Nicotine self-administered directly into the VTA by rats is weakly reinforcing but has strong reinforcement enhancing properties

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Abstract

Rationale Rats will lever press to deliver nanolitre quantities of nicotine or the muscarinic agonist carbachol directly into the ventral tegmental area (VTA). The purpose of these experiments was to investigate further the characteristics of nicotine self-administration directly into the VTA.

Objectives This study aimed to confirm previous data relating to intra-VTA self-administration of nicotine and carbachol and then test two hypotheses: (a) that pre-sensitisation of nicotinic receptors is needed for robust intra-VTA self administration and (b) that rats will lever press for intra-VTA nicotine if pre-trained to associate lever pressing with a rewarding outcome.

Methods Rats were equipped with cannulae aimed at posterior VTA and allowed five sessions to self-administer nicotine or carbachol. In different experiments, rats were either pre-sensitised to nicotine by subcutaneous (s.c.) injections or pre-trained to lever press for food and a simultaneous conditioned stimulus light.

Results We confirmed that carbachol had strong activating effects when self-administered into the VTA; selective responding for nicotine developed over five sessions by reduction in the amount of pressing on an inactive lever. Prior sensitisation did not improve responding for intra-VTA nicotine but training rats to lever press before putting them on the drug regime did potentiate pressing.

Conclusions The action of nicotine in the VTA might be better considered as reinforcement enhancing and that its intrinsic rewarding property here is at best weak. Identification of the VTA as a target for the reinforcement enhancing effects of nicotine is compatible with the reinforcement-related functions of VTA dopamine neurons and their cholinergic inputs.

Keywords Acetylcholine · Dopamine · Intra-cranial self-administration · Nicotine · Pedunculopontine · Reinforcement · Reward · Ventral tegmental area

Introduction

Nicotinic receptors (nAChRs) in the ventral tegmental area (VTA) are commonly associated with nicotine’s reinforcing properties, consistent with the belief that VTA dopamine (DA) neurons have a major role in addiction (Markou 2008). Nicotinic α7 receptors are on somatodendritic portions of VTA DA neurons and terminals of glutamate projections, while α6/β2 and α4/β2 receptors are on DA and GABA neurons (Livingstone and Wonnacott 2009; Yang et al. 2011). Nicotine controls DA neuron burst firing by direct activation and by reducing inhibitory inputs by desensitisation of receptors on GABA neurons (Livingstone and Wonnacott 2009). Acetylcholine (ACh) input to the VTA comes from pedunculopontine and laterodorsal tegmental nuclei (PPTg; LDTg) (Oakman et al. 1995; Omelchenko and Sesack 2005). LDTg stimulation elicits DA release in the ipsilateral nucleus accumbens triphasi-
cally: (1) a fast pulse mediated by VTA nicotinic and ionotropic glutamate receptors; (2) depression of DA release mediated by LDTg M2 muscarinic ACh receptors (mAChRs); and (3) long-lasting increase in DA efflux dependent on VTA M5 mAChRs (Forster and Blaha 2003). The VTA mediates locomotor activity stimulated by nicotine (Clarke and Kumar 1983a, b) and its rewarding properties: nicotine microinjection into VTA promotes positive place preferences for example (Muse and Wise 1994; Laviolle and Van der Kooy 2003). Similarly, intravenous self-administration (IVSA) of nicotine depends on the availability of VTA nicotinic receptors (Corrigall et al. 1994), while lesioning VTA afferents from posterior PPTg also affects this (Alderson et al. 2006). More specifically, rats will self-administer nicotine directly into VTA (Ikemoto et al. 2006) with the most potent effects being elicited from the posterior VTA (which has a pattern of nAChR subunit expression different to that of the anterior VTA; Zhao-Shea et al. 2011). Intracranial self-administration (ICSA) is a powerful technique in which nanoliter amounts of drug are delivered voluntarily by rats to stereotaxically targeted locations in their own brains. In psychopharmacological studies in animals, ICSA is the most direct measure of whether a drug has reinforcing properties at specific brain locations. Although ICSA studies show that rats will deliver nicotine into VTA (Ikemoto et al. 2006), there are questions to resolve. In a two lever discrimination, rats showed increasing responding for 25 mM nicotine in the posterior VTA over four sessions (having been allowed to self-administer only vehicle on the first session)—lever pressing for nicotine increased by ~50% between first and second nicotine test sessions (Ikemoto et al. 2006, Fig. 2). It might be suggested that intra-VTA nicotine was not immediately recognised as a reward (on first exposure it did not provide incentive to bar press) nor was it immediately reinforcing (nicotine delivery did not promote repetition of the action preceding delivery). In contrast, carbachol (a mAChR agonist) was self-administered straight away, as was the cholinesterase inhibitor neostigmine (Ikemoto and Wise 2002).

We have attempted to understand better the mechanism of action of intra-VTA nicotine by replicating existing data concerning ICSA of nicotine and carbachol and then asking two questions. (1) Repeated injections of nicotine change receptor states: Is intra-VTA ICSA of nicotine enhanced by prior sensitisation? (2) Several authors argue that the effects of systemic nicotine are reinforcement enhancing—that is, nicotine potentiates the reinforcing properties of other reinforcers, working to invigorate ongoing seeking behaviour that was established previously (Chaudhri et al. 2006; Lof et al. 2007; Palmatier et al. 2006; Palmatier et al. 2007; Paterson 2009). Consequently, we examined ICSA of nicotine into VTA in rats pre-trained to bar press for food reward [with an associated conditioned stimulus light (CS)] to determine whether they would more readily press for nicotine using a lever that already had an association with reward delivery in the absence of the primary food reward.

Materials and methods

Subjects

Ninety-three male Lister Hooded rats (Harlan Olac Ltd., UK) were used, weighing 350–410 g at surgery. Rats were housed in temperature- and humidity-controlled rooms with lights on a 12-h cycle (on, 0700 hours; off, 1900 hours). Testing was carried out during the light phase. Rats were pair housed on arrival in the vivarium but were separated prior to surgery and remained single housed after. Following recovery from surgery, they were maintained on a food restriction regime such that they gained weight each week by ~10 g. In order to achieve this, 20 g food was given per rat per day, always at the end of the testing procedure or at the equivalent time on non-testing days. Water was freely available in the home cage throughout. All experiments were conducted with the authority of the appropriate UK Home Office Licences and adhered to guidelines set out in the Animals (Scientific Procedures) Act (1986) and International [European Communities Council Directive of 24 November 1986 (86/609/EEC)] legislation governing the maintenance of laboratory animals and their use in scientific experiments.

Surgery

Rats were anaesthetised using isoflurane (Abbot Laboratories Ltd, Maidenhead, UK) in an induction box and placed in a stereotoxic frame (David Kopf, Tujunga, CA, USA); anaesthesia was maintained via a facemask mounted on the incisor bar (1–3% isoflurane, 1.4 l/min O2). Pre-surgery analgesia was given [0.05 ml/rat subcutaneously (s.c.) “Rimadyl” (5% w/v carprofen); Pfizer Ltd, Kent, UK]. All rats were implanted with a unilateral guide cannula (Plastics One, Roanoke, VA, USA; 24 ga; 1.0 mm above the VTA) normally occluded by a stylet extending 0.5 mm beyond the tip. The coordinates were as follows: anterior—posterior, +3.2 mm from interaural line; mediolateral, distance from midline was not measured—the midline sinus was exposed and cannulae inserted adjacent to it; dorsoventral, cannulae were cut so that there was 7.8 mm below the pedestal when it was on the skull surface. Behavioural testing began 10 days after surgery.
Drugs

(–)-Nicotine hydrogen tartrate salt and carbamylcholine chloride (carbachol, a predominantly mAChR agonist; Sigma, Poole, Dorset UK) were dissolved in artificial cerebral spinal fluid (aCSF) consisting of 148 mM NaCl, 2.7 mM KCl, 1.2 mM CaCl2, and 0.85 M MgCl2, pH adjusted to 7.4 with NaOH.

Self-administration apparatus

Behavioural testing was carried out in operant chambers (Med-Associates, St Albans, VT, USA) housed in ventilated, sound attenuating cubicles. The operant chambers measured 30.5 cm (L)×24.1 cm (W)×21 cm (H) and on one wall were illuminated by a house light (1.5 w, 28 v) during testing. On the opposite wall were two standard retractable levers 5 cm wide, spaced 11 cm apart. Above each lever (by 7 cm) was a stimulus light (1.5 w, 28 v) programmed to illuminate when the active lever was pressed. The duration of each test session was 90 min with the maximum number of infusions restricted to 60. There was no evidence post mortem of trauma produced by fluid accumulation; to minimise the non-specific damage produced by introducing the injector cannula itself, rats were tested in only five sessions, the injector cannula therefore being placed into the VTA only five times.

Each rats’ 31-ga injector cannula was connected by PE tubing to a head attachable micropump, built according to the method of Ikemoto and Sharpe (2001). The pump was connected to an electrical swivel to facilitate free movement of the rat in the chamber and to prevent cables tangling. This ICSA procedure enables drug to be delivered to the desired site with the minimum delay. It reproducibly delivers a precise volume (75 nl) when the response schedule is met, facilitating the initiation and maintenance of ICSA. During food-reinforced testing, the swivel was removed and a pellet dispenser was positioned between the levers to deliver 45-mg precision pellets (Noyes Precision Pellets, 45 mg, formula A/1, Sandown Scientific, UK) into a pellet receptacle. The measurements taken by the MED-PC program were the number of responses on the active and inactive levers and the total number of infusions or food pellets received. Video data for each session were recorded using standard VHS video recorders.

Histology

At the end of testing, rats were deeply anaesthetised with 0.8 ml “Dolethal” (200 mg/ml Pentobarbitone; various suppliers) and perfused transcardially with 0.1 M phosphate-buffered saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer at 14 ml/min. Brains were removed and stored in 20% sucrose overnight before 50-μm coronal sections were cut on a freezing microtome; 1:4 was stained with cresyl violet. Cannula placement was confirmed by microscopy and mapped to sections from Paxinos and Watson’s stereotaxic atlas.

Behavioural data analysis

Results were collated as means±SEM for each group, and statistical analysis was performed using SPSS v.17.0. Repeated measures ANOVA were made to compare each behavioural measure on a given testing regime; between levers (active or inactive lever, within-subjects factor) across session (testing day, within-subjects factor) and between drug (the various testing conditions, between-subjects factor). A significant between-groups effect was analysed further with least significant difference post hoc testing to determine if groups were statistically different from control. Where there was a statistically significant day×group interaction paired samples t tests were used to assess the effect. Chi-squared analysis was made of the lever pressing ratio to identify if there was selectivity in responding on one or other lever. All effects were considered statistically significant (P≤0.05).

Experiment 1: acquisition of nicotine and carbachol ICSA

Thirty-five rats with no previous operant training (naive) were placed in operant conditioning chambers for a total of five sessions, with sessions separated by 48 h to minimise tissue damage at the injection site. On an FR1 schedule with two retractable levers, a response on the conditioned lever (active) resulted in a 75-nl infusion of drug. A cue light was illuminated above this lever, and both levers retracted for 20 s. A response on the unconditioned lever (inactive) did not deliver an infusion, retract the levers, or illuminate a cue light. The allocation of left and right levers for conditioned or unconditioned responses was counterbalanced between subjects. Rats were randomly assigned to one of three groups to receive infusions of either aCSF, 25 mM nicotine or 1 mM carbachol (drug doses previously shown to be most rewarding in the posterior VTA; Ikemoto et al. 2006).

Experiment 2: acquisition of nicotine ICSA after sensitisation

Prior to ICSA, 17 rats received six daily s.c. injections of 0.4 mg/kg nicotine and 10 rats received vehicle only (0.9% saline); this regime sensitises rats to the effects of nicotine (Balfour et al. 1998). The following day all rats underwent the ICSA testing regime as above. Rats from each group (nicotine or saline pre-treated) were randomly assigned to
lever press for aCSF or 25 mM nicotine. Group sizes were as follows: nicotine sensitisation/nicotine ICSA, 8; nicotine sensitisation/aCSF ICSA, 9; saline pretreatment/nicotine ICSA, 10.

Experiment 3: dose response assessment of nicotine ICSA after sensitisation

Rats were sensitised by daily s.c. administration of 0.4 mg/kg nicotine as described in experiment 2. Rats were then allowed to ICSA various doses of nicotine. Rats were randomly assigned to the following groups: nicotine sensitisation/aCSF ICSA, three rats; nicotine sensitisation/12.5 mM nicotine ICSA, four rats; nicotine sensitisation/25 mM nicotine ICSA, four rats; nicotine sensitisation/50 mM nicotine ICSA, four rats.

Experiment 4: acquisition of nicotine ICSA following lever training for food reward

Previous studies (for example, Donny et al. 2003; Palmatier et al. 2007) demonstrated that IVSA nicotine is reinforcement enhancing. To investigate this in the context of intra-VTA nicotine ICSA, we trained 16 rats to lever press on a food reinforced schedule prior to surgery—five consecutive days of operant testing using the two retractable lever protocol as before. Responses on the conditioned lever rewarded the rat with a food pellet. Sessions lasted 30 min or until 60 pellets were earned. Rats then underwent surgery and, after recovery, were placed in the chambers and tested for intra-VTA ICSA of aCSF or 25 mM nicotine as in experiment 1.

Results

Histological analysis

Figure 1 shows silhouette sections illustrating cannula placements in each experiment. All were appropriately located in or proximal to the posterior VTA, and there was no obvious difference between experiments in cannula positioning. Two points are worth noting: (1) use of the midline sinus as a guide significantly reduces variability in placing cannulae in the VTA; (2) in each experiment, despite the consistency of the cannula placements, there was considerable variability in rats’ lever pressing, as noted previously (Ikemoto et al. 2006).

Experiment 1: acquisition of nicotine and carbachol ICSA

Rats were randomly assigned to one of three groups to receive infusions of aCSF, 25 mM nicotine or 1 mM carbachol. Pressing on active and inactive levers is shown in Fig. 2a, b. On the active lever, there were no significant effects over sessions \(F_{4,128}=0.477\), not significant (NS) and no session×drug interaction \(F_{8,128}=1.081\), NS, but there was a significant effect of drug \(F_{2,32}=11.00\), \(P<0.001\). On the inactive lever, the effect of session approached significance \(F_{4,128}=2.416\), \(P=0.052\). There was no session×drug interaction \(F_{8,128}=1.014\), NS though there was a significant effect of drug \(F_{2,32}=8.403\), \(P<0.001\). Post hoc analyses showed that rats administering carbachol made more presses on the active lever than rats administering either aCSF \((P<0.001)\) or nicotine \((P=0.026)\) and significantly more on the inactive lever than rats administering aCSF \((P<0.001)\). Comparison was made of active lever pressing as a proportion of the total (active lever presses/active+inactive lever presses). Ratios in session 1 were as follows: aCSF, 0.45; carbachol, 0.53; and nicotine, 0.53. Ratios in session 5 were as follows: aCSF, 0.51; carbachol, 0.60; and nicotine, 0.68. Analysis of the numbers of rats in each group with a ratio of 0.5 or less compared with those having a ratio of 0.51 or better (0.5—no selectivity in lever pressing; higher scores being a shift to pressing on the active lever) confirmed that on the first session, there was no selectivity in responding by any group \((\chi^2=1.47\), NS\), but by session 5, rats in the carbachol and nicotine groups preferred the active lever \((\chi^2=7.73, P=0.025)\).

In order to examine drug effects further, we analysed the inter-press interval (IPI) between first and second press on the active lever, shown in Fig. 2c. Measuring the time taken by rats to press a second time after receiving a first infusion of drug is an inferential measure of reinforcement—if a drug is positively reinforcing, rats should (and do) lever press for its delivery a second time. The latency to do so is assumed to reflect the degree to which the drug is reinforcing. Comparing all groups was not possible because of a lack of data in the aCSF group: of 55 possible occasions for lever pressing (11 rats, five sessions), 21 included no presses on either lever (suggesting that aCSF was undetectable in the VTA). Rats in the carbachol group always pressed two or more times across all sessions; only one rat in the nicotine treatment group failed to press at least twice and was excluded from this analysis. ANOVA comparing carbachol and nicotine IPI (log10) showed no effect over sessions (though it approached statistical significance) and no effect of drug, but there was a significant drug×session interaction \((session, F_{4,88}=2.317, P=0.063\); drug, \(F_{1,22}=1.497, P=0.234\), NS; session×drug, \(F_{4,88}=2.565, P=0.044\)). Further analysis (paired samples \(t\) tests) showed significant differences between carbachol and nicotine on session 1 \((P=0.023)\) but not session 5 \((P=0.152)\). On the first session, rats self-administering carbachol made a second lever press more quickly than rats self-administering nicotine, but by session 5, this difference between drugs had disappeared.
Experiment 2: acquisition of nicotine ICSA after sensitisation

Before ICSA, rats were treated with nicotine in a sensitisation regime (Balfour et al. 1998; Ferrari et al. 2001; Pidoplichenko et al. 1997). Rats were randomly assigned to one of three groups: (1) pre-sensitised to nicotine (s.c. injection of 0.4 mg/kg nicotine over six daily sessions) followed by ICSA of 25 mM nicotine; (2) pre-sensitised to nicotine followed by aCSF ICSA; and (3) control pre-sensitisation (six daily injections of isotonic saline) followed by ICSA of 25 mM nicotine. Pre-sensitising rats to nicotine did not influence ICSA: No significant differences between the groups in active or inactive lever pressing were seen over five sessions (see Fig. 3a, b; active lever: drug, $F_{2,24}=0.207$, NS; sessions, $F_{4,96}=0.692$, NS; interaction, $F_{8,96}=0.736$, NS; inactive lever: drug, $F_{2,24}=0.673$, NS; sessions, $F_{4,96}=1.903$, NS; interaction, $F_{8,96}=0.237$, NS.) Selectivity for responding on the active lever was absent (measured as in experiment 1, $\chi^2_2=2.07$, NS in sessions 1 and 5). Measurement of IPI (Fig. 3c) showed no differences (drug, $F_{2,10}=0.320$, NS; sessions, $F_{4,76}=0.171$, NS; interaction, $F_{8,76}=0.619$, NS). Prior sensitisation did not affect the response to ICSA nicotine; the control response (saline pretreatment followed by nicotine ICSA) was weak, but similar to that in the previous experiment (compare Fig. 2a, b with Fig. 3a, b).

Experiment 3: dose response assessment of nicotine ICSA after sensitisation

All rats were sensitised to 0.4 mg/kg nicotine, after which they were assigned to four matched ICSA groups: (1) aCSF, (2) 12.5 mM, (3) 25 mM, or (4) 50 mM nicotine. There was considerable variability in individual rats’ lever pressing, illustrated in Fig. 4a, b. Significant effects of drug administration were seen on active lever pressing over sessions ($F_{4,44}=4.65$, $P=0.003$), but effects of drug ($F_{3,11}=1.949$, NS) and an interaction ($F_{12,44}=1.195$, NS) were absent. Post hoc analyses comparing groups showed no significant effect of nicotine compared to aCSF (12.5 mM, $P=0.305$; 25 mM, $P=0.062$; 50 mM, $P=0.061$). No statistically significant changes were seen in the amount of inactive lever pressing over five sessions (drug, $F_{3,11}=1.68$, NS; sessions, $F_{4,44}=1.124$, NS; interaction, $F_{12,144}=1.692$, NS). Rats self-administering 12.5, 25, and 50 mM nicotine demonstrated no significant selectivity towards the active lever from session 1 ($\chi^2_2=3.01$, NS) to session 5 ($\chi^2_2=1.46$, NS). Willingness to self-administer nicotine varied

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between doses (Fig. 4c), but analysis of IPI showed no significant differences in pressing for nicotine (drug, $F_{1.7}=0.368$, NS; sessions, $F_{4,28}=0.345$, NS; interaction, $F_{12,28}=0.372$, NS).

**Experiment 4: acquisition of nicotine ICSA following lever training for food reward**

Does it make a difference in ICSA responding if the lever delivering drug is already associated with reinforcement? Rats were trained to lever press over 5 days (tests separated by 48 h) using the operant chambers in which ICSA would later be studied. Responses on the active lever were rewarded with a 45-mg food pellet; responses on the inactive lever had no programmed consequence. All rats successfully learned this and were then surgically prepared for ICSA experiments. One group was allowed to self-administer aCSF into the VTA, the other 25 mM nicotine, as in experiment 1.

High levels of responding were seen on session 1 in food pre-trained animals—both groups were still anticipating food reward (Fig. 5a, b). Statistically significant effects were seen in lever pressing across sessions (active lever, $F_{4.56}=8.10$, $P<0.001$; inactive lever, $F_{4.56}=3.29$, $P=0.017$), but session×drug interactions were not significant (active lever, $F_{4.56}=0.61$, NS; inactive lever, $F_{4.56}=1.391$, NS). Analysis of responses on the active lever showed a drug effect ($F_{1.14}=6.36$, $P=0.024$): Nicotine clearly produced more responding than aCSF (Fig. 5a). Analysis of the proportion of active lever presses was skewed by the fact that on session 1, rats were anticipating food reward rather than nicotine, so on the first test session, there was a significant difference between rats administering carbachol compared to those administering nicotine ($P=0.023$). Full details of statistical analyses are provided in the text.
Fig. 3 Mean number of active (a) and inactive (b) lever presses across five sessions, each separated by 48 h. Rats were assigned to one of three groups: pre-sensitised to nicotine followed by ICSA of 25 mM nicotine, pre-sensitised to nicotine followed by ICSA of aCSF, and pre-sensitised to saline followed by ICSA of 25 mM nicotine. Pre-sensitising rats to nicotine had no statistically significant effect on drug self-administration. c Measurement of the time interval between the first and second response on the active lever [first inter press interval (IPI) in minutes]. Sessions 1 and 5 are shown; there were no statistically significant effects. Full details of all statistical analyses are provided in the text.

Comparison with experiment 1: Fig. 2c shows that 25 mM nicotine had a long IPI on session 1, but this reduced by session 5. Figure 5c shows a much shorter IPI on session 1 (when food was anticipated) and a longer one on session 5.

Comparison of the effects of nicotine with and without food pre-training

The effects of nicotine ICSA in food pre-trained rats differed to those seen in experiment 1, where rats were not pre-trained. We made direct comparison of lever pressing for nicotine and aCSF in experiments 1 and 4; the results are shown in Fig. 6. Analysis was made of active and inactive lever pressing (two levers) in sessions 1 and 5 (two sessions) of rats self-administering 25 mM nicotine or aCSF in either food trained or non-pre-trained rats (four drug/training conditions). Overall ANOVA showed effects of session ($F_{1,30}=18.95$, $P<0.001$), lever ($F_{1,30}=32.52$, $P<0.001$) and training condition ($F_{3,30}=11.23$, $P<0.001$). Of the two-way interactions, session x training condition ($F_{3,30}=1.76$, NS) and session x lever ($F_{1,30}=3.41$, NS) were not statistically significant, but lever x training condition was ($F_{3,30}=19.70$, $P<0.001$). The three-way interaction, session x lever x training condition was also significant ($F_{3,30}=4.28$, $P=0.013$).

We examined activity on active and inactive levers independently in the two sessions. For the inactive lever, there was no effect of training condition ($F_{3,30}=2.13$, NS) and no session x training condition interaction ($F_{3,30}=0.53$, NS), but there was a main effect of session ($F_{1,30}=15.18$, $P=0.001$). Rats in every group showed less pressing on the inactive lever in session 5 compared to session 1, but other than this, no effects were present. In contrast, pressing on the active lever showed significant effects (session, $F_{1,30}=14.87$, $P<0.001$; training condition, $F_{3,30}=16.91$, $P<0.001$) and a session x training condition interaction ($F_{3,30}=3.07$, $P=0.043$). Figure 6 shows that rats pre-trained to lever press for food maintained high levels of responding on session 1, with a clear preference for the active lever. Differences between pre-trained and trained rats are unsurprising in this session precisely because some rats were responding in the expectation of food while the others were entirely naive with respect to the operant boxes.
Session 5 was radically different: ANOVA of all session 5 data showed a significant effect of lever ($F_{1,30}=12.722$, $P<0.001$), training condition ($F_{3,30}=6.001$, $P<0.002$) and a lever×training condition interaction ($F_{3,30}=6.824$, $P<0.001$). Separate analysis of performance on each lever showed no effect on the inactive lever ($F_{3,30}=1.561$, NS) but a significant effect on the active lever ($F_{3,30}=7.355$, $P<0.001$). Post hoc tests revealed a number of effects: (1) there was no difference in responding on the active lever between pre-trained and non-pre-trained rats working for aCSF ($P=0.773$) This is important: It shows that by session 5, the aCSF pre-trained rats were not responding any differently to untrained rats, meaning that the association of active lever pressing with food reward was extinguished: The continued presence of the light CS was not sufficient to maintain responding. (2) Post hoc tests of responding on the active lever showed that lever pressing by pre-trained rats working for nicotine was significantly greater than that of the pre-trained rats responding for aCSF ($P=0.002$) or the non-pre-trained rats responding for aCSF ($P<0.000$) or nicotine ($P=0.013$). No other post hoc tests showed statistical significance, including non-pre-trained nicotine vs. non-pre-trained aCSF ($P=0.221$).

This appears to be enhancement of responding. This can be seen if the effects on reinforced lever pressing of ICSA nicotine are expressed as a percentage of responding for aCSF in the matched control group. In session 1, for nicotine compared to aCSF, pre-trained rats pressed the active lever 189.75%, non-pre-trained 190.03%. Despite the pre-trained rats pressing more often, the ratio was obviously not different—nearly two presses on the lever delivering nicotine compared to the one delivering aCSF (see Figs. 2a and 5a). However, in session 5, pre-trained rats pressed 460.00% compared to 279.71% for non-pre-trained. Over sessions all rats improved responding for nicotine compared to aCSF, but this effect was much enhanced in pre-trained rats, an effect not explained by previous experience of food or the presence of the light CS—neither of these were sufficient to maintain performance in control rats.
Discussion

We confirm that rats will self-administer nicotine and carbachol directly into the VTA, with a clear distinction between them. In experiment 1, carbachol had activating effects: Even on session 1, the interval between first and second lever press was short, and high rates of pressing on both levers were maintained across sessions, with a preference for the active lever. On the other hand, on first exposure to nicotine, there were relatively low rates of pressing, but over sessions, the proportion of reinforced presses increased, by a reduction in pressing on the inactive lever rather than an increase in pressing on the active. Given that the VTA is a major target for nicotine, why are the effects of ICSA relatively weak? Because sensitisation follows repeated administration of nicotine, we hypothesised rats’ performance might depend on the development of this in VTA, and that its acceleration might improve ICSA, but there was no improvement in pre-sensitised rats. However, nicotine was much more readily self-administered by rats pre-trained to work for food and an associated light CS. In the absence of food (but with the CS present), rats responded more vigorously for nicotine than those not food pre-trained, an effect that was significantly enhanced by session 5. The conclusion is that while intra-VTA nicotine has variable but always modest effects in naive rats, it has a substantial effect when paired with another reinforcing stimulus, even when the primary one is no longer present (and when its memory was insufficient to maintain responding, as shown by rats administering aCSF, who extinguished within five sessions).

The idea that nicotine has reinforcement-enhancing properties is based on IVSA studies (Palmatier et al. 2006, 2007; Paterson 2009). In IVSA studies using rats previously trained to lever press, systemic nicotine was a weak reinforcer with little more potency than a visual stimulus independently activated by lever pressing (Palmatier et al. 2006). However, when available together, non-pharmacological reinforcers and nicotine synergised, producing a more robust effect than either alone (Palmatier et al. 2006; see also Lof et al. 2007; Palmatier et al. 2009; Sorge et al. 2009). Nicotine’s reinforcement enhancing effects have also been shown to be dependent on the intensity of the primary reinforcer.
whose effects are being enhanced (Palmatier et al. 2007) and on factors such as the physical response requirement (Clemens et al. 2010). Taken together, these data indicate that the effects of nicotine can vary dependent on training regimes (and quite possibly on route of administration—the data described in these papers are from IVSA studies, rather than the ICSA reported here). Moreover, reinforcement-enhancing effects echo human experience of nicotine use, which is frequently associated with reinforcers including food, alcohol and sexual activity (and the close association of nicotine with other reinforcing stimuli will necessarily make craving difficult to avoid). The present nicotine effects in food-trained rats can also be discussed in light of the fact that VTA nicotine administration activates the mesolimbic DA system, which has been known for some time to be involved in invigorating responding reinforced by salient stimuli (Taylor and Robbins 1984, 1986). A more recent study (Shin et al. 2010) examining amphetamine effects argues that in the ventral striatum “the interaction between drugs and sensory cues could be critically important for understanding the acquisition of drug-taking habit, leading to addiction” (p. 9).

Two alternative explanations to reinforcement enhancement can be considered. (1) The extra lever pressing experience in training was sufficient to enhance responding. In this case, one would have to argue that, given time rats that were not pre-trained would increase pressing for nicotine, but in experiment 1, there was no hint of this. (2) Shifting from a high value reinforcer (food) to a lower (nicotine) produces elevated levels of lever pressing. This is likely on the first session but should not persist: A low value reinforcer substituted for a high one will not maintain responding. Pre-trained rats receiving nicotine kept responding at high rates while those receiving aCSF extinguished.

How does nicotine act in the VTA to enhance reinforcement? The actions of nicotine appear designed to regulate DA containing neurons, which express homomeric α7 and heteromeric α4/β2 receptors. In addition, α7 receptors regulate release of glutamate onto DA neurons and α4/β2 and α6/β2 receptors are on GABA neurons—these receptors desensitise preferentially, releasing inhibition from DA neurons. Data suggest that VTA α4 and α6 subunits rather than α7 are critical to nicotine IVSA in KO mice and in mice where the KO has been ameliorated by viral vector techniques.
(Maskos et al. 2005; Gotti et al. 2010; Pons et al. 2008). More particularly, again in KO mice, nicotine ICSA and burst firing of DA neurons have been shown to depend on α4 containing subunits but not α6 (Exley et al. 2011). This might also be critical in considering the role of sensitisation because of the known role of α4/β2 nAChRs in this. However, the observation here that pre-sensitisation had no effect suggests that this might not be a mechanism of significance in the reinforcement-enhancing properties of intra-VTA nicotine. Indeed, the critical contrast here is between the effects of nicotine in rats that were trained before ICSA and those that were not. It is possible that in both conditions, the same degree of desensitisation of nAChRs occurred, but the behavioral outcomes were radially different.

While understanding the role of particular nAChR subunits in the VTA is of importance, it is also the case that the interaction between nicotinic and glutamatergic activity is likely of significance. Glutamate activity is strongly linked to addiction (Kalivas 2009). Excitatory glutamatergic inputs arrive in the VTA from multiple sources, primarily but not exclusively subcortical (Omelchenko and Sesak 2007); from the mesopontine tegmentum [also the source of the cholinergic input to VTA (Maskos 2008; Mena-Segovia et al. 2008)], prefrontal cortex and the bed nucleus of the stria terminalis (BNST) (Georges and Aston-Jones 2002). Cortical and BNST inputs to the VTA deliver highly processed information, and the effectiveness of the input to VTA from BNST is strengthened when rats lever press for rewards (Dumont et al. 2005). Moreover, it is known that burst firing of VTA DA neurons is potentiated in rats self-administering intravenous nicotine but not yoked rats passively on the same drug regime: This is associated with increased activity in infralimbic cortex and BNST (Caillé et al. 2009).

Our hypothesis is that in the VTA nicotine enhances reinforcement when there is concurrent activation of cortical or subcortical inputs signalling the presence of a primary or conditioned reinforcer. Nicotine acts on a complex interaction in the VTA, where DA neuron activity is regulated by descending inputs from sites such as the BNST and prefrontal cortex and by cholinergic input from the mesopontine tegmentum. Indeed, the role of cholinergic systems appears crucial to this. (1) ACh released from terminals of PPTg and LDTg neurons in the VTA has multiple properties. There is evidence that DA release in the nucleus accumbens following stimulation in the LDTg is mediated by different nAChR and mAChR processes in the VTA: nAChRs (and ionotropic glutamate receptors) mediate a fast, relatively brief release of DA, but mAChRs mediate a larger and more prolonged release of DA (Forster and Blaha 2003). We can hypothesise that reinforcement enhancement is mediated through nAChRs (that is, nicotine potentiates the reinforcing properties of other reinforcers, working to invigorate ongoing seeking behaviour that was established previously), which is consistent with the potentiating effect of PPTg activation on already-stimulated activity in the VTA (Floresco et al. 2003). It is likely that a different process is mediated by VTA mAChRs—possibly one that is more generally activating, giving the large and prolonged elevation of DA release. (2) It is also consistent with experiments showing that excitotoxic lesions of posterior PPTg (a source of cholinergic input to VTA) but not anterior PPTg impair learning reinforced by natural rewards or drugs (Alderson et al. 2008; Wilson et al. 2009; Winn et al. 2009). (3) Electrophysiological data in primates show that PPTg neurons respond in a proportionally graded manner either to signals predicting reward or to reward delivery (Okada et al. 2009)—two pieces of information critical to forming predictive associations. We can therefore present a hypothesis for investigation: that in VTA, nicotine is best thought of not as having rewarding properties per se, but that it contributes to a process of reinforcement enhancement in which cholinergic activity synergises with glutamatergic activity to drive the DA activity crucial for normal learning.

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References

Alderson HL, Latimer MP, Winn P (2006) Intravenous self-administration of nicotine is altered by lesions of the posterior, but not anterior, pedunculopontine tegmental nucleus. Eur J Neurosci 23:2169–2175

Alderson HL, Latimer MP, Winn P (2008) A functional dissociation of the anterior and posterior pedunculopontine tegmental nucleus: excitotoxic lesions have differential effects on locomotion and the response to nicotine. Brain Struct Funct 213:247–253

Balfour DJK, Benwell MEM, Birrell CE, Kelly J, Al-Aloul M (1998) Sensitization of the mesoaccumbens dopamine response to nicotine. Pharmacol Biochem Behav 59:1021–1030

Caillé S, Guillem K, Cador M, Manzoni O, Georges F (2009) Voluntary nicotine consumption triggers in vivo potentiation of cortical excitatory drives to midbrain dopaminergic neurons. J Neurosci 29:10414–10415

Chaudhri N et al (2006) Complex interactions between nicotine and nonpharmacological stimuli reveal multiple roles for nicotine in reinforcement. Psychopharmacol 184:353–366

Clarke PBS, Kumar R (1983a) The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br J Pharm 78:329–337

Clarke PBS, Kumar R (1983b) The effects of nicotine on locomotor activity in non-tolerant and tolerant rats. Br J Pharm 78:329–337
Clarke PBS, Kumar R (1983b) Characterization of the locomotor stimulant action of nicotine in tolerant rats. Br J Pharm 80:587–594
Clemens KJ, Caillé S, Cador M (2010) The effects of response operandum and prior food training on intravenous nicotine self-administration in rats. Psychopharmacol 211:43–54
Corrigall WA, Coen KM, Adamson KL (1994) Self-administered nicotine activates the mesolimbic dopamine system through the ventral tegmental area. Brain Res 653:279–284
Donny EC et al (2003) Operant responding for a visual reinforcer in rats is enhanced by non-contingent nicotine: implications for nicotine self-administration and reinforcement. Psychopharmacol 169:68–76
Dumont EC, Mark GP, Mader S, Williams JT (2005) Self-administration enhances excitatory transmission in the bed nucleus of the stria terminalis. Nature Neurosci 8:413–414
Exley R et al (2011) Distinct contributions of nicotinic acetylcholine receptor subunit α4 and subunit α6 to the reinforcing effects of nicotine. Proc Nat Acad Sci (US) 108:7577–7582
Ferrari R, Le Novere N, Picciotto MR, Changeux JP, Zoli M (2001) Acute and long-term changes in the mesolimbic dopamine pathway after systemic or local single nicotine injections. Eur J Neurosci 15:1810–1818
Floresco SB, West AR, Ash B, Moore H, Grace AA (2003) Afferent modulation of dopamine neuron firing differentially regulates tonic and phasic dopamine transmission. Nat Neurosci 9:968–973
Forster GL, Blaha CD (2003) Pedunculopontine tegmental stimulation evokes striatal dopamine efflux by activation of acetylcholine and glutamate receptors in the midbrain and pons of the rat. Eur J Neurosci 17:751–762
Georges F, Aston-Jones G (2002) Activation of ventral tegmental area cells by the bed nucleus of the stria terminalis: a novel excitatory amino acid input to midbrain dopamine neurons. J Neurosci 22:5173–5187
Gotti C et al (2010) Nicotinic acetylcholine receptors in the mesolimbic pathway: primary role of ventral tegmental α6/β2 receptors in mediating systemic nicotine effects on dopamine release, locomotion and reinforcement. J Neurosci 30:5311–5325
Ikemoto S, Qin M, Liu ZH (2006) Primary reinforcing effects of nicotine are triggered from multiple regions both inside and outside the ventral tegmental area. J Neurosci 26:723–730
Ikemoto S, Sharpe LG (2001) A head-attachable device for injecting nanoliter volumes of drug solutions into brain sites of freely moving rats. J Neurosci Methods 110:135–140
Ikemoto S, Wise RA (2002) Rewarding effects of the cholinergic agents carbachol and neostigmine in the posterior ventral tegmental area. J Neurosci 22:9895–9904
Kalivas PW (2009) The glutamate homeostasis hypothesis of addiction. Nat Rev Neurosci 10:561–568
Laviolette SR, Van der Kooy D (2003) Blockade of mesolimbic dopamine transmission dramatically increases sensitivity to the rewarding effects of nicotine in the ventral tegmental area. Mol Psychiat 8:50–59
Livingstone PD, Wonnacott S (2009) Nicotinic acetylcholine receptors and the ascending dopamine pathways. Biochem Pharmacol 78:744–755
Loef E et al (2007) Nicotinic acetylcholine receptors in the ventral tegmental area mediate the dopamine activating and reinforcing properties of ethanol cues. Psychopharmacol 195:333–343
Markou A (2008) Neurobiology of nicotine dependence. Phil Trans Roy Soc B 363:3159–3168
Maskos U (2008) The cholinergic mesopontine tegmentum is a relatively neglected nicotinic master modulator of the dopaminergic system: relevance to drugs of abuse and pathology. Br J Pharmacol 153(suppl 1):S438–S445
Maskos U et al (2005) Nicotine reinforcement and cognition restored by targeted expression of nicotinic receptors. Nature 436:103–107
Mena-Segovia J, Winn P, Bolam JP (2008) Cholinergic modulation of midbrain dopamine systems. Brain Res Rev 58:265–271
Muse E, Wise RA (1994) Place preference conditioning with ventral tegmental injections of cytosine. Life Sci 55:1179–1186
Oakman SA, Faris PL, Kerr PE, Cozzari C, Hartman BK (1995) Distribution of pontomesencephalic cholinergic neurons projecting to substantia nigra differs significantly from those projecting to ventral tegmental area. J Neurosci 15:5859–5869
Okada K, Toyama K, Inoue Y, Isa T, Kobayashi Y (2009) Different pedunculopontine tegmental neurons signal predicted and actual task rewards. J Neurosci 29:4858–4870
Omelchenko N, Sesack SR (2005) Laterodorsal tegmental projections to identified cell populations in the rat ventral tegmental area. J Comp Neurol 483:217–235
Omelchenko N, Sesak SR (2007) Glutamate synaptic inputs to ventral tegmental area neurons derive from subcortical sources. Neuroscience 146:1259–1274
Palmatier MI et al (2006) Dissociating the primary reinforcing and reinforcement-enhancing effects of nicotine using a rat self-administration paradigm with concurrently available drug and environmental reinforcers. Psychopharmacol 184:391–400
Palmatier MI et al (2007) The reinforcement enhancing effects of nicotine depend on the incentive value of non-drug reinforcers and increase with repeated drug injections. Drug Alcohol Depend 89:52–59
Palmatier MI et al (2009) Bupropion and nicotine enhance responding for nondrug reinforcers via dissociable pharmacological mechanisms in rats. Psychopharmacol 207:381–390
Paton NE (2009) The neuropharmacological substrates of nicotine reward: reinforcing versus reinforcement-enhancing effects of nicotine. Behav Pharmacol 20:211–225
Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, 4th edn. Academic, New York
Pidoplichenko VI, DeBiasi M, Williams JT, Dani JA (1997) Nicotine activates and desensitizes midbrain dopamine neurons. Nature 390:401–404
Pons S et al (2008) Crucial role of α4 and α6 nicotinic acetylcholine receptor subunits from ventral tegmental area in systemic nicotine self-administration. J Neurosci 28:12318–12327
Shin R, Cao J, Webb SM, Ikemoto S (2010) Amphetamine administration into the ventral striatum facilitates behavioural interaction with unconditioned visual signals in rats. PLoS One 5(1):e8741
Sorge RE, Pierre VJ, Clarke PBS (2009) Facilitation of intravenous nicotine self-administration in rats by motivationally neutral sensory stimulus. Psychopharmacol 207:191–200
Taylor JR, Robbins TW (1984) Enhanced behavioral control by conditioned reinforcers following microinjections of d-amphetamine into the nucleus accumbens. Psychopharmacol 84:405–412
Taylor JR, Robbins TW (1986) 6-Hydroxypamine doses of the nucleus-accumbens, but not of the caudate-nucleus, attenuate enhanced responding with reward-related stimuli produced by intra-accumbens d-amphetamine. Psychopharmacol 90:390–397
Wilson DIG, MacLaren DAA, Winn P (2009) Bar pressing for food: differential consequences of lesions to anterior versus posterior pedunculopontine. Eur J Neurosci 30:504–513
Winn P, Wilson DIG, Redgrave P (2009) Subcortical connections of the basal ganglia. In: Steiner H, Tseng K-Y (eds) Handbook of basal ganglia structure and function: a decade of progress. Academic (Elsevier), San Diego, pp 397–408
Yang K et al (2011) Functional nicotinic acetylcholine receptors containing α6 subunits are on GABAergic neuronal boutons adherent to ventral tegmental area dopamine neurons. J Neurosci 31:2537–2548
Zhao-Shea R et al (2011) Nicotine-mediated activation of dopaminergic neurons in distinct regions of the ventral tegmental area. Neuropsychopharmacol 36:1021–1032