variation than would be the case with the SC route, and (3) it is physically easier to deliver than the SC route when the animal is in the scanner.

2.5.2. Imaging procedure
In order to scan fully conscious rats, they must be restrained in the scanner [16]. To reduce physiologic stress and motion artifact during imaging, the animals were acclimated to the restraint and imaging procedures for three consecutive days by being restrained and placed for 60 min in a mock scanner with recorded scanner sounds. We have shown that repeatedly placing animals in the restraint in a simulated magnet quickly habituates them, as indicated by normalization of physiologic and neuroendocrine measures, decreased motion artifact, and improved contrast to noise ratio [17].

The data were collected during a single 30-min scanning session, with the injection given after 5 min of baseline functional data collection. The baseline period prior to the injection controls for factors such as restraint, stress, and scanner noise. The animals were restrained in a multi-concentric, dual-coil, animal restrainer (Insight Neuroimaging Systems, LLC, Worcester, MA). They were lightly anesthetized with 2.5% isoflurane just long enough to secure them in the restrainer. The setup procedure took 2–3 min, by which time the animals were fully conscious.

The restrained animals were placed in a BrukerBioSpec 4.7T/40 cm horizontal scanner (Oxford Instrument, Oxford, UK) equipped with a BiospecBruker console (Bruker, Billerica, MA). Once the animal was secured in the magnet, high-resolution anatomical images were obtained using fast spin echo pulse sequences (echo time, 48 ms; repetition time, 2000 ms; field of view, 30 mm; 1.2 mm slice thickness; 256 x 256 data matrix; rapid acquisition relaxation enhanced (RARE) factor, 8). After the anatomical images were obtained, BOLD fMRI images were continuously acquired over a 30 min period to include a 5 min baseline and a 25 min period of administration of the challenge dose. The functional images were obtained with a fast spin echo pulse sequence (field of view, 30 mm; 1.2 mm slice thickness; echo time, 56 ms; repetition time, 2000 ms; 64 x 64 data matrix; RARE factor, 16).

2.5.3. fMRI data analysis
The processing of fMRI data was performed using STIMULATE software [18]. Motion artifact was assessed by qualitative analysis of time series movies looking for pixel displacement and analysis of raw data time series for course spikes. The time series movies correlated with course spike activity. The multiple data sets collected from these imaging sessions showed very little motion artifact using these criteria. There were no discernable differences in motion artifact as a function of stimulation type. On the rare occasions when a course spike was detected (usually caused by swallowing) the data for the corresponding image were excluded.

The percent change in BOLD activation was calculated on a pixel-by-pixel basis by comparing the average time course data obtained during the 5 min baseline period prior to the injection controls for factors such as restraint, stress, and scanner noise. After day 1, differences between varenicline doses and saline did not reach statistical significance. On day 3, the animals treated with varenicline 0.04 mg/kg outperformed those treated with nicotine 0.4 mg/kg (F(1, 13) = 4.66, p < 0.05) and this difference was of borderline significance on day 6 (p = 0.06).

There were no significant differences between nicotine and saline on days 1 and 2, but by day 3 the nicotine-treated animals began to fall behind the saline controls (p = 0.06) and showed significantly worse performance than controls on days 5 (F(1, 13) = 4.66, p < 0.05) and 6 (F(1, 13) = 4.66, p < 0.01).

2.5.4. Statistical methods
Statistical tests were performed on each subject with control and stimulation windows appropriately specified. The t-test statistics used a 95% confidence level, two-tailed distributions and heteroscedastic variance assumptions. These settings provided conservative estimates for significance. On the activation maps, we reset the activation of pixels that were not statistically significant to zero to display only significant activations. Single factor ANOVA was used to compute the BOLD percentage change between groups, with 0.05 as the level of significance. The methods used for these experiments were identical to those used in our prior nicotine experiments except that the effect of repeated dosing was assessed in conjunction with the fifth dose in the nicotine study and the sixth dose for varenicline. Also the doses were different: varenicline 0.04 mg/kg versus nicotine 0.4 mg/kg. Some results from the nicotine study are presented here for comparison purposes [6].

2.5.5. Results
The intensity of the BOLD response is reflected by the percent change. As seen in Fig. 3, the initial dose of varenicline 0.04 mg/kg produced BOLD activation in the same brain structures as did nicotine 0.4 mg/kg. The activation produced by varenicline exceeded that of nicotine in the temporal and insular cortices. While the repeated dosing of nicotine increased activation in several areas in our prior study (Fig. 3), a significant decrease in the percent BOLD activation was seen with repeated dosing of varenicline in the prefrontal and insular cortices, as well as the hippocampus (Fig. 3). Decreases in BOLD activation that did not reach significance were observed in all other areas of interest except the striatum.

Fig. 4 compares the time course of BOLD activation in response to the initial and repeated doses of varenicline and nicotine for representative areas of the HP and PFC. Repeated nicotine caused sensitization [6] while repeated varenicline caused tolerance. In the hippocampus, retrosplenial and insular cortex, the repeated dose of varenicline did not produce as large an initial peak BOLD response as the initial dose, and the effect of the repeated dose appeared to wane more rapidly. In the VTA, there were no significant differences in the time course for the initial and repeated doses.

3. Discussion

As an α4β2 nAChR partial agonist, varenicline has the potential to mimic some of the effects of nicotine that are mediated via this nAChR subtype. We compared the effects of varenicline and nicotine in relation to brain activation, locomotion and spatial learning. Our data indicate that varenicline produces a pattern of regional brain activation similar to that which we have previously observed with nicotine [6]. However, since both studies examined a priori brain regions of interest, there may be undetected differences in the pattern of activation produced by these drugs. The brain areas that were activated by varenicline include those of the limbic system.
that are involved with addiction, and cortical areas associated with
cognition. The involvement of the limbic system is consistent with
varenicline’s proven efficacy as a smoking cessation drug [26]. We
are not aware of any human imaging study using BOLD to exam-
ine the effect of varenicline. One study used arterial spin labeling
to study smokers’ responsivity to cues after three weeks of either
varenicline or placebo. Subjects smoked just prior to scanning so
the results cannot be compared to ours [27].

Prior research has established that varenicline has approx-
imately 20-fold higher affinity for the human α4β2 nicotinic
receptor than nicotine [1]. At a dose of 0.04 mg/kg, the first admin-
istration of varenicline produced a pattern of regional activation
comparable to that of a first dose of nicotine 0.4 mg/kg (base), but
with greater activation in the temporal and insular cortices [6].

With repeated dosing at 24 h intervals, nicotine and other addic-
tive drugs produce behavioral sensitization [9]. Our prior study
demonstrated neural sensitization to nicotine as manifested by
activation of increased magnitude (Fig. 3) and duration (Fig. 4). Even
though the initial dose of varenicline produced activation equal to
or exceeding that of nicotine in all regions (Fig. 3), no sensitized
response was observed with repeated administration of varenicline
either in terms of BOLD activation, or in the assessment of locomo-
tor sensitization. The lack of sensitization with varenicline cannot
be explained based on the switch from subcutaneous administra-
tion with the five daily pretreatments to IV administration with the
challenging dose as this was the same procedure used to demonstrate
nicotine sensitization.

It has been proposed that varenicline might stabilize the nico-
tinic receptor in its deactivated conformation [1]. When varenicline
is administered to nicotine-sensitized rats minutes before a chal-
lenge dose of nicotine, the expression of locomotor sensitization
is blocked [28]. In humans, the half-life of nicotine (about 90 min)
is much shorter than that for varenicline (17–24 h) [28–30]. The
same is true for rats but the difference is not as marked, (nico-
tine $T_{1/2}$ = about 90 min, varenicline $T_{1/2}$ = 4 h [31,32]. Our repeated
dosing at 24 h intervals would not be expected to result in accumu-
lation of either nicotine or varenicline. However, in patients
taking 1 mg varenicline every 12 h there is a cumulative increase
in varenicline levels until a steady state is reached by day 4 [30].
Although our spacing of varenicline should have allowed it to clear
before the challenge dose was administered, additional experi-
ments with wider dosing intervals should be conducted before
concluding that varenicline is incapable of producing sensitization.
Our data suggest that tolerance and not sensitization to vareni-
cline would be expected at the dosing interval used in clinical
practice.

When prescribed, varenicline is started at a reduced dosage of
0.5 mg daily to minimize its main side effect, nausea, and the dose
is then increased in steps to 1 mg twice daily at the end of the first
week. Thus, patients are required to take the medication at 24 h
intervals to develop a tolerance to this side effect which then allows
the dose to be advanced. In our study, repeated doses of vareni-
cline at 24 h intervals produced a less intense activation that also
appeared to be diminished in duration. Thus, under the conditions

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**Fig. 3.** Percent change in positive BOLD in response to the initial dose or the repeated dose. Top panel—results for nicotine (0.4 mg/kg) adapted from Li et al. Shown are results for experiments involving an initial dose of nicotine administered to naïve animals, the response to the 6th dose of saline, and the 5th dose of nicotine. *p < 0.05 by t-test comparing the initial dose and repeated doses of nicotine. Repeated nicotine dosing increased BOLD reactivity in several regions. Bottom panel—results for varenicline (0.04 mg/kg). Repeated varenicline dosing decreased BOLD reactivity in several regions. VSC – visual cortex, RTC – retrosplenial cortex, TMC – temporal cortex, PRC – parietal cortex, ISC – insular cortex, CGC – cingulate cortex, PFC – prefrontal cortex, HP – hippocampus.
of our experiment, we observed tolerance to the neural activating effect of varenicline, consistent with the clinical experience.

As the animals treated with varenicline showed superiority over saline controls in the water maze experiment only on day one, this may reflect effects of varenicline on anxiety, attentional, or working memory performance rather than spatial memory. After a positive effect on day 1, nicotine-treated animals showed a trend to impaired performance in comparison to the saline control animals. As daily doses of nicotine 0.4 mg/kg as used in this study have been previously shown to produce locomotor sensitization [9] we speculate that locomotor stimulation might have in some way impaired performance, masking any cognitive enhancing properties of nicotine under the conditions of this experiment.

Prior studies of the effects of nicotine on spatial learning have yielded varying results of nicotine's effects on cognition perhaps due to methodological differences in strains, doses, and treatment schedules [33–35]. Studies have found cognitive enhancement on spatial learning in animals previously sensitized to nicotine, but testing on the MWM was not conducted in the presence of nicotine. Our administration of nicotine prior to testing would better model the effects of nicotine in smokers. Another study employing methods similar to those used here also found no effect of nicotine over three testing days [35].

4. Conclusion

In conclusion, the first administration of varenicline produced a pattern of neural activation that was similar in magnitude and regional distribution to that produced by nicotine. In contrast to the sensitizing effect of nicotine, with repeated dosing at the interval used in clinical settings, varenicline produced decidedly less neural activation in many regions than was seen with the initial dose. Under the conditions of our study varenicline did not produce locomotor sensitization, but appeared to improve performance on the first day of testing in the Morris Water Maze.

Conflicts of interest

Dr. DiFranza has consulted for Pfizer. Pfizer had no control over the content of this article. None of the other authors report any conflicts of interest.

Acknowledgements

Funding for this project was provided by from the National Institute of Drug Abuse (R01DA021846) and Pfizer to Dr. King and Dr. DiFranza. Its contents are solely the responsibility of the authors and do not represent the official views of NIDA or Pfizer.

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