Jackson A, Silk S, Buhidma Y, Shoaib M.

Varenicline, the clinically effective smoking cessation agent, restores probabilistic response reversal performance during withdrawal from nicotine.

Addiction Biology 2016, 22(5), 1316-1328.

Copyright:

This is the peer reviewed version of the following article: Jackson A, Silk S, Buhidma Y, Shoaib M. Varenicline, the clinically effective smoking cessation agent, restores probabilistic response reversal performance during withdrawal from nicotine. *Addiction Biology* 2016, 22(5), 1316-1328, which has been published in final form at <https://doi.org/10.1111/adb.12423>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

DOI link to article:

<https://doi.org/10.1111/adb.12423>

Date deposited:

14/12/2017

Embargo release date:

20 July 2017

Title:

Varenicline, the clinically effective smoking cessation agent, restores probabilistic response reversal performance during withdrawal from nicotine.

Running Title:

Nicotine withdrawal and probabilistic reversal

Authors:

Anne Jackson, PhD², Sarah Silk¹, Yazeed Buhidma¹ and Mohammed Shoaib, PhD¹✉

Affiliations:

¹ Institute of Neuroscience, The Medical School, University of Newcastle, Newcastle upon Tyne, NE2 4HH, UK.

² School of Pharmacy and Biomolecular Sciences, University of Brighton, Moulsecoomb, Brighton, East Sussex, BN2 4GJ, UK.

Contact:

✉ Mohammed.Shoaib@newcastle.ac.uk

Abstract

There is recognition that cognitive problems can contribute to renewed drug taking in former addicts. Our previous work has indicated that current smokers show reduced performance on a probabilistic reversal learning (PRL) task, relative to former smokers (Butler et al, 2011). To further explore PRL performance and its relevance to smoking, in addition to the role of nicotine, we developed a model of nicotine withdrawal-induced deficits in rodents. A second goal was to test varenicline, an $\alpha 4\beta 2$ partial agonist, for its ability to restore any cognitive impairment. Acute effects of nicotine and varenicline on PRL performance in non-dependent animals were minimal and confined to speed of responding. When rats were made dependent on nicotine via osmotic minipumps implanted for 7 days (3.16mg/kg/day), repeated tests at specified withdrawal time points revealed PRL disruption peaking at 12 and 24 hours following surgical removal of minipumps. Withdrawal was characterised by significant deficits in the number of reversals ($p < 0.05$) speed of responding ($p < 0.01$) and increases in omissions ($p < 0.05$). Nicotine (0.2 mg/kg SC) or varenicline (0.3 & 1.0 mg/kg SC) administered 10-min prior to PRL test sessions during withdrawal, relieved the performance deficits. At 24 hrs withdrawal, nicotine and varenicline (1 mg/kg) prevented decrements in reversals, in addition to ameliorating slower speed of responding. The high dose of varenicline only, reduced omissions. These results confirm the role of nicotine in withdrawal-induced disruption of PRL performance and suggest that the model may be useful for investigating efficacy of potential new treatments for smoking cessation.

Introduction

Smoking cessation is typically accompanied by a variety of withdrawal symptoms including mood changes (West & Russell, 1988) emotional reactivity (Postma *et al*, 2001; West & Russell, 1988) and craving (Hughes *et al*, 1991). There is also now a recognition that cognitive deficits can play a role, perhaps acting as drivers of relapse or by reducing resistance to factors that trigger relapse, in former smokers. In some studies, deficits have been found to be predictors of relapse (e.g., Powell *et al*, 2010) whilst in others, superior cognitive performance has been associated with successful abstinence (e.g., Nestor *et al*, 2011). Amelioration of cognitive problems experienced while attempting to stop smoking may therefore constitute a valid target for improving cessation rates, which stand at less than 50% a year after treatment with even the most successful of currently available therapies (Kralikova *et al*, 2013).

In order to target cognition as a therapy, it is necessary to understand the specific problems that occur during withdrawal and the underlying (perturbations of) brain mechanisms. Cognitive problems in smokers that are now well documented include disturbances in attention (e.g., Foulds *et al*, 1996) working memory (e.g., Mendrek *et al*, 2006) and inhibitory control (e.g., Dawkins *et al*, 2009). Another aspect of executive control that appears to be important is performance monitoring. Performance monitoring refers to the ability to monitor feedback in order to guide behaviour. Two studies suggest that this type of cognition is relevant to successful abstinence. Firstly, Nestor and colleagues (2011) found that ex-smokers showed greater prefrontal neural activity during error monitoring, compared with current smokers. Secondly, Butler *et al* (2011) used a probabilistic reversal learning task and found evidence for better performance monitoring in former smokers compared with current smokers. The task used was based on Budhani *et al* (2006) where participants were required to indicate which stimuli of several pairs was correct. Selection of correct and incorrect stimuli were given positive or negative feedback respectively, in the form of points won or lost. Correct and incorrect stimuli were sometimes

reversed and variation of contingencies resulted in false feedback for some responses. In this study, performance monitoring deficits were detected as a greater reaction to false feedback during reversals. In addition, slowing that normally occurs in response to the commission of an error was also impaired in smokers (Butler, 2013) consistent with a reduced ability to efficiently monitor feedback during ongoing performance of the task.

Although these studies imply that good performance monitoring is helpful for successful abstinence from tobacco smoking, it is not possible to know if the results arose because of fundamental individual differences between current and ex-smokers, or because prolonged nicotine intake alters brain mechanisms underlying performance monitoring. Homomeric $\alpha 7$ nicotinic receptors, thought to be particularly dense in the cortex (Gotti et al, 2009) have been detected in both rodent (Murakami et al, 2013) and human cingulate cortices (Wong et al, 2014) and $\alpha 4\beta 2$ receptors have also been detected in the cingulate (Picard et al, 2013). Cortical sites are thought to mediate some of the cognitive effects of nicotine (Stolerman, 1996; Hahn et al 2002) and the cingulate is an area of particular interest in terms of performance monitoring (Iannaccone et al 2015; Garavan et al 2003); it is therefore feasible that chronic intake of nicotine, as a result of smoking, alters the normal functioning of the cingulate cortex.

In order to investigate if deficits could be related to prolonged self-dosing with nicotine (as opposed to other substances in tobacco or pre-existing differences in cognition) we turned to animal studies, as there are clear ethical problems associated with prolonged drug dosing in human volunteers. We used the probabilistic reversal learning task (PRL) developed for rodents as, like the human task we used previously, it measures reaction to positive and negative feedback; in addition, reward contingencies can be varied and reversed (Cools et al 2007; Hornak et al 2004). Drugs modulating 5-HT function have been shown to have analogous effects in both the rat task (Bari et al, 2010) and in human studies (Chamberlain et al, 2006).

Investigations of cholinergic mechanisms in rodent reversal learning are few and have been restricted to “non-probabilistic” tasks (e.g., Roberts et al, 1992; Chen et al, 2004). Therefore, to gain further information about the action of nicotine on performance monitoring and its relevance to smoking, we first studied a range of acutely administered doses of nicotine, in addition to the acute effects of the $\alpha 4\beta 2$ nicotinic-receptor partial agonist varenicline, in animals trained to perform the PRL task. Varenicline was used as a tool to gain insight to possible underlying receptor mechanisms as well as to evaluate a clinically effective smoking cessation treatment in the task. Secondly, we used an established method for inducing dependence on nicotine in rats (Malin et al 1988; Shoaib & Bizarro 2005) to examine the consequences of spontaneous withdrawal on performance of the PRL task. In our final experiment, we investigated whether or not acute nicotine and varenicline would ameliorate withdrawal-induced deficits. To our knowledge, this is the first study to examine the effects of nicotinic ligands in the PRL task; we report here our findings in full, some of which have previously been published in abstract form (Silk & Shoaib 2014).

Materials and Methods

Subjects

Male hooded Lister rats (Harlan UK) were housed in groups of 4 per cage (RB3, North Kent Cages). They were maintained on a 12 h light-dark cycle with lights off at 1900 h. Ambient temperature was maintained between 20 – 22 °C. Rats were allowed to acclimatise to conditions for minimum 7 days before they were placed on a food restriction schedule for 5 days prior to training. *Ad libitum* access to water in the home cage in addition to access to 15g of chow per rat, ensured that they maintained 90% of their free-feeding weight. All procedures complied with local and national ethical requirements in accordance with the UK Animals (Scientific Procedures) Act, 1986, in addition to the EU Directive

2010/63/EU on the protection of Animals for Scientific Purposes, under a project license issued by the UK Home Office.

Apparatus

Experimental apparatus consisted of 8 standard operant conditioning boxes (MED-Associates, Georgia, VT, USA) housed in fan-ventilated, light- and sound-attenuating chambers. Each box was fitted with a house light, a food hopper on the right-hand wall (standard 45 mg dustless pellets; TestDiet Inc., Richmond, Indiana) plus two apertures with LED lights that permitted detection of nose-poke entries on the left-hand wall. Scheduling of contingencies and recording of data were controlled by personal computer using MED-PC software (MED-Associates, Georgia, VT, US).

Drugs

Nicotine hydrogen tartrate (Sigma-Aldrich, UK) was dissolved in 0.9% saline and the pH was adjusted to 7 with NaOH solution. Varenicline tartrate (Tocris, UK) was dissolved in 0.9% saline. For the osmotic minipumps, nicotine solutions were dissolved in 0.9% saline and did not require any pH adjustment. All doses of drugs are expressed as those of the base.

Training

The training procedure used for the PRL was adapted from that used by Bari *et al*, (2010). Initially, rats were shaped to collect food pellets from the hopper every 30 seconds under a VI-30 schedule. When rats reliably collected the pellets during 60-minute daily sessions, the task was changed to reinforce each nose-poke response with a single food pellet. The rats were presented with one hole illuminated per trial, assigned randomly to a hole, left or right. A nose-poke in the correct (illuminated) hole resulted in delivery of a single food pellet to the hopper on the opposite wall. Each new nose-poke trial began when the food pellet earned on the previous trial had been retrieved. Approximately 20 magazine training sessions were required to reach stable performance.

After stable levels of accuracy were reached, rats were presented with two nose-poke holes, simultaneously illuminated. One of these was the correct location for 80% of the trials, a nose-poke

resulting in delivery of a food pellet, and the other was incorrect, resulting in reinforcement for 20% of trials. Holes were illuminated for 30 seconds and if there was no response within this time, the trial was deemed an omission which triggered a 5-second time-out. There was no consequence to a nose poke during time-out. A nose-poke in the correct hole on eight consecutive occasions resulted in the status of the holes being reversed – the correct hole became incorrect and *vice versa*. These sessions and subsequent post-training test sessions ended after 199 trials had been completed, or after 40 minutes, whichever occurred first. The house light remained on during both training and test stages.

Experiment 1: Acute nicotine and varenicline.

Eight subjects trained on the PRL task met the stability criteria and were tested with nicotine and varenicline acutely. Using a repeated measures design, rats were administered each dose of nicotine or varenicline in a randomised order, with one day of normal training between to allow between-session washout. Doses of either nicotine or varenicline were injected SC ten-minutes before each session; rats remained in the home cage during this pre-session period. This pretreatment time is based on previous pharmacokinetic data derived from nicotine discrimination experiments and plasma nicotine determinations indicating near peak levels of absorption and penetration into the brain 10 min after injection (Pratt et al 1983).

Doses of nicotine were: vehicle, 0.025, 0.05, 0.1, 0.2 and 0.4 mg/kg. Doses of varenicline were: vehicle, 0.03, 0.1, 0.3 and 1 mg/kg.

Experiment 2: Withdrawal from chronic nicotine treatment

Subjects were 16 rats weighing 280-300g at the start of the experiment and pre-trained on the PRL task. Following training, rats underwent surgery to implant Alzet osmotic minipumps (2ML2) for chronic nicotine administration. Thirty to sixty minutes before implantation, minipumps were primed to deliver 3.16 mg/kg/day nicotine or saline solution and incubated in physiological saline. Rats were then anaesthetised using isoflurane and an incision made at the top left of the back. The minipump was

inserted subcutaneously and the incision closed using surgical clips and pain relief was administered systemically (carprofen 5mg/kg IP). Following surgery, rats were placed in an incubator while recovering; meloxicam for pain relief and enrofloxacin (antibiotic) were administered post-surgery where necessary. Minipumps remained implanted for 7 days, when they were removed under isoflurane anaesthesia. Topical analgesia was applied using lidocaine and bupivacaine following the removal of the osmotic minipumps. Rats subsequently underwent PRL test sessions at 6, 12, 24, 48, 72 and 96 hours from the time the osmotic pumps were removed. Following PRL test session conducted at 12 hours post withdrawal, all rats received further pain relief administered systemically (carprofen 5mg/kg IP). We have previously shown that nicotine delivered via these osmotic minipumps in this dose (3.16mg/kg/day) for 7 days induces a robust level of physical withdrawal from nicotine as measured on somatic signs (Wing & Shoaib 2007) and also on attentional processes in the 5-choice serial reaction time task (Bizarro & Shoaib 2005).

Experiment 3: Nicotine and varenicline administered during withdrawal

Subjects were 32 rats weighing 280—300g at the start, who were trained and implanted with nicotine-primed osmotic minipumps, as described under Experiment 2. Minipumps remained implanted for 7 days and were subsequently removed under isoflurane anaesthesia. Following pump removal, rats were allocated to one of four treatment groups – withdrawal + saline vehicle (control), withdrawal + nicotine 0.2 mg/kg (nicotine), withdrawal + varenicline 0.3 mg/kg (varenicline low dose), withdrawal + varenicline 1.0 mg/kg (varenicline high dose). They then completed PRL test sessions at 12 and 24 hours post-pump removal, following acute subcutaneous injections (t - 10 mins) according to their allocated treatment group.

Data Analysis

In all experiments data collected were: total number of 'correct' and 'incorrect' responses, total number of omissions (trials where no response was made for 30 seconds), total number of pellets earned, number of reversals completed (eight consecutive correct nose-pokes), total session time, response latency (mean time, in seconds, between aperture light coming on and a nose-poke). Two ratios were calculated: win-stay ratio (number of trials where the same aperture was selected following a rewarded trial/total rewarded trials) and lose-shift ratio (number of trials where the opposite aperture was selected following a non-rewarded trial/total non-rewarded trials).

For the first experiment, normally distributed data were analysed by Repeated Measures Analysis of Variance (RMANOVA) with treatment group as the repeated factor. Significant main effects were further analysed using paired t-tests to compare performance with controls. Where variables were not normally distributed (latencies) data were first analysed by Friedman Test for paired samples and individual comparisons were made using a Wilcoxon Test.

For the two withdrawal experiments, there were no differences between groups at baseline (T=0 hrs) and with the exception of omissions in the first withdrawal study, data were normally distributed. Data were therefore first analysed using RMANOVA with factors of Group and Time (repeated). As we needed to know where changes in performance differed from the baseline point before withdrawal, significant interactions arising from RMANOVA were explored using paired t-tests within each group. This enabled us to compare performance at each post-pump removal time point to baseline. This approach was deemed preferable to using multiple comparisons, such as Bonferroni, which perform all possible comparisons regardless of their relevance to the scientific question and in doing so, increase the possibility of Type II errors (accepting the null hypothesis, when the alternative is true; Perneger, 1998). The use of paired t-tests within each group resulted in five comparisons per group. Therefore, in order to reduce the possibility of Type I errors (rejecting the null hypothesis, when the alternative is

true) p-values were adjusted using Benjamini-Hochberg method to control for false positives (Benjamini & Hochberg, 1995). Unless otherwise stated, all p-values refer to adjusted values. For some variables, significant interactions were also investigated using independent t-tests to examine between group differences at the 12 hr or the 24 hr time points. As data for omissions in the first withdrawal study were not normally distributed, they were analysed by Friedman Test for paired samples and individual comparisons used a Wilcoxon Test.

Results

Experiment 1: Acute nicotine and varenicline

Acute nicotine injections did not affect reversals ($F_{5,35}=1.074$, $p>0.3$) omissions ($F_{5,35}=1.038$, $p>0.4$) errors ($F_{5,35}=0.673$, $p>0.6$) number of pellets earned ($F_{5,35}=0.330$, $p>0.8$) win-stay ($F_{5,35}=0.259$, $p>0.9$) or lose-shift ratios ($F_{5,35}=1.821$, $p>0.1$); see Table 1. Response latencies increased at the higher doses of nicotine and a Friedman Test indicated a difference between the groups overall ($\chi^2_{(5)}=11.929$, $p<0.05$); individual Wilcoxon comparisons with vehicle were not significant ($Z's<-0.1$, $p's>0.05$). Likewise there was a significant main effect of dose for total session time ($F_{5,35}=2.597$, $p<0.05$) but individual comparisons were not significant (all $t_7's<-3.2$, $p's>0.07$). Similarly, varenicline did not alter performance (Table 1) as measured by reversals ($F_{4,28}=0.727$, $p>0.5$) omissions ($\chi^2_{(4)}=6.667$, $p>0.1$) errors ($F_{4,28}=0.293$, $p>0.8$) pellets earned ($F_{4,28}=0.544$, $p>0.7$) win-stay ($F_{4,28}=1.443$, $p>0.2$) or lose-shift ratios ($F_{4,28}=0.538$, $p>0.7$). However, both response ($F_{4,28}=7.728$, $p<0.001$) and total session times ($F_{4,28}=6.964$, $p<0.01$) were faster following varenicline treatment. Individual comparisons revealed that this was true for all doses of varenicline (latency: all $t_7's>3.2$, $p's<0.05$; total time: all $t_7's>3.0$, $p's<0.05$) compared to controls. Therefore, acute injections of nicotine did not significantly alter performance of the PRL task, whereas varenicline **increased response rates** and reduced the time taken to complete the task.

Experiment 2: Withdrawal from chronic nicotine treatment

After pump removal, the number of reversals completed changed over time (main effect: $F_{6,84}=4.162$, $p<0.01$) but there were no overall differences between the groups ($F_{1,14}=3.626$, NS). RMANOVA revealed a significant time-by-group interaction ($F_{6,84}=2.255$, $p<0.05$). This was due to a decrease in performance of the nicotine withdrawal group only; reduced reversals were seen 12 and 24 hrs after pump removal, although this was only significant at 12 hrs ($t_7=4.889$, $p<0.02$; Figure 1A). Response latencies were also disrupted in the nicotine withdrawal group. RMANOVA revealed a significant effect of time ($F_{6,84}=35.709$, $p<0.001$) but not group ($F_{1,14}=3.543$, NS) with a significant interaction ($F_{6,84}=4.809$, $p<0.001$). The interaction was due to considerable slowing of responding in nicotine withdrawn animals such that response times were greater than baseline at 6 ($t_7=-7.194$, $p<0.001$) 12 ($t_7=-5.757$, $p<0.01$) 24 ($t_7=-5.897$, $p<0.01$) and 48 hrs ($t_7=-2.851$, $p<0.05$) after pump removal. There was also some disruption in the control group, but this was of much smaller magnitude and significant only at the 24 hr time point ($t_7=-5.342$, $p<0.01$; Figure 1B). Similarly, the total time to complete the PRL task was prolonged in the nicotine withdrawal group. RMANOVA revealed significant effects of time ($F_{6,84}=32.104$, $p<0.001$) and group differences ($F_{1,14}=6.238$, $p<0.05$) with the nicotine withdrawn animals taking longer overall. Greater disruption in the drug treated group was evidenced by a significant time-by-group interaction ($F_{6,84}=5.340$, $p<0.001$). Paired t-tests indicated a small change in the control group at the 24 hr time point ($t_7=-3.694$, $p<0.05$) but a much larger effect in the nicotine treated group at all time points (all t_7 's > 2.773 , p 's < 0.05) except 72 hrs ($t_7=-1.857$, NS; Figure 1C).

The results for the remaining variables measured are shown in Table 2. Whilst there was no change across time in the control group ($\chi^2_{(5)}=8.489$, NS) the number of omissions increased in the nicotine withdrawal group ($\chi^2_{(5)}=25.502$, $p<0.001$). Increased omissions peaked at 24 ($Z=-2.371$, $p<0.05$) and 48 hrs withdrawal ($Z=-2.375$, $p<0.05$). RMANOVA of data for the number of pellets collected indicated a significant time-by-group interaction ($F_{6,84}=3.995$, $p<0.01$) although no main effects of time

($F_{6,84}=1.004$, NS) or group ($F_{1,14}=1.682$, NS) were apparent. The interaction was likely due to increases in pellet collection for the control group, but decreases for the nicotine withdrawal group, although paired t-tests were not significant (all t_7 's $< \pm 2.500$, p 's > 0.05). An independent groups t-test however, at the 24 hr time point, indicated that pellet collection in the withdrawal group was significantly lower than the control group ($t_{14}=3.010$, $p < 0.01$). Similarly, for errors that animals made, there was a significant time-by-group interaction ($F_{6,84}=2.369$, $p < 0.05$) but no main effect of time ($F_{6,84}=1.688$, NS) or group ($F_{1,14}=0.880$, NS). Again, paired t-tests were not significant at any time point in either treatment group (all t_7 's $< \pm 2.800$, p 's > 0.05) but at 12hrs, an independent t-test indicated significantly more errors in the withdrawal group, compared with controls ($t_{14}=-2.233$, $p < 0.05$). Win-stay behaviour was also disrupted by nicotine withdrawal. There was a significant main effect of time ($F_{6,84}=5.128$, $p < 0.05$) and group by time interaction ($F_{6,84}=2.520$, $p < 0.05$) but no overall group difference ($F_{1,14}=3.886$, NS). Paired t-tests were not significant in either group (all t_7 's $< \pm 3.300$, p 's > 0.05) although independent t-tests indicated significantly lower performance in the withdrawal group, relative to controls, at 12 ($t_{14}=2.524$, $p < 0.05$) and 24 hrs ($t_{14}=2.401$, $p < 0.05$). There were no significant changes in either group, for lose-shift behaviour (Group: $F_{1,14}=0.503$, NS; Time: $F_{6,84}=0.715$, NS; Interaction: $F_{6,84}=1.374$, NS). Thus withdrawal from nicotine disrupted performance by increasing omissions, errors and response times. It also decreased the number of reversals completed, the number of pellets collected and reduced win-stay behaviour.

Experiment 3: Nicotine and varenicline administered during withdrawal

Following removal of the pumps, the number of reversals completed changed over time (main effect: $F_{5,140}=16.34$, $p < 0.01$). Although there were no overall differences between the groups ($F_{3,28}=0.829$, NS) RMANOVA revealed a significant time-by-group interaction ($F_{15,140}=1.765$, $p < 0.05$). In the saline treated group the number of reversals completed decreased over time, to reach a low point 24 hrs after pump removal ($t_7=5.134$, $p < 0.01$; Fig 2A); thereafter reversals recovered to baseline levels. In the group

treated with nicotine, reversals were not significantly different from baseline at any time point (all t_7 's < 2, p 's > 0.2) and the same was true for the group given the larger dose of varenicline (all t_7 's < 3.6, p 's > 0.05). In animals given the smaller dose of varenicline, performance followed a similar pattern to controls, in that significant reductions in reversals were seen at 24 hrs (t_7 =4.333, p <0.05) and also at 30 hrs (t_7 =3.543, p <0.05). Thus both nicotine and the larger dose of varenicline only, prevented decrements in reversals seen 24 hrs into withdrawal.

RMANOVA of response latencies indicated significant time ($F_{5,140}$ =30.189, p <0.0001) and time-by-group interactions ($F_{15,140}$ =2.334, p <0.01) but no overall differences between groups ($F_{3,28}$ =2.112, NS). Responding slowed significantly in the group treated with vehicle, at all time points after baseline (all t_7 's > 2.54, p 's < 0.05) with the exception of the final time point, where latencies were similar to baseline and not significantly different (t_7 =-1.655, NS; Fig 2B).

A similar pattern was seen in the other treatment groups where responding slowed at all time points (t_7 's > 2.93, p 's < 0.05) except the 12hr point, where latencies were not different from baseline (t_7 's < 0.6, p 's > 0.5). However, as withdrawal slowing varied considerably in the controls at 12 and 24 hrs, these data were further analysed with between group t-tests. In Figure 2B it can be seen that at 12 hrs, there was significant reversal of withdrawal slowing by nicotine (t_{14} =2.325, p <0.05) and by the larger dose of varenicline (t_{14} =2.831, p <0.05) but not by the lower dose (t_{14} =1.212, p >0.2). Likewise, at 24 hrs withdrawal, nicotine (t_{14} =2.760, p <0.05) and varenicline 1 mg/kg (t_{14} =3.032, p <0.01) reversed slowing, but not varenicline 0.3 mg/kg (t_{14} =0.429, p >0.6). Similarly, overall omissions also increased over time ($F_{5,140}$ =35.547, p <0.0001) and there was a significant time-by-group interaction ($F_{15,140}$ =2.161, p <0.05) but no overall group differences ($F_{3,28}$ =1.638, NS). Figure 2C shows that withdrawal-induced increases in omissions were significant in controls at 6, 24 and 30 hrs (t_7 's > 4.31, p 's < 0.01). At 24 hrs withdrawal, treatments reduced omissions somewhat, but this was only significant for the group treated with the largest dose of varenicline (t_{14} =2.505, p <0.05).

The results for the remaining variables are shown in Table 3. The total number of pellets collected varied across time points ($F_{5,140}=30.929$, $p<0.0001$) and there was a significant time-by-group interaction ($F_{15,140}=1.917$, $p<0.05$); between groups factor was not significant ($F_{3,28}=1.096$, NS). At 24 and 30 hrs, pellets collected were considerably reduced as withdrawal progressed in the control group (respectively: $t_7=5.468$, $p<0.01$; $t_7=4.619$, $p<0.01$). At 24 hrs, none of the other treatment groups were significantly different from baseline, indicating reversal of the withdrawal effect (t_7 's <2.9 , p 's >0.05). Reductions in pellets collected were still evident at 30 hrs in the group treated with the smallest dose of varenicline ($t_7=5.292$, $p<0.01$) but not with the highest dose ($t_7=1.990$, $p>0.1$) suggesting that varenicline at 1 mg/kg had a prolonged effect on this measure.

Errors were affected overall during withdrawal (time: $F_{5,140}=26.355$, $p<0.0001$). RMANOVA revealed a significant time-by-group interaction ($F_{15,140}=2.913$, $p<0.001$) but no between group differences ($F_{3,28}=0.746$, NS). At 24 hrs, there was a significant reduction in errors committed in the vehicle control group ($t_7=5.126$, $p<0.01$) and this was also true of the group administered 0.3 mg/kg varenicline ($t_7=6.020$, $p<0.01$). Reductions in the nicotine treated group ($t_7=1.276$, $p>0.3$) and those given the larger dose of varenicline ($t_7=1.928$, $p>0.2$) were not significantly different from baseline, indicating that these two treatments prevented the withdrawal effect on errors.

For total session time, win-stay and lose-shift ratios, RMANOVA revealed significant overall time effects (respectively: $F_{5,140}=47.717$, $p<0.001$; $F_{5,140}=2.793$, $p<0.02$; $F_{5,140}=3.094$, $p<0.02$). However, there were no significant interactions (respectively: $F_{15,140}=1.697$, NS; $F_{15,140}=0.758$, NS; $F_{15,140}=1.002$, NS) or between group effects (respectively: $F_{3,28}=1.904$, NS; $F_{3,28}=0.483$, NS; $F_{3,28}=0.895$, NS) so these measures were not further analysed. Both nicotine and varenicline were therefore able to ameliorate withdrawal-induced disruption in performance of the PRL task, by preventing decrements in reversals,

speed of responding and pellet collection; they also reversed withdrawal-induced changes in errors and omissions.

Discussion

We carried out a series of experiments to investigate the effect of nicotine and withdrawal following chronic nicotine treatment on PRL responding in rats and also to determine if a clinically effective treatment would ameliorate any withdrawal-induced deficits. The acute effects of nicotine and varenicline on PRL performance were minimal and confined to speed of responding. In the case of nicotine, total time on task tended to increase (albeit non-significantly) at the highest doses. In contrast, varenicline increased response rates and reduced total time on task at all doses tested. Withdrawal from chronic nicotine treatment disrupted performance of PRL as seen by a reduced number of reversals, prolonged response latencies and time on task, accompanied by increased omissions. Disruption peaked at 12 – 24 hrs after pump removal, with a small increase in errors and with win-stay behaviour also being reduced somewhat. A reduction in pellet collection was seen at 24 hrs withdrawal. After 96 hrs, PRL performance was largely returned to baseline levels.

The time course of the withdrawal effect is interesting in comparison with human studies of performance monitoring. Nestor and colleagues (2011) found differences in neural activity during error monitoring between former and current smokers. In their study, volunteers smoked as usual before beginning the experimental procedure, but it was not possible to exclude the probability that volunteers entered early withdrawal during the experimental procedure. In the PRL study by Butler et al (2011) again differences were found between former and current smokers. In this study however, volunteers either smoked just prior to testing or had been abstinent from 11pm the night before and the *largest* difference from former smokers that was seen, was in those who smoked prior to testing. The current study clearly shows withdrawal-induced disruption peaking around 24 hrs, but results of

human studies however, are ambiguous, as neither have studied the effect of withdrawal in a controlled manner, over the same time course, or over an extended period of abstinence. Human studies now need to establish the time course of any withdrawal-induced disruptions and if there is any recovery within a time period similar to that observed here. Long-term abstinence-induced deficits in cognition are important modulators of cessation success but given that most people who attempt to stop smoking will relapse during the very early stages of withdrawal (Hughes et al, 2004) early treatment could have considerable impact on cessation rates (Powell et al, 2010).

In the final study, disruption of PRL performance by withdrawal from chronic nicotine treatment was replicated, with the exception that significant alterations in total session time and win-stay ratios were not seen. Both nicotine and the largest dose of varenicline prevented withdrawal-induced decrements in reversals, slowing of responding, increased omissions and changes in the number of errors. Reduction of pellet collection was ameliorated by all three treatments (nicotine and both doses of varenicline) with the highest dose of varenicline having the most prolonged effect. Both nicotine and varenicline therefore were able to restore PRL performance after withdrawal-induced disruption; the results generally validate the model as suitable for further studies investigating potential treatments for smoking cessation.

Clear withdrawal-induced disruptions were seen in the number of reversals that animals made, suggesting deficits in cognitive flexibility associated with abstinence. This contrasts with results obtained by Nesic et al (2011) who found impaired set-shifting in satiated smokers, compared with abstinent smokers, using the Intra-Extra Dimensional Set-Shift (IED) test. There are two possibilities to explain this apparent discrepancy. Firstly, effects of smoking/withdrawal on cognitive flexibility may depend on degree of dependence. Negative correlational relationships between smoking dependence

and cognitive flexibility have been reported (Martin et al 2000, Kalmijn et al 2002) and in the Nesic et al study (2011), impaired set shifting following smoking was seen only in volunteers with a high degree of dependence, not those with less dependence. Secondly, whether or not a reward-based task is used may be a factor. As in the current task, animal models of cognitive flexibility necessarily use food reward to train animals to perform (e.g., Allison & Shoaib 2013). Tests used in human studies are not always reward based – for example, the Wisconsin Card Sorting Test and the IED test used by Nesic et al (2011) involved using feedback in the form of “correct” or “wrong”, rather than rewards such as points or money. Indeed there is some evidence that in certain populations, problems of cognitive flexibility are only manifest as reward-based inflexibility (Boog et al, 2014). Further studies in human smokers using reward-based tasks might therefore be informative. Both nicotine and varenicline reversed the withdrawal induced deficit in reversals in this study; it would therefore be of interest also, to determine the effects of varenicline on cognitive flexibility in human smokers.

Speed of responding in the PRL was also disrupted during withdrawal from chronic nicotine treatment. This deficit was also ameliorated by both nicotine and varenicline. Interestingly, restoration of response speed was unlikely due to direct psychomotor stimulant effects of either nicotine or varenicline. Nicotine did not alter response speeds (except possibly to produce slowing at the highest dose) when administered to naïve animals. In the case of varenicline, **increased response rates in naïve animals were seen at all doses tested**, but only the largest dose tested was able to restore performance during withdrawal. The inefficacy of lower doses in withdrawal suggests that some tolerance developed during chronic treatment. This would be consistent with work in humans indicating that smokers can tolerate higher doses of varenicline than non-smokers (Faessel et al, 2006). It also suggests that the chronic dosing regimen used in this model produced at least some adaptation in brain mechanisms appropriate to the human situation, where smokers have often been ‘self-dosing’ with nicotine for many years.

Win-stay ratios (sensitivity to positive feedback) were reduced in the first withdrawal experiment, but in the second study, these ratios showed only a non-significant tendency to be disrupted (at 24 hrs). Withdrawal-induced alterations in lose-shift ratios (sensitivity to negative feedback) were also anticipated, but not seen. In recent years, evidence has accumulated for disrupted sensitivity to feedback in depressed patients. Using a variety of tasks, changes in sensitivity to positive- or negative-feedback, or both, have been reported (Chase et al, 2010). In some studies, abnormal reaction to feedback in patients has been correlated with anhedonia (Steele et al, 2007). Whilst it is not possible to measure affect directly in rodents, changes in win-stay/lose-shift ratios might have occurred due to the aversive nature of withdrawal altering sensitivity to feedback. Indeed, in an earlier study in rats on reward responsiveness, Pergadia et al (2014) saw reduced reward-related response bias during nicotine withdrawal. These authors also found a similar reduction in human smokers, although approximately half of these volunteers had a history of depression. Finally, in a study in human smokers that excluded history of depression, using the Cambridge Gambling Task, Austin et al (2014) showed that in early abstinence from smoking, reactivity following gains was reduced; this would be consistent with an expectation that withdrawal should reduce sensitivity to positive feedback. Unfortunately, because of the lack of a statistically significant disruption in our final study, it is not possible to comment further on any effect of nicotine or varenicline.

The reasons for the lack of sensitivity of these measures to withdrawal are not completely clear. Firstly however, it should be noted that performance on the task was very good, with win-stay ratios of around 80% (compared to, for example, around 60% in Bari et al, 2010) possibly making it hard to disrupt performance. Alternatively, it may be that longer dosing with nicotine is required to see robust disruptions in win-stay or lose-shift ratios. As previously noted, changes in cognition occurring during smoking cessation may depend on the degree of dependence (Brooks & Jackson, 2008; Nesic et al, 2011).

We used varenicline, not only to validate the model in terms of current treatments, but also as a tool to gain some insight into nicotinic receptor mechanisms. Interestingly, a “partial” effect that might have been anticipated from a partial agonist was not seen; where withdrawal-induced disruption of performance was evident, varenicline was clearly as effective as the full agonist nicotine. Interestingly, some drug discrimination studies report full generalisation of the nicotine stimulus (Rollema 2007; Jutkiewicz et al 2011; Cunningham 2012) to varenicline, rather than partial generalisation (Smith et al 2007; LeSage et al 2009; Cunningham & McMahon 2013) as might be expected of a partial agonist. Whilst the degree of generalisation seen in drug discrimination studies depends partly on how it is measured, partly on the training methods used and the doses of nicotine and varenicline, Jutkiewicz et al (2011) have suggested that some nicotine discriminative stimuli can be based on mainly $\alpha 4\beta 2$ receptors, but others can be based on multiple nicotinic receptor mechanisms. This is an entirely plausible explanation for the varied results with varenicline in the drug discrimination assay. In a similar vein, our results with varenicline may have arisen because the particular chronic dosing regimen with nicotine and subsequent withdrawal, affected more than just $\alpha 4\beta 2$ receptors. There is evidence that varenicline is a partial agonist at $\alpha 4\beta 2$ nicotinic receptor subtypes (Coe et al, 2005; Rollema et al, 2007) but also that it has affinity for and efficacy at others. Mihalak et al (2006) investigated functional activity at rat neuronal nicotinic receptors and reported efficacy values of 13.4% at $\alpha 4\beta 2$, 93% at $\alpha 7$ and 75% at $\alpha 3\beta 4$ subtypes. Potency values (EC_{50}) were respectively 2.3 μM , 18 μM and 55 μM . Grady et al (2010) also report considerable efficacy of varenicline at $\alpha 7$ and $\alpha 3\beta 4$ subtypes, although their data were derived from mice. Thus on the basis of potency and efficacy, $\alpha 7$ receptors become a possible target for the action of varenicline in our study. In addition, $\alpha 7$ receptors do not appear to undergo lasting inactivation by chronic nicotine exposure and are expressed particularly in the cortex (Gotti et al, 2009). We therefore tentatively suggest that ligands with specific action at $\alpha 7$ subtypes

might be particularly suitable for ameliorating withdrawal-induced deficits in performance monitoring, although clearly further studies would be required to establish this.

The time course of withdrawal-induced disruptions to PRL performance were similar to those previously observed using a visuospatial attention task. Removal of osmotic minipumps from nicotine-dependent subjects elicited peak deficits in number of omission errors and increases in latency to respond (Shoaib & Bizarro 2005). In contrast to the current results however, these withdrawal effects may not have involved $\alpha 7$ nicotinic receptors, as the selective antagonist methyllycaconitine did not induce attentional deficits, but a non-selective antagonist did (dihydro- β -erythroidine; Shoaib & Bizarro 2005). Taken together with the results of our studies using PRL, this would seem to suggest that nicotine may be able to exert effects on different cognitive domains via dissociable mechanisms. Whilst preclinical studies have pointed to a role for dopamine in the effects of nicotine on attention (Cole & Robbins, 1989; Shoaib & Bizarro, 2005), nothing is known about its action on PRL performance. It is therefore important to investigate this further, not only from the point of view of developing new treatments to help smoking cessation, but also because of the growing evidence for differential involvement of nicotinic receptor subtypes in healthy and disordered human cognition (e.g., Martin-Ruiz et al 1999; Reinvang et al 2010; Evans et al, 2013).

Finally, whilst the use of osmotic minipumps for nicotine administration may not mimic absolutely the peaks and troughs of nicotine self-dosing in human smokers, it provides a simple means of dosing rats with nicotine for periods of 1 to 4 weeks in order to produce “physiological” (or somatic) dependence, demonstrated either by spontaneous withdrawal or precipitated withdrawal. It is the nature of the slow, prolonged nicotine exposure, that allows adaptations to develop, and which do not develop as easily with repeated systemic injections of nicotine (Corrigall, et al 1989). In addition, the constant infusion of nicotine via minipumps achieves plasma levels comparable to those found in smokers

(Henningfield, London, & Benowitz, 1990; Jansson, Andersson, Fuxe, Bjelke, & Eneroth, 1989). It therefore provides a relatively straightforward model that can be used in conjunction with cognitive tasks that are translatable to the human situation.

In conclusion, we have developed an animal model of nicotine withdrawal-induced deficits in probabilistic reversal learning, an aspect of executive control that appears to be impaired in human smokers. Performance deficits peaked around 24 hours into withdrawal and were largely recovered by 96 hours. Both nicotine and varenicline were able to restore withdrawal-induced deficits. Our results suggest that the model may be useful for investigating the efficacy of putative new treatments for smoking cessation.

Funding and Disclosure

These experiments were funded by the University of Newcastle. The authors have no competing financial interests.

Author contributions

AJ conceived the original idea for the nicotine withdrawal study, prepared the publication and contributed towards design of the experiments. MS conceived the original idea for varenicline studies, had the main role in designing and executing the studies and contributed towards the final publication. SS and YB assisted in running the experiments.

Acknowledgements

A special thanks to Dr Leigh Panlillio from the National Institute on Drug Abuse, Baltimore, US who provided the necessary programming skills to provide the PRL program in Med-State Notation.

References

- Allison C, Shoaib M (2013). Nicotine improves performance in an attentional set shifting task in rats. *Neuropharmacology* **64**: 314-320.
- Austin AJ, Duka T, Rusted J, Jackson A (2014). Effect of varenicline on aspects of inhibitory control in smokers. *Psychopharmacology* **231**: 3771-3785.
- Bari A, Theobald DE, Caprioli D, Mar A C, Aidoo-Micah A, Dalley JW, Robbins TW (2010) Serotonin modulates sensitivity to reward and negative feedback in a probabilistic reversal learning task in rats. *Neuropsychopharmacology* **35**: 1290-1301.
- Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Methodological)* **57**: 289-300.
- Bizarro L, Shoaib M (2005) Deficits in a sustained attention task following nicotine withdrawal in rats. *Psychopharmacology* **178**: 211-22
- Boog M, Höppener P, v. d. Wetering BJM, Goudriaan AE, Boog MC and Franken IHA (2014) Cognitive inflexibility in gamblers is primarily present in reward-related decision making. *Front. Hum. Neurosci.* **8**: 1-6.
- Brooks DDS, Jackson A (2008) Behavioural differences in impulsivity amongst chippers and regular smokers. *J Psychopharmacology* **22 (5, suppl)**: A75.
- Budhani S, Richell RA, Blair RJR (2006) Impaired reversal but intact acquisition: probabilistic response reversal deficits in adult individuals with psychopathy. *J. Abnorm. Psychol.* **115**: 552–558.
- K Butler, J Rusted, P Gard, A Jackson (2011) Probabilistic Reversal Learning Behaviour in Current, Former and Never Smokers. *13th Annual European Meeting of the Society for Research on Nicotine and Tobacco (SRNT-E) Turkey*. Sept 2011.
- Butler KA (2013). Effects of smoking status on punishment sensitivity and cognitive control. Unpublished PhD Thesis, University of Brighton, Brighton, UK. p144.
- Chamberlain SR, Muller U, Blackwell AD, Clark L, Robbins TW, Sahakian BJ (2006) Neurochemical modulation of response inhibition and probabilistic learning in humans. *Science* **311**: 861–863.
- Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, Sands SB, Davis TI, Lebel LA, Fox CB, Shrikhande A, Heym JH, Schaeffer E, Rollema H, Lu Y, Mansbach RS, Chambers LK, Rovetti CC, Schulz DW, Tingley FD 3rd, O'Neill BT (2005) Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation. *J Med Chem* **48**: 3474–3477.
- Chase HW, Frank MJ, Michael A, Bullmore ET, Sahakian BJ, Robbins, TW (2010) Approach and avoidance learning in patients with major depression and healthy controls: Relation to anhedonia. *Psychological Medicine* **40**: 433–440.

Chen, K.C., Baxter, M.G. & Rodefer, J.S. (2004) Central blockade of muscarinic cholinergic receptors disrupts affective and attentional set-shifting. *Eur. J. Neurosci.* **20**, 1081-1088.

Corrigall WA, Coen KM (1989) Nicotine maintains self-administration in rats on a limited- access schedule. *Psychopharmacology* **99**: 473-478

Cunningham CS, Javors MA, McMahon LR (2012) Pharmacologic Characterization of a Nicotine-Discriminative Stimulus in Rhesus Monkeys. *JPET* **341**: 840-849

Cunningham CS, McMahon LR (2013) Multiple nicotine training doses in mice as a basis for differentiating the effects of smoking cessation aids. *Psychopharmacology* **228**: 321–333

Dawkins L, Powell JH, Pickering A, Powell J, West R (2009) Patterns of change in withdrawal symptoms, desire to smoke, reward motivation and response inhibition across 3 months of smoking abstinence. *Addiction* **104**: 850-858.

Evans S, Gray MA, Dowell NG, Tabet N, Tofts PS, King SL, Rusted JM (2013) APOE E4 Carriers Show Prospective Memory Enhancement Under Nicotine, and Evidence for Specialisation Within Medial BA10. *Neuropsychopharmacology* **38**: 655–663;

Faessel HM, Smith BJ, Gibbs MA, Gobey JS, Clark DJ, Burstein AH (2006) Single-Dose Pharmacokinetics of Varenicline, a Selective Nicotinic Receptor Partial Agonist, in Healthy Smokers and Nonsmokers. *J. Clin. Pharmacol.* **46**: 991-998.

Foulds J, Stapleton J, Swettenham J, Bell N, McSorley K, Russel MAH (1996) Cognitive performance effects of subcutaneous nicotine in smokers and never-smokers. *Psychopharmacology* **127**: 31-38.

Garavan H, Ross TJ, Kaufman J, Stein EA (2003) A midline dissociation between error-processing and response-conflict monitoring. *NeuroImage* **20**: 1132–1139.

Gotti C, Clementi F, Fornari A, Gaimarri A, Guiducci S, Manfredi I, Moretti M, Pedrazzi P, Pucci L, Zoli M (2009) Structural and functional diversity of native brain neuronal nicotinic receptors. *Biochemical Pharmacology* **78**: 703–711.

Grady SR, Drenan RM, Breining SR, Yohannes D, Wageman CR, Fedorov NB, McKinney S, Whiteaker P, Bencherif M, Lester HA, Marks MJ (2010) Structural differences determine the relative selectivity of nicotinic compounds for native $\alpha 4\beta 2^{*-}$, $\alpha 6\beta 2^{*-}$, $\alpha 3\beta 4^{*-}$ and $\alpha 7$ -nicotine acetylcholine receptors. *Neuropharmacology* **58**: 1054-1066

- Henningfield JE, London ED, Benowitz NL (1990) Arterial-venous differences in plasma concentrations of nicotine after cigarette smoking. *JAMA*: **263**(15):2049-50.
- Hornak J, O'Doherty J, Bramham J, Rolls ET, Morris RG, Bullock PR, Polkey CE (2004) Reward-related reversal learning after surgical excisions in orbito-frontal or dorsolateral prefrontal cortex in humans. *J Cogn Neurosci* 16:463-478
- Hughes JR, Gust SW, Skoog K, Keenan RM, Fenwick JW (1991) Symptoms of tobacco withdrawal – a replication and extension. *Arch Gen Psychiatry* **48**: 52-59.
- Hughes JR, Keely J, Naud S (2004) Shape of the relapse curve and long-term abstinence among untreated smokers. *Addiction*, **99**: 29–38.
- Iannaccone R, Hauser TU, Staempfli P, Walitza S, Brandeis D, Brem S (2015) Conflict monitoring and error processing: New insights from simultaneous EEG–fMRI. *NeuroImage* **105**: 395–407.
- Jansson A, Andersson K, Fuxe K, Bjelke B, Eneroth P (1989) Effects of combined pre- and postnatal treatment with nicotine on hypothalamic catecholamine nerve terminal systems and neuroendocrine function in the 4-week old and adult male and female diestrous rat. *J Neuroendocrinol*. **1**(6):455-64.
- Jutkiewicz EM, Brooks EA, Kynaston AD, Rice KC, Woods JH (2011) Patterns of Nicotinic Receptor Antagonism: Nicotine Discrimination Studies *JPET* **339**: 194–202.
- Kalmijn S, van Boxtel MP, Verschuren MW, Jolles J, Launer LJ (2002) Cigarette smoking and alcohol consumption in relation to cognitive performance in middle age. *Am J Epidemiol* **156**: 936–944.
- Kralikova E, Kmetnova A, Stepankova L, Zvolaska K, Davis R, West R (2013) Fifty-two-week continuous abstinence rates of smokers being treated with varenicline versus nicotine replacement therapy. *Addiction* **108**: 1497-1502.
- LeSage MG, Shelley D, Ross JT, Ivy Carroll F, Corrigan WA (2009) Effects of the nicotinic receptor partial agonists varenicline and cytisine on the discriminative stimulus effects of nicotine in rats. *Pharmacology, Biochemistry & Behavior* **91**: 461–467.
- Malin DH, Lake JR, Newlin-Maultsby P, Roberts LK, Lanier JG, Carter VA, Cunningham JS, and Wilson OB (1992) Rodent model of nicotine abstinence syndrome. *Pharmac Biochem Behav* **43** :779-784.
- Martin CA, Rayens MK, Kelly T, Hartung C, Leukefeld C, Haigler E (2000) Card Perseveration Task performance and post-task feeling states: relationship to drug use in adolescents. *Am J Drug Alcohol Abuse* **26**:325–33.
- Martin-Ruiz CM, Court JA, Molnar E, Lee M, Gotti C, Mamalaki A, Tsouloufis T, Tzartos S, Ballard C, Perry RH, Perry EK (1999) Alpha4 but not alpha3 and alpha7 nicotinic acetylcholine receptor subunits are lost from the temporal cortex in Alzheimer's disease. *Journal of Neurochemistry*, **73**: 1635–1640.
- Mendrek A, Monterosso J, Simon SL, Jarvik M, Brody A, Olmstead R, Domier CP, Cohen MS, Ernst M, London ED (2006): Working memory in cigarette smokers: Comparison to non- smokers and effects of abstinence. *Addict Behav* **31**: 833– 844.

- Mihalak KB, Carroll FI, Luetje CW (2006) Varenicline is a partial agonist at $\alpha 4\beta 2$ and a full agonist at $\alpha 7$ neuronal nicotinic receptors. *Mol Pharmacol* **70**: 801–805
- Murakami K, Ishikawa Y, Sato F (2013) Localization of $\alpha 7$ nicotinic acetylcholine receptor immunoreactivity on gabaergic interneurons in layers i–iii of the rat retrosplenial granular cortex. *Neuroscience* **252**: 443–459.
- Nesic J, Rusted J, Duka T, Jackson A (2011) Degree of dependence influences the effect of smoking on cognitive flexibility. *Pharmacology, Biochemistry and Behavior* **98**: 376–384.
- Nestor L, McCabe E, Jones J, Clancy L, Garavan (2011) Differences in “bottom-up” and “top-down” neural activity in current and former cigarette smokers: Evidence for neural substrates which may promote nicotine abstinence through increased cognitive control. *NeuroImage* **56**: 2258–2275.
- Pergadia ML, Der-Avakian A, D’Souza MS, Madden PAF, Heath AC, Shiffman S, Markou A, Pizzagalli DA (2014) Association Between Nicotine Withdrawal and Reward Responsiveness in Humans and Rats. *JAMA Psychiatry* **71**(11): 1238-1245.
- Perneger TV (1998) What’s wrong with Bonferroni adjustments. *BMJ* **316**: 1236–8.
- Picard F, Sadaghiani S, Leroy C, Courvoisier DS, Maroy R, Bottlaender M (2013) High density of nicotinic receptors in the cingulo-insular network. *NeuroImage* **79**: 42–51.
- Postma P, Kumari V, Sharma T, Hines M, Gray JA (2001) Startle response during smoking and 24h after withdrawal predicts successful smoking cessation. *Psychopharmacology* **156**: 360 – 367.
- Powell J, Dawkins L, West R, Powell J, Pickering A (2010). Relapse to smoking during unaided cessation: clinical, cognitive and motivational predictors. *Psychopharmacology* **212**: 537–549.
- Pratt JA, Stolerian IP, Garcha HS, Giardini V, Feyerabend C (1983) Discriminative stimulus properties of nicotine: further evidence for mediation at a cholinergic receptor. *Psychopharmacology (Berl)*. **81**(1):54-60.
- Reinvang I, Lundervold AJ, Wehling E, Rootwelt H, Espeseth T (2010) Epistasis between *APOE* and nicotinic receptor gene *CHRNA4* in age related cognitive function and decline. *Journal of the International Neuropsychological Society* **16**: 424–432.
- Roberts AC, Robbins TW, Everitt BJ, Muir JL (1992) A specific form of cognitive rigidity following excitotoxic lesions of the basal forebrain in marmosets. *Neuroscience* **47**(2):251-64.
- Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, Lu Y, Mansbach RS, Mather RJ, Rovetti CC, Sands SB, Schaeffer E, Schulz DW, Tingley FD 3rd, Williams KE (2007) Pharmacological profile of the $\alpha 4\beta 2$ nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. *Neuropharmacology* **52**: 985–994.
- Shoaib M, Bizarro L (2005) Deficits in a sustained attention task following nicotine withdrawal in rats. *Psychopharmacology (Berl)* **178**(2-3):211-22.

- Silk S, Shoaib M (2014) Capturing cognitive deficits associated with nicotine withdrawal using a probabilistic reversal-learning paradigm in rats *J Psychopharm* **28(8)**: Supplement A3
- Smith JW, Mogg A, Tafi E, Peacey E, Pullar IA, Szekeres P, Tricklebank M (2007) Ligands selective for $\alpha 4\beta 2$ but not $\alpha 3\beta 4$ or $\alpha 7$ nicotinic receptors generalise to the nicotine discriminative stimulus in the rat. *Psychopharmacology* **190**: 157–170.
- Steele JD, Kumar P, Ebmeier KP (2007) Blunted response to feedback information in depressive illness. *Brain* **130**: 2367–2374.
- Stolerman IP (1996) Multiple brain sites of action for nicotine. *J Psychopharmacology* **10**:173.
- West R, Russell MAH (1988) Loss of acute nicotine tolerance and severity of cigarette withdrawal. *Psychopharmacology* **94**: 563 – 565.
- Wing VC, Shoaib M (2007) Examining the clinical efficacy of bupropion and nortriptyline as smoking cessation agents in a rodent model of nicotine withdrawal. *Psychopharmacology* **195**:303-13.
- Wong DF, Kuwabara H, Pomper M, Holt DP, Brasic JR, George N, Frolov B, Willis W, Gao Y, Valentine H, Nandi A, Gapasin L, Dannals RF, Horti AG (2014) Human Brain Imaging of $\alpha 7$ nAChR with [18F]ASEM: a New PET Radiotracer for Neuropsychiatry and Determination of Drug Occupancy. *Molecular Imaging and Biology* **16**: 730-738.

Table 1. The effect of nicotine and varenicline on probabilistic response reversal (PRL) in rats.

	nicotine (mg/kg)					
	0	0.025	0.05	0.1	0.2	0.4
<i>Reversals (n)</i>	4.44 ± 0.3	4.5 ± 0.7	4.38 ± 0.8	5.25 ± 0.6	4.38 ± 0.5	5.38 ± 0.6
<i># Response latency (s)</i>	3.46 ± 0.2	2.93 ± 0.7	3.38 ± 0.9	3.01 ± 1.4	3.71 ± 1.8	3.67 ± 2.2
<i>Omissions (n)</i>	0.82 ± 0.3	0.38 ± 0.3	0.0 ± 0.0	1.13 ± 1	1.75 ± 1.8	2.75 ± 1.6
<i>Pellets (n)</i>	117.8 ± 1.0	119.2 ± 2.9	120.0 ± 3.1	121.0 ± 2.8	120.8 ± 2.2	121.6 ± 3.2
<i>Errors (n)</i>	71.4 ± 2.3	66.1 ± 2	68.6 ± 3	66.2 ± 4.3	68.6 ± 1.8	68.1 ± 3.1
<i>Session Time (s)</i>	1249.8 ± 60.9	1178.3 ± 76.4	1252.3 ± 91.1	1378.5 ± 125.6	1440.5 ± 147.9	1552.7 ± 124.3
<i>Win-Stay Ratio</i>	0.810 ± 0.02	0.825 ± 0.03	0.813 ± 0.04	0.813 ± 0.04	0.825 ± 0.02	0.804 ± 0.02
<i>Lose-Shift Ratio</i>	0.573 ± 0.06	0.610 ± 0.05	0.619 ± 0.06	0.586 ± 0.04	0.560 ± 0.06	0.496 ± 0.05

	varenicline (mg/kg)				
	0	0.03	0.1	0.3	1
<i>Reversals (n)</i>	5.5 ± 0.8	5.13 ± 1.1	6.38 ± 0.9	6.88 ± 0.8	6.25 ± 0.9
<i>Response latency (s)</i>	3.02 ± 0.2	2.43 ± 0.2*	2.35 ± 0.2*	2.25 ± 0.2*	2.29 ± 0.2*
<i># Omissions (n)</i>	0.0 ± 1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
<i>Pellets (n)</i>	120.2 ± 3.5	120.2 ± 4.6	124.8 ± 2.2	125.0 ± 3.9	123.7 ± 2.3
<i>Errors (n)</i>	61.0 ± 3.1	64.8 ± 5	63.2 ± 2.3	62.1 ± 3.8	61.7 ± 2.3
<i>Session Time (s)</i>	1100.6 ± 30.9	970.5 ± 36.4*	964.9 ± 27.2*	938.8 ± 43.3*	937.3 ± 32.4*
<i>Win-Stay Ratio</i>	0.888 ± 0.02	0.846 ± 0.03	0.866 ± 0.02	0.888 ± 0.02	0.867 ± 0.03
<i>Lose-Shift Ratio</i>	0.627 ± 0.05	0.566 ± 0.03	0.598 ± 0.06	0.602 ± 0.05	0.610 ± 0.06

Data are means (± s.e.m.) for n= 8 rats, except where data were not normally distributed, # so are medians (± semi-quartile range).

*p<0.05 w.r.t. corresponding vehicle controls.

Table 2. The effect of withdrawal from chronic nicotine treatment, across time, on probabilistic response reversal performance.

Omissions (n)							
	T=0 hrs	T=6 hrs	T=12 hrs	T=24 hrs	T=48 hrs	T=72 hrs	T=96 hrs
<i>vehicle</i>	1 ± 0.25	1 ± 1	1.5 ± 1.75	3 ± 5.75	1.5 ± 3.25	1.5 ± 2	0 ± 0.5
<i>nicotine</i>	0 ± 1	4.5 ± 3.5	2 ± 2.75	10.5 ± 10.5 *	3 ± 2.5 *	1.5 ± 3	1 ± 2.5

Total Pellets (n)							
	T=0 hrs	T=6 hrs	T=12 hrs	T=24 hrs	T=48 hrs	T=72 hrs	T=96 hrs
<i>vehicle</i>	116.7 ± 3.1	118.8 ± 2.71	120.3 ± 3.5	124.1 ± 2.38	122.7 ± 2.23	121.3 ± 2.07	116.1 ± 2.44
<i>nicotine</i>	123.1 ± 2.2	120.2 ± 3.7	114.7 ± 2.56	105.2 ± 5.81 **	118.1 ± 2.87	119.7 ± 3.42	120.2 ± 2.68

Errors (n)							
	T=0 hrs	T=6 hrs	T=12 hrs	T=24 hrs	T=48 hrs	T=72 hrs	T=96 hrs
<i>vehicle</i>	64.2 ± 2.79	66.8 ± 3.46	65.1 ± 2.84	63.5 ± 2	62 ± 3.55	66.2 ± 2.33	70.7 ± 3.31
<i>nicotine</i>	64.1 ± 2.75	72.8 ± 3.68	74.5 ± 3.1 †	63.3 ± 4.88	69.8 ± 4.7	70.3 ± 2.47	62.3 ± 2.81

Win Stay Ratio							
	T=0 hrs	T=6 hrs	T=12 hrs	T=24 hrs	T=48 hrs	T=72 hrs	T=96 hrs
<i>vehicle</i>	0.855 ± 0.013	0.809 ± 0.025	0.855 ± 0.016	0.855 ± 0.024	0.865 ± 0.022	0.866 ± 0.018	0.834 ± 0.017
<i>nicotine</i>	0.851 ± 0.012	0.739 ± 0.037	0.772 ± 0.029 †	0.773 ± 0.031 †	0.823 ± 0.027	0.78 ± 0.031	0.829 ± 0.028

Lose Shift Ratio							
	T=0 hrs	T=6 hrs	T=12 hrs	T=24 hrs	T=48 hrs	T=72 hrs	T=96 hrs
<i>vehicle</i>	0.563 ± 0.018	0.531 ± 0.051	0.523 ± 0.034	0.544 ± 0.051	0.506 ± 0.039	0.572 ± 0.053	0.544 ± 0.041
<i>nicotine</i>	0.488 ± 0.046	0.441 ± 0.046	0.52 ± 0.073	0.479 ± 0.088	0.619 ± 0.06	0.523 ± 0.058	0.468 ± 0.052

Data are means (±s.e.m.) for n=8 rats per treatment group, except for omissions, where data are medians (±semi-quartile range). ** p<0.01, * p<0.05 w.r.t. baseline (T=0 hrs). ** p<0.01, † p<0.05 w.r.t vehicle group.

Table 3. The effect of nicotine and varenicline on probabilistic response reversal (PRL) performance across time, during withdrawal from chronic nicotine treatment.

Total Pellets (n)						
	T=0 hrs	T=6 hrs	T=12 hrs	T=24 hrs	T=30 hrs	T=36 hrs
<i>vehicle</i>	120.3 ± 3.9	103.6 ± 7.7	120.0 ± 3.1	63.8 ± 8.2 **	76.2 ± 9.4 **	113.9 ± 4.9
<i>nicotine (0.2 mg/kg)</i>	110.9 ± 4.4	107.4 ± 7.5	118.3 ± 2.3	100.6 ± 8.5	69.6 ± 11.8 \$	113.5 ± 6.1
<i>varenicline (0.3 mg/kg)</i>	113.3 ± 4.3	99.2 ± 9.9	119.6 ± 2.6	89.7 ± 9.9	67.0 ± 8.2 **	106.6 ± 5.3
<i>varenicline (1.0 mg/kg)</i>		104.0 ± 3.6				
	119.0 ± 2.5	**	120.1 ± 3.4	96.5 ± 9.8	94.3 ± 11.9	119.9 ± 3.4
Errors (n)						
	T=0 hrs	T=6 hrs	T=12 hrs	T=24 hrs	T=30 hrs	T=36 hrs
<i>vehicle</i>	67.0 ± 3.4	59.9 ± 5.0	63.5 ± 1.5	40.9 ± 5.8 **	49.3 ± 5.9 #	63.0 ± 3.7
<i>nicotine (0.2 mg/kg)</i>	68.1 ± 2.6	61.3 ± 3.0	68.8 ± 3.8	61.0 ± 6.6	31.5 ± 4.2 **	54.8 ± 3.3 *
<i>varenicline (0.3 mg/kg)</i>	73.3 ± 5.1	66.5 ± 3.4	67.6 ± 2.8	46.1 ± 4.7 **	40.2 ± 3.6 **	59.3 ± 3.5
<i>varenicline (1.0 mg/kg)</i>	66.0 ± 2.7	69.8 ± 3.4	61.6 ± 2.2	54.2 ± 4.9	53.0 ± 5.4	63.8 ± 2.1
Total Session Time (s)						
	T=0 hrs	T=6 hrs	T=12 hrs	T=24 hrs	T=30 hrs	T=36 hrs
<i>vehicle</i>	1923.2 ± 101.6	2288.5 ± 111.5	2137.8 ± 131.7	2400.0 ± 0.0	2390.1 ± 9.8	2178 ± 87.50
<i>nicotine (0.2 mg/kg)</i>	1757.5 ± 125.4	2195.1 ± 103.2	1758.8 ± 73.99	2190.5 ± 101.5	2400.0 ± 0.0	2257.3 ± 84.80
<i>varenicline (0.3 mg/kg)</i>	1793.8 ± 91.0	2203.0 ± 97.5	1898.2 ± 94.7	2400.0 ± 0.0	2400.0 ± 0.0	2388.2 ± 11.7
<i>varenicline (1.0 mg/kg)</i>	1681.6 ± 90.1	2179.5 ± 103.7	1708.3 ± 98.9	2103.0 ± 160.0	2381.2 ± 17.2	2151.6 ± 86.3
Win Stay Ratio						
	T=0 hrs	T=6 hrs	T=12 hrs	T=24 hrs	T=30 hrs	T=36 hrs
<i>vehicle</i>	0.79 ± 0.03	0.74 ± 0.03	0.80 ± 0.01	0.71 ± 0.04	0.77 ± 0.04	0.83 ± 0.02
<i>nicotine (0.2 mg/kg)</i>	0.81 ± 0.02	0.78 ± 0.03	0.82 ± 0.03	0.78 ± 0.03	0.78 ± 0.06	0.83 ± 0.04
<i>varenicline (0.3 mg/kg)</i>	0.78 ± 0.03	0.73 ± 0.06	0.80 ± 0.03	0.83 ± 0.01	0.75 ± 0.05	0.78 ± 0.04
<i>varenicline (1.0 mg/kg)</i>	0.83 ± 0.02	0.78 ± 0.01	0.83 ± 0.02	0.82 ± 0.01	0.80 ± 0.02	0.83 ± 0.02
Lose Shift Ratio						
	T=0 hrs	T=6 hrs	T=12 hrs	T=24 hrs	T=30 hrs	T=36 hrs
<i>vehicle</i>	0.52 ± 0.05	0.45 ± 0.05	0.57 ± 0.04	0.58 ± 0.06	0.53 ± 0.06	0.58 ± 0.04
<i>nicotine (0.2 mg/kg)</i>	0.60 ± 0.03	0.60 ± 0.04	0.68 ± 0.03	0.56 ± 0.05	0.51 ± 0.05	0.62 ± 0.03
<i>varenicline (0.3 mg/kg)</i>	0.53 ± 0.03	0.47 ± 0.03	0.65 ± 0.03	0.50 ± 0.04	0.56 ± 0.03	0.53 ± 0.07
<i>varenicline (1.0 mg/kg)</i>	0.53 ± 0.04	0.54 ± 0.04	0.57 ± 0.03	0.59 ± 0.04	0.50 ± 0.06	0.55 ± 0.04

Data are means (± s.e.m.) for n= 8 rats per treatment group. *p<0.05, **p<0.01 w.r.t. corresponding baseline (t=0 hrs). # p=0.033, adjusted p=0.085. \$ p=0.01, adjusted p=0.05.

Figure 1. Performance of a probabilistic response reversal task during withdrawal from chronic nicotine treatment or chronic saline. Across time for each treatment group: Panel A shows the number of reversals, Panels B shows response latencies and Panels C shows the total time on task. N=8 rats per treatment group. * $p<0.05$, ** $p<0.01$ w.r.t. baseline before withdrawal (T=0 hrs).

Figure 2. The effect of nicotine and varenicline on performance of the probabilistic response reversal task during withdrawal from chronic nicotine treatment. Acute injections of drug (or vehicle) were administered at 12 hrs and again at 24 hrs withdrawal. Panel A shows the number of reversals across time for each treatment group. * $p<0.05$, ** $p<0.01$ w.r.t. corresponding baseline. Panels B shows response latencies across time for the vehicle treatment group (left) as well as results for each treatment group at 12 hrs and 24hrs withdrawal. * $p<0.05$, ** $p<0.01$ w.r.t. corresponding vehicle control group. Panels C shows omissions across time for the vehicle treatment group (left) and results for all treatment groups at 24hrs withdrawal. * $p<0.05$, ** $p<0.01$ w.r.t. corresponding vehicle control group. N=8 rats per treatment group.

Figure 1

Figure 2