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ORIGINAL INVESTIGATION

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Sensitization of cortical acetylcholine release by repeated administration of nicotine in rats

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Abstract *Rationale:* The integrity of cortical cholinergic transmission is vital to attentional processing. A growing literature suggests that alterations in attentional processing accompany addictive drug use. This study examined the effects of acute and repeated administration of nicotine on cortical acetylcholine release. *Objectives:* The effects of repeated systemic nicotine administration on cortical acetylcholine (ACh) efflux in the frontal cortex were determined to test the hypothesis that repeated administration of nicotine results in a potentiated or sensitized increase in ACh efflux. *Methods:* Animals were injected with nicotine (0.4 mg/kg, i.p.) or vehicle twice daily for 4 days. Cortical ACh efflux was measured using repeated microdialysis sampling on four occasions: on day 1, during the first exposure to nicotine or vehicle, on day 5 during a final exposure to nicotine, on day 8 during a nicotine challenge, and again on day 10 following saline administration. *Results:* Acute nicotine administration on day 1 produced a 90% increase in cortical ACh efflux. Repeated exposure to nicotine resulted in a larger increase in cortical ACh efflux on day 5 (200%) and day 8 (210%) relative to ACh levels measured on day 1, and relative to animals that received vehicle during the initial treatment period. Cortical ACh efflux following acute nicotine administration was blocked by mecamylamine (1.0 mg/kg, i.p.). However, the sensitized efflux of cortical ACh on day 8 was only partially attenuated by mecamylamine (1.0 or 5.0 mg/kg, i.p.), suggesting a mecamylamine-insensitive component of the sensitized response to repeated nicotine administration. *Conclusions:* Repeated administration of nicotine results in a sensitized increase in cortical ACh release. Sensitized cortical ACh release may mediate, in part, the cognitive components of nicotine addiction.

Keywords Nicotine · Prefrontal cortex · Mecamylamine · Acetylcholine · Sensitization · Microdialysis

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Introduction

The effects of nicotine on the central nervous system, while complex, seem to share fundamental properties with other psychostimulant drugs of abuse, such as amphetamine and cocaine (Koob et al. 1998). Repeated systemic exposure to drugs of abuse, including nicotine, has been shown under certain conditions to enhance, or sensitize, their effects on locomotor activity and accumbens dopamine release (Robinson and Berridge 1993; Balfour et al. 1998). It has been postulated that sensitization of dopaminergic transmission within the nucleus accumbens (NAC) mediates abnormal motivational/cognitive processes that may account for the intense craving and repeated compulsive use of addictive drugs (Robinson and Berridge 1993), particularly following extensive withdrawal periods (Paulson and Robinson 1995).

While the literature regarding the effects of repeated administration of nicotine on locomotor behavior and dopamine release in the NAC is extensive (Balfour et al. 1998, 2000; Di Chiara 2000), the effects of nicotine administration on in vivo ACh release have been studied less extensively. The majority of these studies have focused on the effects of acute administration of nicotine on ACh release in the hippocampus and cortex (Toide and Arima 1989; Summers et al. 1994, 1996; Summers and Giacobini 1995; Tani et al. 1998). The one study that has examined the effects of repeated administration of nicotine on cortical ACh release, using epidural cups to measure ACh, suggested that chronic exposure to nicotine increases basal levels of ACh (Nordberg et al. 1989). Furthermore, the studies by Nordberg et al. (1989) and others (Kisr et al. 1985; Marks et al. 1985; Peng et al. 1994; Shoaib et al. 1997) indicated that repeated admin-

istration of nicotine can result in the upregulation of neuronal nicotinic ACh receptors (nAChR), suggesting a possible mechanism for increased effects of nicotine on neurotransmission, including the release of ACh. The dysregulation of cortical cholinergic transmission by psychostimulants may play a role in the altered cognitive processes that characterize compulsive drug use, by mediating the preferential processing of stimuli that become associated with drug acquisition and drug use, such that these stimuli gain behavioral control and limit behavioral alternatives (Deller and Sarter 1998; Nelson et al. 2000; Robinson and Berridge 1993; Sarter and Bruno 1999).

The first goal of the present series of experiments was to test the hypothesis that repeated daily administration of nicotine results in a potentiated or sensitized stimulation of cortical ACh release, relative to the acute nicotine-stimulated increase in ACh release. This hypothesis was also based on the previous finding that repeated administration of another psychostimulant, amphetamine, sensitizes cortical ACh efflux (Nelson et al. 2000). To measure cortical ACh efflux in these experiments, a microdialysis probe was placed in the dorsomedial prefrontal cortex, a terminal region of corticopetal cholinergic neurons (Zaborszky et al. 1999), and an area linked to attentional processing (Arnold et al. 2002; Gill et al. 2000). The second aim of the current studies was to examine the effects of nAChR blockade, using the antagonist mecamylamine (MEC), on the potentiated release of cortical ACh produced by repeated administration of nicotine. For comparison, the current experiments also replicated previous studies that have shown that MEC blocks the release of cortical ACh following acute administration of nicotine (Summers et al. 1994; Summers and Giacobini 1995; Tani et al. 1998).

Materials and methods

Subjects

Adult male Fisher-344/Brown Norway F1 hybrid rats (Harlan Sprague-Dawley, Indianapolis, Ind., USA), weighing between 250 and 350 g, served as subjects in these experiments. Animals were allowed access to food and water ad libitum and were housed in a temperature- and humidity-controlled colony room kept on a 12:12 light:dark cycle (lights on at 6:30 a.m.). Animals were housed in pairs in stainless steel hanging cages until the day prior to guide cannula implantation, when animals were moved to individual plastic cages (50×23×20 cm; l×w×h) with hardwood shavings, where they were housed for the duration of the experiment. All animal care and experiments were performed in accordance with protocols approved by the University Institutional Laboratory Animal Care and Use Committee of Ohio State University and were consistent with the NIH Guide for the Care and Use of Laboratory Animals.

Guide cannula surgery

Animals were anesthetized with ketamine (100.0 mg/kg, i.p.) and xylazine (3.0 mg/kg, i.p.) prior to stereotaxic surgery. A microdialysis guide cannula was implanted (0.5 mm O.D.; Bioanalytical Systems, W. Lafayette, Ind., USA) into the medial prefrontal cortex (hemisphere counterbalanced across animals) positioned 2.7 mm anterior to bregma, 0.8 mm lateral to the midline, and 1.0 mm below dura mater (coordinates according to the atlas of Paxinos and Watson 1986). Following surgery animals were allowed to recover for 3 days prior to the first microdialysis session.

Microdialysis sessions

On each of the 4 days immediately prior to and the 3 days following implantation of the guide cannula, animals were placed in concentric dialysis bowls (35×38 cm; h×d; CMA, Stockholm, Sweden) for 6–7 h each day in the testing room. Microdialysis was conducted using a repeated perfusion paradigm in which each rat received four separate microdialysis sessions over a 10-day period with at least two full days between each session. This repeated perfusion paradigm allows the assessment of the effects of multiple treatments, including control conditions, in the same animal and has been validated for measurement of cortical ACh efflux (Moore et al. 1995) as well as for striatal ACh efflux (Johnson and Bruno 1995) and striato-nigral GABA efflux (Byrnes et al. 1997), by showing that neither basal efflux nor drug effects interact significantly with the order of the dialysis sessions (see also Bruno et al. 1999). On each microdialysis day, animals were placed in the testing chambers 30 min prior to the insertion of concentric microdialysis probes (0.35 mm O.D., 2.0 mm membrane length; Bioanalytical Systems) through the guide cannula. The probe was perfused at 1.25 μl/min with artificial CSF (aCSF; pH=7.1) containing the following (in mM): NaCl, 166.5; $NaHCO_3$, 27.5; KCl, 2.4; Na_2SO_4, 0.5; KH_2PO_4, 0.5; $CaCl_2$, 1.2; $MgCl_2$, 0.8; glucose, 1.0; and the acetylcholinesterase inhibitor neostigmine bromide (0.025 μM) to promote recovery of detectable basal levels of ACh. The probes were attached to a dual channel liquid swivel (Instech, Plymouth Meeting, Pa., USA) and perfused for 3 h before collection of dialysate began, an interval that results in stable basal ACh efflux that is highly (>95%) dependent on axonal depolarization (Moore et al. 1992).

Drugs

(–)-Nicotine di-(+)tartrate salt (0.4 mg/kg, i.p., calculated as the free base) was dissolved in 0.9% sterile saline and the pH was adjusted to 7.2–7.4 with sodium hydroxide. The acute administration of this dose of nicotine increases cortical and hippocampal ACh efflux (Tani et al. 1998) and, following repeated administration, this dose sensitizes behavioral activity and NAC dopamine efflux (Balfour et al. 1998). Mecamylamine hydrochloride (1.0 or 5.0 mg/kg, i.p.) was dissolved in 0.9% sterile saline. The lower dose of MEC was shown to be sufficient to precipitate a nicotine withdrawal syndrome following chronic nicotine administration (Carboni et al. 2000). Based on pilot studies, a higher dose was also selected to ensure maximal receptor channel blockade. Both chemicals were purchased from Sigma Chemical (St Louis, Mo., USA) and delivered in a volume of 1.0 ml/kg.

Experimental procedure

Experiment 1: effects of repeated administration of nicotine on cortical ACh efflux

To characterize the effects of repeated administration of nicotine on cortical ACh efflux, two groups of rats underwent four microdialysis sessions over a 10-day period. On days 1 through 4, rats were injected twice daily (morning and afternoon) with either saline (1.0 ml/kg) or nicotine (0.4 mg/kg, i.p.). Cortical ACh efflux was measured for each subject during day 1 following the first exposure to nicotine (NIC group; n=6) or a saline injection (SAL group; n=5). Cortical ACh efflux was assessed a second time on day 5; during this session both groups (NIC and SAL) were injected with

nicotine (0.4 mg/kg). Both groups of rats were injected twice daily with saline during days 6 and 7. During a third microdialysis session, on day 8, ACh efflux was again assessed following injection of nicotine (0.4 mg/kg) in both groups.

Environmental cues such as the testing room, dialysis bowl, and the injection procedure could become associated with repeated drug administration and thus contribute to any observed changes in cortical ACh efflux. In order to assess potential changes in cortical ACh efflux as a result of such variables, both groups underwent a final microdialysis session on day 10 in which they received an injection of saline (1.0 ml/kg, i.p.). Each session began with four 15-min dialysate collections to establish baseline levels of ACh efflux. At the conclusion of the baseline period, rats were injected with nicotine (or saline) and dialysate was collected every 15 min for an additional 3 h.

In order to characterize the behavioral effects of systemic injections of nicotine, motoric activity was rated on an 11-point scale modeled after Ellinwood and Balster (1974). This rating scale was as follows: (1) lying down, eyes closed; (2) lying down, eyes open; (3) standing (or crouching); (4) in-place activities (i.e. grooming, chewing, sniffing); (5) locomotion about the bowl, with occasional sniffing and rearing; (6) excessive locomotion about bowl; (7) hyperactive movements (i.e. running, jerky); (8) repetitive exploration with normal activity; (9) repetitive exploration with hyperactivity; (10) restricted/stereotypy; (11) dyskinesia. Behavior was recorded at the end of each 15-min collection interval; if more than one behavior was observed the behavior that predominated during that interval was recorded.

Effects of MEC pretreatment on cortical ACh efflux following acute nicotine administration

To characterize the effects of the nAChR antagonist MEC on the increase in cortical ACh efflux produced by a single acute injection of nicotine, two separate groups of rats, pretreated with either saline or MEC, were compared. In both groups (n=5/group), cortical ACh efflux was measured for each subject during the first exposure to nicotine. At the conclusion of the baseline period, half the rats were injected with MEC (1.0 mg/kg, i.p.) and the other half were injected with an equal volume of 0.9% saline (1.0 ml/kg, i.p.). Thirty minutes (two collection intervals) after the initial injection, both groups of rats were injected with nicotine (0.4 mg/kg, i.p.) and dialysate was collected every 15 min for an additional 3 h.

Effects of MEC pretreatment on cortical ACh efflux following repeated nicotine administration

To characterize the effects of MEC on cortical ACh efflux following repeated exposure to nicotine (0.4 mg/kg, i.p.) a within-subjects design was used in which each subject underwent four different microdialysis sessions over a 10-day period. As in experiment 1, rats were exposed to a twice-daily injection regimen of saline or nicotine (0.4 mg/kg) on day 1 through day 4. Cortical ACh efflux was measured for each subject during the first exposure to nicotine on day 1, and during the injection of nicotine on day 5. During the third microdialysis session on day 8, one of two doses of MEC (1.0 or 5.0 mg/kg, i.p.) was injected 30 min prior to the administration of nicotine. Finally, in order to assess potential increases or decreases in cortical ACh efflux as a result of MEC alone, rats that were repeatedly exposed to nicotine underwent a final session on day 10 in which they were given an injection of saline (1.0 ml/kg, i.p.) 30 min following MEC administration.

At the conclusion of the baseline period on days 1 and 5, rats were injected with 0.9% saline (1.0 ml/kg, i.p.). Following the baseline period on day 8 and 10 rats were injected (i.p.) with either 1.0 (n=4) or 5.0 (n=5) mg/kg MEC (each rat received the same dose of MEC on both day 8 and 10). Thirty minutes (two collection intervals) following the initial injection (saline or MEC), rats were injected with nicotine (0.4 mg/kg, i.p.) and dialysate was collected every 15 min for an additional 3 h.

Neurochemical analysis

ACh levels in microdialysis samples were determined by high-performance liquid chromatography with electrochemical detection. From each sample collected, 12 µl were injected. ACh and choline were separated by a C-18 carbon polymer column (250×3 mm; ESA, Inc., Chelmsford, Mass., USA) using a sodium di-phosphate mobile phase (100 mM Na_2HPO_4, 5 mM TMACI, 2.0 mM 1-octanesulfonic acid, pH=8.0). ACh was hydrolyzed on a post-column enzyme reactor (ESA, Inc.) and converted to hydrogen peroxide (Potter et al. 1983) that was detected using a "peroxidase-wired" (Huang et al. 1995) ceramic glassy carbon electrode (ESA, Inc.) with the potential set at –200 mV. The detection limit for ACh under these conditions was approximately 5.0 fmol/12 µl injection.

Histology

Following the last microdialysis session, animals were given an overdose of sodium pentobarbital and transcardially perfused with 0.2% heparin in 0.9% saline followed by 10% formalin. Brains were stored in 10% formalin at 4°C for at least 24 h and then transferred to 30% sucrose phosphate buffer until sectioning at least 3 days later. Histological verification of dialysis probe placement was made using 45 µm cresyl-violet stained sections. Figure 1 depicts coronal brain sections showing representative placements of the microdialysis probe in the frontal cortex. The dialysis membranes were located within the ventral portion of the anterior cingulate cortex and in the prelimbic cortex. Rats with dialysis probes that fell between hemispheres or extended into corpus callosum were excluded from subsequent data analysis.

Statistical analyses

To determine the stability of basal ACh efflux during each microdialysis session, as well as over the repeated dialysis sessions, baseline collections for each session were compared using two-factor (Session×Time), or three factor (Group×Session×Time) repeated measures analysis of variance (ANOVA). These data are expressed in picomoles (pmol)/12 µl sample. Demonstrating that basal efflux for each treatment is similar across sessions permits unbiased expression of the data as a percent change from basal levels. Thus, for each subject the mean of the four baseline collections was calculated and the rest of the statistical analyses were performed on data expressed as a percent change from the mean baseline for each session.

For each experiment, a repeated measures ANOVA was conducted over the last collection prior to the injection of nicotine (or saline on day 10) through the final collection period. To further delineate the source of significant interactions additional ANOVAs were conducted over relevant factors. In experiments 2 and 3 an additional repeated measures ANOVA over session and time was conducted over the final baseline (60 min) through the collection at 90 min to determine potential differences in pre-nicotine injection levels of ACh efflux following saline or MEC injections.

Ratings of motoric activity yielded non-parametric data and were analyzed using the Wilcoxon signed ranks test, adjusted for tied ranks (Keppel 1991). In an effort to control for type I error, only selected time points were analyzed.

For all experiments, pairwise comparisons following a statistically significant main effect or interaction were performed. Based on Keppel's suggestion (Keppel 1991) that the error term for follow-up comparisons in repeated measure designs should be the error of the two conditions in question, means were compared using paired t tests. In recognition of the potential for increases of familywise error, the number of such comparisons was minimized and only used to probe the source of statistically significant main effects or interactions revealed by ANOVAs (α=0.05 for all tests). When follow-up comparisons exceeded the "natural limits" (the degrees of freedom in the numerator of the ANOVA), α was subjected to a modified Bonferroni correction ($\alpha_{MB}=\alpha\times$degrees of

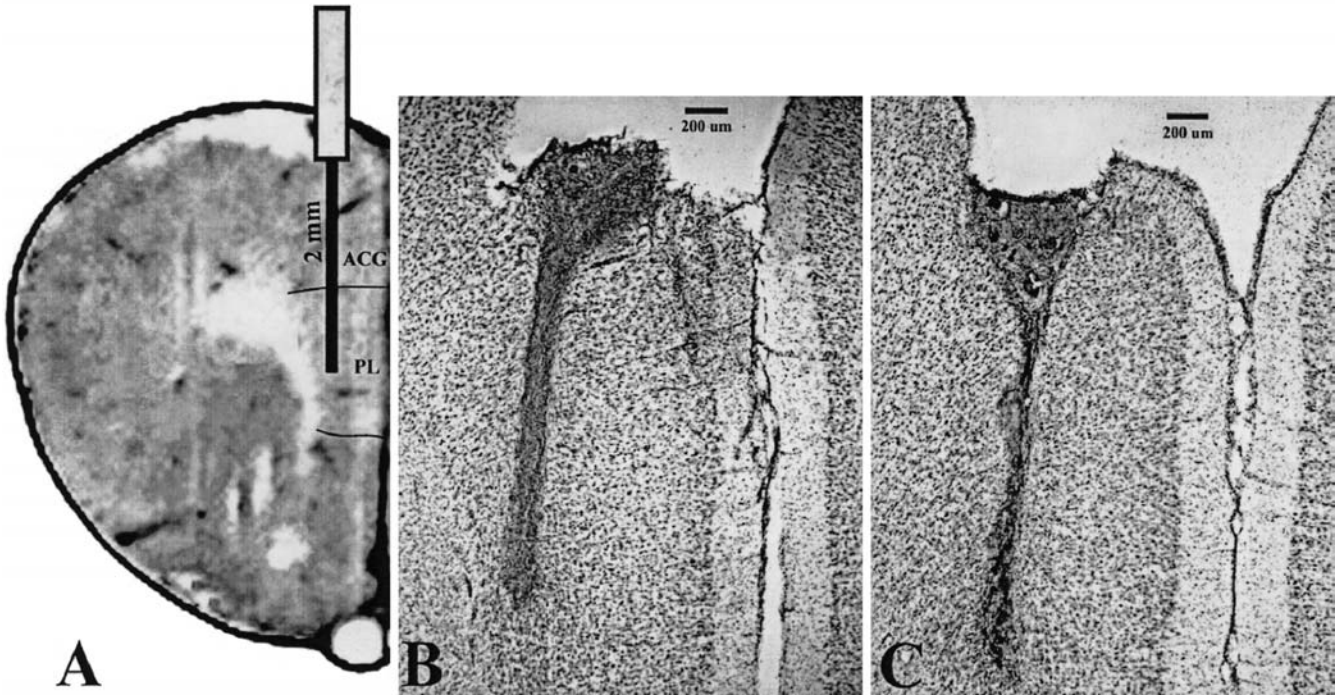

Fig. 1A–C Placement of the microdialysis probes in the medial frontal cortex. **A** Schematic representation of the placement of the dialysis probes. The 2.0 mm microdialysis probes collected ACh from the anterior cingulate cortex (*ACG*) and the prelimbic area (*PL*). **B** and **C** Photomicrographs of cresyl violet-stained coronal sections depicting the actual placements of two probes (200 μm scale inserted at the top of the photographs). The damage produced by the guides is visible at the top of the photographs

freedom for the numerator/actual number of comparisons) (Keppel 1991). When this correction is used the modified significance level (α_{MB}) is reported. All statistical analyses were completed using SPSS (V 10.0.5; SPSS, Inc., Chicago, Ill., USA). The level of significance for all ANOVAs was defined as $P<0.05$.

Results

Experiment 1: effects of repeated administration of nicotine or vehicle on cortical ACh efflux

Basal levels of cortical ACh efflux

Basal levels of cortical ACh did not change as a result of repeated microdialysis sampling. A three-factor ANOVA did not reveal an effect of session, an effect of time across the four baseline collections, or between the two treatment groups. There were also no interactions between session, time, and group (all $P>0.1$). The mean (±SEM) basal values (pmol/12 μl) for each session (collapsed across group and time) were: 0.054±0.011, 0.051±0.008, 0.047±0.009, 0.030±0.005, on day 1, 5, 8 and 10, respectively.

Effects of acute nicotine administration on cortical ACh efflux (day 1)

The acute administration of nicotine resulted in the elevation of cortical ACh efflux levels relative to rats injected with saline for over an hour following the injection (see Fig. 2, upper left). A mixed-factor ANOVA between the two treatment groups over the repeated measure of time (the last baseline at 60 min through the end of the session) confirmed a significant interaction between group and time [$F(12,108)=3.792$, $P<0.001$]. Specifically, rats injected (following the collection at 60 min) with nicotine (termed "NIC:nic" in Fig. 2) had significantly higher ACh efflux levels than rats injected with saline (SAL:sal) during the collections taken at 90 min [$t(9)=3.46$, $P=0.007$), 105 min [$t(9)=4.21$, $P=0.002$], 120 min [$t(9)=2.73$, $P=0.023$], and 135 min [$t(9)=3.89$, $P=0.004$].

Effects of repeated nicotine administration on cortical ACh efflux (day 5)

In animals that had been repeatedly exposed to nicotine (NIC:nic) on days 1–4, nicotine administration on day 5 stimulated cortical ACh efflux (Fig. 2, upper right) to an extent significantly greater than those observed in rats given comparable repeated exposure to saline and injected with nicotine for the first time on day 5 (SAL:nic). The

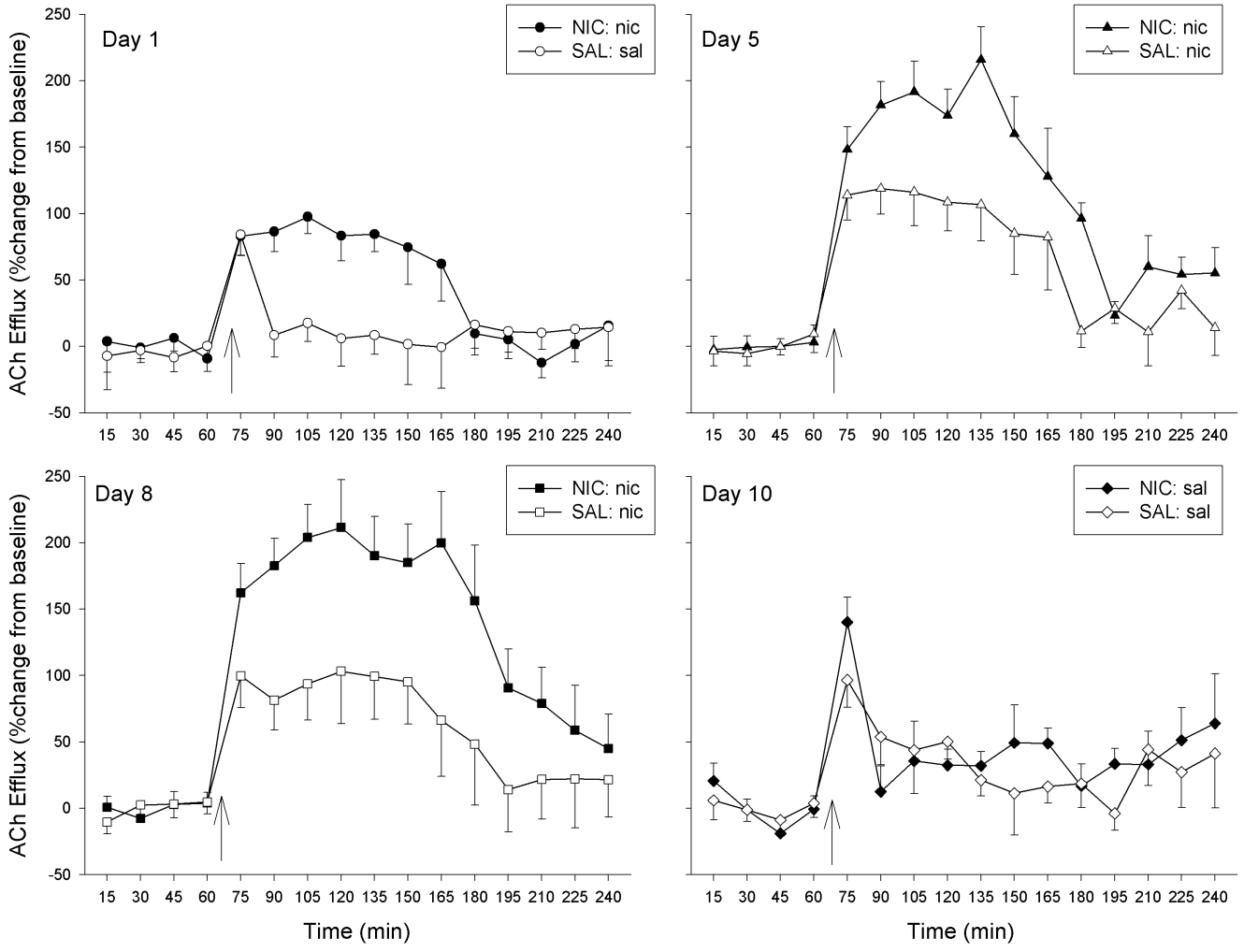

Fig. 2 Mean (±SEM) levels of ACh efflux (percent change from baseline) during each of the four microdialysis sessions on days 1, 5, 8, and 10. The two treatment groups are distinguished in the legend by the abbreviations in all capital letters with rats (n=6) exposed to nicotine (0.4 mg/kg) on days 1–4 designated "*NIC*" and rats (n=5) treated with saline (1.0 ml/kg) on days 1–4 as "*SAL*." The lower case abbreviation following the colon denotes the drug treatment (*nic* nicotine; *sal* saline) given during that particular microdialysis session. The up arrow in each panel between 60 and 75 min denotes the time of the injection procedure. Cortical ACh efflux increased after the acute administration of nicotine (day 1). The increase in cortical ACh efflux produced by nicotine was enhanced after repeated exposure to nicotine as revealed by differences between NIC and SAL groups on day 5 and day 8. Injection of saline on day 10 produced only a brief increase in cortical ACh efflux following the injection procedure in both treatment groups

mixed-design, two-factor ANOVA between the two groups, on collections from 60 min until the end of the session, revealed a significant effect of treatment group [$F(1,9)=10.876$, $P=0.009$] and a significant effect of time [$F(12,108)=15.914$, $P<0.001$]; however, the interaction between group and time was not significant.

A completely within-subjects ANOVA performed on the data across day 1 and day 5 confirmed that cortical ACh efflux levels in the nicotine exposed rats on day 5 were elevated relative to that observed in these same rats after acute administration of nicotine on day 1, [session, $F(1,5)=81.169$, $P<0.001$; time, $F(12,60)=19.403$, $P<0.001$]; these two sessions did not interact over time.

Effects of a nicotine challenge on cortical ACh efflux (day 8)

As shown in Fig. 2 (lower left), nicotine administration resulted in increases in cortical ACh efflux levels on day 8. These increases were significantly higher in rats repeatedly exposed to nicotine on days 1–4 (NIC:nic) than that observed in rats given saline on days 1–4 and injected with nicotine for only the second time on day 8 (SAL:nic). The mixed-design, two-factor ANOVA revealed main effects of group [$F(1,9)=5.092$, $P=0.050$] and time [$F(12,108)=14.056$, $P<0.001$], as well as a significant interaction between group and time [$F(12,108)=1.885$, $P=0.044$]. Pairwise comparisons confirmed that rats repeatedly treated with nicotine (NIC:nic) had

significantly higher ACh efflux than rats treated with saline during the pre-exposure phase (SAL:nic) at collections taken at 90 min [$t(9)=3.34$, $P=0.009$], 105 min [$t(9)=2.97$, $P=0.016$], and 165 min [$t(9)=2.34$, $P=0.044$].

Again, a completely within-subjects analysis on cortical ACh efflux levels on rats pretreated with nicotine on days 1–4 (NIC:nic) confirmed that ACh efflux levels were higher on day 8 in these rats than the levels observed in these same rats on day 1. The ANOVA revealed main effects of session [$F(1,5)=11.326$, $P=0.020$] and time [$F(12,60)=13.755$, $P<0.001$]. The effects of session and time interacted significantly [$F(12,60)=2.713$, $P=0.005$].

Furthermore, the increase in nicotine-stimulated cortical ACh efflux observed on day 8 was similar to that observed on day 5 (compare filled symbols in Fig. 2 on day 5 and day 8). That is, a within-subjects ANOVA examining cortical ACh efflux levels observed on day 5 and day 8 in the nicotine exposed subjects (NIC:nic) revealed only a significant effect of time [$F(12,60)=26.392$, $P<0.001$], but no effect of session nor a session by time interaction.

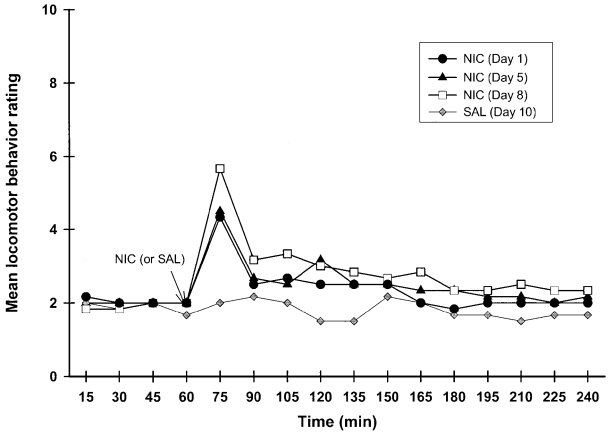

Fig. 3 Mean level of locomotor activity in rats ($n=6$) exposed to the nicotine treatment on day 1–4 of experiment 1. Systemic administration of 0.4 mg/kg nicotine increased motor activity levels, relative to those seen in saline controls, following the first acute administration (day 1). This nicotine-induced increase in motor activity was also observed on both day 5 and day 8. Injection of saline (1.0 ml/kg) on day 10 did not increase locomotor activity. See Materials and Methods for explanation of the behavioral rating scale

Effects of saline administration on day 10

On day 10 (Fig. 2, lower right) animals in both groups received a saline injection. A mixed factor two-way ANOVA (from 60 min to 240 min) revealed that cortical ACh efflux varied over time [$F(12,108)=3.505$, $P<0.001$], probably due to the increase of cortical ACh efflux triggered by the injection procedure and lasting only for one collection interval. However, there was no effect of treatment group and there was not an interaction between group and time.

Effects of repeated nicotine administration on motor activity

Figure 3 illustrates the mean locomotor activity for rats given repeated exposure to nicotine on days 1–4 during each of the four microdialysis sessions on days 1, 5, 8 and 10. As shown in Fig. 3, there were no differences in basal activity levels among the four sessions. Typically, animals were awake and lying still in the test bowl during the baseline period prior to nicotine (or saline) administration. Nicotine produced an increase in motor activity at 75 min, compared to 60 min, during the first 15-min interval following nicotine administration on day 1 ($Z=-2.27$, $P=0.023$), day 5 ($Z=-2.23$, $P=0.026$), and day 8 ($Z=-2.27$, $P=0.023$); there were no differences in activity among these three sessions (all $P>0.05$). Immediately following the injection of nicotine, rats moved about the microdialysis bowl in a cautious manner, stretching their abdomen, and occasionally extending their hind limbs. During the subsequent collection intervals the rats did not return to the lying down posture they had exhibited during the baseline period, but settled in to a standing or crouching position, with little or no ambulation about the microdialysis bowl. There was no increase in motor activity following the saline injection on day 10; rats returned to their previous position in the bowl and continued to lie on their sides, apparently asleep during the remainder of the session.

Experiment 2: effects of MEC on cortical ACh efflux following acute nicotine administration

Basal levels of cortical ACh efflux

Basal cortical ACh efflux was similar in each group and stable across the four baseline collections; there was also no interaction between these factors. The basal ACh efflux values (pmol/12 μl; mean±SEM) for the two groups ($n=5$) were: SAL+NIC, 0.057±0.010 and MEC+NIC, 0.039±0.010.

Effects of MEC on basal cortical ACh efflux

The injection of saline or MEC resulted in an immediate and transient increase in cortical ACh efflux over basal levels (shown in Fig. 4). An ANOVA over the collections at 60, 75 and 90 min confirmed a main effect of time [$F(2,16)=18.450$, $P<0.001$]. Importantly, during these three collections prior to the administration of nicotine, the two groups did not differ from one another, nor was there a significant interaction between group and time, suggesting that the increase in cortical ACh efflux can be attributed to stimuli related to handling during the

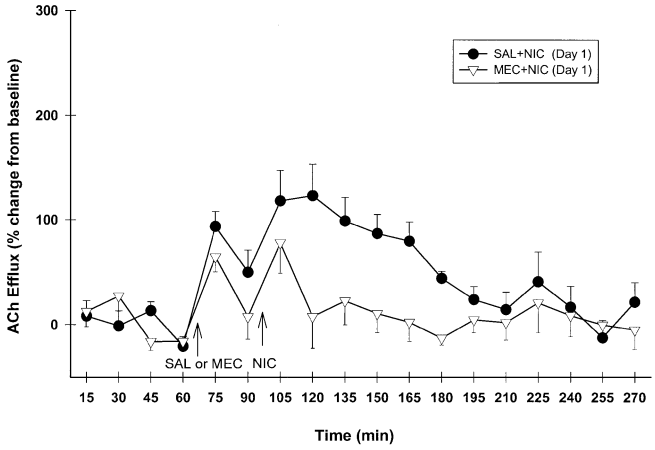

Fig. 4 Mean (±SEM) levels of ACh efflux (percent change from baseline) in two separate groups of animals (both *n*=5/group) during their first microdialysis session and first exposure to nicotine. One group (*MEC+NIC*) was pretreated with the nAChR antagonist MEC (1.0 mg/kg) prior to the administration of nicotine (0.4 mg/kg), while the other group (*SAL+NIC*) was pretreated with an equal volume of 0.9% saline (1.0 ml/kg) prior to nicotine administration. The *arrow on the left* indicates when the pretreatment injection was administered while the *arrow on the right* indicates the time at which nicotine was given. Administration of MEC blocked the nicotine-induced increase in cortical ACh efflux

injection procedure and not specifically linked to the properties of the drug being injected.

Effects of MEC on acute nicotine-induced cortical ACh efflux

Pre-treatment with MEC (Fig. 4; MEC+NIC) prevented the nicotine-induced increase in cortical ACh efflux. Similar to the initial injection (MEC or saline), both groups demonstrated an increase in cortical ACh efflux immediately after the injection of nicotine. An ANOVA over the collections from 90 min until the last collection at 270 min revealed a significant effect of time [$F(12,96)=5.539$, $P<0.001$]. Cortical ACh efflux remained elevated in the saline pretreated group (SAL+NIC), while levels returned to basal values in the MEC pretreated group (MEC+NIC), as revealed by an effect of group [$F(1,8)=6.007$, $P=0.040$], and a significant interaction between time and group [$F(12,96)=2.088$, $P=0.025$]. Follow-up comparisons revealed that this interaction was the result of higher levels of cortical ACh in the saline pre-treated rats compared to the MEC pre-treated rats during the collections at 120 min [$t(8)=2.70$, $P=0.027$], 135 min [$t(8)=2.38$, $P=0.045$], 150 min [$t(8)=2.97$, $P<0.018$], 165 min [$t(8)=2.99$, $P=0.017$] and 180 min [$t(8)=6.08$, $P<0.001$]. By 195 min, ACh efflux was no longer significantly different between the two treatment groups.

Experiment 3: effects of MEC pretreatment on cortical ACh efflux following repeated nicotine administration

Basal ACh levels prior to MEC (1.0 mg/kg)

As in experiment 1, basal levels of cortical ACh efflux did not change across the four microdialysis sessions and did not vary over the four baseline collections nor was there an interaction between session and time. The mean basal ACh efflux values (pmol/12 µl; mean±SE) for the four sessions (collapsed across time) were: day 1, 0.049±0.013, day 5, 0.047±0.016, day 8, 0.038±0.011, and day 10, 0.048±0.023.

Effects of MEC (1.0 mg/kg) or saline on basal cortical acetylcholine efflux

The injection of saline (days 1 and 5) or MEC (days 8 and 10) after the collection at 60 min resulted in an increase in cortical ACh efflux over basal levels (see Fig. 5A). An ANOVA over the collections at 60, 75 and 90 min confirmed a main effect of time [$F(2,6)=27.172$, $P=0.001$]. While the injection produced an increase in cortical ACh efflux, these three time points prior to nicotine administration were similar in all four microdialysis sessions and there was no interaction between session and time. Again, this suggests that the increase in cortical ACh efflux seen following the injection (saline or MEC) can be attributed to handling and the injection procedure.

Effects of mecamylamine (1.0 mg/kg) on cortical ACh efflux following repeated administration of nicotine

Cortical ACh efflux during the four microdialysis sessions is shown in Fig. 5A. A within-subjects repeated measures ANOVA over the data from 90 min through 270 min revealed that ACh efflux varied significantly among these four sessions [session, $F(3,9)=8.300$, $P=0.006$; time, $F(12,36)=6.116$, $P<0.001$; session×time, $F(36,108)=2.132$, $P=0.001$]. Subsequent within-subjects one- and two-way ANOVAs were conducted to further delineate the nature of this interaction.

Acute administration of nicotine increased cortical ACh efflux on day 1 [$F(12,36)=5.463$, $P<0.001$]. Pairwise comparisons confirmed that ACh efflux was elevated during the collection at 105 min [$t(3)=–3.726$, $P=0.034$], the first collection following the nicotine injection relative to basal levels at 60 min.

Consistent with the results of experiment 1, nicotine-stimulated increases in cortical ACh efflux on day 5 following repeated exposure to nicotine were sensitized relative to nicotine-stimulated increases in ACh observed after acute administration on day 1 [session, $F(1,3)=41.64$, $P=0.008$; time, $F(12,36)=8.154$, $P<0.001$] and there was a significant interaction between the effects

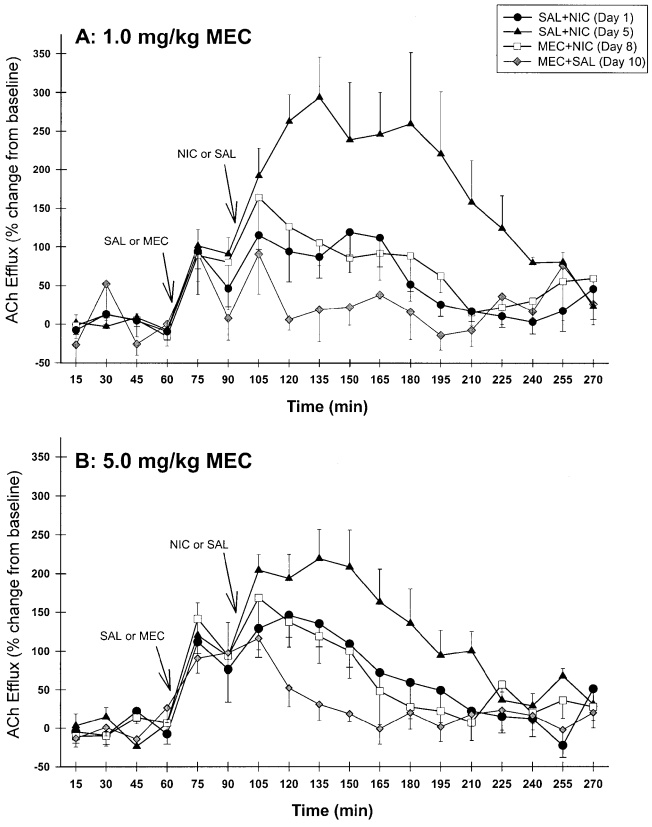

Fig. 5A,B The effects of two doses of the nAChR antagonist MEC on nicotine-stimulated cortical ACh levels after repeated exposure to nicotine. **A** Data for rats (n=4) that were treated with 1.0 mg/kg MEC on day 8; **B** data for a separate group of rats (n=5) treated with 5.0 mg/kg MEC on day 8. Data are the mean (±SEM) level of ACh efflux (percent change from baseline) in rats implanted with a microdialysis probe in the mPFC. In both sets of subjects, acute administration of nicotine (0.4 mg/kg, i.p.) increased cortical ACh efflux (SAL+NIC, day 1). Nicotine-stimulated increases in cortical ACh were sensitized during the second microdialysis session (SAL+NIC, day 5). The systemic administration of either dose of MEC (1.0 or 5.0 mg/kg) on day 8 (MEC+NIC) attenuated, but did not block, the sensitized release of cortical ACh efflux following nicotine administration. ACh levels during day 8 were increased to levels similar to those observed following acute administration of nicotine on day 1. Injection of MEC alone (MEC+SAL, day 10) did not change cortical ACh efflux beyond that typically seen following saline injections. The *arrow on the left* in each panel indicates the time at which the pretreatment injection (saline or MEC) was given while the *arrow on the right* indicates the time at which nicotine was administered

of session and time [F(12,36)=2.259, P=0.029]. This interaction was the result of ACh levels during day 5 being higher than those observed during the same time point on day 1 during collections at 120 min [t(3)= –10.200, P=0.002], 135 min [t(3)=–6.837, P=0.006], 165 min [t(3)=–5.793, P=0.010], and 240 min [t(3)= –9.432, P=0.003], while all other time points were not statistically different (all P>0.05).

The administration of MEC (1.0 mg/kg, i.p.) prior to nicotine on day 8 significantly attenuated the nicotine-stimulated increases in cortical ACh efflux, relative to levels observed following nicotine administration on day 5 in these rats. Thus, an ANOVA revealed that ACh efflux was higher during day 5 than that observed following MEC on day 8 [F(1,3)=25.033, P=0.015]; ACh levels were changing over time [F(12,36)=4.664, P<0.001], but these changes over time did not interact with session.

Despite the significant attenuation of nicotine-stimulated cortical ACh levels by pretreatment with MEC, the antagonist did not completely block the effect of nicotine on cortical ACh following the repeated administration of nicotine as it did after acute administration in experiment 2 (see Fig. 4). Thus the increase in ACh efflux was similar on day 1 (acute administration) and day 8 (MEC pretreatment) following the repeated administration of nicotine, as an ANOVA over these two sessions revealed only a significant effect of time [F(12,36)=4.218, P<0.001], but no effect of session or a session×time interaction.

Finally, analysis of the session on day 10, during which rats were pretreated with MEC and then given a saline injection in place of nicotine, revealed that cortical ACh efflux did not significantly vary over time.

Basal levels of cortical ACh efflux prior to MEC (5.0 mg/kg)

Again, basal cortical ACh efflux did not differ across the four collections during the four sessions. The mean basal ACh efflux values (pmol/12 µl; mean±SEM) for the four sessions (collapsed across time) were: day 1, 0.049±0.006, day 5, 0.052±0.007, day 8, 0.042±0.008, and day 10, 0.032±0.006.

Effects of MEC (5.0 mg/kg) or saline injection on basal efflux

A within-subjects repeated measures ANOVA of the four sessions comparing the last baseline (60 min) and two collections following initial injection (MEC or saline) revealed a main effect of time [F(2,8)=17.847, P=0.001] as a result of the increase in cortical ACh (Fig. 5B) following the handling and injection. Importantly, prior to the administration of nicotine, the percent change in cortical ACh efflux was similar during all four sessions and there was no interaction between session and time.

Effects of MEC (5.0 mg/kg) on cortical ACh efflux following repeated administration of nicotine

Cortical ACh efflux during these four microdialysis sessions is shown in Fig. 5B. As in rats treated with the lower dose of MEC (Fig. 5A), a within-subjects repeated measures ANOVA over the data from 90 min through 270 min revealed that ACh efflux varied significantly among the four sessions [F(3,12)=7.301, P=0.005] and

over the 13 collection intervals [$F(12,48)=14.787$, $P<0.001$]. Finally, there was an interaction between session and time [$F(36,144)=2.061$, $P=0.001$]. Thus, subsequent one- and two-way ANOVAs were conducted to delineate further the nature of this interaction.

As before, acute nicotine administration stimulated cortical ACh efflux on day 1 [$F(12,48=5.182$, $P<0.001$]. Pairwise comparisons confirmed that ACh efflux was elevated over basal (60 min) levels at 75 min [$t(4)=-8.193$, $P=0.001$], after the injection of MEC, and after injection of nicotine at 105 min [$t(4)=-4.545$, $P=0.010$], 120 min [$t(4)=-6.958$, $P=0.002$], 135 min [$t(4)=-5.232$, $P=0.006$], and 150 min [t(4)=–3.932, $P=0.017$].

Nicotine-stimulated increases in cortical ACh efflux on day 5 following repeated exposure to nicotine were sensitized relative to nicotine-stimulated increases in ACh observed after acute nicotine on day 1 [$F(1,4)=9.175$, $P=0.039$]. ACh levels were falling over the course of the session resulting in a significant effect of time [$F(12,48)=12.502$, $P<0.001$]; the ANOVA over these two sessions did not result in an interaction between session and time.

The administration of MEC (5.0 mg/kg, i.p.) prior to nicotine on day 8 did not eliminate the increase in cortical ACh efflux following nicotine administration, but did significantly attenuate the nicotine-stimulated increases in cortical ACh efflux, relative to levels observed following nicotine administration on day 5 [F(1,4)=24.437, $P=0.008$]; these results are essentially identical to the effects of the lower dose of MEC (1.0 mg/kg). The ANOVA also revealed a main effect of time [$F(12,48)=7.756$, $P<0.001$] and an interaction between session and time [$F(12,48)=2.888$, $P=0.004$]. Pairwise comparisons confirmed that ACh efflux on day 8 was significantly lower than that on day 5 in collections taken at 120 min [$t(4)=-3.280$, $P=0.030$], 135 min [$t(4)=-8.822$, $P=0.001$], 150 min [t(4)=–4.412, $P=0.012$], 165 min [$t(4)=-3.901$, $P=0.018$], and 210 min [t(4)=6.105, $P=0.004$].

Thus, the increase in ACh efflux was similar on day 1 (acute administration) and day 8 (MEC pretreatment) following the repeated administration of nicotine, as an ANOVA over these two sessions revealed only a significant effect of time [$F(12,48)=8.572$, $P<0.001$], but no effect of session nor a session×time interaction.

Finally, examination of the data from the rats pretreated with MEC on day 10 (from 90 min to 270 min) that received a saline injection in place of nicotine revealed that cortical ACh efflux varied over time [F(12,48)=3.903, $P<0.001$]. Pairwise comparisons revealed that relative to the collection at 60 min, ACh was significantly elevated over baseline at 105 min [$t(4)=-2.781$, $P=0.050$] just after the nicotine injection, but ACh levels were no different from basal levels at any other time point (all $P>0.05$).

Discussion

Collectively, these experiments demonstrate that the repeated administration of nicotine over a 4-day injection regimen results in a potentiated release of cortical ACh in rats relative to the increase in ACh release observed following the initial administration of nicotine. In experiment 1, there was a moderate increase in motor activity following acute administration of nicotine on day 1, but no sensitization or tolerance of motor activity was observed following nicotine administration on day 5 or day 8. Consistent with previous studies examining the effects of MEC on acute nicotine-induced increases in ACh release (Summers et al. 1994; Tani et al. 1998), the effects of acute nicotine administration were completely blocked by pre-treatment with the non-competitive nAChR antagonist MEC. However, the potentiated or sensitized increase in cortical ACh release produced by repeated exposure to nicotine was not fully blocked by MEC.

Potential neuronal mechanisms mediating the sensitization of cortical ACh efflux

At present, one can only speculate about potential neural mechanisms mediating the effects of repeated administration of nicotine on cortical ACh release. Several possible mechanisms through which repeated administration of nicotine could result in sensitized release of cortical ACh are discussed below. Nicotine is known to bind to neuronal nAChRs that are composed of several subunits to form a ligand-gated ion channel. These subunits form several different isoforms, the predominant (~90%) isoform in the brain being the $\alpha_4\beta_2$ receptor (Mihailescu and Drucker-Colin 2000), which has a high affinity for nicotine (Flores et al. 1992). In considering the potential neural mechanisms which may mediate the effects of repeated nicotine on the sensitized release of ACh, it is important to remember that while pretreatment with either dose of the nAChR channel-blocker MEC (1.0 or 5.0 mg/kg) attenuated the sensitized increase in cortical ACh efflux observed following chronic administration of nicotine, neither dose of the antagonist completely blocked the sensitized response.

It is tempting to speculate that receptor (Kisr et al. 1985; Peng et al. 1994; Shoaib et al. 1997) or functional upregulation (Buisson and Bertrand 2002), particularly of the $\alpha_4\beta_2$ subtype, contribute to the sensitized release of cortical ACh. There is also some evidence for upregulation of α_7 receptors following chronic administration of nicotine (Marks et al. 1985), although the current understanding is that higher doses of nicotine are needed to upregulate α_7 subunit-containing nAChRs than $\alpha_4\beta_2$ isoforms. However, the functional consequences of chronic nicotine exposure, including increased receptor number, is still controversial (see Marks et al. 1993; Peng et al. 1994; Ke et al. 1998), so there is no clear

explanation for enhanced cortical ACh release in this regard.

MEC (even at a relatively high dose) did not fully block the sensitized increase in ACh following repeated administration of nicotine reported here. This could suggest that the $\alpha_4\beta_2$ subunit-containing isoform is not able to account for the full effects of repeated nicotine administration on cortical ACh release. Although MEC blocks all types of nAChR receptors, it is much more potent at $\alpha_4\beta_2$ subunits than other isoforms, such as the α_7 subunit-containing nAChRs (Mihailescu and Drucker-Colin 2000). Alternatively, alterations to the receptors could have occurred resulting in MEC no longer acting as a full antagonist under these conditions. Another intriguing possibility is that of a change in the calcium dependence of sensitized cortical ACh efflux. A change in calcium dependence may have resulted in the altered response to MEC in the current study. For example, the enhanced release of DA in the NAC induced by amphetamine following repeated cocaine has been demonstrated to have a calcium-dependent component (Pierce and Kalivas 1997), and glutamate release following nicotine has been demonstrated to be partially calcium-independent (Reid et al. 2000). Further experiments will be required to provide an adequate explanation.

Sensitized increases in NAC dopamine transmission following chronic systemic nicotine exposure (Balfour et al. 1998) could also be indirectly increasing cortical ACh release. The basal forebrain cholinergic neurons are believed to be trans-synaptically regulated (at least in part) by a GABAergic projection from the NAC (Zaborszky and Cullinan 1992; Sarter and Bruno 1994). Anatomical and neuropharmacological evidence supports the general assumption that dopamine receptor stimulation reduces this GABAergic inhibition of basal forebrain neurons, including the corticopetal cholinergic projections (Yang and Mogenson 1989; Bourdelais and Kalivas 1990; Zaborszky and Cullinan 1992; Ferre et al. 1994). Therefore, sensitized increases in dopamine levels in the NAC following chronic exposure to nicotine may be releasing the basal forebrain cholinergic neurons from GABAergic inhibition from the NAC, which could result in increased cortical ACh release (see also Moore et al. 1999). With respect to the failure of MEC to block fully the sensitized release of cortical ACh release after repeated nicotine administration, the suggestion that both MEC-sensitive receptors in the VTA and MEC-insensitive receptors in the NAC mediate the full effects of nicotine-stimulated dopamine release in the NAC (Fu et al. 2000) may explain the partial attenuation of the effects of repeated nicotine by MEC. Additionally, as part of this distributed system, mesocortical dopamine release could be contributing to the effects of repeatedly administered nicotine on cortical ACh release through interactions with the basal forebrain cholinergic neurons either locally within the cortex, or through modulation of cortical efferents projecting back to somatodendritic aspects of the basal forebrain cholinergic system and to VTA dopaminergic neurons (Nomikos et al. 2000).

Finally, recent findings regarding the effects of nicotine on glutamate levels suggest that nicotine could be exerting its effects on cortical ACh release through changes in glutamate as well as glutamatergic regulation of other transmitters either locally in the cortex or in brain regions that may trans-synaptically modulate cortical ACh release, including the nucleus accumbens or basal forebrain. For example, the *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 blocks behavioral sensitization and nAChR upregulation produced by repeated nicotine administration (Shoaib et al. 1997). It has been suggested that nicotine may modulate the release of glutamate through MEC-insensitive α_7 subunit-containing nAChRs located pre-synaptically on glutamatergic terminals (Schilstrom et al. 1998; Fu et al. 2000) or through effects on glutamate transporters (Reid et al. 2000).

Motor activity following repeated administration of nicotine

The administration of nicotine produced an increase in locomotor activity that was most evident in the 15-min period immediately following the injection procedure, consistent with other reports of locomotor activity following repeated administration of nicotine (Nisell et al. 1996; Reid et al. 1996, 1998). Sensitization of motor activity was not evident in the current experiments, as the behavioral ratings on day 1 (after acute administration of nicotine) did not differ from behavioral ratings on days 5 and 8 (after the repeated exposure regimen). The absence of sensitized motor behavior may not be unexpected given the complex interactions between environmental/behavioral variables and different nicotine administration regimens (Kisr et al. 1987; Benwell and Balfour 1992; Shoaib et al. 1997). For example, using a 5-day pretreatment regimen similar to the one used in the current studies, Reid, Ho, and Berger (1996) demonstrated that in order for nicotine to result in sensitized locomotor behavior, conditioning to the environmental context was required. In their study, those subjects that showed sensitized increases in activity were introduced to the test box for only 15 min prior to nicotine administration, whereas rats that received nicotine administration in their home cages did not show an increase in activity during the nicotine challenge. In the current study, rats were exposed to the microdialysis bowl for 7 h each day beginning 7 days prior to the first exposure to nicotine. In addition, these rats received two nicotine injections each day, the first in the testing bowl, the second upon returning to their home cage in the colony room. This protracted exposure to the testing environment, coupled with the multiple injection environments, may account for the absence of sensitized effects on locomotor ratings in the current study.

The absence of sensitized locomotor behavior also has implications for the potential relationship between motor activity and cortical ACh release, as manipulations that

increase motor activity may also increase cortical ACh release (Acquas et al. 1998; Day and Fibiger 1992; Bruno et al. 1999). However, motor activity following nicotine did not vary in the current studies from day 1 to day 5, while significant differences in cortical ACh release were observed between these two sessions, suggesting that motor activity alone can not account for the changes in cortical ACh release. Studies examining amphetamine-sensitized cortical ACh release (Nelson et al. 2000) have similarly argued that changes in locomotor behavior alone, following repeated psychostimulant administration, are not sufficient to account for the full range of observed effects on cortical ACh release.

Cortical acetylcholine release after repeated administration of nicotine

The increase in cortical ACh release observed following the repeated administration of nicotine is not likely to be the result of conditioned increases in cortical ACh release due to repeated exposure to the testing environment and injection procedures. Although the increase in cortical ACh release during the first collection after the injection of saline on day 10 could be interpreted as consistent with such an effect, as can be seen in Fig. 2, the increase in cortical ACh release was as high following the saline injection (relative to the injection on day 1) as it was following the nicotine injection on days 5 and 8. However, if the environmental cues were producing increases in transmitter release, then one would predict increasing basal levels of ACh efflux with an increasing number of injections. Basal levels during the current experiments did not change across the length of the experiment and ACh levels quickly returned to baseline following the saline injection. Coupled with the failure to see evidence for conditioned increases in motor activity (see below) the current data do not support the notion of environmentally supported conditioned increases in cortical ACh release.

One additional point to consider is the addition of a cholinesterase inhibitor to the perfusion medium. It is possible that increased stimulation of autoreceptors could dampen drug effects, or even produce effects opposite to those in the absence of an inhibitor. For example, DeBoer and Abercrombie (1996) demonstrated that striatal ACh release was differentially affected by administration of dopamine ligands, depending on the level of neostigmine present. More relevant to the present manuscript, we have recently observed that the magnitude of stimulated cortical ACh efflux accompanying potassium depolarization (Herzog et al. 2002, unpublished observations) or performance in a sustained attention task (Arnold et al. 2001, unpublished observations) is not significantly affected by the presence or absence of neostigmine. Therefore, it is not likely that the neostigmine produced the results seen in this set of experiments.

Implication of sensitized cortical ACh release for compulsive drug use

Research on the neural mechanisms mediating the use of addictive drugs, such as nicotine, has begun to focus on potential cellular adaptations postulated to be important for the compulsive nature of addictive behaviors (Wise 2000; Brown and Kolb 2001; Robinson et al. 2001). In addition, many authors have suggested that sensitization of mesoaccumbens dopamine pathway may serve as a common pathway mediating the compulsive use of psychostimulant drugs (Robinson and Berridge 1993; Berridge and Robinson 1998; Koob et al. 1998; Ikemoto and Panksepp 1999). As discussed above, neural transmission within the nucleus accumbens contributes to the regulation of cortical ACh release, and thus long-term neuroadaptions to this system may underlie sensitization of cortical ACh release. The well-documented role of the basal forebrain cholinergic system in attentional processing (Sarter and Bruno 2000) suggests that the sensitization of cortical ACh release is a necessary correlate of sensitized mesolimbic dopaminergic systems and increases the degree to which drug-related stimuli control behavior. These recent conceptualizations of the efferent consequences of abnormal neural transmission within the nucleus accumbens may help unravel the complex cognitive mechanisms underlying the experience of "reward" and "craving" associated with addiction, and consequently help elucidate the neural mechanisms that mediate the compulsive use of drugs such as nicotine.

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References

Acquas E, Wilson C, Fibiger HC (1998) Pharmacology of sensory stimulation-evoked increases in frontal cortical acetylcholine release. Neuroscience 85:73–83

Arnold HM, Burk JA, Hodgson E, Sarter M, Bruno JP (2002) Differential increases in cortical acetylcholine release in rats performing a sustained attention task versus behavioral control tasks that do not explicitly tax attention. Neuroscience 114:451–460

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

Balfour DJK, Wright AE, Benwell MEM, Birrel CE (2000) The putative role of extra-synaptic mesolimbic dopamine in the neurobiology of nicotine dependence. Behav Brain Res 113:73–83

Benwell MEM, Balfour DJK (1992) The effects of acute and repeated nicotine treatment on nucleus accumbens dopamine and locomotor activity. Br J Pharmacol 105:849–856

Berridge KC, Robinson TE (1998) What is the role in reward: hedonic impact, reward learning, or incentive salience? Brain Res Rev 28:309–369

Bourdelais A, Kalivas PW (1990) Amphetamine lowers extracellular GABA concentration in the ventral pallidum. Brain Res 516:132–136

Brown RW, Kolb B (2001) Nicotine sensitization increases dendritic length and spine density in the nucleus accumbens and cingulate cortex. Brain Res 899:94–100

Bruno JP, Sarter M, Arnold HM, Himmelheber AM (1999) In vivo neurochemical correlates of cognitive processes: methodological and conceptual challenges. Rev Neurosci 10:25–48

Buisson B, Bertrand D (2002) Nicotine addiction: the possible role of functional upregulation. Trends Pharmacol Sci 23:130–136

Byrnes EM, Reilly A, Bruno JP (1997) Effects of AMPA and D_1 receptor activation on striatal and nigral GABA efflux. Synapse 26:254–268

Carboni E, Bortone L, Giua C, DiChiara G (2000) Dissociation of physical abstinence signs from changes in extracellular dopamine in the nucleus accumbens and in the prefrontal cortex of nicotine dependent rats. Drug Alcohol Depend 58:93–102

Day J, Fibiger H C (1992) Dopaminergic regulation of cortical acetylcholine release. Synapse 12:281–286

DeBoer P, Abercrombie ED (1996) Physiological release of striatal acetylcholine in vivo: modulation by D_1 and D_2 dopamine receptor subtypes. J Pharm Exp Ther 277:775–783

Deller T, Sarter M (1998) Effects of repeated administration of amphetamine on behavioral vigilance: evidence for "sensitized" attentional impairments. Psychopharmacology 137:410–414

Di Chiara G (2000) Role of dopamine in the behavioural actions of nicotine related to addiction. Eur J Pharmacol 393:295–314

Ellinwood EH, Balster RL (1974) Rating the behavioral effects of amphetamine. Eur J Pharmacol 28:35–41

Ferre S, O'Connor WT, Snaprud P, Ungerstedt U, Fuxe K (1994) Antagonistic interaction between adenosineA_{2a} receptors and dopamine D_2 receptors in the ventral striopallidal system. Implications for the treatment of schizophrenia. Neuroscience 63:765–773

Flores CM, Rogers SW, Pabreza LA, Wolfe BB, Kellar KJ (1992) A subtype of nicotinic cholinergic receptor in rat brain is composed of a_4 and b_2 subunits and is up-regulated by chronic nicotine treatment. Mol Pharmacol 41:31–37

Fu Y, Matta SG, Gao W, Sharp BM (2000) Local a-bungarotoxin-sensitive nicotinic receptors in the nucleus accumbens modulate nicotine-stimulated dopamine secretion in vivo. Neuroscience 101:369–375

Gill TM, Sarter M, Givens B (2000) Sustained visual attention performance-associated prefrontal neuronal activity: evidence for cholinergic modulation. J Neurosci 12:4745–4757

Huang T, Yang L, Gitzen J, Kissinger PT, Vreeke M, Heller A (1995) Detection of basal acetylcholine in rat brain microdialysate. J Chromatogr B 670:323–327

Ikemoto S, Panksepp J (1999) The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward seeking. Brain Res Rev 31:6–41

Johnson BJ, Bruno JP (1995) Dopaminergic modulation of striatal acetylcholine release in rats depleted of dopamine as neonates. Neuropharmacology 34:191–203

Ke L, Eisenhour CM, Bencherif M, Lukas RJ (1998) Effects of chronic nicotine treatment on expression of diverse nicotinic acetylcholine receptor subtypes. I. Dose- and time-dependent effects of nicotine treatment. J Pharmacol Exp Ther 286:825–840

Keppel G (1991) Design and analysis: a researcher's handbook. Prentice Hall, Englewood Cliffs, N.J.

Kisr C, Hakan RL, Hal, DP, Kellar KJ (1985) Exposure to nicotine enhances the behavioral stimulant effect of nicotine and increases [^{3}H]acetylcholine to nicotinic receptors. Neuropharmacology 24:527–531

Kisr C, Hakan RL, Kellar KJ (1987) Chronic nicotine and locomotor activity: influences of exposure dose and test dose. Psychopharmacology 92:25–29

Koob GF, Sanna PP, Bloom FE (1998) Neuroscience of addiction. Neuron 21:467–476

Marks M, Stitzel J, Collins A (1985) Time course study of the effects of chronic nicotine infusion on drug response and brain receptors. J Pharmacol Exp Ther 235:619–628

Marks MJ, Grady SR, Collins AC (1993) Downregulation of nicotinic receptor function after chronic nicotine infusion. J Pharmacol Exp Ther 266:1268–1276

Mihailescu S, Drucker-Colin R (2000) Nicotine, brain nicotinic receptors, and neuropsychiatric disorders. Arch Med Res 31:131–144

Moore H, Dudchenko P, Bruno JP, Sarter M (1992) Toward modeling age-related changes of attentional abilities in rates: simple and choice reaction time tasks and vigilance. Neurobiol Aging 13:759–772

Moore H, Sarter M, Bruno JP (1995) Stimulation of cortical acetylcholine efflux by FG 7142 measured with repeated microdialysis sampling. Synapse 21:324–331

Moore H, Fadel J, Sarter M, Bruno JP (1999) Role of accumbens and cortical dopamine receptors in the regulation of cortical acetylcholine release. Neuroscience 88:811–822

Nelson CL, Sarter M, Bruno JP (2000) Repeated pretreatment with amphetamine sensitizes increases in cortical acetylcholine release. Psychopharmacology 151:406–415

Nisell M, Nomikos GG, Hertel P, Panagis G, Svensson TH (1996) Condition-independent sensitization of locomotor stimulation and mesocortical dopamine release following chronic nicotine treatment in the rat. Synapse 22:369–381

Nomikos GG, Schilstrom B, Hildebrand BE, Panagis G, Grenhoff J, Svensson TH (2000) Role of a_7 nicotinic receptors in nicotine dependence and implications for psychiatric illness. Behav Brain Res 113:97–103

Nordberg A, Ramanelli L, Sundwall A, Bianchi C, Beani L (1989) Effect of acute and subchronic nicotine treatment on cortical acetylcholine release and on nicotinic receptors in rats and guinea-pigs. Br J Pharmacol 98:71–78

Paulson PE, Robinson TE (1995) Amphetamine-induced time-dependent sensitization of dopamine neurotransmission in the dorsal and ventral striatum: a microdialysis study in behaving rats. Synapse 19:56–65

Paxinos G, Watson C (1986) The rat brain in stereotaxic coordinates, 2nd edn. Academic Press, New York

Peng X, Gerzanich V, Anand R, Whiting PJ, Lindstrom J (1994) Nicotine-induced increase in neuronal nicotinic receptors results from a decrease in the rate of receptor turnover. Mol Pharmacol 46:523–530

Pierce RC, Kalivas PW (1997) Repeated cocaine modifies the mechanism by which amphetamine release dopamine. J Neurosci 17:3254–3261

Potter PE, Meek JL, Neff NH (1983) Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J Neurochem 41:188–193

Reid MS, Ho LB, Berger SP (1996) Effects of environmental conditioning on the development of nicotine sensitization: behavioral and neurochemical analysis. Psychopharmacology 126:301–310

Reid MS, Ho LB, Berger SP (1998) Behavioral and neurochemical components of nicotine sensitization following 15-day pretreatment: studies on contextual conditioning. Behav Pharmacol 9:137–148

Reid MS, Fox L, Ho LB, Berger SP (2000) Nicotine stimulation of extracellular glutamate levels in the nucleus accumbens: neuropharmacological characterization. Synapse 35:129–136

Robinson T, Gorny G, Mitton E, Kolb B (2001) Cocaine self-administration alters the morphology of dendrites and dendritic spines in the nucleus accumbens and neocortex. Synapse 3:257–266

Robinson TE, Berridge KC (1993) The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev 18:247–291

Sarter M, Bruno JP (1994) Cognitive functions of cortical ACh: lessons from studies on trans-synaptic modulation of activated efflux. Trends Neurosci 17:217–221

Sarter M, Bruno JP (1999) Abnormal regulation of corticopetal cholinergic neurons and impaired information processing in neuropsychiatric disorders. Trends Neurosci 22:67–74

Sarter M, Bruno JP (2000) Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents. Neuroscience 95:933–952

Schilstrom B, Svensson HM, Svensson TH, Nomikos GG (1998) Nicotine and food induced dopamine release in the nucleus accumbens of the rat: putative role of a_7 nicotinic receptors in the ventral tegmental area. Neuroscience 85:1005–1009

Shoaib M, Schindler CW, Goldberg SR, Pauly JR (1997) Behavioural and biochemical adaptations to nicotine in rats: influence of MK801, an NMDA receptor antagonist. Psychopharmacology 134:121–130

Summers KL, Giacobini E (1995) Effects of local and repeated systemic administration of (–)nicotine on extracellular levels of acetylcholine, norepinephrine, dopamine, and serotonin in rat cortex. Neurochem Res 20:753–759

Summers KL, Cuadra G, Naritoku D, Giacobini E (1994) Effects of nicotine on levels of acetylcholine and biogenic amines in rat cortex. Drug Dev Res 31:108–119

Summers KL, Lippiello P, Giacobini E (1996) A microdialysis study of the effects of nicotinic agonist RJR-2403 on cortical release of acetylcholine and biogenic amines. Neurochem Res 21:1181–1186

Tani Y, Saito K, Imoto M, Ohno T (1998) Pharmacological characterization of nicotinic receptor-mediated acetylcholine release in rat brain–an in vivo microdialysis study. Eur J Pharmacol 351:181–188

Toide K, Arima T (1989) Effects of cholinergic drugs on extracellular levels of acetylcholine and choline in rat cortex, hippocampus and striatum studied by brain dialysis. Eur J Pharmacol 173:133–141

Wise RA (2000) Addiction becomes a brain disease. Neuron 26:27–33

Yang CR, Mogenson GJ (1989) Ventral pallidal neuronal responses to dopamine receptor stimulation in the nucleus accumbens. Brain Res 489:237–246

Zaborszky L, Cullinan WE (1992) Projections from the nucleus accumbens to cholinergic neurons of the ventral pallidum: a correlated light and electron microscopic double-immunolabeling study in rat. Brain Res 570:92–101

Zaborszky L, Pang K, Somogyi J, Nadasdy Z, Kallo I (1999) The basal forebrain corticopetal system revisited. Ann NY Acad Sci 877:339–367