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Effects of Short-Term Abstinence from Escalating Doses of D-Amphetamine on Drug and Sucrose-Evoked Dopamine Efflux in the Rat Nucleus Accumbens

Giada Vacca[1], Soyon Ahn[1] and Anthony G Phillips*[,1]

[1]Department of Psychiatry, Institute of Mental Health, University of British Columbia, Vancouver, BC, Canada

Abstinence from high doses of psychostimulant drugs, in both humans and rodents, is linked to adverse psychological effects including anhedonia, a core symptom of major depression, manifested behaviorally as decreased responding for rewarding stimuli. The present study used brain microdialysis in freely moving rats to examine the effect of D-amphetamine (D-amph) withdrawal on changes in extracellular dopamine (DA) levels in the nucleus accumbens (NAc) evoked by D-amph or behavior related to sucrose consumption. D-amph was administered intraperitoneally (i.p.) according to an escalating dose (ED) schedule (from 1 to 10 mg/kg, 3 doses/day). We first confirmed the development of tolerance by monitoring DA efflux in the NAc in response to 5 and 10 mg/kg doses of D-amph administered during the ED schedule of drug administration and again in response to the 5 mg/kg dose of D-amph 72 h following the last 10 mg/kg D-amph injection. In a separate study, DA efflux in the NAc was first shown to be increased significantly during both preparatory and consummatory phases of responding for a 4% sucrose solution. Withdrawal from the ED schedule of D-amph caused a selective attenuation of DA efflux only during the preparatory phase of the sucrose test. These results provided convincing evidence of neurochemical adaptation within the mesocorticolimbic DA pathway during and following the administration of an ED schedule of D-amph as well as suppressed neurochemical responses to a psychostimulant drug and cues associated with a natural reward after withdrawal from drug treatment. Accordingly, these findings support the hypothesis that downregulation of mesocorticolimbic DA function maintained during D-amph withdrawal may account for the selective disruption of motivated behavior reported in studies employing psychostimulant drug withdrawal as a model of depression in rodents.
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INTRODUCTION

Several studies have shown that short-term abstinence from sustained exposure to high doses of psychostimulant drugs such as cocaine or D-amphetamine (D-amph) may precipitate psychological (Gawin and Kleber, 1986; Weddington *et al*, 1990; Kampman *et al*, 1998) and somatic (Srisurapanont *et al*, 1999) effects that share many of the features of major depressive disorder (MDD) in humans. Preclinical studies of the effects of cessation of repeated administration of psychostimulants in rodents have demonstrated many behavioral changes that conform to key symptoms of MDD in humans, including the core symptom of anhedonia (see Barr *et al*, 2002, for a review). Anhedonia may be defined as 'a markedly diminished interest or pleasure in all or most activities' (Gardner, 2000) and has been modeled in experimental animals as a failure to maintain responding for natural or artificial reward stimuli (Wise, 1978).

Rats recently withdrawn from chronic treatment with psychostimulant drugs display decreased responding for rewarding electrical brain stimulation reward, which is attributed to diminished activity in the neural substrates of reward (Kokkinidis *et al*, 1980; Markou and Koob, 1991; Lin *et al*, 1999). Recent findings from our laboratory have shown that during withdrawal from an escalating dose (ED) schedule of D-amph administration (1–10 mg/kg), rats also exhibit reduced motivation to obtain natural reinforcers, including a sucrose solution (Barr and Phillips, 1999) or a sexually receptive conspecific (Barr *et al*, 1999), for up to 5 days following the termination of drug treatment. Furthermore, rats withdrawn from the same regimen of D-amph injections showed increased successive negative contrast (Barr and Phillips, 2002), and failed to display successive

*Correspondence: Dr AG Phillips, Department of Psychiatry, Institute of Mental Health, University of British Columbia, 2255 Wesbrook Mall, Vancouver, BC, Canada V6T 2A1, Tel: + 1 604 822 4624, Fax: + 1 604 822 7756, E-mail: aphillips@psych.ubc.ca
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positive contrast (Vacca and Phillips, 2005). These results provide compelling evidence that, in addition to deceased motivation to respond for artificial and natural reward stimuli, animals withdrawn from an ED schedule of psychostimulant drug treatment also have a generalized incapacity to respond to unexpected gains or reductions in reward value.

A major brain reward pathway arises from dopaminergic neurons in the ventral tegmental area of the midbrain and projects mainly to the nucleus accumbens (NAc) and medial prefrontal cortex (Mogenson and Phillips, 1978; Phillips and Fibiger, 1978). This mesocorticolimbic dopamine (DA) pathway plays a critical role in many aspects of reward, including motivational states triggered by natural incentive stimuli (eg, food, sex, and social interaction) or pharmacological stimuli that can maintain drug self-administration (Everitt *et al*, 1999; Koob *et al*, 1998; Wise, 1998). *In vivo* microdialysis studies in rats report increased DA efflux in the NAc during preparatory and consummatory phases of feeding and sexual behaviors (Phillips *et al*, 1993; Wilson *et al*, 1995; Fiorino *et al*, 1997; Ahn and Phillips, 1999). Furthermore, virtually all drugs of abuse increase DA transmission in the NAc, which partly mediates their rewarding effects (Koob *et al*, 1998; Wise, 1998). Therefore, it is possible that some of the depressive-like symptoms observed during psychostimulant withdrawal in rodents, including anhedonia and decreased motivation, may result from reduced activity in the mesocorticolimbic DA system in the form of decreased efflux of DA in the NAc.

The hypothesis that DA efflux in the NAc, evoked by pharmacological stimuli and natural rewards, is attenuated following withdrawal from an ED schedule of D-amph was tested using *in vivo* microdialysis coupled with high-performance liquid chromatography with electrochemical detection (HPLC-EC). In the first experiment, rats were challenged with a 5-mg/kg injection of D-amph 72-h after the ED treatment. The development of tolerance to the indirect DA agonist property of D-amph (5 and 10 mg/kg) was also monitored during the ED schedule of D-amph injections. A second experiment examined the effect of withdrawal from D-amph on DA efflux in the NAc associated with sucrose reward, during preparatory, consummatory, and post-consummatory phases of responding for this natural reward.

MATERIALS AND METHODS

Subjects

Male Long–Evans rats (Charles River, Quebec, Canada), weighing 250–300 g, were pair-housed for a minimum of 1 week before surgery and then housed individually following surgery in a temperature-controlled colony (21°C) with reverse light/dark cycle conditions (lights on 0400–1600). Food and water were available *ad libitum*, unless otherwise indicated. All training and testing sessions were conducted during the dark cycle. All experimental procedures were performed in accordance with the standards of the Canadian Council on Animal Care and approved by the Committee on Animal Care, University of British Columbia.

Surgery

All rats were anesthetized with intraperitoneal (i.p.) injections of ketamine hydrochloride (100 mg/kg) and xylazine (7 mg/kg) and implanted bilaterally with stainless-steel guide cannulae (19 G, 15 mm length) positioned 1 mm below dura, directly over the NAc (+ 1.7 mm AP and $\pm$1.1 mm ML from bregma). Coordinates were determined according to Paxinos and Watson brain atlas (1997). Animals were allowed to recover for 1 week before being assigned to *Experiment 1* or *Experiment 2*.

ED Protocol of D-Amph Administration

Different doses of D-amph sulfate (Sigma, St Louis, MO) were prepared daily by dissolving the appropriate amount of drug in 1 ml isotonic saline (0.9% NaCl). The injection volume for each rat was adjusted on a daily basis according to the rat's weight. In all experiments, D-amph was administered (i.p.) using an ED protocol shown to minimize the chance of acute toxicity associated with high doses of D-amph (Ryan *et al*, 1990). Rats received injections at 0800, 1500, and 2200 for 5 consecutive days. On Day 1, rats received an initial dose of 1 mg/kg and on Days 2–4, nine subsequent doses that each increased by 1 mg/kg (last dose 10 mg/kg). On Day 5, rats received two additional doses of 10 mg/kg.

Microdialysis Procedure and HPLC

Microdialysis probes were concentric in design with silica inlet–outlet lines. The active surface consisted of a semipermeable membrane 2 mm in length (340 μm outer diameter; 65 000 molecular weight cutoff; Filtral 12; Hospal, Neurnberg, Germany). Typical *in vitro* probe recoveries of DA conducted at room temperature were 18$\pm$1% of a standard DA solution. Before implantation, probes were connected to an Instech dual-channel liquid swivel (Plymouth Meeting, PA) and continuously perfused at 1 μl/min with artificial cerebrospinal fluid (aCSF). The aCSF consisted of a 10.0 mM sodium phosphate buffer with 147.0 mM NaCl, 3.0 mM KCl, 1.0 mM $MgCl_2$, and 1.2 mM $CaCl_2$ (pH 7.4). Probes were implanted in the NAc via the guide cannulae, with the dialyzing membrane extending from −5.8 to −7.8 mm DV from dura, and perfused overnight ($\sim$14–16 h) at 1 μl/min with aCSF.

DA content in NAc dialysates was analyzed using HPLC-EC. The HPLC system consisted of an ESA 582 pump (ESA Inc., Bedford, MA), a pulse damper (Scientific Systems Inc., State College, PA), a Rheodyne Inert manual injector (model 9125i, 20 μl injection loop; Rohnert Park, CA), a Tosoh Bioscience Super ODS TSK column (2 μm particle, 2 × 10 mm; Montgomeryville, PA) and an Antec Leyden Intro Electrochemical detector and VT-03 flow cell with a Ag/AgCl reference electrode ($V_{applied}$ = + 700 mV; Leiden, The Netherlands). The mobile phase (70 mM sodium acetate, 40 mg/l EDTA and 50 mg/l of sodium octyl sulfate (adjustable), pH 4.0, 12% methanol) flowed through the system at 0.18 ml/min. EZChrome Elite software (Scientific Software, Pleasanton, CA) was used to acquire and analyze chromatographic data.

Experiment 1: Changes in DA Efflux during and Following an ED Schedule of D-Amph Injections

One week following the implantation of bilateral NAc guide cannulae, one group of rats received ED of D-amph (ED-treated group) as described above (see Table 1). A separate group of rats was treated with isotonic saline (saline-treated group) under the same schedule as rats in the ED-treated group. The ED-treated group was tested with microdialysis in two of the following D-amph injection conditions: during (i) the fifth injection (5 mg/kg), (ii) the 12th injection (10 mg/kg), and (iii) the challenge injection (5 mg/kg) 72 h following the completion of the ED schedule of D-amph treatment. The saline-treated group was tested in two of the following saline injection conditions: (i) the first injection (acute saline group), (ii) the fifth injection, (iii) the 12th injection, and (iv) challenge injection 72 h following the final scheduled saline injection. Each rat was implanted with a probe in one NAc (either the right or left hemisphere) for the first microdialysis test, and then the contralateral NAc for the second test.

Two separate groups of rats, without prior treatment history, received a single injection of either 5 or 10 mg/kg D-amph (i.p.) during a microdialysis experiment (acute groups). Acute groups received only one microdialysis session.

On each test day, samples were collected at 30-min intervals and analyzed immediately for DA using HPLC. When three consecutive baseline samples showed less than 10% fluctuation, rats were administered the appropriate injection (D-amph or saline) and dialysates were collected for a further 240 min.

Experiment 2: Effect of D-Amph Withdrawal on Sucrose-Evoked DA Efflux

Rats in *Experiment 2* were divided into two groups: the ED-treated group received ED of D-amph as described in Table 2. The control group received matched saline injections in accordance with the timing of the ED schedule (saline-treated group). All rats were trained to drink a 4% sucrose solution during a single 20-min period in the testing chamber. A removable Plexiglas screen perforated with holes (1 cm diameter) divided each chamber into a smaller ($16 \times 38 \times 38$ cm) and a larger compartment ($25 \times 38 \times 38$ cm). Rats were food deprived for 12 h before each training session. All the training sessions occurred between 0900 and 1000. Each day, 4% (w/v) sucrose solutions were freshly prepared and made available via a drinking spout protruding into the smaller compartment of the cage. Each training session consisted of two phases. During the preparatory phase, rats were confined to the larger compartment by the screen for 10 min and could not make contact with the sucrose. The consummatory phase began with the removal of the screen, giving access to the sucrose solution for 20 min. Removal of the drinking spout marked the beginning of a 30-min post-consummatory phase.

Once latencies to initiate sucrose intake following removal of the screen had stabilized (in most cases by Day 3), the next day, a sucrose drinking test was conducted in conjunction with concurrent microdialysis sampling from the NAc (see Table 2). Samples were collected every 5 min and immediately analyzed for DA by HPLC. When three consecutive baseline samples showed less than 5% fluctuation, the spout containing sucrose was inserted through the back wall of the smaller compartment, initiating the preparatory phase. The perforated screen was removed after 10 min and dialysates were collected for 20 min with sucrose available and for an additional 30 min following the end of the consummatory phase. On Days 5–9, one group of rats was administered D-amph, and one group saline, according to the ED protocol. On Day 12, 72 h after the last D-amph or saline injection, rats were tested with microdialysis during the preparatory, consummatory, and post-consummatory phases of sucrose consumption.

Table 1 Experiment 1 Protocol

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
	ED schedule of D-amph injections (mg/kg, i.p.)					Withdrawal		
0800		2	5 Dial 1	8	10			
1500		3	6	9	10 Dial 2	24 h	48 h	72 h Dial 3
2200	1	4	7	10				

Table 2 Experiment 2 Protocol

					Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12
	Day 1	Day 2	Day 3	Day 4	ED schedule of D-amph injections (mg/kg, i.p.)					Withdrawal		
0800	Train	Train	Train	Dial 1		3	6	9	10	24 h	48 h	72 h Dial 2
1500					1	4	7	10				
2200					2	5	8	10				

Histology

Following the last microdialysis experiment, rats were deeply anesthetized with sodium pentobarbital (100 mg/ml) and perfused intracardially with 0.9% NaCl. Brains were removed immediately and stored in 20% (w/v) sucrose and 4% paraformaldehyde solution for 1 week, sliced into 50 μm coronal sections, and then stained with cresyl violet. Placements of probes were verified according to the atlas of Paxinos and Watson (1997). Only animals with tracts in the shell/core region of the NAc were included in the statistical analysis.

Data Analyses

The mean concentrations of DA in the final three baseline samples before treatment were normalized (0%) and all data were expressed as % change from this baseline value. Statistical significance of the neurochemical data was evaluated using a one- or two-way repeated measures analysis of variance (ANOVA) with Time as the within- and Group as the between-subjects factor. This was followed, when appropriate, by the Dunn (for between-groups comparisons) or Dunnett (within-group comparisons) method of multiple comparisons. In the latter test, the baseline sample preceding experimental manipulation was used as the control value. The Huynh–Feldt correction for nonsphericity was applied to the degrees of freedom for all within-subject analyses. Analyses were performed using Systat statistical software.

RESULTS

Experiment 1: Changes in DA Efflux during and Following an ED Schedule of D-Amph Injections

As shown in Figure 1a, injection of a 5 mg/kg (i.p.) dose of D-amph resulted in a significant change in DA efflux in the NAc of rats that received D-amph for the first time (acute group, $n=5$), as well as those that received the dose as the fifth injection of the ED schedule (ED-treated group, $n=7$), but DA efflux was altered neither by an acute saline injection ($n=4$) nor by the fifth injection of saline in saline-treated rats ($n=3$) ($F_{(27,135)}=32.498$, $p<0.001$). The peak magnitude of D-amph-evoked DA efflux (+972% above baseline, observed 60-min post-injection) in the ED-treated group was significantly smaller than the maximum effect observed in the acutely treated rats (+3324% above baseline; Dunn's $p<0.05$). The D-amph-evoked increases remained significantly elevated above baseline for ~3 h in both groups (Dunnett's, $p<0.05$).

During a separate series of microdialysis sessions similar group differences were observed in the maximum increase in DA efflux evoked by a 10 mg/kg (i.p.) dose of D-amph, as either the final dose of the ED schedule ($n=6$) or as an acute dose of D-amph ($n=5$), but DA efflux was not altered by an acute injection of saline ($n=4$), or by the 12th saline injection in saline-treated rats ($n=3$) ($F_{(27,126)}=31.436$, $p<0.001$; Figure 1b). These significant increases in DA efflux in the NAc remained elevated for over 3 h (Dunnett's, $p<0.05$), and the peak increase observed 60-min post-injection in the ED-treated group (+1004% above baseline)

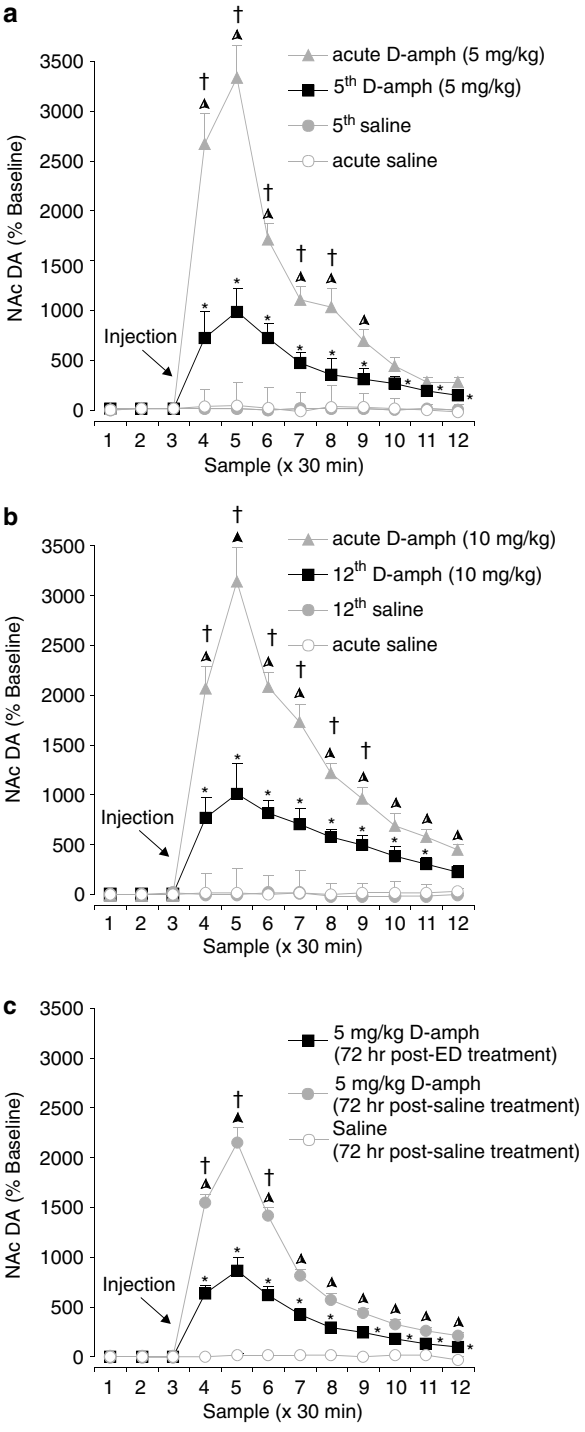

Figure 1 NAc DA efflux evoked by D-amph during or following an ED schedule of D-amph treatment. DA efflux was sampled by microdialysis and measured by HPLC-EC (mean + SEM) from rats receiving D-amph according to the ED schedule of drug treatment (black squares), ED schedule-matched saline injections (gray circles), acute D-amph injections of 5 or 10 mg/kg (gray triangles), and acute saline injections (white circles). DA efflux was monitored during the (a) fifth scheduled or acute injection of D-amph (5 mg/kg, i.p.), (b) 12th scheduled or acute injection (10 mg/kg, i.p.), or (c) a 5 mg/kg (i.p.) challenge injection or saline injection 72 h following the final ED scheduled or saline treatment. Significant difference from baseline for the *ED D-amph group or $^{\Delta}$acute group (Figure 1a and b; Dunnett's, $p<0.05$). Significant difference from baseline *saline-treated group (Figure 1c; Dunnett's, $p<0.05$). †Significant difference between groups (Dunn's, $p<0.05$).

was again significantly blunted compared to a +3132% increase in the acute group given a single injection of 10 mg/kg D-amph (Dunn's $p<0.05$).

The final series of microdialysis sessions monitored differences in basal and D-amph-(5 mg/kg)-evoked DA efflux in the NAc, 72 h following the last injection of D-amph or saline of the ED schedule (Figure 1c). In saline-treated rats, the average basal concentration of DA was 1.9 nM ± 0.21/9 µl sample (uncorrected for probe recovery). This parameter was not significantly altered by prior administration of the ED schedule to the ED-treated group (2.5 nM ± 0.23 per 9 µl sample). Injection of a 5 mg/kg (i.p.) dose of D-amph resulted in a significant change in DA efflux in both ED- ($n=7$) and saline-treated ($n=9$) groups ($F_{(18,189)}=179.727$, $p<0.001$). D-amph-induced DA efflux increased significantly above baseline in both groups (Dunnett's, $p<0.05$); however, the maximum change in DA efflux was significantly attenuated in the ED-treated group (+838%) compared to the saline-treated group (+2100%; Dunn's, $p<0.05$). Administration of a saline injection to saline-treated rats ($n=8$) failed to induce any changes in DA efflux in the NAc.

Experiment 2: Effect of Withdrawal from an ED D-Amph Schedule on Sucrose-Evoked DA Efflux

The average basal concentration of DA in the presaline and preamph groups was 1.82 nM ± 0.30/5 µl sample (uncorrected for probe recovery). This parameter was not significantly altered by the administration of either the ED schedule to the ED-treated group (2.1 ± 0.26 nM/5 µl sample) or saline to the saline-treated group (1.97 ± 0.20 nM/5 µl sample).

As shown in Figure 2a, during the initial microdialysis sessions conducted before being treated on the ED schedule of D-amph injections ($n=6$), or saline injections ($n=5$), significant changes in DA efflux in the NAc were observed during the preparatory and consummatory phases of the sucrose test ($F_{(12,108)}=4.657$, $p<0.001$). In rats assigned to receive either ED or saline treatment (preamph or presaline groups, respectively), significant increase in DA efflux in the NAc associated with the preparatory phases was only observed in the first 5 min sample, whereas the enhanced DA efflux during sucrose consumption remained elevated for 15 min in both groups (Dunnett's, $p<0.05$). The latency to approach the drinking spout was 11.05 ± 2.04 s, whereas the volume of sucrose solution consumed during the 20-min test was 7 ± 1.16 ml.

Seventy-two hours following withdrawal from treatment with the ED schedule of D-amph ($n=7$) or saline ($n=5$), significant changes in DA efflux were observed during both the preparatory and consummatory phases of the sucrose test (see Figure 2b) ($F_{(12,132)}=4.059$, $p<0.001$). As noted in Figure 2b, significant increase in DA efflux from baseline was observed during the preparatory and consummatory phases of sucrose intake (Dunnett's, $p<0.05$) in both the ED- and saline-treated rats. The latency to approach the drinking spout was 17.23 ± 2.60 s, whereas the volume of sucrose solution consumed during the 20-min test was 6.25 ± 1.52 ml.

A more detailed comparison of the magnitude of change in DA efflux during the preparatory and consummatory

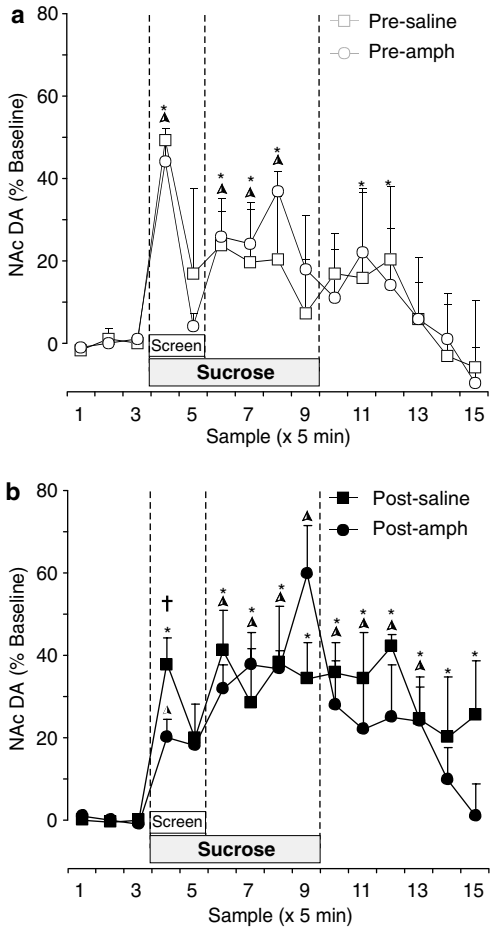

Figure 2 Changes in NAc DA efflux during a two-stage access to a 4% sucrose solution, (a) before (white circles and white squares) and (b) 72 h following the final ED-scheduled injection of D-amph (1–10 mg/kg, i.p.) (black circles) or the final saline injection (black squares). *Dashed lines* highlight the initial preparatory phase in which sucrose was presented behind a perforated screen (Samples 4 and 5) and the consummatory phase, when rats were given access to the sucrose following removal of the screen (Samples 6–9). Significant difference from baseline for $^{\Delta}$ED- rats and *saline-treated rats (Dunnett's, $p<0.05$). †Significant difference between ED- and saline-treated groups (Dunn's, $p<0.05$).

phases of the sucrose test in the ED- and saline-treated rats were conducted with separate two-way repeated measure ANOVAS. Results indicated a significant interaction of Group × Time only in the preparatory phase ($F_{(3,20)}=3.697$, $p<0.029$). *Post hoc* analysis revealed that the increase in DA efflux during the first 5 min of the preparatory phase in the D-amph-treated rats was significantly lower than the increase in DA efflux observed in the saline-treated rats (+38 *vs* +20%; Dunn's, $p<0.05$).

Histology

As illustrated in Figure 3, tracts of microdialysis probes were located close to the border of the shell/core regions of the NAc (+1.6 to 2.2 mm AP). In Experiment 1, neurochemical data from six NAc sites were excluded from statistical analyses owing to probe misplacement. In

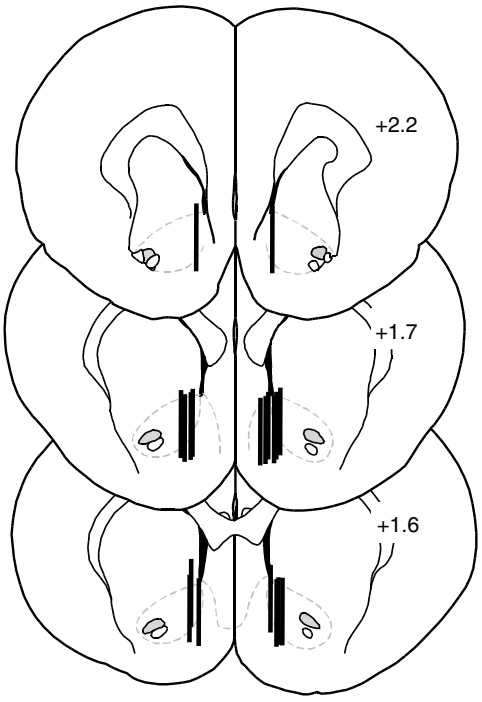

Figure 3 Placement of microdialysis probes in the NAc for those rats included in statistical analyses of neurochemical data. *Vertical lines* represent the 2 mm length of dialysis membrane. Distance from bregma is indicated (mm).

Experiment 2, data from three NAc site was excluded owing to failure of the rat to drink sucrose.

DISCUSSION

The present study provided neurochemical data that are complimentary to a growing body of behavioral studies showing marked reduction in responding for natural or artificial rewards shortly after withdrawal from an ED schedule of D-amph. Specifically, DA efflux in the NAc evoked by a pharmacological reward stimulus or cues presented during the preparatory but not consummatory phase of a sucrose test was attenuated in ED-treated rats, compared to saline-treated rats (Figure 1). This may reflect neurochemical adaptation within DA neurons of the mesocorticolimbic DA pathway, which in turn may underlie the development of tolerance to the indirect DA agonist property of D-amph. Interestingly, tolerance to the effect of D-amph on DA efflux developed rapidly, and was observed in response to fifth dose of the ED regimen (5 mg/kg, i.p.), and maintained in response to the final dose of D-amph (10 mg/kg, i.p.). Decreased extracellular levels of DA in the NAc have also been observed following withdrawal from extended treatment with cocaine (Weiss *et al*, 1992). In the present study, tolerance was maintained for 72 h following withdrawal from the drug, a time when symptoms of anhedonia and decreased motivation are observed in models of depression in rats using withdrawal from similar ED schedule of treatment with psychostimulants (Barr *et al*, 1999; Barr and Phillips, 1999, 2002). Using a chronic mild stress model of depression, Di Chiara *et al* (1999) similarly observed blunted mesolimbic DA efflux in response to appetitive rewarding stimuli. Collectively, these data are consistent with the hypothesis that synaptic transmission in the mesocorticolimbic DA system is significantly reduced following exposure to repeated treatment with high doses of psychostimulants, which in turn may account for the behavioral symptoms related to depression.

A direct comparison of the magnitude of DA efflux in the NAc evoked by a 5 mg/kg dose of D-amph in saline-treated rats 72 h after the last saline injection (see Figure 1c) was approximately $\frac{1}{3}$ less than the magnitude of DA efflux evoked by the same dose administered to drug-naïve animals that had minimal handling and no prior injection history (see Figure 1a). These data imply a possible interaction between the effects of handling and/or injection stress and the pharmacological effects of D-amph on DA efflux in the NAc. Given the possible contribution of habituation to the stress of the injection protocol to the magnitude of DA efflux evoked by D-amph 72 h after withdrawal, it is important to note that the magnitude observed in ED D-amph condition (+838%) was still significantly less than the value measured in control rats (+2100%) with a similar treatment history.

A second experiment confirmed a significant increase in DA efflux in the NAc during both the preparatory and consummatory phases of sucrose intake by drug-naïve rats (Figure 2). In control tests conducted before treatment with either saline or an ED schedule of D-amph, the maximal increase in DA efflux occurred during the first 5 min of the preparatory phase preceding sucrose drinking (preamph group +45%, presaline group +49%). A similar pattern of DA efflux also was observed previously only in the first 5 min of the preparatory phase of feeding behavior (Wilson *et al*, 1995). Together, these findings are consistent with the hypothesis that preparatory aspects of feeding behaviors are preferentially associated with changes in DA metabolism and/or release in the NAc (Blackburn *et al*, 1989). Preparatory behaviors, including searching for food and water, are flexible patterns of responding that lead to and facilitate consummatory behaviors. These latter behaviors have rigidly defined patterns of motor activity, can be described objectively, and occur after an animal has made contact with a reward stimulus such as food (Konorski, 1967; Phillips *et al*, 1993).

Following withdrawal from an ED schedule of D-amph, DA efflux was significantly attenuated only during the initial 5-min period of the preparatory phase of the sucrose test when compared to the preD-amph treatment (Figure 2c). In contrast, the increase observed during the first 5 min of the consummatory phase (+32%) of the sucrose test was comparable to values obtained before treatment with D-amph (+27%). These neurochemical data may explain our previous finding that withdrawal from an ED schedule of D-amph decreased selectively the preparatory components of sexual behavior in sexually experienced male rats (Barr *et al*, 1999). Previous *in vivo* microdialysis studies by our group have reported an immediate increase in DA levels in the NAc of sexually experienced male rats in the presence of an inaccessible receptive female (Fiorino *et al*, 1997; Fiorino and Phillips, 1999), which suggests that activity within the mesocorticolimbic DA pathway may be especially

important for the initiation of motivated behavior. Furthermore, it is this rapid rise in DA efflux in the NAc that may be especially sensitive to the disruptive effects of withdrawal from extended exposure to psychostimulant drugs.

In addition to effects on sexual behavior, withdrawal from an ED schedule of D-amph has significant effects on other sensitive tests of motivated behavior. These include a significant reduction in break points under a progressive ratio schedule for a sucrose reward (Barr and Phillips, 1999; Orsini *et al*, 2001). It must be noted however that Russig *et al* (2003), in a study that restricted the ED schedule of D-amph to a maximum dose of 5 mg/kg, failed to replicate this effect. Amphetamine withdrawal also causes an elevation in brain reward thresholds in rats as measured by intracranial self-stimulation (Geyer and Markou, 1995). Furthermore, rats treated with an ED regimen of D-amph failed to display successive positive contrast when shifted from 4 to 32% sucrose (Vacca and Phillips, 2005), a finding that parallels the enhancement of successive negative contrast observed in rats withdrawn from the same drug treatment (Barr and Phillips, 2002). Genn *et al* (2004) have recently shown that DA efflux in the NAc was significantly attenuated when rats unexpectedly experience a reward of a lesser value in a negative contrast paradigm. Together, these data indicate that short-term abstinence from psychostimulants has a significant effect on the motivation to respond for natural rewards. This in turn may represent a preclinical analogue of the anhedonic state observed in humans during short-term abstinence from repeated high doses of D-amph or cocaine.

In a recent review of the role of the brain reward system in depression, Naranjo *et al* (2001) expanding on an earlier proposal by Blackburn *et al* (1992), argue that the mesolimbic DA pathway, rather than mediating hedonic responses *per se*, serves instead to induce approach (or preparatory) behaviors essential for consumption, as well as positive reinforcement and learning. Accordingly, DA neurons may be active during the encoding and retrieval of the positive value of an object, behavioral act, or internal physical state, essential for behavioral adaptation in a complex environment. According to this perspective, anhedonia may be redefined as state in which motivation is severely compromised. On the basis of the present data, we can also add that anhedonia induced by withdrawal from psychostimulant drugs may be a direct consequence of a reduced release of DA evoked by cues associated with natural reward stimuli or drug rewards. In conclusion, this study supports the continued use of preclinical psychostimulant withdrawal models of depression and emphasizes the need for further research on the role of brain DA systems in mood disorders.

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