

SYSTEMIC AND INTRA-ACCUMBENS ADMINISTRATION OF AMPHETAMINE DIFFERENTIALLY AFFECTS CORTICAL ACETYLCHOLINE RELEASE

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Abstract—The present experiments tested the hypothesis that the amphetamine-induced increase in dopamine release in the nucleus accumbens represents a necessary and sufficient component of the ability of systemically administered amphetamine to stimulate cortical acetylcholine release. The effects of systemic or intra-accumbens administration of amphetamine on accumbens dopamine release and cortical acetylcholine release were assessed simultaneously in awake animals equipped with dialysis probes inserted into the shell of the nucleus accumbens and the medial prefrontal cortex. Additionally, the ability of intra-accumbens administration of dopamine D₁ and D₂ receptor antagonists to attenuate the effects of systemic amphetamine on cortical acetylcholine was tested. The effects of all treatments were assessed in interaction with a stimulus-induced activation of cortical acetylcholine release to account for the possibility that the demonstration of the trans-synaptic effects of accumbens dopamine requires pre-activation of basal forebrain circuits. Systemic amphetamine resulted in increases in basal cortical acetylcholine and accumbens dopamine efflux. Intra-accumbens administration of amphetamine substantially increased accumbens dopamine efflux, but did not significantly affect cortical acetylcholine efflux. Furthermore, intra-accumbens administration of sulpiride or SCH 23390 did not attenuate the systemic amphetamine-induced increase in cortical acetylcholine efflux.

Collectively, the present data suggest that increases in accumbens dopamine release are neither sufficient nor necessary for the effects of systemically administered amphetamine on cortical acetylcholine release. The systemic amphetamine-induced increase in cortical acetylcholine may be mediated via multiple, parallel pathways and may not be attributable to a single afferent pathway of the basal forebrain. © 2000 IBRO. Published by Elsevier Science Ltd.

Key words: acetylcholine, dopamine, amphetamine, basal forebrain, prefrontal cortex, nucleus accumbens.

The cholinergic neurons originating in the basal forebrain project to all cortical areas and layers.⁴⁷ Cortical cholinergic inputs mediate fundamental aspects of cognitive functions, specifically attentional processes (for reviews, see Refs. 13 and 39). Consequently, aberrations in the functioning of these neurons and in the afferent regulation of their excitability have been implicated in the manifestation of the cognitive symptoms of major neuropsychiatric disorders, including schizophrenia, dementia and compulsive addictive drug use.⁴⁰

Systemically administered psychostimulants, including amphetamine, potently increase cortical acetylcholine (ACh) efflux.^{6,10,20,37} Day *et al.*¹¹ provided indirect support of the hypothesis that this effect of amphetamine is mediated mainly via mesotelencephalic dopaminergic projections by demonstrating that forebrain dopamine (DA)-depleting lesions partially attenuated the ability of systemically administered amphetamine to increase cortical ACh efflux. The present experiments were designed to test directly the hypothesis that DA release in the nucleus accumbens (NAC) represents a necessary and sufficient component in the neuronal mediation of the effects of systemic amphetamine on cortical ACh efflux. In addition to the data of Day *et al.*,¹¹ this hypothesis was based on anatomical and neuropharmacological evidence, which suggests that NAC DA receptor stimulation reduces the GABAergic inhibition of basal forebrain neurons, including the corticopetal cholinergic projections.^{16,45,48,49} Consistent with this hypothesis, blockade of D₂ receptors in the NAC was demonstrated to attenuate the

increases in cortical ACh efflux that were produced by the systemic administration of a negative GABA modulator.³⁰

The present experiments assessed the effects of systemic or intra-accumbens administration of amphetamine on DA efflux in the NAC and ACh efflux in the medial prefrontal cortex (mPFC) simultaneously in awake animals with concentric dialysis probes inserted into both areas. While both the systemic and intra-accumbens administration of amphetamine results in robust increases in NAC DA release,^{1,9} the extremely high levels of NAC DA release following the perfusion of amphetamine into the NAC⁷ would be expected to stimulate cortical ACh efflux if NAC DA receptor stimulation represents a sufficient step in increasing cortical ACh efflux. Conversely, the increase in cortical ACh efflux produced by systemic amphetamine was also expected to be attenuated by intra-accumbens infusions of a DA receptor D₁ (SCH 23390) or D₂ (sulpiride) antagonist, thereby demonstrating the necessity of NAC DA receptor stimulation in the effects of systemic amphetamine on cortical ACh efflux. As the current neuronal hypothesis suggests that the demonstration of the effects of NAC DA receptor stimulation on cortical ACh efflux requires activation of basal forebrain corticopetal cholinergic projections to reveal the effects of a DA-mediated disinhibition (for review see Ref. 38), the effects of these manipulations were studied in conjunction with a stimulus known to activate cortical ACh efflux (exposure to darkness paired with the opportunity to consume sweetened cereal^{29,31,32}).

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Abbreviations: ACh, acetylcholine; aCSF, artificial cerebrospinal fluid; DA, dopamine; EDTA, disodium ethylenediaminetetra-acetate; mPFC, medial prefrontal cortex; NAC, nucleus accumbens; NE, norepinephrine.

EXPERIMENTAL PROCEDURES

Subjects

Adult male Fisher-344/Brown Norway F1 hybrid rats (Harlan

Sprague–Dawley, Indianapolis, IN, U.S.A.), weighing between 250 and 350 g, served as subjects in each experiment. 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-h/12-h light–dark cycle (lights on at 6.30 a.m.). Prior to guide cannula implantation, animals were housed in pairs in stainless steel hanging racks. On the day prior to surgery, animals were moved to individual standard plastic cages with pine 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. Two stainless steel microdialysis guide cannulae (0.72 mm o.d.) were implanted, one into the mPFC and the other into the shell region of the ipsilateral NAC. To place the guide cannula into the mPFC, the carrier arm of the stereotaxic apparatus was angled 10° away from vertical towards anterior, and the cannula was positioned 3.0 mm anterior to bregma, 0.7 mm lateral to the midline and 1.0 mm below dura mater (all coordinates according to the atlas of Paxinos and Watson³⁶). For placement into the ipsilateral shell of the NAC, the carrier arm was angled 15° away from vertical towards posterior, and the cannula was positioned 0.3 mm posterior to bregma, 1.1 mm lateral of the midline and 6.0 mm below dura. Following surgery, animals were allowed to recover in their home cages for three days prior to the first dialysis session.

Habituation

On each of the 10 days immediately prior to cannula surgery, animals were habituated to concentric dialysis bowls (35 cm height × 38 cm depth; CMA, Stockholm, Sweden) for 5–7 h each day. Between the fourth and fifth hours in the bowl, the animals were exposed to sudden darkness by turning off the room lights (experimenter provided illumination with a 60-W red light bulb). Immediately after the lights were extinguished, rats were presented with a single piece of sweetened cereal (Fruit Loop, Kellogg's, Battle Creek, MI, U.S.A.). After several days of exposure to these conditions (referred to as the "darkness/cereal" stimulus), animals became active immediately after lights were extinguished and rapidly approached and consumed the cereal, usually within 30–60 s. This stimulus has been repeatedly shown to transiently activate cortical ACh release^{29,31,32} and, as such, was employed to characterize the ability of amphetamine to modulate stimulated ACh release.

Microdialysis sessions

Microdialysis was conducted using a repeated perfusion paradigm in which each rat received a different pharmacological treatment on each of four days, with an "off day" between each microdialysis session. This repeated perfusion paradigm allows the assessment of the effects of multiple treatments, including control conditions, in the same animal. This procedure has been validated previously for measurement of cortical ACh efflux,³³ as well as for striatal ACh efflux²¹ and striatonigral GABA efflux,⁵ by showing that neither basal release nor drug effects interact significantly with the order of the dialysis sessions. On each microdialysis day, animals were placed in the testing chambers at least 30 min prior to the insertion of concentric dialysis probes (0.35 mm o.d., 2.0 mm membrane length; Bioanalytical Systems, W. Lafayette, IN, U.S.A.) through the guide cannulae. Each probe was perfused with an artificial cerebrospinal fluid (aCSF; pH 7.0) containing the following (in mM): NaCl, 166.5; NaHCO₃, 27.5; KCl, 2.4; Na₂SO₄, 0.5; KH₂PO₄, 0.5; CaCl₂, 1.2; MgCl₂, 0.8; glucose, 5.0. The probes were attached to a dual channel liquid swivel (Instech, Plymouth Meeting, PA, U.S.A.) and perfused for 3 h before collection of dialysates began—an interval that results in stable basal ACh efflux that is highly (>95%) dependent on neuronal release.²⁹

Drugs

Systemic injections of the indirect dopaminergic agonist D-amphetamine sulfate (2.0 mg/kg, i.p.; Sigma, St Louis, MO, U.S.A.) were

delivered in 0.9% saline. This dose of amphetamine has been shown to increase reliably cortical ACh efflux.^{10,11} Amphetamine was also administered locally (via the NAC dialysis probe) using aCSF as the vehicle solution. The selection of the doses of locally perfused amphetamine (10, 100 or 250 μM) was based on several recent studies on the effects of intra-accumbens amphetamine on locomotor behavior^{9,19} or local neurotransmitter release.^{22,24} The D₁ antagonist SCH 23390 (100 μM) and the D₂ antagonist L-sulpiride (100 μM; both drugs from RBI, Natick, MA, U.S.A.) were also delivered via the dialysis probe in the NAC using aCSF as the vehicle solution. Intra-accumbens perfusions of either drug, at this dose, block the increase in cortical ACh efflux produced by systemic administration of the benzodiazepine partial inverse agonist FG 7142.³⁰

Experiment 1: effects of intra-accumbens and systemically administered amphetamine on cortical acetylcholine efflux and motoric activity

In this experiment, each subject received the following four microdialysis sessions: (i) aCSF as a vehicle control; (ii) intra-accumbens administration of 100 μM amphetamine perfused through the NAC probe; (iii) intra-accumbens administration of 250 μM amphetamine perfused through the NAC probe; and (iv) systemic injection (i.p.) of 2.0 mg/kg amphetamine. The sessions were presented in a pseudo-random order to each subject. The dialysis probe in the mPFC was perfused at 2.5 μl/min with aCSF containing the acetylcholinesterase inhibitor neostigmine bromide (0.5 μM); collections were taken every 5 min. The probe in the shell of the NAC was perfused at 1.25 μl/min and collections were taken every 10 min in vials containing 5.0 μl of a perchloric acid (0.05 N) solution containing sodium bisulfite (200 μM) and EDTA (1.0 mM) as an antioxidant. During sessions in which amphetamine was delivered via the NAC probe, the inlet line to the probe was switched to a syringe containing the amphetamine (in aCSF) immediately after the baseline period, where it remained for the duration of the session. Dialysate was not collected for the first 15 min following this switch to account for dead volume in the swivel, inlet and outlet lines. Control (aCSF vehicle) and systemic amphetamine sessions were run identically, but the NAC inlet was moved to a syringe containing only aCSF to control for the brief interruption of perfusion fluid with the change in syringes for the drug treatment group. Systemic amphetamine was administered at the end of the 15-min discard period. Dialysates were collected for an additional 20 min after the onset of the drug treatments. At the end of this 20-min period, room lights were extinguished and the darkness/cereal stimulus was presented. Dialysates were collected for an additional 30 min while the room lights remained off.

Based on initial experiments indicating that intra-accumbens amphetamine resulted in extremely high levels of NAC DA efflux but no significant change in cortical ACh efflux, the effects of a lower dose of intra-accumbens amphetamine (10 μM) was tested in a separate group of rats (*n* = 7). This experiment was designed to test the possibility that relatively small increases in NAC DA levels, more closely approximating the effects of systemic amphetamine (also see Ref. 22), may be more effective in increasing cortical ACh efflux. These animals were treated identically to the animals described above, with the exception that one session (first or fourth) was an aCSF vehicle control session and the other session (fourth or first) was intra-accumbens administration of 10 μM amphetamine perfused through the NAC probe. During sessions 2 and 3, the animals were treated identically to a vehicle control session and were perfused but no dialysates were collected.

Experiment 2: effects of intra-accumbens dopamine antagonists on systemic amphetamine-induced acetylcholine efflux and motoric activity

The general methods for this experiment were identical to those described above, with the following exceptions. First, the concentration of neostigmine used in the aCSF of the cortical dialysis probe was reduced from 0.5 to 0.05 μM in order to minimize any potential effects of this drug on cortical ACh efflux. To maintain sensitivity to the resulting lower levels of ACh under this lowered concentration of neostigmine, the dialysis collection interval was increased from 5 to 15 min. In addition, the flow rate for the mPFC probe was lowered so that both probes (mPFC and NAC) were perfused at 1.25 μl/min. Finally, DA efflux in the NAC was not measured in this experiment. The NAC probes were used solely for the local perfusion of the DA antagonists.

Three separate groups of rats were tested in two microdialysis

sessions each. The first group ($n = 6$) was tested with aCSF and the D_1 antagonist SCH 23390 (100 μM), whereas the second group ($n = 6$) was tested with aCSF and the D_2 antagonist sulpiride (100 μM). Finally, a "mixed antagonist group" ($n = 4$) was tested with aCSF and both SCH 23390 (100 μM) and sulpiride (100 μM) delivered simultaneously.

Following four baseline collections, the inlet to the NAC probe was switched to a line containing a DA antagonist or aCSF. After 30 min (two collections), subjects were injected with amphetamine (2.0 mg/kg, i.p.) and another two collections were taken prior to the onset of the darkness/cereal stimulus. An additional four collections were taken following the onset of this stimulus.

Measurement of motoric activity

Motoric activity was rated on a nine-point scale in order to characterize the behavioral effects of systemic injections and intra-accumbens perfusions of amphetamine. At the end of each collection interval, prior to removing a collection vial from the liquid swivel, the experimenter rated ongoing motoric activity according to a well-characterized rating scale.¹² The behavioral ratings were: 1, lying down, eyes closed; 2, lying down, eyes open; 3, normal grooming/chewing cage litter; 4, locomoting about the cage, with occasional sniffing and rearing; 5, hyperactive movements (jerky); 6, repetitive exploration of the cage; 7, repetitive exploration of the cage with hyperactivity; 8, licking, chewing and gnawing stereotypies; 9, dyskinetic movements.

Neurochemical analyses

Acetylcholine. ACh levels in dialysates collected from the mPFC were determined by high-performance liquid chromatography with electrochemical detection using procedures described previously.³⁰ Briefly, 10 μl from each collection were injected, and ACh and choline were separated by a C-18 carbon polymer column (530 mm \times 1 mm; Bioanalytical Systems) using a sodium phosphate mobile phase (50 mM NaH_2PO_4 , 0.1 mM EDTA, 10 mM NaCl, pH 8.4). ACh was hydrolysed on a post-column enzyme reactor and converted to hydrogen peroxide, which was detected using a "peroxidase-wired" glassy carbon electrode. The detection limit for ACh under these conditions was approximately 20 fmol/10- μl injection.

Dopamine. DA levels in dialysate collected from the NAC were also determined using high-performance liquid chromatography with electrochemical detection. From each sample, 10 μl was injected and DA was detected via a dual electrode coulometric detector (ESA, Chelmsford, MA, U.S.A.) with the potential for electrode 1 = -75.0 mV and for electrode 2 = 130.0 mV. DA was separated on a C-18 carbon polymer column (150 mm \times 5 mm; MD-150, ESA) using a sodium phosphate mobile phase (75 mM NaH_2PO_4 , 1.7 mM octanesulfonic acid, 25 mM EDTA, 100 μl triethylamine and 9.5% acetonitrile, pH 3.0). The detection limit for DA was approximately 0.5 fmol/10- μl injection.

Verification of probe placement

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 24 h and then transferred to 30% sucrose phosphate buffer until sectioning at least three days later. Histological verification of dialysis probe placement was made using 50- μm Cresyl Violet-stained sections. Only subjects that had both probe placements clearly within the target regions (mPFC and shell region of the NAC) were included in these analyses.

Statistical analyses

Basal efflux levels for each subject in each dialysis session were defined as the median of the baseline collections. In order to determine whether basal ACh or DA efflux changed over the course of multiple dialysis sessions, and in order to justify the expression of the data as percentage change from baseline, basal efflux, across all drug treatment groups, was compared among the four different dialysis sessions using a repeated measures ANOVA. These data are expressed in pmol/10- μl sample for ACh and as fmol/10- μl sample for DA.

For the neurochemical data, the remainder of the statistical analyses were performed on data expressed as a percentage change from the

median baseline. To determine whether the darkness/cereal stimulus enhanced efflux, a repeated measures ANOVA over Time (dialysate collections during the sessions) was conducted on the entire aCSF control session for both ACh and DA. Based on Keppel's suggestion²³ that the error term for follow-up comparisons in repeated measure designs should be the error of the means in question, means were compared using paired *t*-tests. In recognition of the possibilities of familywise error, these comparisons were minimized and used only to probe the source of statistically significant main effects or interactions revealed by ANOVAs.

To determine whether systemically administered amphetamine increased basal efflux levels, a repeated measures ANOVA with two factors was conducted over the last baseline and first 20 min following the drug injection (prior to darkness/cereal); the two factors were Drug (aCSF session vs systemic amphetamine) and Time (four collections for ACh, two collections for DA). To assess the effects of systemic amphetamine during the stimulation period, a second ANOVA (Drug, Time) was conducted over the last 30 min of the session, beginning with the first collection following the darkness/cereal manipulation.

To determine whether intra-accumbens amphetamine increased basal levels of ACh and DA efflux, a repeated measures ANOVA was conducted over the last baseline and first 20 min following the drug delivery (prior to darkness/cereal); again, Drug (aCSF, 10, 100 and 250 μM) and Time (four collections for ACh, two collections for DA) were the two factors. To assess the effects of intra-accumbens amphetamine during the stimulation period (i.e. following the darkness/cereal manipulation), a second ANOVA (Drug, Time) was conducted over the last 30 min of the session, beginning with the first collection following the darkness/cereal manipulation.

In order to determine whether the perfusion of D_1 or D_2 antagonists affected basal ACh efflux, a repeated measures ANOVA was conducted with Drug (aCSF, D_1 , D_2 or mixed antagonist) and Time (last baseline, first two collections after the onset of antagonist perfusion) as factors. In order to determine whether intra-accumbens administration of the D_1 , D_2 or mixed antagonist attenuated the increase in cortical ACh efflux produced by systemic amphetamine, a repeated measures ANOVA (Drug, Time) was conducted over the last baseline and all remaining collection intervals.

Ratings of motoric activity yielded non-parametric data and were analysed using the Wilcoxon signed ranks test, adjusted for tied ranks.⁴³ In an effort to control for Type I error, a minimum number of comparisons was conducted on these data. To test whether the darkness/cereal stimulus increased motoric activity, data from the aCSF vehicle control session just prior to the stimulus (the 25-min collection interval) were compared to the ratings taken during the first collection period after the onset of the darkness/cereal stimulus (the 30-min interval). To determine whether Drug treatment influenced the behavioral ratings, the aCSF session was compared to the 100 and 250 μM intra-accumbens amphetamine and the systemic amphetamine sessions at a single time, during the collection at 30 min, just after presentation of the darkness/cereal stimulus. Preliminary observations suggested that this time interval was associated with the greatest degree of motoric activity across the various drug treatments. Finally, to assess the effects of different routes of amphetamine administration on behavioral ratings, the effects of 250 μM intra-accumbens amphetamine were compared to the effects of systemic amphetamine at the 30-min time-point. Statistical analyses were completed using SPSS (V. 8.0, SPSS, Chicago, IL, U.S.A.). The level of significance was defined as $P < 0.05$.

RESULTS

Placements of microdialysis probes

Figure 1 depicts the placement of microdialysis probes in the mPFC (top) and the shell region of the NAC (bottom) from animals in Experiment 1. For all animals, at least two-thirds of the dialysis membrane was located in the mPFC or NAC, respectively.

Basal levels of transmitter efflux in Experiment 1

Basal efflux of cortical ACh was stable across all four microdialysis sessions. The mean (\pm S.E.M.) values (pmol/10 μl) by order of session were: 0.10 ± 0.02 , 0.10 ± 0.01 ,

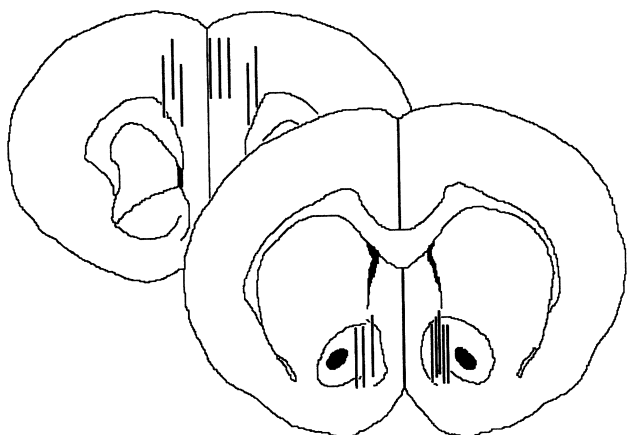


Fig. 1. Drawings of frontal sections indicating microdialysis probe placements in the mPFC (top section) and shell of the NAC (bottom section). The bars in each section indicate the position of the dialysis membrane for each of the animals in Experiment 1 ($n = 8$). Probe placements for animals in the other experiments were comparable.

0.09 ± 0.02 and 0.10 ± 0.02 for sessions 1–4, respectively ($F_{3,21} = 0.10$, $P = 0.958$). Likewise, mean (\pm S.E.M.) basal ACh efflux levels, collapsed across sessions, were not different for the four Drug treatments: aCSF control, 0.11 ± 0.01 ; $100 \mu\text{M}$ amphetamine, 0.09 ± 0.02 ; $250 \mu\text{M}$ amphetamine, 0.08 ± 0.01 ; systemic amphetamine, 0.10 ± 0.02 ($F_{3,21} = 0.66$, $P = 0.584$).

Basal efflux of DA in the NAC also did not differ across the four microdialysis sessions. The mean (\pm S.E.M.) values (fmol/ $10 \mu\text{l}$) were: 6.15 ± 1.54 , 7.23 ± 1.38 , 4.79 ± 0.94 and 5.84 ± 1.37 for sessions 1–4, respectively ($F_{3,21} = 1.17$, $P = 0.35$). Moreover, the mean (\pm S.E.M.) basal values (fmol/ $10 \mu\text{l}$) for the four Drug treatments, collapsed across sessions, did not differ from one another: aCSF control, 6.49 ± 1.47 ; $100 \mu\text{M}$ amphetamine, 5.50 ± 0.82 ; $250 \mu\text{M}$ amphetamine, 5.63 ± 1.42 ; systemic amphetamine, 6.36 ± 1.57 ($F_{3,21} = 0.27$, $P = 0.85$). The similarities in basal release across session and

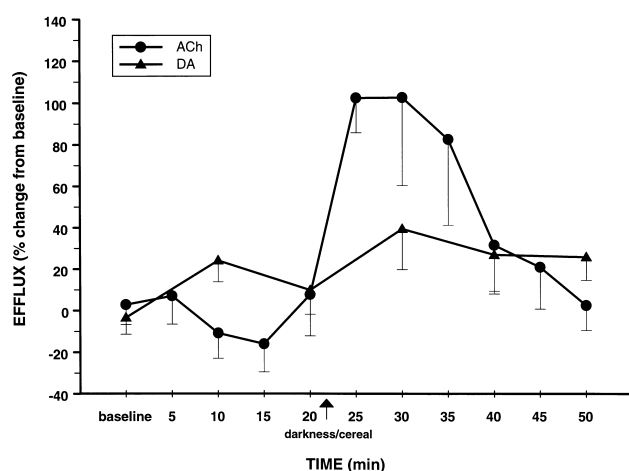


Fig. 2. Mean (\pm S.E.M.) efflux of ACh in the mPFC and DA in the shell region of the NAC from animals ($n = 8$) implanted with dual microdialysis probes. The overall median baseline efflux for ACh in the mPFC was 0.11 ± 0.01 pmol/ $10 \mu\text{l}$ (10-min collections) and for DA in the NAC was 6.49 ± 1.47 fmol/ $10 \mu\text{l}$ (5-min collections). Immediately after the 20-min collection interval, the lights of the testing room were extinguished and the animals were provided access to a piece of sweetened cereal (darkness/cereal). This exposure produced a transient elevation in cortical ACh efflux without any significant change in NAC DA efflux.

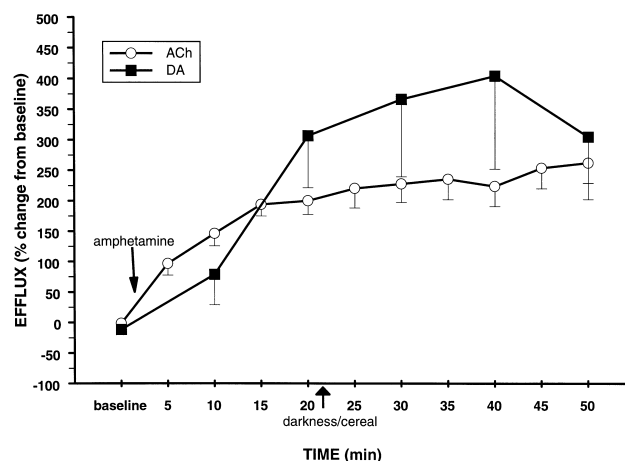


Fig. 3. Mean (\pm S.E.M.) mPFC ACh and NAC DA efflux in animals ($n = 6$) with dual microdialysis probes following systemic administration of amphetamine (2.0 mg/kg , i.p.). Animals received the amphetamine injection immediately after collection of the final baseline (indicated on the abscissa). The darkness/cereal stimulus was presented after the 20-min collection and remained in effect throughout the remainder of the session. Systemic administration of amphetamine produced a significant increase in ACh and DA efflux that appeared soon after the injection and remained elevated throughout the dialysis session.

drug condition (for both ACh and DA) allowed for subsequent analyses on the effects of drug treatments on stimulated release to be conducted on data expressed as percentage change from these basal values.

Effects of exposure to darkness/cereal on cortical acetylcholine and accumbens dopamine efflux

As can be seen in Fig. 2, cortical ACh efflux was relatively stable from the last baseline through the first 20 min of the aCSF control session. The presentation of the darkness/cereal stimulus resulted in a transient increase in cortical ACh release to approximately 100% above baseline levels (Time: $F_{6,42} = 5.86$, $P < 0.001$). Pairwise comparisons confirmed that cortical ACh efflux was elevated from baseline following the darkness/cereal manipulation at 25 min ($t_7 = 4.37$, $P = 0.003$) and at 30 min ($t_7 = 2.39$, $P = 0.048$), but returned to near basal levels by 35 min ($t_7 = 1.93$, $P = 0.096$). However, in contrast to its effects on cortical ACh efflux, the darkness/cereal stimulus did not produce an increase in DA efflux in the NAC; DA levels were relatively stable across the entire session (Time: $F_{3,21} = 0.15$, $P = 0.928$).

Systemic amphetamine stimulates cortical acetylcholine efflux

The systemic administration of amphetamine (2.0 mg/kg , i.p.) produced a large and rapid increase in basal cortical ACh release (Drug: $F_{1,7} = 46.44$, $P < 0.001$). Control levels of ACh remained stable (Fig. 2, baseline to 20 min) while, as seen in Fig. 3, ACh levels increased over time following systemic amphetamine (Time: $F_{4,28} = 28.44$, $P < 0.001$; Drug \times Time: $F_{4,28} = 24.11$, $P < 0.001$). Pairwise comparisons revealed that ACh efflux was significantly higher than control levels at 10 min ($t_7 = 7.23$, $P < 0.001$), 15 min ($t_7 = 9.27$, $P < 0.001$) and 20 min ($t_7 = 6.05$, $P = 0.001$) following the amphetamine injection.

Cortical ACh efflux remained elevated following the darkness/cereal stimulus in animals injected systemically with

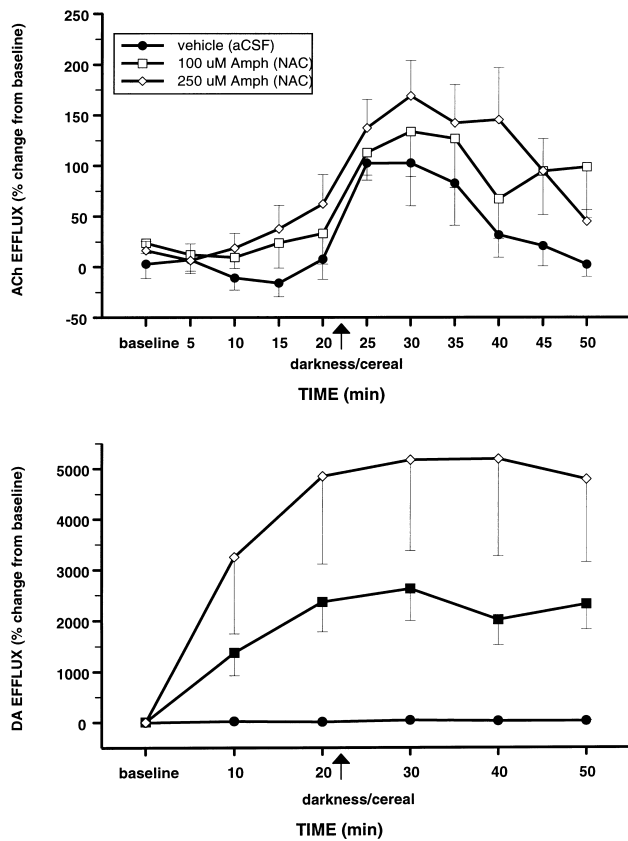


Fig. 4. Mean (\pm S.E.M.) efflux of mPFC ACh (top panel) and NAC shell DA (bottom panel) following local perfusion of the NAC with aCSF (control procedure), 100 μ M amphetamine or 250 μ M amphetamine. All animals ($n=6$) were implanted with dual microdialysis probes and received each of these drug treatments in a counterbalanced design. Local perfusion of amphetamine began immediately after the last baseline and continued throughout the session. The onset of the darkness/cereal stimulus immediately after the 20-min collection interval resulted in a transient increase in cortical ACh efflux during the aCSF control session (top panel). This increase was not significantly affected by the local perfusion of amphetamine into the NAC. The darkness/cereal stimulus did not affect DA efflux during the aCSF session (also see Fig. 1). Local perfusion of amphetamine resulted in dose-dependent increases in DA efflux both before and after the darkness/cereal stimulus.

amphetamine. An ANOVA comparing the final six collections during the aCSF control session (Fig. 2) and the systemic amphetamine session (Fig. 3) revealed main effects of Drug ($F_{1,7}=20.48$, $P=0.003$) and Time ($F_{5,35}=3.25$, $P=0.016$). The darkness/cereal manipulation transiently increased cortical ACh efflux during the aCSF control session (as shown in Fig. 2). However, elevated levels of cortical ACh efflux were maintained throughout the systemic amphetamine session, relative to the control data in Fig. 2 (Drug \times Time: $F_{5,35}=6.76$, $P<0.001$). Pairwise comparisons revealed significant increases in ACh release above control levels at 35 min ($t_7=2.59$, $P=0.036$), 40 min ($t_7=4.18$, $P=0.004$), 45 min ($t_7=6.21$, $P<0.001$) and 50 min ($t_7=7.94$, $P<0.001$).

Systemic amphetamine stimulates dopamine efflux in the nucleus accumbens

While basal DA efflux in the NAC remained stable in the aCSF control session (Fig. 2), DA levels increased following systemic administration of amphetamine (Fig. 3; Drug \times Time: $F_{2,14}=8.86$, $P=0.003$). Although DA efflux increased

70% during the first 10 min following amphetamine injection, it was not significantly elevated relative to the aCSF control session at this time ($t_7=0.99$, $P=0.356$). However, DA efflux continued to increase to just over 300% above baseline 20 min after amphetamine administration, which differed from aCSF control levels ($t_7=3.46$, $P=0.011$).

Overall, DA efflux in the NAC during the systemic amphetamine session (Fig. 3) was elevated following presentation of the darkness/cereal stimulus relative to the aCSF control session (Fig. 2; Drug: $F_{1,7}=7.51$, $P=0.029$). Visual comparison between the mean DA efflux in Figs 2 and 3 illustrates the potent (i.e. 250–400%) and long-lasting (for 30 min) increases induced by systemic amphetamine. For both sessions, the DA levels were fairly stable following the darkness/cereal stimulus (Time: $F_{2,14}=0.43$, $P=0.656$; Drug \times Time: $F_{2,14}=0.53$, $P=0.601$).

Intra-accumbens amphetamine perfusion does not affect cortical acetylcholine efflux

Basal levels of cortical ACh efflux did not change following amphetamine delivered directly into the NAC through the microdialysis probe. As shown in Fig. 4 (top panel), the baseline and first 20 min after the baseline did not differ among the vehicle (aCSF) control session and either session of locally perfused amphetamine (100 or 250 μ M) (Time: $F_{4,28}=2.58$, $P=0.059$; Drug: $F_{2,14}=1.14$, $P=0.348$; Drug \times Time: $F_{4,28}=0.63$, $P=0.752$). A lower dose of amphetamine (10 μ M) also did not increase basal ACh efflux (Fig. 5, top panel) relative to the control session (Drug: $F_{1,6}=1.70$, $P=0.239$; Time: $F_{4,24}=2.09$, $P=0.114$; Drug \times Time: $F_{4,24}=1.48$, $P=0.239$).

Following the darkness/cereal stimulus, intra-accumbens perfusion of amphetamine (100 or 250 μ M) did not stimulate cortical ACh efflux beyond that seen during the control session (see Fig. 4, top panel). In each of these three sessions there was a transient increase in ACh efflux following the darkness/cereal stimulus that gradually returned to basal values (Time: $F_{5,35}=8.36$, $P<0.001$). There was a trend toward an interaction between Drug \times Time ($F_{5,35}=1.89$, $P=0.062$) that could be viewed in support of the possibility that the local amphetamine perfusion slowed the rate at which activated levels of ACh returned to baseline.

Comparison of the lower dose of amphetamine (10 μ M) to the aCSF control (Fig. 5, top panel) revealed that ACh efflux transiently increased following the darkness/cereal stimulus and then returned to basal levels for both sessions (Time: $F_{5,30}=5.03$, $P=0.002$). This stimulated cortical ACh efflux was not potentiated by local perfusion of 10 μ M amphetamine. In fact, the ANOVA suggests that this lower dose of amphetamine actually dampened stimulated cortical ACh release (Drug: $F_{1,6}=8.45$, $P=0.027$). Multiple comparisons revealed that cortical ACh was lower during the 10 μ M intra-accumbens amphetamine session relative to the control session at 25 min ($t_6=12.81$, $P<0.004$), but not at any other time-point (all other $P>0.05$).

Intra-accumbens amphetamine markedly increases basal dopamine efflux

Although intra-accumbens administration of amphetamine had no significant effect on cortical ACh efflux, amphetamine perfused directly into the NAC (100 or 250 μ M) produced, as

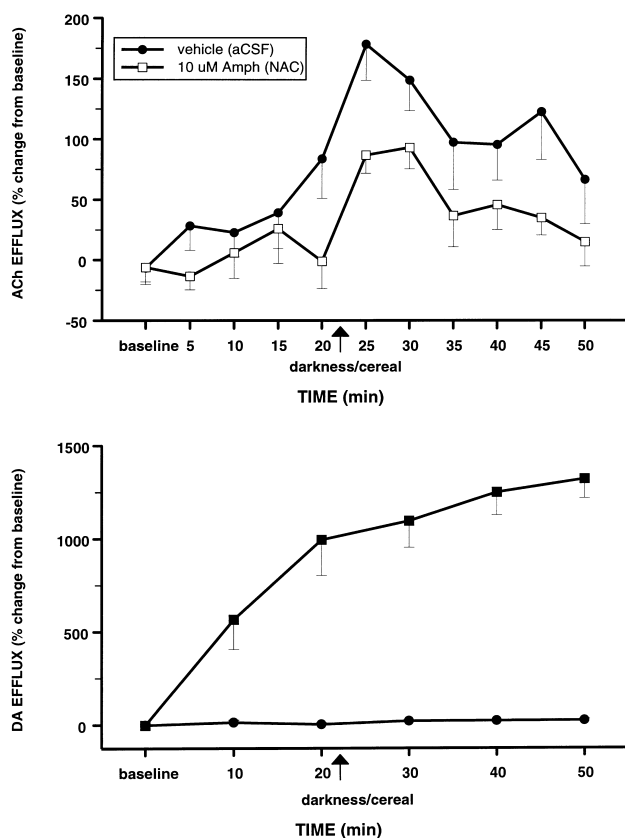


Fig. 5. Mean (\pm S.E.M.) efflux of mPFC ACh (top panel) and NAC shell DA (bottom panel) following local perfusion of the NAC with aCSF (control procedure) or 10 μ M amphetamine. All animals ($n=6$) were implanted with dual microdialysis probes and received each of the two drug treatments in a counterbalanced design. Local perfusion of amphetamine began immediately after the last baseline and continued throughout the session. The onset of the darkness/cereal stimulus immediately after the 20-min collection interval resulted in a transient increase in cortical ACh efflux during the aCSF control session (top panel). Local perfusion of amphetamine significantly reduced the magnitude of this stimulated ACh efflux. DA efflux in the NAC (bottom panel) was significantly increased following the local perfusion of 10 μ M amphetamine both before and after the darkness/cereal stimulus. DA efflux was unaffected by this stimulus during the aCSF control session.

expected, large dose-dependent increases in basal DA efflux within the NAC (Fig. 4, bottom panel). The ANOVA, conducted on the last baseline, 10- and 20-min collections, revealed main effects of Drug ($F_{2,14} = 5.76$, $P = 0.015$), Time ($F_{2,14} = 8.89$, $P = 0.003$) and a significant Drug \times Time interaction ($F_{4,28} = 6.15$, $P = 0.001$). Multiple comparisons revealed that 100 μ M intra-accumbens amphetamine increased basal DA efflux relative to the aCSF control session in the first 10 min ($t_7 = 3.00$, $P = 0.02$) and at 20 min ($t_7 = 4.04$, $P = 0.005$). Perfusion of 250 μ M amphetamine produced even higher overall mean levels of DA efflux relative to those seen in the control session, with a trend at 10 min ($t_7 = 2.16$, $P = 0.068$) and a significant difference at 20 min ($t_7 = 2.80$, $P = 0.027$). Local perfusion of amphetamine (100 vs 250 μ M) into the NAC did not reveal dose-related differences in DA efflux at 10 min ($t_7 = 1.66$, $P = 0.14$) or 20 min ($t_7 = 1.97$, $P = 0.09$). Local perfusion of the lower dose of amphetamine (10 μ M) produced a smaller, but marked, increase in basal accumbens DA efflux (Fig. 5, bottom panel). The ANOVA for animals tested in the low dose condition, relative to the control session, revealed main effects of

Drug ($F_{1,6} = 21.98$, $P = 0.003$), Time ($F_{2,12} = 18.42$, $P < 0.001$) and a significant interaction ($F_{2,12} = 18.68$, $P < 0.001$). Pairwise comparisons revealed that DA efflux during the amphetamine session was higher than during the aCSF control session at both 10 min ($t_6 = 3.50$, $P = 0.013$) and 20 min ($t_6 = 5.11$, $P = 0.002$).

During the final 30 min of the session, following the darkness/cereal stimulus, DA efflux was higher during the two sessions in which animals were given intra-accumbens amphetamine (100 or 250 μ M) than during the aCSF control session (Drug: $F_{2,14} = 6.93$, $P = 0.008$). These increases remained stable across the remainder of the dialysis session (Time: $F_{2,14} = 1.42$, $P = 0.275$). There was also a significant interaction between Drug and Time ($F_{4,28} = 2.84$, $P = 0.043$). Perfusions of 100 μ M intra-accumbens amphetamine increased DA efflux to levels around 2000% above aCSF session levels, and this increase was significantly higher than the aCSF control session at 30 min ($t_7 = 4.12$, $P = 0.004$), 40 min ($t_7 = 3.89$, $P = 0.006$) and 50 min ($t_7 = 4.54$, $P = 0.003$). The higher dose of amphetamine (250 μ M) resulted in increases (around 5000% above baseline levels) that were significantly higher than levels during the aCSF control session during all three time-points: 30 min ($t_7 = 2.87$, $P = 0.024$), 40 min ($t_7 = 2.68$, $P = 0.031$) and 50 min ($t_7 = 2.90$, $P = 0.023$). Finally, while the mean DA efflux between the two doses of amphetamine strongly suggests dose-related differences, the variability following 250 μ M precluded significant comparisons at each of the time-points (all $P > 0.05$).

Accumbens DA efflux during the 10 μ M amphetamine session also remained elevated relative to the aCSF control session following the darkness/cereal stimulus (Drug: $F_{1,6} = 139.74$, $P < 0.001$). However, there was no effect of Time ($F_{2,12} = 2.13$, $P = 0.161$) nor a Drug \times Time interaction ($F_{2,12} = 1.86$, $P = 0.198$). Pairwise comparisons revealed that amphetamine elevated DA efflux at 30 min ($t_6 = 7.60$, $P < 0.001$), 40 min ($t_6 = 10.21$, $P < 0.001$) and 50 min ($t_6 = 12.81$, $P < 0.004$).

The effects of amphetamine on motoric activity

The darkness/cereal stimulus produced an increase in motoric activity that corresponded temporally to the increase in cortical ACh efflux (see Fig. 6). During the aCSF vehicle session, the darkness/cereal stimulus (25 min) led to an increase in grooming, gnawing behavior and locomotion compared to the pre-stimulus baseline activity (20 min, $z = -2.53$, $P = 0.011$). Inspection of Fig. 6 also reveals that the behavioral ratings during sessions in which animals received local NAC perfusions of amphetamine (100 or 250 μ M) were nearly identical to the ratings seen during aCSF control sessions. The 250- μ M intra-accumbens amphetamine session did not result in increases in motor ratings relative to the aCSF control session at 30 min ($z = -1.41$, $P = 0.157$). Systemic administration of amphetamine (2.0 mg/kg, i.p.), at the 25-min interval, resulted in stereotypic explorations of the test bowl as well as occasional bouts of oral stereotypies. This level of activation was markedly different from the motoric activation seen at 25 min during the aCSF control session ($z = -2.55$, $P = 0.011$). Systemic amphetamine also produced significantly more activity than that observed during the 250 μ M

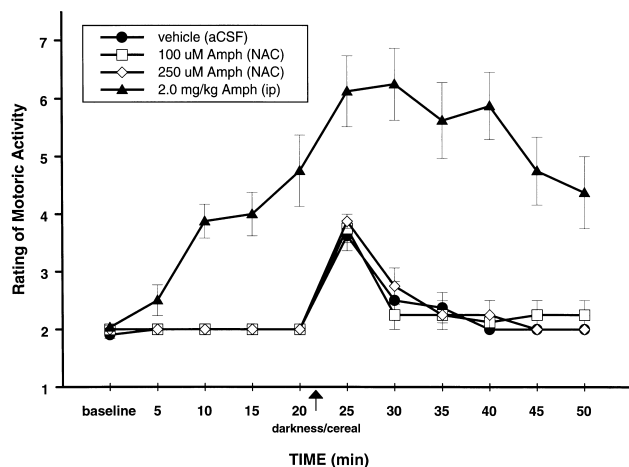


Fig. 6. Mean (\pm S.E.M.) ratings of motoric activity (see Experimental Procedures for description of scale) in rats during control sessions (aCSF) and following systemic injections or local (NAC) perfusions of amphetamine. All animals ($n=6$) received each of these manipulations in counterbalanced order. The injection or local perfusion of amphetamine began immediately after the last baseline and continued throughout the session. Only the systemic administration of amphetamine (2.0 mg/kg, i.p.) increased basal (i.e. pre-darkness/cereal stimulus) levels of motoric activity. The onset of the darkness/cereal stimulus produced comparable, transient increases in activity during the aCSF control session and during local (NAC) perfusions of amphetamine. Motor activity following systemic amphetamine remained elevated and significantly greater than that seen during the other test days throughout the extent of the dialysis session.

intra-accumbens amphetamine session at this time-point ($z = -2.26$, $P = 0.024$).

Basal levels of cortical acetylcholine in Experiment 2

Mean (\pm S.E.M.) basal efflux (pmol/10 μ l) of cortical ACh did not differ between sessions for animals that received one session of aCSF/systemic amphetamine (0.05 ± 0.01) and the session in which the animals received SCH 23390/systemic amphetamine (0.07 ± 0.01 ; $t_5 = 0.85$, $P = 0.434$). Likewise, there were no differences between mean basal ACh efflux for animals that experienced one session of aCSF/systemic amphetamine (0.07 ± 0.02) and sulpiride/systemic amphetamine (0.11 ± 0.06) ($t_5 = 0.73$, $P = 0.499$) in the other session. Finally, there were no differences in mean basal efflux between sessions for animals given one session of aCSF/systemic amphetamine (0.07 ± 0.02) and the mixed antagonists/systemic amphetamine (0.06 ± 0.03) ($t_3 = 0.296$, $P = 0.786$) in the other session. As in Experiment 1, the absence of significant session effects on basal efflux allow for the subsequent expression and analysis of drug effects as a percentage change from baseline.

Intra-accumbens perfusion of SCH 23390 does not affect cortical acetylcholine efflux

Basal efflux of cortical ACh was not changed by the intra-accumbens perfusion of 100 μ M SCH 23390 (see Fig. 7). An ANOVA comparing the aCSF/amphetamine session to the SCH 23390/amphetamine session over the last baseline and first 30 min of SCH 23390 delivery did not yield any significant effects (Drug: $F_{1,5} = 0.30$, $P = 0.607$; Time: $F_{2,10} = 0.40$, $P = 0.681$; Drug \times Time: $F_{2,10} = 0.94$, $P = 0.423$).

Systemic administration of amphetamine induced a marked increase in cortical ACh efflux relative to the last baseline

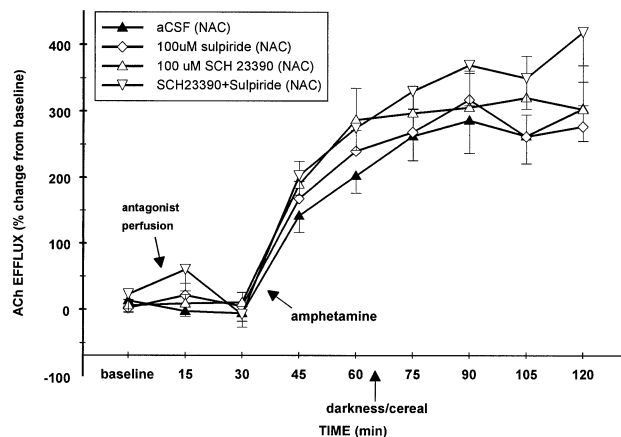


Fig. 7. The effects of systemic amphetamine and local perfusion of dopamine antagonists on mean (\pm S.E.M.) cortical ACh efflux in the mPFC under basal and stimulated conditions. The first group of animals ($n=6$) received intra-accumbens perfusions of aCSF (control condition) and the D_1 antagonist SCH 23390 (100 μ M). The second group of animals ($n=6$) received aCSF and the D_2 antagonist sulpiride (100 μ M). The final group of animals ($n=4$) received aCSF and both antagonists (100 μ M) simultaneously. Drug treatment in each group was counterbalanced between the aCSF and antagonist dialysis sessions. For the purposes of visual presentation, data from the aCSF control session were combined in the three groups of animals ($n=16$). As seen in previous figures, systemic injection of amphetamine (2.0 mg/kg, i.p.) markedly stimulated cortical ACh efflux both before and after the darkness/cereal stimulus. Local NAC perfusion of the D_1 , D_2 or D_1/D_2 antagonists had no effect on basal ACh efflux (i.e. prior to darkness/cereal) and did not attenuate the ability of systemic amphetamine to stimulate cortical ACh efflux.

(Time: $F_{8,40} = 43.95$, $P < 0.001$). The magnitude of the increase (pooled between the three aCSF groups for illustration only) was similar to that observed in Experiment 1 (Fig. 3). Intra-accumbens perfusion of 100 μ M SCH 23390 had no effect on amphetamine-induced increases of cortical ACh (Drug: $F_{1,5} = 0.09$, $P = 0.773$), nor was there a significant Drug \times Time interaction ($F_{8,40} = 0.27$, $P = 0.972$). The motoric ratings were quite similar during the two Drug sessions (data not shown). The Wilcoxon signed ranks test at 75 min (first collection following darkness/cereal) did not reveal any difference in activity between the vehicle/amphetamine session compared to the SCH 23390/amphetamine session ($z = 1.28$, $P = 0.202$).

Intra-accumbens perfusion of sulpiride does not affect cortical acetylcholine efflux

Basal efflux of cortical ACh was not changed by the intra-accumbens perfusion of 100 μ M sulpiride (see Fig. 7). An ANOVA comparing the aCSF/amphetamine session to the sulpiride/amphetamine session over the last baseline and first 30 min of sulpiride perfusion did not yield any significant effects (Drug: $F_{1,5} = 0.30$, $P = 0.608$; Time: $F_{2,10} = 1.52$, $P = 0.264$; Drug \times Time: $F_{2,10} = 1.40$, $P = 0.292$).

Again, systemic administration of amphetamine (2.0 mg/kg, i.p.) potently increased cortical ACh efflux (Time: $F_{8,40} = 14.51$, $P < 0.001$). Intra-accumbens perfusion of the D_2 antagonist sulpiride did not attenuate the effect of amphetamine on cortical ACh efflux (Drug: $F_{1,5} = 0.007$, $P = 0.938$), nor was the interaction significant ($F_{8,40} = 0.47$, $P = 0.873$). As was seen following SCH 23390, the motoric activation following systemic amphetamine was unaltered by administration of sulpiride into the NAC (data not shown). The

Wilcoxon signed ranks test at 75 min did not reveal any difference in activity between these two sessions ($z = 0.69$, $P = 0.492$).

Intra-accumbens co-perfusion of SCH 23390 and sulpiride does not affect cortical acetylcholine efflux

Basal efflux of cortical ACh was not systematically changed by the intra-accumbens perfusion of the mixed antagonists (also in Fig. 7). An ANOVA comparing the aCSF/amphetamine session to the D₁/D₂ antagonist/amphetamine session over the last baseline and first 30 min of sulpiride perfusion did not yield any main effects (Drug: $F_{1,3} = 1.06$, $P = 0.379$; Time: $F_{2,6} = 0.514$, $P = 0.622$). There was a significant Drug \times Time interaction ($F_{2,6} = 10.501$, $P = 0.011$), where ACh efflux increased briefly during the first 15 min of combined drug delivery (up to 60% above baseline) while decreasing during the same time period during the session in which only vehicle was delivered (-40% below baseline levels); however, this was a transient effect as ACh efflux returned to baseline during both sessions during the second 15-min collection.

As for each of the two antagonists alone, although systemic administration of amphetamine increased cortical ACh efflux (Time: $F_{8,24} = 91.37$, $P < 0.0001$), intra-accumbens perfusion of the mixed antagonists failed to attenuate this effect (Drug: $F_{1,3} = 0.717$, $P = 0.459$; Drug \times Time: $F_{8,24} = 1.728$, $P = 0.143$). Like the single antagonists, the motoric activation following systemic amphetamine was not influenced by administration of the mixed D₁/D₂ antagonists into the NAC (data not shown). The Wilcoxon signed ranks test at 75 min did not reveal any difference in activity between these two sessions ($z = 0.736$, $P = 0.461$).

DISCUSSION

The results of these experiments extend our analysis of the modulation of cortical ACh release. Consistent with our previous studies, we have shown that exposure to the darkness/cereal stimulus transiently increases cortical ACh efflux.^{14,15,32} Moreover, the present data confirm that systemic injections of amphetamine produce large and long-lasting increases in cortical ACh efflux,^{10,11} as well as increases in DA efflux within the NAC.^{1,9} The current experiments also produced two unanticipated results. First, the ability of systemic amphetamine to stimulate cortical ACh efflux could not be reproduced with perfusions of amphetamine directly into the NAC, despite the drug's ability to markedly stimulate NAC DA efflux. Thus, a large increase in NAC DA efflux is not a sufficient condition for increasing basal ACh efflux or potentiating stimulated ACh efflux. Second, the stimulated ACh release following systemic amphetamine was unaffected by local perfusions of D₁ or D₂ antagonists into the NAC. Thus, stimulation of D₁ and/or D₂ receptors in the NAC is not necessary for the effects of systemic amphetamine on cortical ACh efflux.

The discussion below focuses on two issues related to the interpretation of these results: (i) the differential effects of systemic and intra-accumbens amphetamine on cortical ACh release; and (ii) the psychobiological issues critical for revealing the potential dopaminergic modulation of NAC–basal forebrain–mPFC circuits.

The differential effects of systemic injections and intra-accumbens perfusions of amphetamine on cortical acetylcholine release

Systemically administered amphetamine produced large and long-lasting increases in cortical ACh efflux, whereas several concentrations of amphetamine perfused directly into the NAC did not. The inability of locally applied amphetamine to stimulate cortical ACh efflux was not due to an insufficient increase in NAC DA efflux. On the contrary, intra-accumbens perfusions of 100 or 250 μ M amphetamine resulted in enormous increases in NAC DA (far greater than those seen following systemic amphetamine), yet had no significant effect on ACh efflux. One might argue that these increases in NAC DA were too large and, via oppositional effects on multiple DA receptor subtypes, somehow precluded the reproduction of the ACh-stimulating effects of systemic amphetamine. In this regard, different concentrations of amphetamine, perfused into the NAC, are associated with bi-directional effects on local ACh efflux in the NAC.²⁴ However, following perfusion of a lower dose of amphetamine (10 μ M), which approached the increases in NAC DA seen following systemic administration, a small but significant decrease in ACh efflux was observed. Moreover, we have recently determined that local perfusions of an even smaller dose of amphetamine (0.25 μ M) increase NAC DA to an extent similar to that seen following systemic injections, yet still do not stimulate cortical ACh efflux (Arnold H. M. *et al.*, unpublished observations). Thus, the mechanisms underlying the differential effectiveness of systemic and intra-accumbens amphetamine to stimulate cortical ACh efflux lie beyond a simple consideration of the magnitude of increases in NAC DA efflux.

The inability of local perfusions of the D₁ antagonist SCH 23390 or the D₂ antagonist sulpiride to attenuate the effects of systemic amphetamine on cortical ACh efflux strongly suggests that increases in NAC DA efflux are not even necessary for the drug's ability to stimulate ACh release. It is unlikely that the inability of the antagonists to reduce the effects of amphetamine reflected an insufficient dose (100 μ M), as this same dose of these two drugs blocked local amphetamine-induced changes in glutamate²² or ACh²⁴ efflux within the NAC. In addition, the speculation that amphetamine-induced increases in D₁ or D₂ receptor activity are sufficient to stimulate cortical ACh efflux is not supported by the current data, as the co-perfusion of D₁ and D₂ antagonists also does not attenuate amphetamine-induced increases in cortical ACh release.

It should be noted that the previous links between amphetamine-induced increases in NAC DA and stimulation of cortical ACh release have been indirect. First, the sufficiency of transmitter changes within a potentially important non-accumbens region, the frontal cortex, was dismissed because local perfusion of amphetamine did not reproduce the stimulating effects of systemically administered amphetamine¹⁰ (although only a single dose, 10 μ M, was tested). Second, 6-hydroxydopamine-induced depletions of forebrain DA led to a reduction in stimulated ACh following systemic amphetamine.¹¹ However, these near-total depletions in NAC DA produced only a 50% reduction in amphetamine-stimulated ACh efflux. Moreover, given the site of 6-hydroxydopamine injection (medial forebrain bundle), these DA-depleting lesions were not restricted to the NAC, but probably extended

throughout the telencephalon. Finally, data from our own laboratory have demonstrated that increases in cortical ACh efflux stimulated by systemic administration of the benzodiazepine partial inverse agonist FG 7142 were completely blocked by intra-accumbens perfusions of the D₂ antagonist sulpiride and attenuated by the D₁ antagonist SCH 23390.³⁰ However, while systemically administered FG 7142 and amphetamine both increase DA efflux in the NAC,^{9,27} they also have a variety of different effects throughout the accumbens–basal forebrain–cortical network, limiting speculation about common mechanisms between amphetamine and FG 7142 (see the discussion in Ref. 30).

The manipulations of DA receptor activity by administration of intra-accumbens amphetamine or DA antagonists in the current paper were all limited to manipulations of the NAC shell territory, leaving open the possibility that DA receptor activity in the core of the NAC could prove to be sufficient, necessary, or both, for amphetamine-induced changes in cortical ACh efflux. However, though possible, this interpretation is rather unlikely given that it is the shell territory of the NAC that primarily projects to the area of the basal forebrain where the corticopetal neurons are located.^{50,51} In addition, the core more directly targets the substantia nigra reticulata and is less likely to influence directly the corticopetal cholinergic neurons.^{50,51} Thus, while it is not possible to entirely rule out a contribution of the NAC core, the anatomical evidence suggests that this territory of the NAC is less likely to be involved in modulating changes in cortical ACh release than the shell.

These data suggest that there is no single site that is either necessary or sufficient for the effects of systemic amphetamine on cortical ACh release. Rather, this stimulation reflects the synchronous effects of amphetamine on DA release (as well as other transmitter systems) throughout a distributed multisynaptic neuronal system that includes (at least) the NAC, ventral tegmentum, prefrontal cortex, amygdala, hippocampus and basal forebrain. For example, neuronal activation of the amygdala has been shown in response to systemic methamphetamine,⁴⁶ and a large number of neurons from the basolateral amygdaloid nucleus terminate in the prefrontal cortex.⁴¹ Dopamine levels in the prefrontal cortex are also known to increase in response to amphetamine,²⁶ as are norepinephrine (NE) levels in the prefrontal cortex and hippocampus.¹⁷ Although it is important to recognize the potential contributions of NE⁹ and serotonin¹⁸ to the effects of systemic amphetamine, Day and Fibiger¹⁰ reported that the effects of systemic amphetamine were completely attenuated with systemic administration of D₁ antagonists and markedly decreased with D₂ antagonists. Moreover, 6-hydroxydopamine-induced depletions of telencephalic NE did not affect amphetamine-induced cortical ACh efflux.¹¹ Thus, it may be that the multisynaptic changes underlying systemic amphetamine-induced increases in ACh efflux render attempts to identify a single locus of sufficient or necessary changes unproductive.

A recent study by Darracq *et al.*⁹ illustrates the importance of the multisynaptic mediation of another effect of systemic amphetamine, increases in locomotor activity. This hyperactivity was blocked by systemic injection or intra-cortical perfusion of the α_1 NE antagonist prazosin. However, prazosin did not attenuate the increases in NAC DA efflux seen following systemic or intra-accumbens amphetamine administration. They postulate that systemic amphetamine increases

NE in the cortex, which then, via α_1 receptors, increases excitatory cortical efferents and ultimately increases DA release from a functional pool of subcortical DA. Like the present data, no locomotion occurred following the local perfusion of low doses of amphetamine (3 μ M) directly into the NAC, despite large (500%) increases in NAC DA.

At a more complex behavioral level of analysis, evidence for differential effects following systemic and intra-accumbens amphetamine administration has been reported. In a study on the effects of amphetamine on latent inhibition, Killcross and Robbins²⁵ observed that, while low systemic doses of amphetamine attenuated latent inhibition, micro-injections of amphetamine directly into the NAC had no effect on latent inhibition (cf. Ref. 44). Similarly, while systemic injections of amphetamine decreased response latency and increased impulsivity on a five-choice reaction time task, intra-accumbens amphetamine failed to influence the latency of the response.⁸ Thus, the effects of amphetamine on cognitive processes also reflect its complex interactions within a more distributed neuronal system and may not be attributed solely to increases in DA efflux in the NAC.

Toward a more valid assessment of the modulatory role of dopamine within the accumbens–basal forebrain–cortical circuit

It would be premature to conclude that dopaminergic transmission within the NAC does not modulate the activity of basal forebrain cholinergic neurons on the basis that intra-accumbens perfusion of amphetamine did not affect cortical ACh efflux. The current model of accumbens–basal forebrain interactions suggests that DA could potentiate stimulated cortical ACh release via a negative modulation of GABAergic inhibition of basal forebrain neurons.^{4,16,48} The validity of this prediction hinges upon at least two interacting conditions: (i) the activity of NAC and basal forebrain telencephalic afferents; and (ii) the extent of DA receptor activation. While the evidence suggests that mesoaccumbens DA modulates excitatory inputs on medium spiny projection neurons, this dopaminergic modulation is seen only when medium spiny efferents are highly activated.^{34,35} Thus, an optimal scenario for revealing a mesoaccumbens dopaminergic modulation of cortical cholinergic transmission is when excitatory inputs appear coincidentally in the accumbens, ventral tegmentum and basal forebrain. The effects of FG 7142 in Moore *et al.*³⁰ may have sufficed to establish such a situation, therefore allowing intra-accumbens injections of sulpiride to block increases in cortical ACh release.

It should be noted that the darkness/cereal stimulus did not result in an increase in DA efflux in the NAC. This lack of effects corresponds with the hypothesis that the darkness/cereal stimulus did not sufficiently activate DA neurons projecting to the NAC, as it was not designed to act as a conditioned stimulus that gained incentive salience as described by Berridge and Robinson.³ The absence of an increase in NAC DA efflux following darkness/cereal may also reflect the consequences of the repeated exposure to the stimulus. For example, Bassareo and Di Chiara² have reported that previous experience with a salient food stimulus blunted the increase in NAC DA efflux seen following the initial exposure to the stimulus. Moreover, there was no increase in NAC DA efflux in response to an appetitively conditioned stimulus paired with the food stimulus. Consistent

with this view are electrophysiological studies^{28,42} which suggest that DA neurons in the ventral tegmental area increase firing to rewarding food stimuli early in training but do not respond later in training, after an association with a cue predictive of the food stimulus has been well established. Although an increase in NAC DA efflux in response to the darkness/cereal stimulus is not necessary for increases in cortical ACh efflux, to understand under what conditions DA does modulate the activity of the basal forebrain cholinergic system a fuller understanding of the role of DA in the NAC under activated and non-activated conditions will be necessary.

CONCLUSION

Large increases in accumbens DA receptor activity are

neither sufficient nor necessary for the systemic amphetamine-induced stimulation of cortical ACh release, suggesting that this stimulation is based on more complex neuronal mechanisms than previously believed. In addition, future studies, utilizing pharmacological and/or behavioral stimuli, which simultaneously activate several telencephalic projections to the NAC that interact with converging dopaminergic inputs, will be necessary to reveal the modulatory transmitter interactions present in accumbens–basal forebrain–cortical circuits.

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