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Regional Differences in the Effects of Amphetamine Withdrawal on Dopamine Dynamics in the Striatum:

Analysis of Circadian Patterns Using Automated On-Line Microdialysis

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Abstract

The purpose of the study is to determine the relationship between behavioral symptoms ofamphetamine withdrawal and the extracellular concentration of dopamine (DA) in the dorsolateral caudate nucleus and the nucleus accumbens across the entire light-dark cycle. This was accomplished using automated on-line microdialysis sampling in behaving rats. Animals were pretreated with escalating doses of d-amphetamine (or saline) over a 6-week period and then were withdrawn from amphetamine for 3, 7, or 28 days before testing. There were regional differences in the effects of amphetamine withdrawal on the concentrations of DA and DA metabolites in dialysate. Early during withdrawal (3 and 7 days), when animals showed postamphetamine withdrawal behavioral depression (nocturnal hypoactivity), there was a significant decrease in DA and DA metabolites in the dorsolateral caudate nucleus and a disruption in the normal circadian pattern of DA activity. In contrast, there was no effect ofamphetamine withdrawal on DA dynamics in the nucleus accumbens. By 28 days after the discontinuation of amphetamine pretreatment, after basal DA in the caudate returned to normal, there was a significant increase in basal DA metabolism in both the caudate and the accumbens. This increase in DA metabolism may be related to the expression of sensitization, including a hypersensitivity to an amphetamine challenge. It is concluded that the role of the dorsal striatum in psychostimulant drug withdrawal syndromes deserves further consideration.

Keywords

Nucleus accumbens; Caudate nucleus; Basal ganglia; Circadian rhythms; Behavior; Locomotion; Dopamine release; Monoamines; Brain; Rat

The pronounced somatic symptoms associated with withdrawal from some addictive drugs, such as barbiturates or opiates, tend to be relatively specific to the class of drugs used (Jaffe 1990). Withdrawal from psychomotor stimulant drugs, however, is not characterized by pronounced somatic signs but primarily by changes in subjective affective and motivational states. Indeed, there may be a number of symptoms of withdrawal that are common to many drug classes, including anxiety, dysphoria, anhedonia, and drug craving. This raises the possibility that withdrawal symptoms that are common to many drugs are due to drug-induced adaptations in a common neurobiological substrate (Acquas and Di Chiara 1992;Rossetti et al. 1992b;1992c). One substrate candidate is the mesotelencephalic dopamine (DA) system because (1) many different addictive drugs increase DA neurotransmission (Wise and Bozarth 1987;Di Chiara and Imperato 1988); (2) repeated exposure to many different addictive drugs produces both transient and persistent neuroadaptations in DA systems (Kalivas and Stewart

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1991;White and Wolf 1991;Robinson and Berridge 1993); and (3) DA systems are thought to play an important role in mediating a variety of affective and motivational processes (Wise 1982;Fibiger and Phillips 1986;Robbins and Everitt 1992).

There have been a number of recent reports consistent with the hypothesis that a decrease in the synaptic concentrations of DA may contribute to some of the symptoms associated with drug withdrawal syndromes (Rossetti et al. 1992c). For example, the abrupt discontinuation of chronic treatment with ethanol (Rossetti et al. 1992a,1992b;Diana et al. 1993), morphine (Acquas et al. 1991;Pothos et al. 1991;Acquas and Di Chiara 1992;Rossetti et al. 1992c;Crippens and Robinson 1994), amphetamine (Rossetti et al. 1992c), or cocaine (Parsons et al. 1991;Robertson et al. 1991;Imperato et al. 1992;Rossetti et al. 1992c;Weiss et al. 1992) is reported to be followed by a significant decrease in the basal extracellular concentration of DA (EC DA) in the nucleus accumbens (ventral striatum), as assessed with in vivo microdialysis.[1] On the other hand, there have been a number of reports according to which psychomotor stimulant withdrawal (amphetamine or cocaine) is *not* accompanied by a decrease in EC DA in the nucleus accumbens (Segal and Kuczenski 1992a,1992b;Crippens et al. 1993; Klivas and Duffy 1993; Crippens and Robinson 1994;Hooks et al. 1994). The reasons for the apparent discrepancies are unknown, but at least in the case of amphetamine withdrawal, they do not seem to be related to the specific drug pretreatment regimen or the length of the withdrawal period (Crippens and Robinson 1994, for example).

The purpose of the experiment reported here was to explore further the relationship between the behavioral symptoms of amphetamine withdrawal and hypothesized changes in striatal EC DA. The experiment was designed with three specific aims in mind. First, we wanted to determine whether there are regional differences in the effects of amphetamine withdrawal on EC DA in the striatum, because this could potentially explain some of the discrepancies in the literature. The two striatal subregions selected for study were the dorsolateral caudate nucleus (caudate) and the nucleus accumbens (accumbens), because these regions represent the two most anatomically, neurochemically, and functionally-distinct subdivisions of the striatal complex (Heimer et al. 1982;Nauta 1989;Groenewegen et al. 1991). Second, we wanted to assess the relationship between withdrawal-related changes in spontaneous locomotor activity and possible withdrawal-related changes in EC DA across the entire light-dark cycle in rats, because one symptom of amphetamine withdrawal in rats, locomotor hypoactivity, is most pronounced during the night portion of the day-night cycle (Segal 1975;Paulson et al. 1991). Therefore, sampling across the daynight cycle should enhance the probability of detecting any withdrawal-related changes in EC DA. This was accomplished by using the powerful sampling technique afforded by automated on-line microdialysis (Wages et al. 1986;Paulson and Robinson 1994). Third, we wanted to assess the relationship between the time course of withdrawal symptoms and the time course of possible changes in EC DA. It is known that the behavioral symptoms of amphetamine withdrawal persist only for a few days to a couple of weeks after the discontinuation of amphetamine treatment, and if they are mediated by changes in EC DA these should show a similar time course. We studied animals, therefore, both early during withdrawal, when symptoms are present (3 and 7 days), and 28 days after withdrawal, when symptoms have dissipated (Robinson and Camp 1987;Paulson et al. 1991).

[1]The phrase EC DA is used here as a short way of saying, “the concentration of DA in dialysate obtained from sampling the extracellular space.” This does not provide an accurate estimate of the actual extracellular concentration of DA, which requires the use of techniques such as “no net flux” microdialysis (e.g., Crippens et al.1993;Smith and Justice 1994).

MATERIALS AND METHODS

Subjects

Adult male Holtzman rats (N = 111; Harlan, Spargue Dawley, Indianapolis, IN) weighing 250 to 300 g at the start of the experiment were housed individually in wire-hanging cages in a temperature-controlled room maintained on a normal light-dark cycle (14:10 hours; lights on at 6:00 A.M.). The animals had free access to food and water and were given one week to acclimatize to the colony room before beginning the experiment.

Amphetamine Pretreatment Regimen

Animals were pretreated twice daily with either intraperitoneal injections of d-amphetamine sulfate or saline in their home cage, with approximately 8 hours separating the two injections, according to the schedule depicted in Paulson et al. (1991).Briefly, to mimic pattern of “runs” and “crashes” seen in addicts (Kramer et al. 1967) amphetamine injections were given each week day, but not on weekends, and the doses were escalated as follows: days 1-2, 1 mg/kg (weight of the salt); days 3-5, 2 mg/kg; day 8, 3 mg/kg; days 9-12, 4 mg/kg; day 15, 4 mg/kg; days 16-19, 5 mg/kg; day 22, 6 mg/kg; days 23-26, 7 mg/kg; day 29, 8 mg/kg; days 30-33, 9 mg/kg; day 36, 9 mg/kg; days 37-40, 10 mg/kg. Control animals received 1 ml/kg of 0.9% saline injection.

Approximately equal numbers of amphetamine- and saline-pretreated animals were prepared for microdialysis testing, which took place either 3, 7, or 28 days after the discontinuation of pretreatment. There were, therefore, three independent groups of saline-pretreated animals and three independent groups of amphetaminepretreated animals. Half of these had probes located in the dorsolateral caudate nucleus, and the other half had probes located in the nucleus accumbens, for a total of 12 independent groups.

Procedure

On day 34 of pretreatment (3- and 7-day groups) or 18 days after discontinuation of pretreatment (28-day groups) each animal was anesthetized with sodium pentobarbital supplemented with methoxyflurane and placed in a stereotaxic apparatus. Using standard stereotaxic techniques a 26-gauge guide cannula was placed unilaterally on the dural surface above either the dorsolateral caudate nucleus or the nucleus accumbens. Half the animals in each group had a cannula placed in the left hemisphere, and the other half had a cannula in the right hemisphere. In addition, a 15-mm length of 17-gauge stainless steel tubing with a 90° bend at the lower end was positioned about 5 mm posterior to the guide cannula, and this was used later to tether the animal to a liquid swivel. Both the cannula and tubing were fixed in place with dental acrylic. A stainless steel stylet was inserted into the guide cannula to maintain patency until insertion of the dialysis probe.

Seven to ten days after surgery each animal was placed in a 45.7-cm x 45.7-cm x 45.7-cm high Plexiglas habituation chamber. This chamber had a flat 30.5-cm x 30.5-cm blue wooden floor with sides that angled 45° to meet the Plexiglas walls of the chamber. This design prevented the animal from bumping its head assembly on the walls or corners of the chamber. One wall was cut out to allow the rat access to a running wheel (112 cm circumference), which faced the chamber. The animal obtained food by breaking a photocell beam located in a receptacle on the side of the chamber (12.7 cm above the floor). This triggered the release and delivery of a 45-mg dustless precision pellet (BioServe, Inc.). Water was freely available via a sipper tube located next to the food cup. Each animal was left in a habituation chamber overnight to allow it to habituate to this environment. The light-dark cycle was the same as in the home colony

The following afternoon each animal was lightly anesthetized with ether supplemented with methoxyflurane, and a dialysis probe quickly was lowered via the guide cannula. The animal was then placed into a test chamber identical in design to the habituation chamber and attached to a dual-channel liquid swivel (Instech) via a cable connected to the stainless steel tubing on the animal's head. A perfusion medium (128.3 mM NaCl, 1.35 mM $CaCl_2$, 2.6 mM KCl, 2 mM $MgCl_2$, and 0.2 mM ascorbic acid, pH 7.3) was pumped through the probe via the side channel on the swivel. The dialysate from the probe exited the central channel of the swivel to a 56-cm length of fused silica tubing. The perfusion medium was pumped at a rate of 1.5 μl/minute using a 2.5-ml gastight Hamilton syringe mounted on a Harvard Model 22 syringe pump. The animal was left undisturbed in the test chamber overnight.

The following morning timed samples of dialysate were collected into minivials to check the outflow volume of the probe. At least three dialysate samples were collected and manually injected onto the HPLC system. This was followed by the injection of a sample of the perfusion medium and then of three standards containing known concentrations of DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), and of the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA). The output from the probe (fused silica tubing) was then attached to a computer-controlled HPLC injection valve (Valco, model #C8W). During each sampling interval dialysate was collected into a 100-μl sample loop with the valve in the load position. At programmed intervals a Commodore® computer triggered the valve to rotate to the inject position, injecting the sample onto the column. After 30 seconds the valve returned to the load position to collect the next sample. Dialysate from each animal (29.25 μL) was continuously injected by this method over 20-minute intervals for the next 18 hours, during which time the animals were left undisturbed. At 8:00 the next morning, the data were retrieved and the probe outflow volume was checked. If the probe outflow was normal the outlet tubing was reconnected to the injection valve and a second experiment was conducted to determine the response to a challenge injection of amphetamine. Dialysate from the animal was injected in the manner previously described for the next 4 hours. After completion of this experiment the volume from the probe was rechecked, and the animal was removed from the chamber. An additional set of standards was run at this time as well. Results from the amphetamine challenge experiment are reported elsewhere (Paulson and Robinson 1995).

The dialysate from the animal was assayed for DA, DOPAC, HVA, and 5-HIAA using HPLC with electrochemical detection using procedures described previously (Paulson and Robinson 1994).

Probe Design

The microdialysis probes were similar to those described by Robinson and Camp (1991b), with the following modifications. They were a removable concentric-style probes with an outer diameter of 250 μm. Both the inlet and outlet lines consisted of fused silica, and the dialysis membrane extended from the bottom of the guide cannula to the ventral tip of the probe. The dialysis membrane was coated with cyanoacrylate glue (Cyanodent Fast, Ellman International, Hewlett, NY), except for the most ventral 2.25 mm at its tip (i.e., the dialysis surface was 2 mm long). Prior to use, all probes were tested in vitro to determine their ability to recover known concentrations of DA, DOPAC, HVA, and 5-HIAA at 37°C.

Behavior

Motor activity in the chamber (90° movements) was monitored over 20-minute intervals by a device equipped with four photocells located 90° apart, which was mounted on the liquid swivel. This device essentially divided the chamber into four quadrants. Disruption of a photocell beam represented movement from one quadrant to the next. Photocell beam disruptions were registered and stored by a Commodore® computer (McFarlane et al. 1992).

In a previous study (Paulson and Robinson 1994) we found a high degree of correspondence between the 90° movements assessed with this automated device and the number of cage "crossovers" assessed by viewing a videotape of the animals ($r = 0.95$, $p < .001$). Locomotor activity in the running wheel was recorded by a microswitch located on the back of the wheel. Feeding behavior was monitored by recording the number of photocell beam breaks made as the animal reached into the food receptacle for a pellet. Dialysate samples were injected 4 minutes after the start of each behavioral interval to coordinate the behavior with the neurochemistry, because it took that amount of time for the dialysate to reach the injection port.

Histology

At the end of each experiment the animal was removed from the chamber, given an overdose of sodium pentobarbital, and perfused through the heart with 0.9% saline, followed by 10% formalin. The brains were removed and stored in 10% formalin until they were sectioned coronally using a frozen technique, stained with cresyl violet, and examined to determine the exact location of the dialysis probe.

Data Analysis

Dialysate values are expressed in picograms per microliter and are corrected for probe recovery in vitro, which controls for differences in probe efficiency due to minor differences in probe construction. Probe recovery values for DA, DOPAC, HVA, and 5-HIAA, respectively, were 13.12 ± 0.49%; 13.67 ± 0.56; 13.42 ± 0.54%; and 12.96 ± 0.63% for the caudate probes and 12.64 + 0.52%; 12.79 + 0.61%; 13.04 ± 0.51%; and 13.21 ± 0.46% for the accumbens probes. Dialysate values were also corrected to ehminate the slow continuous changes in dialysate concentrations seen with long periods of dialysis (Robinson and Camp 19901a, 1991b). This was done using standard computer-assisted statistical methods to "detrend" the data for each compound in each individual animal. Essentially, the program calculates the slope of the line for each subject (and compound) over the entire 18-hour sample period and then corrects each sample for the "drift" in recovery over the sample period (Wilkinson 1989). Group means were then calculated using these "detrended" data, and group comparisons were performed. This was done on the assumption that the slow decrease in dialysate concentrations of DA and the DA metabolites seen over these long periods of dialysis reflect an artifact of the technique (e.g., reduced recovery), not a change in the physiology of the striatum.

RESULTS

There were two criteria for inclusion of data in this experiment: (1) chromatographic (the relevant daytime basal peaks had to be at least three times greater than background noise); and (2) histological (the probe had to be located in either the dorsolateral caudate nucleus or the nucleus accumbens). Seven animals were lost due to chromatography problems, and two animals had probes outside the target structure. In addition, two animals were eliminated because of equipment malfunction. Thus, data analysis was based on 46 animals with probes located in the dorsolateral caudate nucleus (caudate group) and 54 animals with probes located in the nucleus accumbens (accumbens group). The brain areas traversed by the probes for which data are reported are illustrated in Figure 1 of Paulson and Robinson (1995).

The data were first analyzed to determine whether there were any differences between saline-pretreated groups tested 3, 7, or 28 days after the discontinuation of saline pretreatment on any measure. There was no effect of "withdrawal period" in the saline-pretreated control groups, and therefore they were pooled to form two saline-pretreated control groups: one with probes located in the caudate and one with probes in the accumbens.

Circadian Changes in Behavior and DA Dynamics in Control Animals

Control animals showed changes in motor activity across the day-night cycle similar to those described in an earlier study using the same apparatus and procedures (Paulson and Robinson 1994). The animals showed relatively little motor activity during the day, a slow increase as night approached, a large peak in motor activity immediately following lights off, a moderate decrease in motor activity during the middle of the night (but notto daytime levels), and a second peak in motor activity in the last 1 or 2 hours of the dark period. When the lights came on again motor activity fell to the low levels typical of daytime (data not shown).

Figure 1 shows the concentration of DA and its metabolites in dialysate from the dorsolateral caudate nucleus and the nucleus accumbens of just the control animals. In the caudate nucleus EC DA showed circadian variation, being significantly higher at night than during the day (mean daytime value vs. mean nighttime value, $t = 9.28$, $p < .0001$). The concentration of DA obtained from the nucleus accumbens also showed a small degree of circadian variation, being significantly higher at night ($t = 2.22$, $p = .04$). However, the nocturnal increase in DA seen in the nucleus accumbens was much smaller than that seen in the caudate. In the caudate nucleus DA increased by 28.6 ± 3.17% at night relative to the day, but in the nucleus accumbens DA increased by only 9.5 ± 4.4% at night, which is significantly less than in the caudate (t =3.46, $p < .002$).

DA metabolites in both the caudate and accumbens showed circadian variation, being significantly higher at night (DOPAC: caudate, day vs. night, $t = 3.66$, $p < .002$; accumbens, $t = 2.95$, $p = .008$. HVA: caudate, $t = 4.31$, $p < .0006$; accumbens, $t = 5.1$, $p < .0001$). In contrast to DA, however, the magnitude of the nocturnal increase in DOPAC and HVA was comparable in the two regions. In the caudate DOPAC increased by 9.7 ± 2.4% at night, compared to 7.4 + 1.6% ($t = 0.83$) in the accumbens; in the caudate HVA increased by 16.8 ± 3.8% at night, compared to 10.9 ± 1.6% in the accumbens ($t = 1.55$, $p = .13$).

Changes in Behavior and DA Dynamics Associated with Amphetamine Withdrawal

Behavior—Figure 2A summarizes the effect of amphetamine withdrawal on motor activity. As reported previously (Robinson and Camp 1987;Paulson et al. 1991), withdrawal from escalating dose amphetamine treatment was associated with nocturnal hypoactivity. Animals tested after either 3 or 7 days of withdrawal made significantly fewer 90° movements at night than did control animals ($F = 4.43$, $p < .006$, see Figure 2A). By 28 days of withdrawal noctumal motor activity had returned to control levels. This confirms that under the conditions of this study (i.e., during dialysis) the discontinuation of escalating dose amphetamine treatment produced a withdrawal syndrome.

Figure 2B shows the effect of amphetamine withdrawal on the number of nose pokes into the food receptacle during the day and night periods. Control animals made significantly more nose pokes during the lights-off-period, consistent with previous reports of a circadian pattern of feeding behavior in rats (Zucker 1971). Animals tested after 3 or 7, but not 28, days of withdrawal showed a significant increase in the incidence of feeding during the day relative to control animals ($F = 5.75$, $p < .002$), but there was no effect of amphetamine withdrawal on nocturnal feeding ($F < 1.0$). The incidence of feeding is inferred here by the frequency of photocell beam interruptions, and it is possible that these do not reflect feeding behavior, but investigatory nose pokes. This is unlikely, however, because there was no accumulation of food pellets observed the following day in animals tested after 3 or 7 days of withdrawal. The increased incidence of feeding was not associated with group differences in body weight because there were no significant group differences in body weight on the day of dialysis testing.

Neurochemistry—Figure 3 shows the effects of amphetamine withdrawal on EC DA in the dorsolateral caudate nucleus and the nucleus accumbens. There was a significant overall effect of amphetamine withdrawal on DA concentrations in the caudate (two-way repeated-measures ANOVA on the time course data, effect of group, $F = 10.6$, $p < .0001$; interaction nonsignificant). Relative to control, there was a significant decrease in DA concentrations in animals tested after either 3 or 7 days of withdrawal (two-way repeated-measures ANOVA, control vs. 3 day, effect of group, $F = 34.5$, $p < .0001$; control vs. 7 day, $F = 9.17$, $p = 0.006$). There was no statistical difference between control animals and animals tested after 28 days of withdrawal ($F = 3.3$, $p = .08$). hi contrast to the caudate, there was no significant effect of amphetamine withdrawal on DA concentrations in the nucleus accumbens (overall two-way ANOVA, effect of group, $F = 0.78$, interaction $F = 0.99$).

Figure 4 shows the effects of amphetamine withdrawal on EC DOPAC in the caudate and accumbens. There was a significant effect of amphetamine withdrawal on DOPAC concentrations in the caudate (overall two-wayANOVA, effect of group, $F = 0.41$, $p = 0.75$, interaction $F = 1.23$, $p = .03$) that was due to a significant decrease in DOPAC at night in animals tested after 3 days of withdrawal (control vs. 3 day, effect of group, $F = 0.37$, $p = 0.55$, interaction, $F = 2.28$, $p < .0001$). Indeed, in animals tested after 3 days of withdrawal there was no day-night difference in DOPAC concentrations ($t = 0.1$). On the other hand, there was no difference in DOPAC concentrations between control animals and animals tested after 7 or 28 days of withdrawal (FPs < 1.0). There was also a significant effect of amphetamine withdrawal on DOPAC concentration in the nucleus accumbens (overall two-way ANOVA, effect of group, $F = 2.18$, $p = 0.103$, interaction, $F = 1.2$, $p < .05$). This was due, however, to a significant *increase* in DOPAC in animals tested after 28 days of withdrawal, especially at night (control vs. 28 day, effect of group, $F = 4.7$, $p < .04$, interaction, $F = 2.6$, $p < .0001$). There was no difference in DOPAC concentrations in the accumbens between control animals and animals tested after either 3 or 7 days of withdrawal (F′s < 1.0).

Figure 5 shows the effects of amphetamine withdrawal on EC HVA in the caudate and accumbens. There was a significant effect of amphetamine withdrawal on HVA concentrations in the caudate (overall two-way ANOVA, effect of group, $F = 5.38$, $p = 0.004$, interaction, $F = 1.58$, $p < .0001$) that was due to a significant decrease in HVA at night in animals tested after 3 days of withdrawal (control vs. 3 day, effect of group, $F = 1.11$, p=.30, interaction, $F = 3.17$, $p = 0.0001$), and there was a significant increase in HVA in animals tested after 28 days of withdrawal (control vs. 28 day, effect of group, $F = 11.06$, $p < .003$, interaction nonsignificant). There was no day-night difference in HVA in animals tested after 3 days of withdrawal ($t = 0.29$) either, but there was a significant day-night difference in animals tested after 28 days of withdrawal ($t = 4.52$, There p 0.002). was no significant difference between control animals and animals tested after 7 days of withdrawal (effect of group, $F = 3.23$, $p = 0.085$, interaction, $F = 0.67$).

There was also a significant effect of amphetamine withdrawal on EIVA concentrations in the nucleus accumbens (effect of group, $F = 3.11$, $p = .036$, interaction, $F = 1.99$, $p < .0001$). There was, however, no decrease in HVA in the accumbens in animals tested after 3 days of withdrawal (control vs. 3 days, $F's < 1.0$). As in the caudate, there was significant increase in HVA in the accumbens of animals tested after 28 days of withdrawal, especially at night (control vs. 28 days, effect of group, $F = 8.24$, $p < .008$, interaction, $F = 3.36$, $p < .0001$). There was also a small increase in HVA at night in animals tested after 7 days of withdrawal (effect of groups, $F = 0.58$, $p = 0.45$, interaction, $F = 2.55$, $p < .0001$). All groups showed a significant increase in HVA in the accumbens at night relative to the day ($t's > 4.0$, $p's < .005$).

Figure 6 shows the effects of amphetamine withdrawal on EC 5-HIAA in the caudate and accumbens. There was no effect of amphetamine withdrawal on 5-HIAA concentrations in the

caudate (overall two-way ANOVA, *F's* < 1.0). There was, however, a small effect of amphetamine withdrawal on 5-HIAA concentrations in the accumbens (overall two-way ANOVA, effect of group, $F = 0.99$, interaction, $F = 1.26$, $p < 0.02$) that was due to an increase in 5-HIAA in animals tested after 28 days of withdrawal (control vs. 28 day, effect of group, $F = 2.15$, $p = 0.15$, interaction, $F = 2.13$, $p < .0001$). Therewas no difference in 5-HIAA between control animals and animals tested after 3 or 7 days of withdrawal (*F's* < 1.0).

DISCUSSION

The major effects of amphetamine withdrawal on DA dynamics in the dorsal versus ventral striatum are summarized in Table 1. First, there were marked differences regional in the effects of amphetamine withdrawal on both EC DA and DA metabolism. Early after the discontinuation of escalating dose amphetamine treatnent, when behavioral symptoms of amphetamine withdrawal were present (nocturnal hypoactivity), there was asignificant *decrease* in basal EC DA, DOPAC, and HVA in the dorsolateral caudate nucleus. There was, however, no evidence for withdrawal-related changes in EC DA or DA metabolism in the nucleus accumbens. Second, after the behavioral symptoms of withdrawal dissipated (by 28 days) there was a significant *increase* in basal DA metabolism in both the caudate nucleus and the nucleus accumbens, as indicated by an increase in HVA in the caudate and of DOPAC and HVA in the accumbens. Third, there was also a small increase in basal 5-HIAAconcentrations in the accumbens (but not the caudate) in animals tested after 28 days of withdrawal.

Behavior

The postamphetamine withdrawal behavioral depression (nocturnal hypoactivity) reported heire is consistent with numerous other reports (Tonge 1974;Segal 1975;Lynch and Leonard 1978;Robinson anA Camp 1987;Paulson et al. 1991). Nocturnal hypoactiv:ity can last for 1 to 2 weeks following the discontinuation of amphetamine pretreatment, depending on the pre] treatment regimen, but dissipates within 3 to 4 weeks (Paulson et al. 1991), by a si: be reinstated ngle challenge although it can situations as well, many of which may be indicative of alterations in affective or motivational state (Kokkinidis 1988). For example, during amphetamine withdrawal rats show diminished reactivity to novelty (Schreiber et al. 1976), increased immobility in a forced swim test (Kokkinidis et al. 1986), and a decrease in the rate and increase in the threshold of responding for intracerebral electrical self-stimulation (ICSS) (Leith and Barrett 1976;Kokkinidis and Zacharko 1980;Cassens et al. 1981).

An additional sign of amphetamine withdrawal reported here was a transient (3-7 days) increase in feeding behavior during the daytime. Changes in the circadian pattern of feeding behavior have also been reported in rats given continuous access to methamphetamine (Kraeuchi et al. 1984,1985;Morimasa et al. 1987). This transient diurnal hyperphagia was not the result of animals eating to gain weight lost during the period of amphetamine treatment, because by the time of testing there were no group differences in body weight. Some effects of amphetamine on feeding behavior are thought to involve an action of amphetamine on hypothalamic noradrenergic (NE) systems. It is possible, therefore, that the withdrawal-related changes in feeding are related to changes in hypothalamic NE systems. Consistent with this hypothesis, discontinuation of the amphetamine pretreatment regimen used here results in a significant decrease in the postmortem tissue content of NE in the hypothalamus, which lasts for 3 to 7 days, but returns to normal by 28 days (Paulson et al. 1991).

Neurochemistry

In saline-pretreated control animals there were significant regional differences in the pattern of *circadian variation* in EC DA. In contrast, there were no regional differences in the pattern of circadian variation in DA metabolism. DA increased significantly more at night in the

caudate nucleus than in the nucleus accumbens, whereas DOPAC and HVA increased to the same extent at night in these two striatal subregions. These findings essentially replicate an earlier study using "injectionnaive" subjects, and their implications are discussed in detail in that report (Paulson and Robinson 1994).

The most interesting (and somewhat surprising) finding in the present study was that amphetamine withdrawal was accompanied by a significant decrease in DA, DOPAC, and HVA in the classical "motor striatunm" (i.e., the dorsolateral caudate nucleus) but not in the so-called "mesolimbic" striatum, (i.e., the nucleus accumbens). The absence of amphetamine withdrawal-related changes in basal DA in the nucleus accumbens is consistent with a number of previous reports (Segal and Kuczenski 1992a;Crippens et al. 1993;Wolf et al.1993;Crippens and Robinson 1994). On the other hand, Rossetti et al. (1992c) reported that amphetamine withdrawal is accompanied by a significant decrease in basal EC DA in the nucleus accumbens.

It is important to consider, therefore, whether the absence of withdrawal-related changes in basal EC DA in the nucleus accumbens reflects a "false negative" due to the limits of dialysis sampling. For example, the caudate has a significantly higher density of DA terminals than the accumbens, and perhaps this makes it easier to detect changes in EC DA in the caudate than in the accumbens. This is probably not the case for a number of reasons. First, the basal concentration of DA in dialysate obtained from the nucleus accumbens was well above the limits of sensitivity of our assay and dialysis conditions, and both increases or decreases in the concentration of DA in dialysate are easily detectable under our experimental conditions (Robinson et al. 1994, for example). Second, there is reason to believe that small changes in EC DA should be easier to quantify in the nucleus accumbens than in the caudate nucleus, because the major factor limiting the detectibility of DA with dialysis is the rapid rate of DA clearance from the extracellular space due to high-efficiency DA reuptake (Wightman and Zimmerman 1990). There are, however, two to three times fewer DA uptake sites in the nucleus accumbens than in the caudate nucleus (Marshall et al. 1990), which results in a significantly slower rate of DA clearance in the accumbens than in the caudate (Cass et al. 1992;Stamford et al. 1988). Thus, DA can diffuse farther from release sites into the extracellular space in the accumbens than in the caudate (Cass et al. 1992), presumably making it easier to detect changes in EC DA in the accumbens than in the caudate. Withdrawal-related changes in EC DA were apparent, however, in the caudate, not the accumbens. Third, the concentrations of DA metabolites in dialysate from the striatum are very high relative to DA and are not limited by rapid reuptake. The fact that there was also a withdrawal-related decrease in DA metabolism in the caudate, but not the accumbens, is consistent with the hypothesis that the findings reported here reflect a real regional difference in the influence of amphetamine withdrawal on DA neurotransmission in the striatal complex.

Indeed, the regional differences in the effects of amphetamine withdrawal on basal EC DA reported here may explain why some researchers have found no effect of amphetamine withdrawal on DA in the accumbens (Segal and Kuczenski 1992a;Crippens et al. 1993;Wolf et al. 1993;Crippens and Robinson 1994), whereas others have reported a postamphetamine withdrawal-related depression in DA (Rossetti et al. 1992c). For example Rossetti et al. (1992c) used transverse microdialysis probes, rather than the concentric-style probes used here, and thus, may have sampled a different subregion of the ventral striatum. Similarly, Segal and Kuczenski (1992a) may have found no change in EC DA in either the caudate or accumbens 48 hours after the discontinuation of repeated amphetamine treatment because they sampled the *medial* caudate nucleus, not the lateral caudate, as in the present study (although they also used a much less aggressive treatment regimen). In summary, the available evidence suggests that some of the apparent discrepancies in the literature regarding the effects of amphetamine withdrawal on basal EC DA in the striatal complex may be due to regional differences in the effects of amphetamine withdrawal on DA neurotransmission. A more rigorous test of this

hypothesis will require sampling more discrete subdivisions of the dorsal and ventral striatum than has been achieved to date, including perhaps both the shell and core of the nucleus accumbens.

The decrease in the concentration of DA and DA metabolites in dialysate obtained from the caudate strongly suggests that amphetamine withdrawal is accompanied by a decrease in the extracellular concentration of DA in this region. It is not possible to ascertain from a dialysis study, however, the exact nature of the neurobiological adaptations responsible for a decrease in extracellular DA, and there are many possible mechanisms. One rather uninteresting possibility is that the escalating dose regimen used here was neurotoxic, resulting in a reduction in the number of DA terminals in the caudate (but not the accumbens). This is probably not the case, however, because there is considerable evidence showing that this amphetamine pretreatment regimen is not neurotoxic (Robinson and Camp 1987;Paulson et al. 1991). Furthermore, the magnitude of the PA depletion produced by large neurotoxic doses of amphetamine does not result in a significant decrease in basal EC DA in the caudate (Robinson et al. 1990). Another possibility is that there was an *increase* in DA uptake, resulting in less DA in the extracellular fluid accessible to the dialysis probe. This is unlikely, however, because the few studies on this topic suggest that amphetamine withdrawal is accompanied by either no change (Allard et al. 1990) or small transient *decrease* in DA uptake sites (Ikawa et al. 1994). Furthermore, a change in DA uptake would alter the in vivo recovery of DA (Smith and Justice 1994), and at least in the accumbens there is no amphetamine withdrawal related change in in vivo recovery as assessed with “no net flux” dialysis (Crippens et al. 1993).

The most parsimonious explanation at this point is that there is a transient decrease in DA release in the caudate nucleus (but not the accumbens) during amphetamine withdrawal. How this might occur is unclear, and the literature on withdrawal or sensitizationation-related changes in DA terminal autoreceptor regulation of DA release is small and inconsistent (White and Wolf 1991). Interestingly, White and Wang (1984) report that 24 hours after the discontinuation of repeated amphetamine treatment there is a significant *increase* in both the number of spontaneously active DA cells per track in the ventral tegmental region and in mean firing rate, which is not consistent with a decrease in DA release. It is not known, however, whether this also occurs in the substantia nigra (F. White, personal communication). Thus, elucidation of the cellular mechanism responsible for the postamphetamine withdrawal-related decrease in extracellular DA reported here awaits further study.

It is especially interesting that although there were regional differences in the effects of amphetamine withdrawal on basal EC DA and DA metabolites early after the discontinuation of amphetamine pretreatment, there were no regional differences one month later. That is, in animals tested 28 days after the discontinuation of amphetamine treatment basal DA metabolism was increased in both the caudate nucleus and the nucleus accumbens. Similarly, at this time there is also a sensitization-related enhancement in amphetamine-stimulated DA release in *both* the dorsolateral caudate and the nucleus accumbens (Robinson 1991;Paulson and Robinson 1995). An increase in basal DA metabolism has been reported previously in association with amphetamine sensitization (Robinson and Camp 1987;Camp and Robinson 1988;Robinson et al. 1988;Vezina 1993). It is not clear what accounts for this, but it may be related to a small sensitization-related increase in the discharge rate of DA neurons (Paulson and Robinson 1995;White and Wolf 1991, for a discussion of this point). Whatever the reason, it is intriguing that there are regional differences in the effects of amphetamine pretreatment on DA dynamics in association with postamphetamine withdrawal depression (seen early after withdrawal), but no regional differences in association with the persistent neuroadaptations that accompany sensitization (which are most pronounced long after withdrawal).

Finally, the regional differences in amphetamine withdrawal-related changes in basal EC DA reported here have a number of interesting implications for the hypothesis that a decrease in synaptic DA in the nucleus accumbens mediates some of the symptoms associated with amphetamine withdrawal and for the idea that a decrease in synaptic DA in the nucleus accumbens is a common feature of drug withdrawal syndromes (Rossetti et al. 1992c). Our data suggest that the symptoms of amphetamine withdrawal are not attributable to a simple decrease in EC DA in the nucleus accumbens—perhaps some other striatal subregion, but not that portion of the nucleus accumbens sampled here. It also follows from this that a decrease in EC DA in the nucleus accumbens is not a common feature of all drug withdrawal syndromes. Consistent with the latter point, a number of researchers have reported that cocaine withdrawal is not accompanied by changes in basal EC DA in the nucleus accumbens either (Segal and Kuczenski 1992b;Kalivas and Duffy 1993;Hooks et al. 1994); although others have (Parsons et al. 1991;Robertson et al. 1991;Imperato et al. 1992;Rossetti et al. 1992c;Weiss et al. 1992). Whether the discrepancies in the literature regarding the effects of cocaine withdrawal on DA dynamics also can be attributed to regional differences is not known.

Although the data reported here suggest that the symptoms of amphetamine withdrawal cannot be attributed *solely* to a decrease in EC DA in the nucleus accumbens, it is important to emphasize that this does not mean that changes in DA neurotransmission in this region play no role in mediating the symptoms of amphetamine withdrawal. The microdialysis technique used here limits our conclusion to possible changes in DA neurotransmission due to changes in extracellular, and by inference, synaptic, DA. Amphetamine withdrawal could be accompanied by a number of other changes in DA dynamics in the nucleus accumbens, including a variety of postsynaptic adaptations, and these would not be detected with microdialysis sampling.

In conclusion, the data reported here provide evidence for a decrease in DA neurotransmission in the striatum during amphetamine withdrawal. This is consistent with the idea that a hypodopaminergic state may contribute to some of the symptoms of psychomotor stimulant drug withdrawal. There were, however, striking regional differences in the effects of amphetamine withdrawal on EC DA. The basal concentration of PA was decreased in the dorsolateral caudate nucleus, but not in the nucleus accumbens. There has been a great deal of emphasis in recent years on the role of accumbens DA in mediating the affective and motivational effects of psychomotor stimulant drugs. Perhaps it is time to reconsider the possibility that DA neurotransmission in the dorsal striatum may be involved in complex psychological functions as well.

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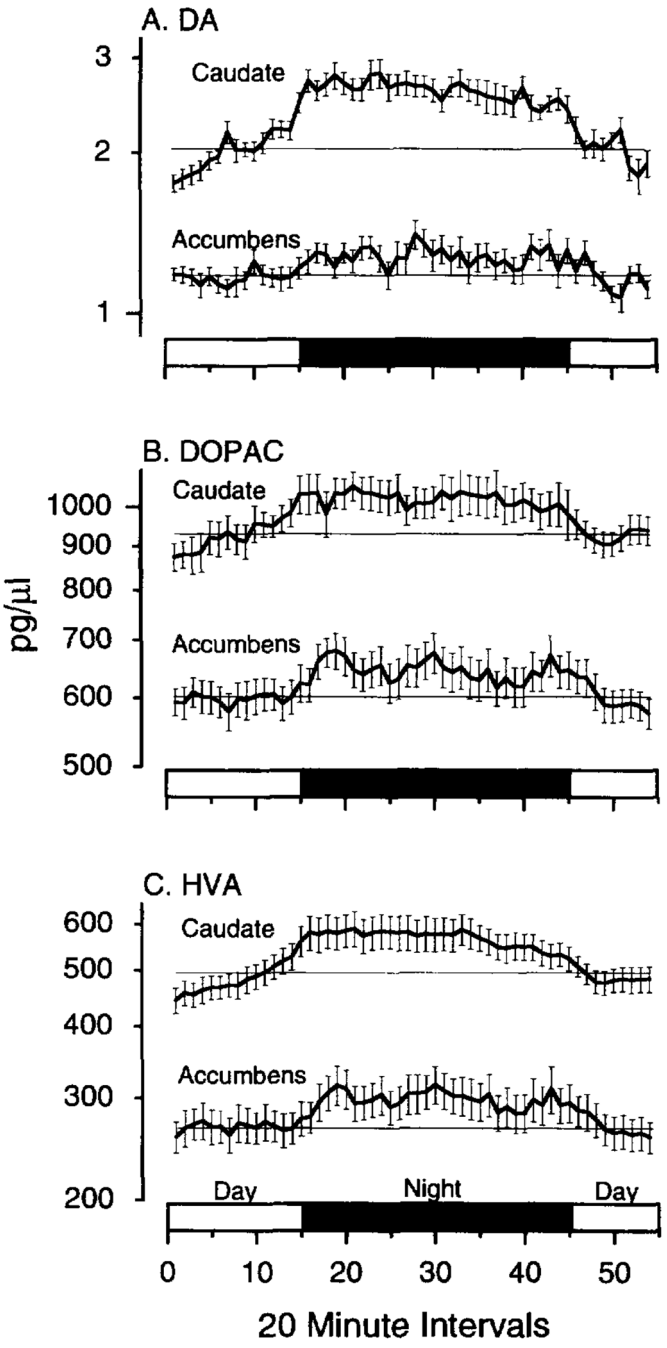

Figure 1.

The mean (± SEM) concentration (pg/µl) of DA, DOPAC, and HVA in 20-minute dialysis samples across the light-dark cycle in saline-pretreated control animals with probes located in either the dorsolateral caudate nucleus *(n = 17)* or the nucleus accumbens *(n = 18)*. The 10-hour lights-off period (night), which began at 8:00 P.M., is indicated by the *solid black* bar on the horizontal axis, and the light-on periods (day) are indicated by the *open bars*. *The solid horizontal lines* represent the average daytime value for each compound (i.e., the average of all the daytime intervals), and these are plotted to facilitate the visual comparison of changes across the light-dark cycle. Note that the vertical axis is a log scale. The extracellular concentrations of DA, DOPAC, and HVA were significantly higher in the caudate than in the

accumbens (two way repeated-measures ANOVAs, effect of group, all F values > 60, $p's$ > .0001). In addition, there was a significant effect of time in all cases, which was due to a significant increase in DA, DOPAC, and HVA at night, relative to the day, in both the dorsolateral caudate nucleus and the nucleus accumbens (two-way repeated-measures (ANOVAs, effect of time, all F values > 6.2, $p's$ < .0001). The magnitude of the nocturnal increase in DA, however, was significantly greater in the caudate than in the accumbens. There were no regional differences in the magnitude of the nocturnal increase in DOPAC orHVA.

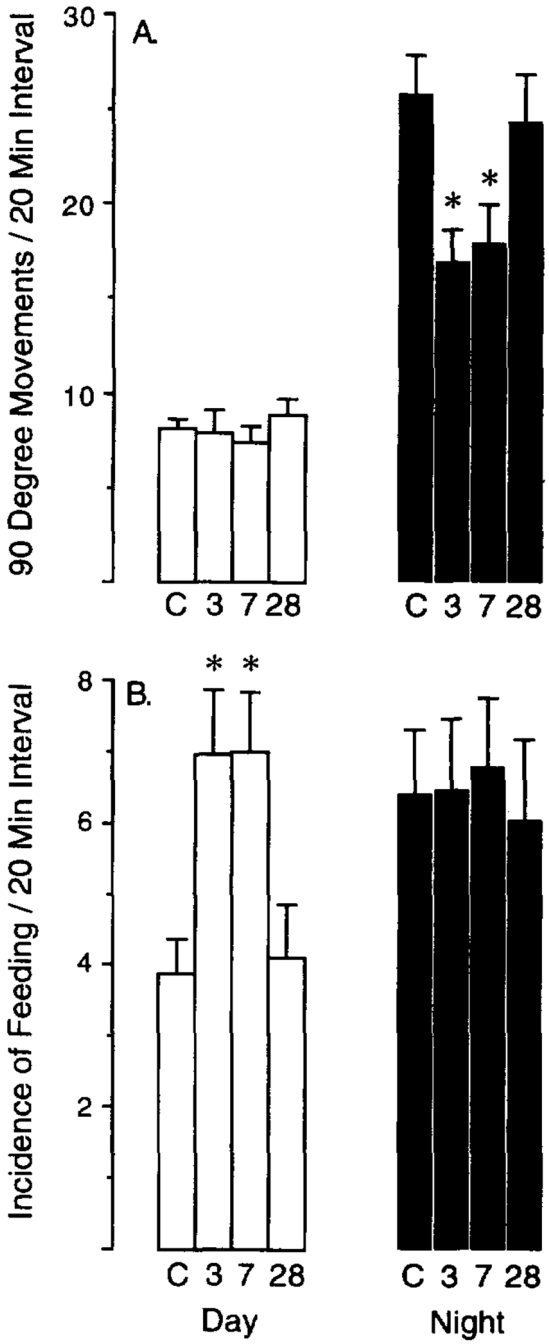

Figure 2.

The effect of amphetamine withdrawal on spontaneous motor activity and the incidence of feeding during the day versus the night. The *bars*, from left to right, represent the mean (± SEM) number of 90° movements in the chamber per 20 minute interval **(A**) or number of noise pokes per 20-minute interval **(B)** made by saline-pretreated control animals (C) and amphetamine-pretreated animals tested 3, 7, or 28 days after the discontinuation of pretreatment (controls *n = 42*; amphetamine pretreated *n = 18-20/*group). Data were averaged over the entire day *(open bars)* or night period *(dark bars*) to simplify data presentation. **(A)** Mean 90° movements: There was a significant effect of amphetamine withdrawal and of the light-dark cycle on motor activity. There was a significant increase in motor activity at night

in all groups (paired *t*-tests comparing the day and night values for each group, all *p′s* < .05). However, the increase in nocturnal motor activity was significantly attenuated in amphetamine-pretreated animals tested after 3 or 7 days of withdrawal relative to controls (one-way ANOVA comparing all four groups at night, $F = 4.43$, $p = .006$; *, indicates groups that differed from control, post-hoc Fisher′s PLSD tests, *p′s* < .01). There were no group differences during the day (one-way ANOVA, $p = .73$). **(B)** Mean incidence of feeding (nose pokes): There was a significant effect of amphetamine withdrawal, and the light-dark cycle, on the incidence of nose pokes into the food receptacle. In control animals and animals withdrawn for 28 days more food pellets were delivered at night than during the day (paired *t*-tests, $p < .05$). In addition, there was a significant increase in the number of food pellets delivered during the day to amphetamine-pretreated animals withdrawn for 3 or 7 days, relative to the control group ($F = 5.8$, $p = .001$; *, indicates groups that differed from control, post-hoc Fisher′s PLSD tests, p′s < .05). There was no significant effect of amphetamine withdrawal on the number of pellets delivered during the night($F = 0.06$, $p = 0.98$)

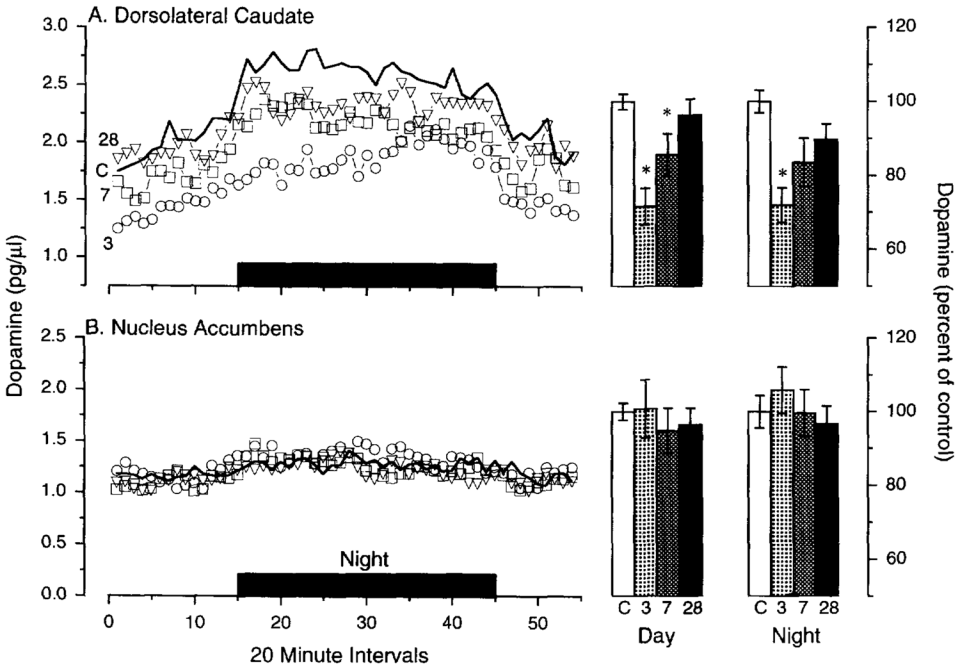

Figure 3.

Effect of amphetamine withdrawal and of the light-dark cycle on the mean concentration of DA in the dorsolateral caudate nucleus **(A)** or the nucleus accumbens **(B)**. The lights-off period, which began at 8:00 P.M., is illustrated by the *solid black* bar on the horizontal axis. The line graphs on the left give the mean concentration of DA (pg/µl) per 20-minute interval. On these graphs the dark *solid line* represents the saline-pretreated control group (same data as in Figure 1), and the amphetamine-pretreated groups are indicated by lines connecting the symbols: 3 days withdrawn*(circles*), 7 days withdrawn*(squares*), 28 days withdrawn*(triangles*). The bar graphs on the right give the mean (± SEM) concentration of DA averaged over the entire day and night periods plotted as a percent of the average control values (i.e., for daytime, the concentration of DA averaged across all the lights-on intervals in control arnimals is equal to 100% and likewise for nighttime values, and the values for amphetamine-pretreated animals are plotted as a percent of this control value. *indicates groups that differed significantly from control; one-way ANOVAs and follow-up Fisher′s PSLD tests. In the dorsolateral caudate nucleus there was a significant effect of amphetamine withdrawal and of the light-dark cycle on EC DA, as indicated by an overall two-way repeated-measures ANOVA. There was a significant decrease in DA in amphetamine-pretreated animals withdrawn for 3 or 7 days relative to control. The effect of amphetamine withdrawal is also apparent in the bar graphs. In the nucleus accumbens, in contrast to the caudate, there was no effect of amphetamine withdrawal on EC DA.

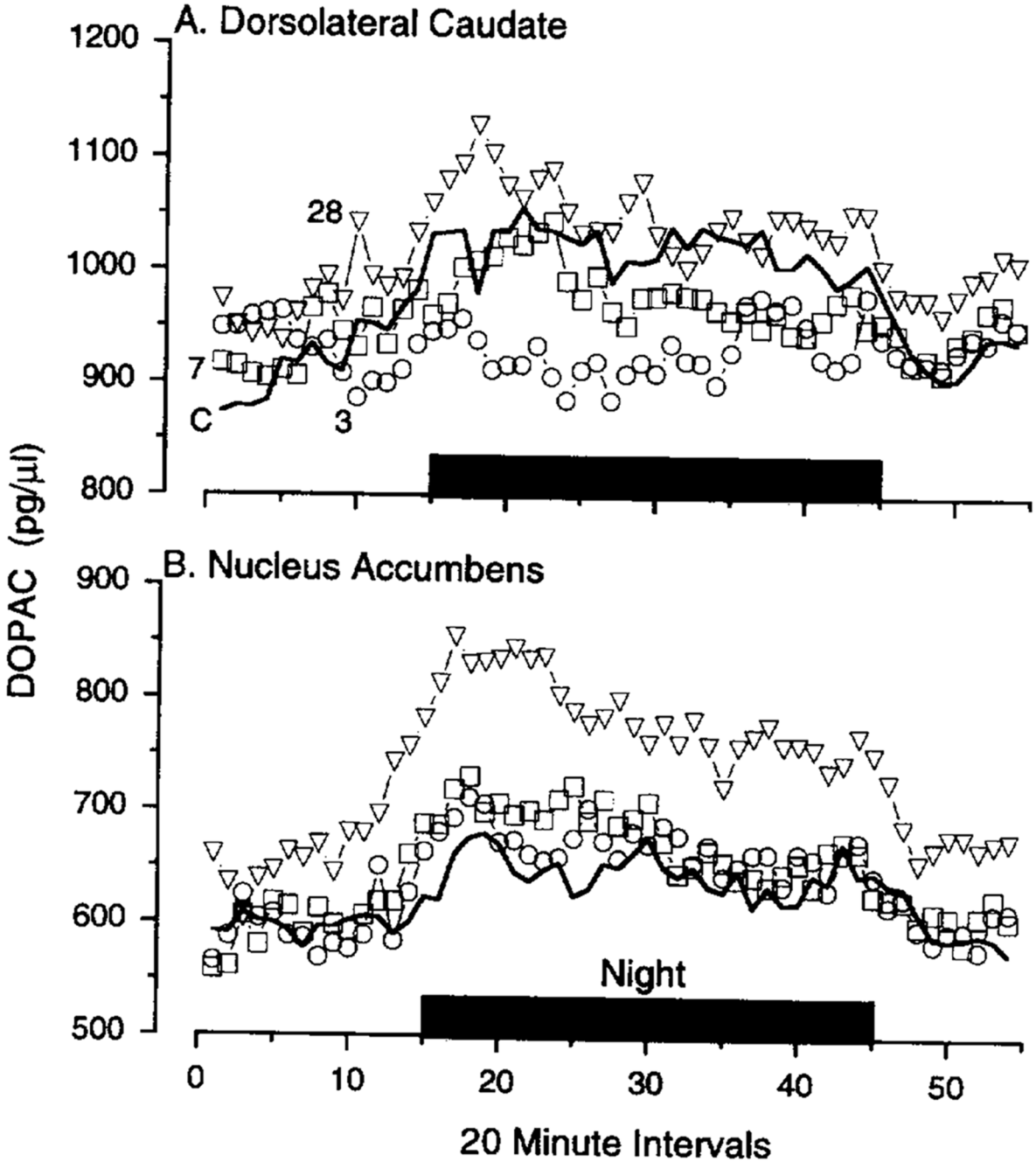

Figure 4.
The effects of amphetamine withdrawl and of the light-dark cycle on the mean concentration of DOPAC in the dorsolateral caudate nucleus **(A)** or the nucleus accumbens **(B)**. The symbols and format are the same as in Figure 3.

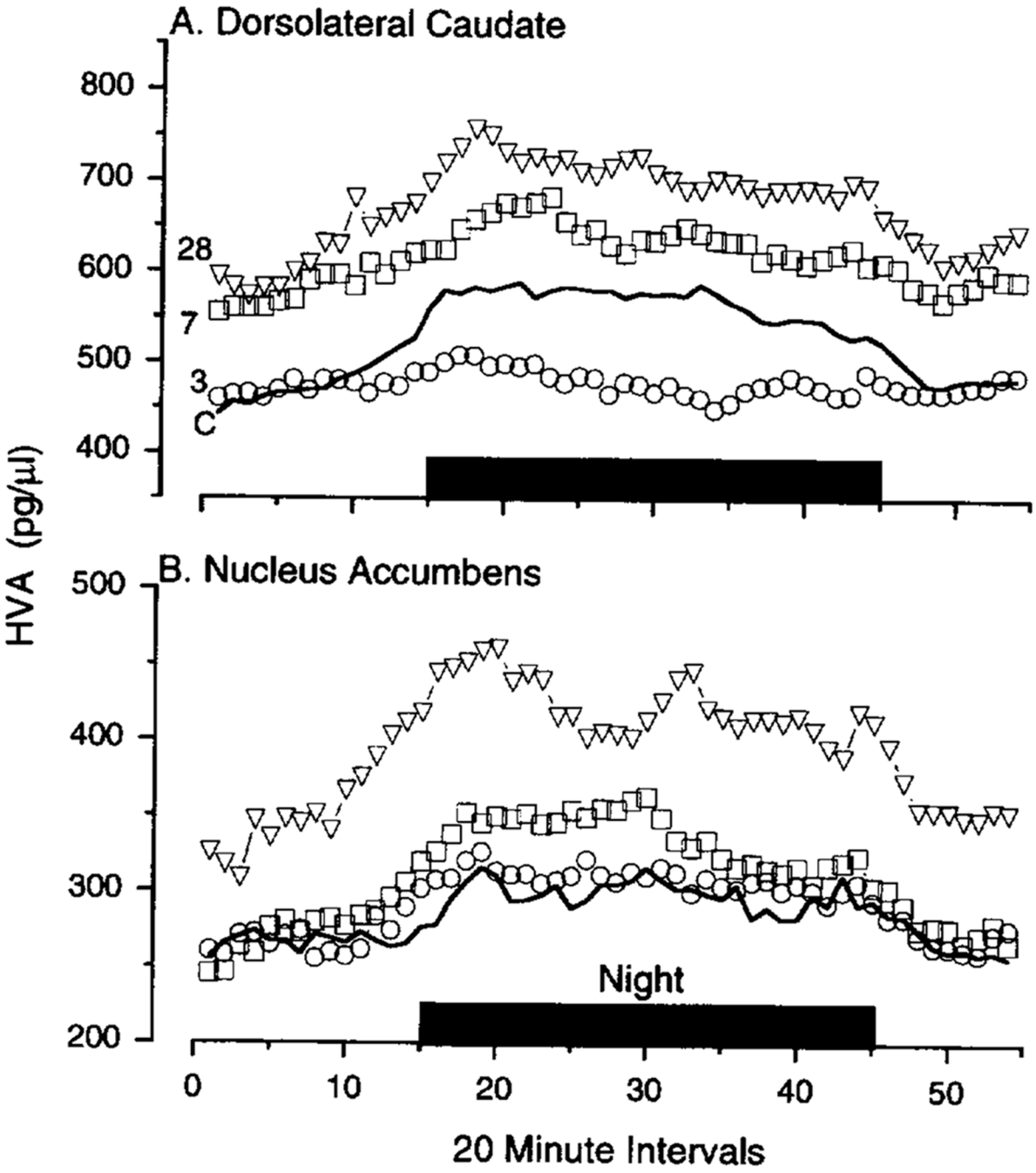

Figure 5.
The effect of amphetamine withdrawal and of the light-dark cycle on the mean extracellular concentration of HVA in the dorsolateral caudate nucleus **(A)** or the nucleus accumbens **(B)**. The symbols and format are the same as in Figure 3.

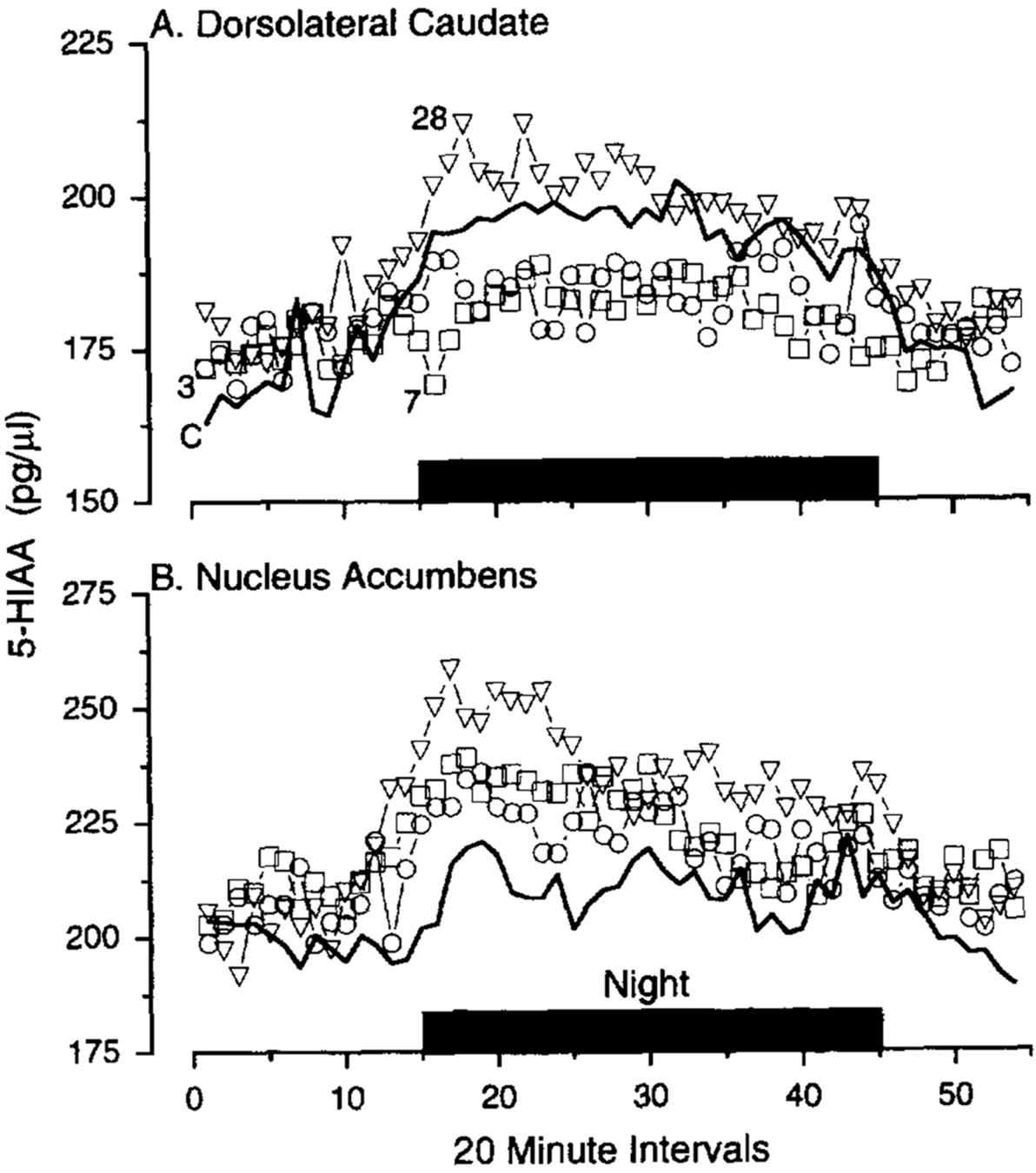

Figure 6.
The effect of amphetamine withdrawal and of the light-dark cycle on the mean concentration of 5-HIAA in the dorsolateral caudate nucleus **(A)** or the nucleus accumbens **(B)**. The symbols and format are the same as in Figure 3.

Table 1

Summary of Regional Differences in the Effects of Amphetamine Withdrawal on DA Dynamics in the Striatum

	Dorsolateral Caudate		Nucleus Accumbens	
	Early (3 days)	Late (28 days)	Early (3 days)	Late (28 days)
DA	↓	—	—	—
DOPAC	↓	—	—	↑
HVA	↓	↑	—	↑
5-HIAA	—	—	—	↑

Dash indicates no change.