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Time course of transient behavioral depression and persistent behavioral sensitization in relation to regional brain monoamine concentrations during amphetamine withdrawal in rats

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Abstract. This experiment was designed to characterize the withdrawal syndrome produced by discontinuation of treatment with escalating, non-neurotoxic doses of *d*-amphetamine (AMPH). AMPH withdrawal was associated with both transient and persistent changes in behavior and postmortem brain tissue catecholamine concentrations. During the first week of withdrawal rats showed a significant decrease in spontaneous nocturnal locomotor activity. This behavioral depression was most pronounced on the first 2 days after the discontinuation of AMPH pretreatment, was still evident after 1 week, but had dissipated by 4 weeks. Behavioral depression was not due to a simple motor deficit, because AMPH-pretreated animals showed a normal large increase in locomotion when the lights initially went out, but they did not sustain relatively high levels of locomotor activity throughout the night, or show the early morning rise in activity characteristic of controls. Behavioral depression was associated with a transient decrease in the concentration of norepinephrine (NE) in the hypothalamus, and a transient decrease in the ability of an AMPH challenge to alter dopamine (DA) concentrations in the caudate-putamen and nucleus accumbens. AMPH pretreatment also produced persistent changes in brain and behavior. The persistent effects of AMPH were not evident in spontaneous locomotor activity, but were revealed by a subsequent challenge injection of AMPH. AMPH pretreated animals were markedly hyper-responsive to the stereotypy-producing effects of an AMPH challenge. This behavioral sensitization was not fully developed until 2 weeks after the discontinuation of AMPH pretreatment, but then persisted undiminished for at least 1 year. It is suggested that the transient changes in brain and behavior described here may represent an animal analogue of the "distress syndrome" seen in humans during AMPH withdrawal, which is associated with symptoms of depression and alterations in catecholamine function. On the other hand, persistent behavioral sensitization may be analogous to the enduring hypersensitivity to the psychotogenic effects of AMPH seen in former AMPH addicts.

Key words: Dopamine – Norepinephrine – Stimulant drugs – Depression – Amphetamine psychosis

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The abrupt discontinuation of chronic amphetamine (AMPH) use has both transient and persistent effects on behavior. In humans, the relatively transient "withdrawal" or "distress" syndrome is characterized by symptoms indicative of depression, including psychomotor alterations, dysphoria, anxiety, anhedonia and anergia (Utena 1966; Kramer et al. 1967; for review see Gawin and Ellinwood 1988). These symptoms usually dissipate in a few weeks, but there are also very persistent sequelae associated with AMPH abuse, characterized by a hypersensitivity (sensitization) to its psychotogenic effects (Utena 1966; Segal and Schuckit 1983). For example, re-exposure to a relatively low dose of AMPH, even after years of abstinence, will often reinstate psychotic symptoms in former AMPH addicts (Sato et al. 1983).

Post-AMPH withdrawal depression and AMPH psychosis are thought to reflect neuronal adaptations to chronic drug treatment, but it is difficult to study these in humans. Thus, animal models of post-AMPH withdrawal depression and AMPH psychosis have been used to identify and study changes in neural activity and behavior precipitated by the discontinuation of AMPH treatment (Segal and Schuckit 1983; Robinson and Becker 1986; Kokkinidis 1988). One popular model involves intermittent injections (1 or 2 times per day or less) of a relatively low (1–5 mg/kg) constant dose of AMPH (Magos 1969; Segal and Mandell 1974; Klawans and Margolin 1975). This paradigm has proven particularly valuable in studying behavioral sensitization and its neurobiological correlates (Robinson and Becker 1986 for review). Nevertheless, the constant low dose regimen has limitations as an animal model of human drug use.

Many AMPH addicts escalate their dose over time, eventually to quite high levels (Kramer et al. 1967; Ellinwood 1972), and the constant low dose regimen does not reflect this pattern of use. It may be important to mimic this pattern of use because the neurochemical effects of high doses of AMPH are altered by pretreatment with gradually escalating doses (Schmidt et al. 1985).

We recently reported that following the discontinuation of treatment with non-neurotoxic escalating doses of AMPH rats showed a depression in spontaneous nocturnal locomotor activity for up to 5–10 days, and a hypersensitivity to a challenge injection of AMPH for up to 21 days (Robinson and Camp 1987; Robinson et al. 1988). This treatment regimen may provide, therefore, a good model for studying both the transient changes in brain and behavior seen early after withdrawal from AMPH, perhaps reflecting the depressive symptomatology reported in humans, as well as the persistent changes in brain and behavior exemplified by sensitization and the susceptibility to AMPH psychosis. The temporal profile of changes in brain and behavior associated with withdrawal from non-neurotoxic escalating dose AMPH treatment has not been well characterized, however, and this was the purpose of the studies reported here.

Materials and methods

Subjects. Adult female Holtzman rats (Holtzman Co., Madison, WI) weighing 200–260 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 05:00 hours). The animals had free access to food and water.

Amphetamine pretreatment regimen. AMPH-pretreated rats received twice daily intraperitoneal injections of *d*-amphetamine sulfate (AMPH) in their home cage, with approximately 8 h separating the two injections. To mimic the pattern of drug use seen in addicts (repeated "runs" followed by a "crash"; Kramer et al. 1967) injections were given each weekday, but not on weekends, and the dose of AMPH escalated from 1 to 10 mg/kg (weight of the salt) over 42 days (30 injection days) according to the schedule illustrated graphically in Fig. 1. Control animals received 1 ml/kg 0.9% saline/injection.

Quantification of behavior. Spontaneous and drug-induced locomotor activity, and drug-induced stereotyped behavior, were quantified. Locomotor activity was quantified using automated activity monitors (41 × 24 × 18 cm) equipped with two pairs of infrared photocells mounted along the long axis of the cage, 5.0 cm above the cage floor and 25.2 cm apart from each other. Disruption of a beam registered a single count, but another count could not be registered by that beam until the second photocell beam at the other end of the cage was disrupted. Therefore, activity counts in this apparatus reflect locomotion ("crossovers") from one end of the cage to the other, and not the repetitive disruption of one beam.

Drug-induced stereotyped sniffing and stereotyped head and limb movements were rated independently by observers blind as to pretreatment condition, using the following five point rating scale: 0) normal in place activity, 1) mild, discontinuous stereotyped behavior (sniffing or repetitive head and limb movements), 2) moderate, discontinuous stereotyped behavior, 3) moderate, continuous stereotyped behavior and 4) intense, continuous stereotyped behavior directed at one place. The two observers assigned the same rating on 91.2% of the rating intervals.

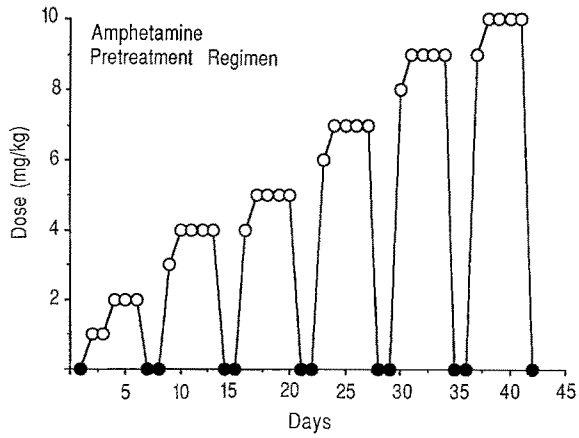

Fig. 1. Graphic representation of the escalating dose amphetamine (AMPH) pretreatment regimen (see Methods). Each *open circle* represents: (1) a day on which AMPH pretreated animals received two injections of *d*-AMPH sulfate with each injection separated by at least 8 h, and (2) the dose of each injection. On days when the dose equals zero *(closed circles)* animals did not receive injections. Control animals received saline injections according to the same schedule. This regimen mimics to some extent the pattern of "runs" and "crashes" seen in addicts (Kramer et al. 1967)

Experiment 1: Withdrawal from AMPH: effects on the behavioral response to a subsequent AMPH challenge. At 2, 6, 13, 27, 89 or 179 days following the cessation of AMPH pretreatment independent groups of animals ($N = 5$–7/group) were placed individually in the activity monitors described above, and allowed to habituate overnight. The next morning at 08:30 hours baseline locomotor activity was recorded for 1 h, after which all animals received a challenge injection of 2.6 mg/kg AMPH. AMPH-induced locomotion was measured over 5-min intervals for the next 3 h, and stereotyped behavior was rated at 10 min following drug injection and every 20 min thereafter, until ten rating scores had been obtained. A challenge dose of 2.6 mg/kg was used because pilot studies established this was the highest dose that produced primarily heightened locomotion (and not focused stereotyped behavior) in naive animals. With this challenge dose, therefore, behavioral sensitization is apparent as a *qualitative* change in behavior, i.e., the emergence of focused stereotyped behavior in AMPH pretreated animals (Segal 1975). A separate group of animals were also tested 1 year after the discontinuation of pretreatment (see Results). We did not "challenge" a group of animals with saline because in previous experiments and pilot studies using this apparatus we found that this had negligible effect (e.g., see Fig. 3 in Camp and Robinson 1988), and that baseline scores provided just as meaningful a comparison.

Experiment 2: Withdrawal from AMPH: effects on spontaneous day/night locomotor activity, the response to an AMPH challenge and the postmortem tissue concentrations of monoamines and monoamine metabolites. In a second experiment animals were pretreated with AMPH or saline, as described above, or left unhandled. One subgroup of animals was placed into the activity monitors immediately after receiving their last pretreatment injection and spontaneous locomotor activity was monitored continuously for 21.5 h per day over 30-min intervals, for either the next 3 or 7 days (separate subgroups). A third group was placed into the monitors 22 days after the cessation of drug treatment and spontaneous locomotor activity monitored between days 23 and 28 (inclusive). After this, half the animals in each of the three subgroups received a challenge injection (IP) of 2.6 mg/kg AMPH, and half received an injection of saline (between 08:45 and 10:00 hours; N = at least 8/group). Stereotyped behavior was rated only once, 40 min after the injection. All animals were then killed immediately by decapitation and brain tissue obtained for neurochemical analysis. Animals were

killed 40 min after the drug challenge because it was determined in experiment 1 that AMPH had its peak behavioral effect at this time.

Postmortem tissue assay. Following decapitation the brain was rapidly removed and placed into ice cold saline for 30–45 s. It was then placed into a chilled cutting block and coronal sections were obtained as described by Heffner et al. (1980). The following regions were dissected in ice-cold saline: (a) medial frontal cortex, consisting of the DA-rich anteromedial portion of the frontal cortex from the genu of the corpus callosum to the frontal pole; (b) nucleus accumbens, removed with a 2 mm diameter micropunch; (c) caudate-putamen, the corpus of the striatum was removed with a 3 mm micropunch; and (d) hypothalamus: the entire hypothalamus was removed by making a horizontal cut at the level of the rhinal fissure and vertical cuts lateral to the optic tracts (Heffner et al. 1980). Tissue from the left and right hemispheres was pooled, weighed and placed into tubes containing 0.05 N $HClO_4$, with dihydroxybenzylamine added as an internal standard. The tissue was assayed for norepinephrine (NE), dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), 5-hydroxytryptamine (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA), using HPLC and electrochemical detection, although all compounds were not detectable in all structures.

Data analysis. Locomotor and neurochemical data were analyzed using parametric statistics, including analyses of variance and Fisher's Least Significant Difference (LSD) test for follow-up pairwise comparisons (Winer 1971). Because stereotyped behavior rankings only comprise an ordinal scale, these were analyzed using nonparametric statistics, including the Kruskal-Wallis and Mann-Whitney *U* tests. To control for multiple Mann-Whitney *U* tests a conservative significance level of $\alpha = 0.01$ was used, and all comparisons were based on two-tailed probabilities.

Results

Withdrawal from AMPH: Effects on the behavioral response to a subsequent AMPH challenge

The ability of an AMPH challenge to produce stereotyped behavior at different points in time after the discontinuation of chronic AMPH treatment is illustrated in Fig. 2. The same results were obtained for stereotyped sniffing as for stereotyped head and limb movements, and therefore only the ratings for stereotyped head and limb movements are shown. The AMPH challenge did not produce continuous (focused) stereotyped behavior at any time following the termination of pretreatment in saline pretreated rats (i.e., the peak stereotypy rating was always <2). Three days after the discontinuation of pretreatment an AMPH challenge also failed to induce focused stereotyped behavior in AMPH-pretreated rats, and there was no significant difference between the two groups at this point in time (Fig. 2). By 7 days of withdrawal there was a small, but statistically significant, difference in the stereotypy ratings of saline- and AMPH-pretreated animals, although AMPH-pretreated rats still did not show focused stereotypy. In contrast, a marked effect of AMPH pretreatment was apparent by 14 days, and this persisted undiminished for at least 6 months. During this latter period (14–180 days after discontinuation of pretreatment) the AMPH challenge produced intense, focused stereotyped behavior in AMPH-pretreated animals, but not in controls (Fig. 2).

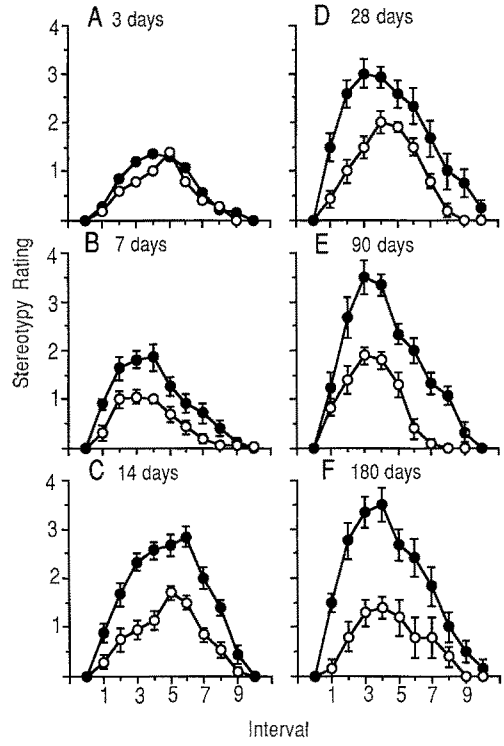

Fig. 2A–F. Mean (±SEM) ratings for stereotyped head and limb movements for saline *(open symbols)*- and AMPH *(closed symbols)*- pretreated rats before (interval 0) and after a challenge injection of 2.6 mg/kg AMPH given 3, 7, 14, 28, 90 or 180 days after the discontinuation of pretreatment ($N = 5$–7/group). Mann-Whitney *U* tests were used to compare the cumulative rating for each AMPH-pretreated group to its respective control group. There was no difference in AMPH-induced stereotyped behavior between saline and AMPH-pretreated animals 3 days after discontinuation of AMPH pretreatment ($U = 14$, $P = 0.6$). By 7 days of withdrawal AMPH-pretreated animals had significantly higher stereotypy ratings than did saline-pretreated animals ($U = 13$, $P < 0.01$). Significant group differences were also found 14, 28, 90 and 180 days after the discontinuation of AMPH pretreatment (U's = 0–3, $P < 0.005$). ● Amphetamine – pretreatment; ○ control

Similar group differences were apparent in the automated measure of locomotor activity. Fig. 3 shows that the AMPH challenge increased locomotion in saline-pretreated rats at all times after the discontinuation of pretreatment. Similarly, at 3 and 7 days after the discontinuation of pretreatment the AMPH challenge produced only locomotor hyperactivity in AMPH-pretreated rats, and there were no significant group differences. But by 14 days a marked effect of AMPH pretreatment was apparent, and this persisted undiminished for at least 180 days (Figs. 3 and 4). Between 14 and 180 days AMPH-pretreated rats showed complex multiphasic changes in locomotor activity in response to an AMPH challenge, characterized in part by an initial increase in locomotion (5–15 min) followed by a marked decline in locomotion (20–60 min). During the period of reduced locomotion AMPH-pretreated animals engaged in focused stereotyped behavior (compare Figs. 2 and 3). Thus, the magnitude of the decrease in locomotor activity during the "stereotypy phase" provides an objective indicator of the ability of an AMPH challenge to produce

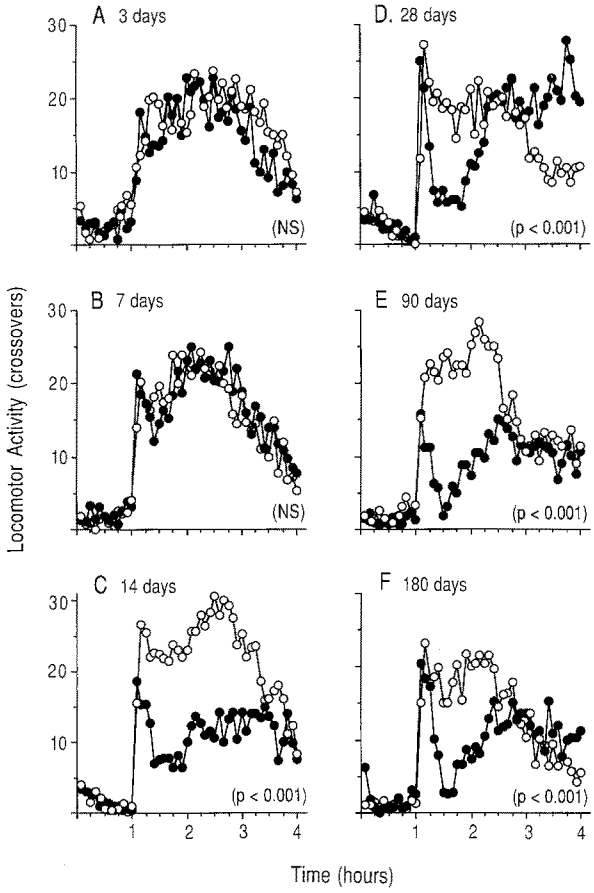

Fig. 3A–F. The average number of crossovers (locomotion from one side of the cage to the other) cumulated over 5-min intervals during 1 h of baseline and for 3 h after a challenge injection of 2.6 mg/kg AMPH, in saline *(open symbols)*- and AMPH *(closed symbols)*-pretreated animals tested 3, 7, 14, 28, 90 or 180 days after the discontinuation of pretreatment ($N=6$–7/group). There was no difference in baseline locomotor activity between saline- and AMPH-pretreated animals at any time following the cessation of AMPH pretreatment (2-way ANOVAs). Two-way ANOVAs (with repeated measures) were also used to compare AMPH-stimulated locomotor activity for each AMPH-pretreated group to its respective control group. There was no effect of AMPH pretreatment on AMPH-induced locomotor activity 3 or 7 days after the discontinuation of AMPH pretreatment (F's <0.5; NS = nonsignificant). Significant group differences were apparent by 14 days of withdrawal ($F=2.03$, $P<0.001$), because AMPH-pretreated rats showed a significant decline in locomotor activity during the period from about 20–60 min after drug injection, during which time they engaged in focused stereotyped behavior (all significant F values represent a group by time interaction; see text and Fig. 2). Significant group differences were also found 28 ($F=3.4$, $P<0.001$), 90 ($F=3.4$, $P<0.001$) and 180 days ($F=3.67$, $P<0.001$) after the discontinuation of AMPH pretreatment. ● Amphetamine – pretreated; ○ control

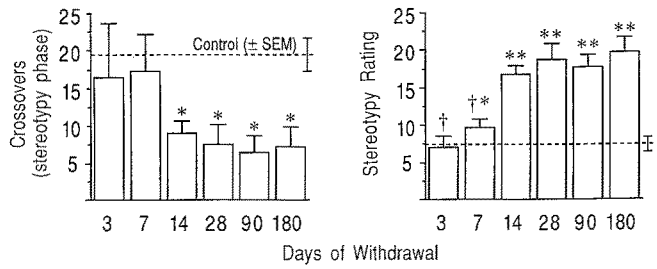

Fig. 4. Summary of the effect of pretreatment with saline or AMPH on stereotypy phase locomotor activity *(left)* and stereotypy ratings *(right)* produced by a challenge injection of 2.6 mg/kg AMPH given 3, 7, 14, 28, 90 or 180 days after discontinuation of pretreatment. *Left:* Bars in the left panel depict the average (+SEM) number of crossovers cumulated during the stereotypy phase (10–60 min post-injection) for AMPH-pretreated animals withdrawn for 3–180 days. The *horizontal dashed line* represents the average number of crossovers for the pooled control group ($n=38$) and the vertical line to the right ± SEM for the controls. A one-way analysis of variance comparing all groups was significant ($F=3.58$, $P<0.004$). The *asterisks* (*) indicate that relative to the control group AMPH-pretreated animals showed a significant decrease in stereotypy phase locomotor activity 14, 28, 90 and 180 days, but not 3 or 7 days, after the cessation of AMPH pretreatment ($P<0.05$, Fisher's LSD tests). No other comparisons were statistically significant. *Right:* Bars in the right panel depict the average (+SEM) rating for stereotyped head and limb movements cumulated over the entire test session following an AMPH challenge in AMPH-pretreated rats withdrawn for 3–180 days. For ease of comparison the average stereotypy rating for the pooled control group is indicated by the horizontal dashed line and ± SEM by the vertical line to the far right. The *asterisks* (*) indicate that AMPH-pretreated rats had significantly higher stereotypy ratings than controls between 7 and 180 days of withdrawal, but not after 3 days of withdrawal (* $P<0.01$; ** $P<0.005$,; see Fig. 2 for U values). The dagger (†) indicates that animals withdrawn for 3 or 7 days (which did not differ from one another, $U=24$) had a significantly lower cumulative stereotypy rating than did those withdrawn for 14–180 days (Kruskal-Wallis test across the six AMPH-pretreated groups, $H=27$, $df=5$, $P<0.001$; follow-up Mann-Whitney U tests, U's = 1–9, $P<0.01$)

focused stereotyped behavior (Segal 1975). The effects of an AMPH challenge on both stereotypy ratings and "stereotypy phase" locomotor activity are summarized as a function of time after the discontinuation of pretreatment in Fig. 4.

A third characteristic of AMPH-induced locomotor activity often seen in AMPH-pretreated animals is the phenomenon of post-stereotypy hyperactivity (Segal 1975). This refers to a second period of increased locomotion seen following the stereotypy phase. Interestingly, only AMPH-pretreated rats withdrawn for 28 days showed significant post-stereotypy hyperactivity (see figure 3D). This pattern of behavior was not seen in control animals at any time, or in AMPH-pretreated animals 3–14 days or 3–6 months after the discontinuation of pretreatment (also see Leith and Kuczenski 1982).

Finally, an independent group of animals, as part of a separate experiment, received the same AMPH pretreatment regimen described above, and were then left undisturbed for 1 year. After the last pretreatment injection these animals were housed in pairs to minimize the stress of isolation. One year following the cessation of AMPH pretreatment the animals received a challenge injection of 2.6 mg/kg (IP) of AMPH, and behavior was recorded as described above. AMPH-pretreated rats showed a significantly greater response to the AMPH challenge than did saline-pretreated controls, as indicated by both stereotypy ratings and a period of reduced locomotor activity 20–90 min after the AMPH challenge (Fig. 5). Furthermore, the enhanced response was as great as that seen between 2 weeks and 6 months

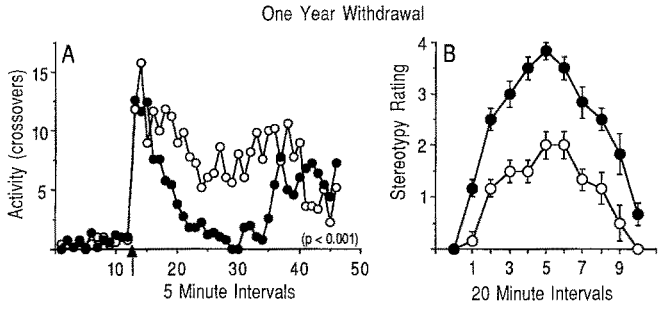

Fig. 5A, B. The effect of pretreatment with saline (*open symbols*, $n=6$) or AMPH (*closed symbols*, $n=6$) on crossovers (locomotor activity) and stereotyped behavior after a challenge injection of 2.6 mg/kg AMPH (IP) given 1 year after the cessation of pretreatment. *Left:* The left panel shows the average number of crossovers cumulated over 5-min intervals during baseline and for 2 h and 50 min after the AMPH challenge (given when indicated by the *arrow*). AMPH-pretreated rats differed significantly from saline pretreated rats, primarily because the former group showed a large decrease in locomotion about 30 min after the challenge injection (2-way ANOVA with repeated measures, group by time interaction, $F=2.09$, $P<0.001$). *Right:* The right panel depicts the mean (±SEM) stereotypy ratings (head and limb movements) obtained during baseline (interval 0) and then repeatedly after the challenge injection of AMPH (see Methods). AMPH-pretreated animals showed significantly more intense stereotypy than saline-pretreated animals (Mann-Whitney U test on the cumulative ratings, $U=0$, $P<0.002$). ● Amphetamine – pretreated; ○ control

after the discontinuation of pretreatment (compare Fig. 5 with Figs 2, 3 and 4).

Withdrawal from AMPH: Effects on spontaneous day/night locomotor activity

Data from this experiment were first analyzed to determine if there was any effect of saline pretreatment, i.e., the nonhandled and saline-pretreated controls were compared. There was no difference between nonhandled and saline-pretreated animals on any measure, and therefore these groups were pooled to form one control group for all subsequent analyses. In addition, to simplify data presentation, spontaneous locomotor activity in AMPH-pretreated and control rats were averaged over days 2–3, days 4–7 or days 24–28 to form just three AMPH "withdrawal" groups.

Figure 6 shows the average spontaneous locomotor activity counts ("crossovers") for control and AMPH-

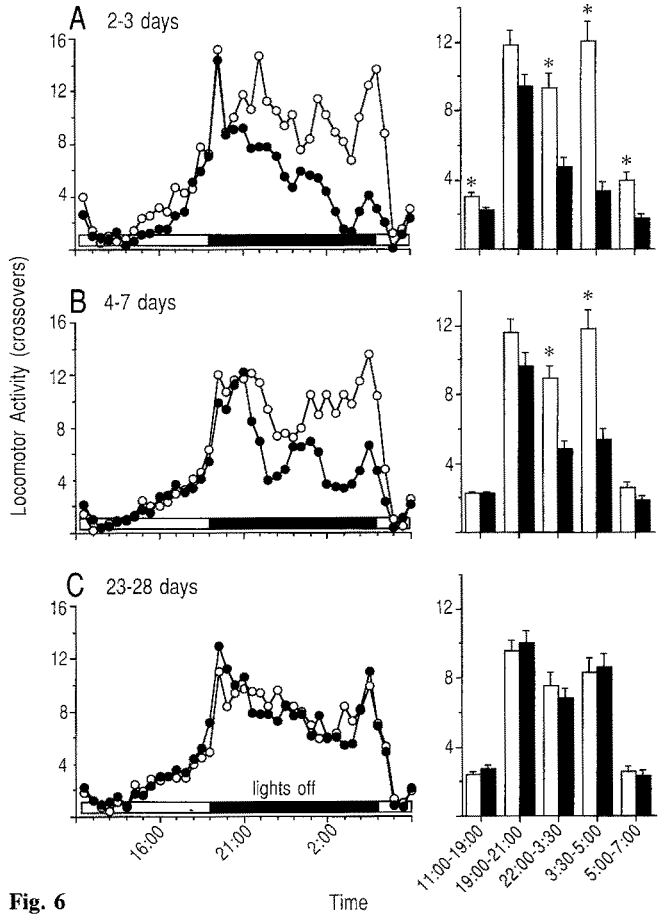

Fig. 6

and the 2 h after the lights came back on (5:00–7:00 hours). Data for the remaining 3.5 daytime hours were excluded because of the disturbance associated with data collection and animal care that occured at this time. **A** 2–3 days. AMPH-pretreated animals were significantly less active than controls both during the daytime (2-way ANOVA with repeated measures on the initial 10 h lights-on period, effect of group, $F=5.38$, $P<0.024$, effect of time, $F=24.8$, $P<0.001$, interaction non-significant), and at night (ANOVA on lights-off period, group $F=15.8$, $P<0.001$, time $F=11.2$, $P<0.001$ and interaction $F=3.4$, $P<0.001$). The significant group by time interaction for nocturnal activity indicates that AMPH-pretreated animals did not differ from controls throughout the entire night, and this is best illustrated in the bar graph to the right. In the *right panel asterisks* (*) indicate times at which AMPH-pretreated animals differed significantly from controls. For the initial daytime period $t=2.32$, $P=0.024$, but during the initial lights-off period (19:00–22:00 hours) there were no significant group differences ($t=1.82$). During the rest of the night AMPH-pretreated rats were significantly less active than controls (22:00–3:30 hours, $t=3.93$, $P<0.001$; 3:30–5:00, $t=5.85$, $P<0.001$), and they remained less active after the lights came on again (5:00–7:00, $t=3.01$, $P=0.004$). **B** 4–7 days. AMPH-pretreated animals were not significantly less active than controls during the lights-on period (ANOVA) but were significantly hypoactive at night (ANOVA on nocturnal activity, group $F=17.7$, $P<0.001$, time $F=5.42$, $P<0.001$, interaction $F=2.07$, $P<0.004$). Again, the panel to the *right* shows that there were no significant group differences during the initial lights-off period (19:00–22:00 hours, $t=2.01$), but during the rest of the night AMPH-pretreated rats were significantly less active than controls (22:00–3:30 hours, $t=4.47$, $P<0.001$; 3:30–5:00 hours, $t=4.05$, $P<0.001$). **C** 24–28 days. By 24–28 days there was no longer any effect of AMPH pretreatment on either daytime or nocturnal activity. For nocturnal activity, the group $F<1.0$ and the group by time interaction $F=1.13$, $P=0.32$. ● ■ Amphetamine – pretreated; ○ □ control

Fig. 6A–C. Mean spontaneous locomotor activity in AMPH-pretreated *(closed symbols)* and control animals *(open symbols)*: **A** 2–3 days ($N=32$ and 24, respectively), **B** 4–7 days ($N=16$/group) and **C** 23–28 days ($N=16$/group) after the discontinuation of pretreatment. The panels on the *left* represent the average number of crossovers cumulated over 30-min intervals across the day/night cycle. The 10 h lights off period, which began at 19:00 hours, is illustrated by the *solid black bar* on the horizontal axis, and daytime hours by the *open bar*. The panels on the *right* show the average (+SEM) number of crossovers cumulated over 7 h before the lights went off (11:00–19:00 hours), the initial 3 h after the lights went off (19:00–22:00 hours), the middle of the night (22:00–3:30 hours), the last 1.5 h prior to the lights going on (3:30–5:00 hours)

Table 1. The mean (± SEM) postmortem tissue concentrations of monoamines and their metabolites, expressed in ng/mg wet tissue weight, in control animals that received a "challenge" injection of saline

	NE	DA	DOPAC	HVA	5-HT	5-HIAA
Caudate-putamen	0.09 ± 0.01	20.87 ± 0.59	3.20 ± 0.09	1.39 ± 0.08	–	–
Nucleus accumbens	0.44 ± 0.02	12.49 ± 0.40	3.46 ± 0.09	1.48 ± 0.16	–	–
Medial frontal cortex	1.59 ± 0.06	0.23 ± 0.02	–	–	0.91 ± 0.07	0.56 ± 0.07
Hypothalamus	7.8 ± 0.19	0.93 ± 0.05	0.16 ± 0.02	–	1.27 ± 0.12	1.01 ± 0.13

In Figs. 7 and 8 the postmortem tissue values for all other groups are expressed as a percent of these values. A dash indicates the compound was not quantified

Abbreviations: NE, norepinephrine; DA, dopamine; DOPAC, dihydroxyphenylacetic acid; HVA, homovanillic acid; 5-HT, 5-hydroxytryptamine; 5-HIAA, 5-hydroxyindoleacetic acid

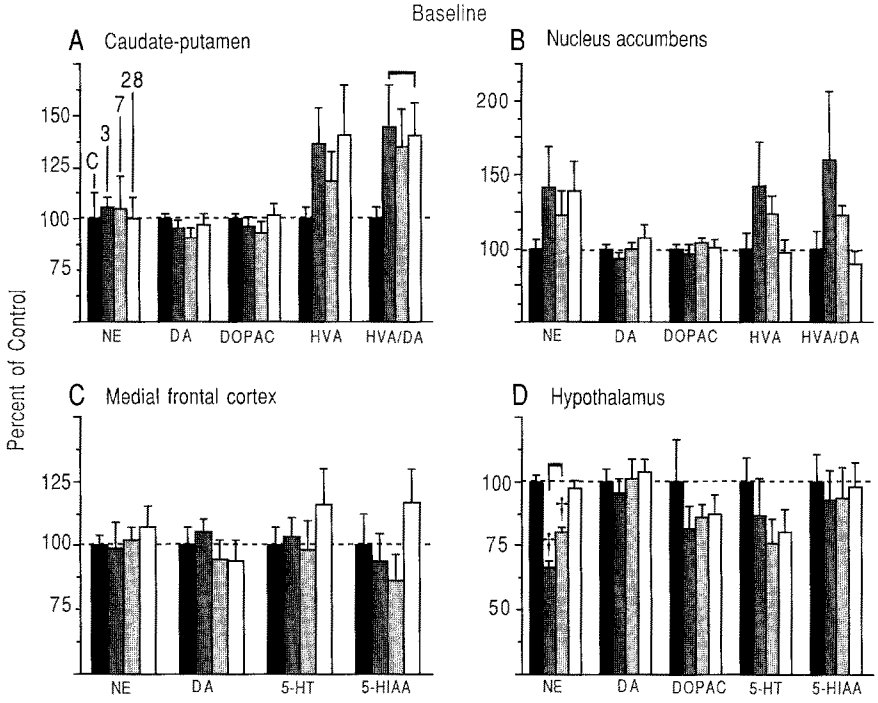

Fig. 7A–D. The effect of pretreatment with escalating doses of AMPH on the basal (steady state) postmortem tissue concentrations of monoamines and their metabolites in selected brain regions (N = at least 8/group). The height of the bars represents the mean (+SEM) concentration of compounds plotted as a percent of the control group that received a saline challenge (see methods and Table 1). The *bars* in each panel, from left to right, indicate control (*C*) or AMPH-pretreated animals 3, 7, or 28 days after the discontinuation of pretreatment. For each compound in each structure statistically significant group differences were determined by conducting a one-way analysis of variance, and if indicated by a significant F value ($P < 0.05$), pairwise comparisons were made using the Fisher's LSD test. A *heavy horizontal line* above some bars indicates significant group differences based on the ANOVA. A *vertical line* extending downwards from the heavy horizontal line indicates those groups that differed significantly from control, based on the Fisher's LSD test ($P < 0.05$). A dagger (†) indicates those groups that differed significantly from AMPH-pretreated animals withdrawn for 28 days, again based on the Fisher's test ($P < 0.05$). **A** Caudate-putamen: the only significant effect was in the ratio of HVA/DA ($F = 2.94$, $P < 0.05$), and only AMPH-pretreated rats withdrawn for 3 or 28 days differed significantly from control ($P < 0.05$). For HVA, $F = 2.1$, $P = 0.12$, and for all other compounds $F < 1.0$. **B** Nucleus accumbens: there was no significant effect of AMPH pretreatment for any compound. **C** Medial frontal cortex: there was no significant effect of AMPH pretreatment for any compound. All F's < 1.0. **D** Hypothalamus: the only significant ANOVA was for NE ($F = 33.2$, $P < 0.001$). AMPH-pretreated rats withdrawn for 3 or 7 days had significantly lower hypothalamic NE concentrations than either controls or rats withdrawn for 28 days ($P < 0.05$). Hypothalamic NE in the 3-day group was also significantly lower than in the 7-day group ($P < 0.05$). The group withdrawn for 28 days did not differ from controls. For all other compounds $F < 1.0$.

pretreated animals across the light-dark cycle. Control animals showed the same pattern of day/night locomotor activity at all times following the discontinuation of pretreatment (Fig. 6). This was characterized by relatively little locomotion during the day, a slow increase in locomotion as night approached, a large peak in activity immediately following lights-off, a decrease in activity during the middle of the night (but not to daytime levels), and finally, a second peak in locomotion 1–2 h prior to lights-on. When the lights came on again locomotor activity fell to very low levels.

AMPH-pretreated animals were significantly less active than controls (Fig. 6). Between 2 and 3 days after the discontinuation of AMPH pretreatment AMPH-pretreated rats were less active than controls during both the day and night periods (Fig. 6A), but by 4–7 days only nocturnal locomotor activity was depressed (Fig. 6B). AMPH-pretreated rats showed a normal large increase

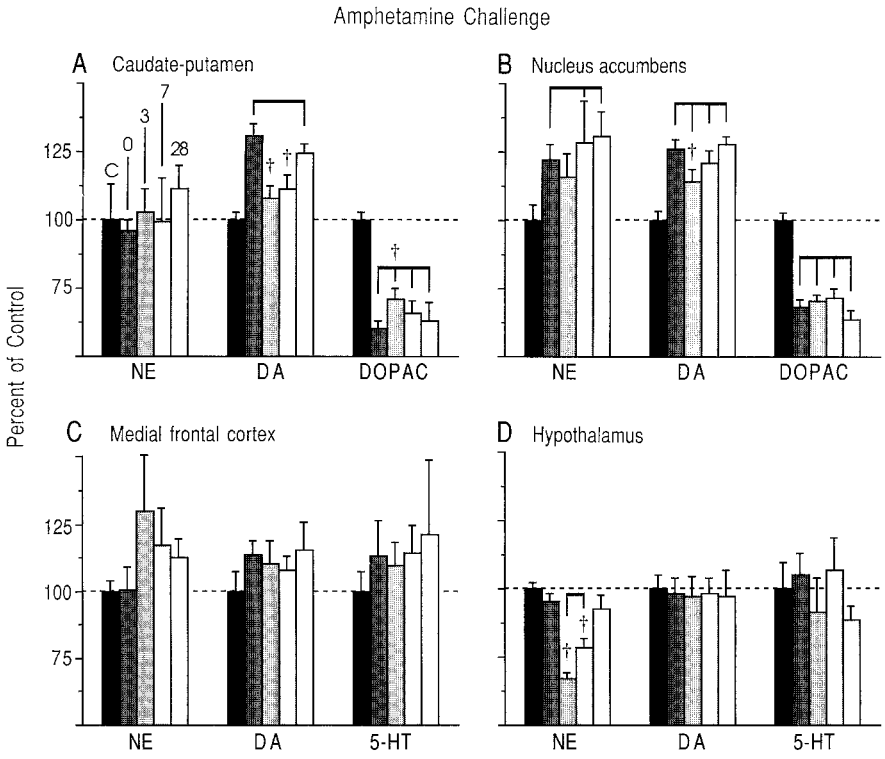

Fig. 8A–D. The effect of pretreatment with escalating doses of AMPH on the postmortem tissue concentrations of monoamines and their metabolites in selected brain regions 40 min following a challenge injection of 2.6 mg/kg AMPH. The height of the bars represents the mean (+SEM) concentration of the compounds plotted as a percent of control animals that did not receive an AMPH challenge (see Table 1). The bars in each panel, from left to right, indicate: (*1*) control animals that did not receive an AMPH challenge (group C; $N = 16$); (*2*) *control animals that received an AMPH challenge (group 0;* $N = 15$); and AMPH-pretreated animals that received a challenge injection of AMPH, (*3*) 3 days (group 3; $N = 8$); (*4*) 7 days (group 7; $N = 8$); or (*5*) 28 days (group 28; $N = 8$) after the discontinuation of pretreatment. A *heavy horizontal line* above some bars indicates there were significant group differences for that compound, based on a one-way ANOVA. A *vertical line* extending downwards from the heavy horizontal line indicates groups that differed significantly from control animals that received saline (Fisher's test, $P < 0.05$). The daggers (†) indicate groups that differed significantly from the control animals that received AMPH (group 0). **A** Caudate-putamen: for NE, $F < 1.0$ (non-significant). For DA, $F = 9.06$, $P < 0.001$. The challenge injection of AMPH significantly increased DA concentrations in control animals, and AMPH-pretreated animals withdrawn for 28 days; but not in AMPH-pretreated rats withdrawn for 3 or 7 days. DA concentrations in AMPH-pretreated rats withdrawn for 3 or 7 days were significantly less than in control animals challenged with AMPH. The 3-day group also differed significantly from the 28-day group ($P < 0.05$). For DOPAC, $F = 31.5$, $P < 0.001$. The AMPH challenge significantly decreased DOPAC in all groups ($P < 0.05$), but in AMPH-pretreated rats withdrawn for 3 days the effect of the AMPH challenge was significantly less than in control animals. **B** Nucleus accumbens: for NE, $F = 2.54$, $P < 0.05$. The AMPH challenge significantly increased NE concentrations in control rats, and in AMPH-pretreated rats withdrawn for 7 or 28 days. There was no significant effect of AMPH pretreatment. For DA, $F = 9.33$, $P < 0.001$. The AMPH challenge significantly elevated DA in all groups, but to a significantly lesser extent in the 3-day group than in controls. For DOPAC, $F = 31.1$, $P < 0.001$. The AMPH challenge significantly decreased DOPAC in all groups, and there was no effect of AMPH pretreatment. **C** Medial frontal cortex: there was no significant effect of AMPH pretreatment or the AMPH challenge on any compound. **D** Hypothalamus: for NE, $F = 19.1$, $P < 0.001$. The AMPH challenge did not have a significant effect on NE concentrations in control animals, or the 28-day group. However, NE was significantly decreased in the 3- and 7-day groups, relative to the saline challenge control group, the AMPH challenge control group, and the 28-day withdrawn group ($P < 0.05$). In addition, the 3- and 7-day groups differed significantly from one another (Fisher's LSD tests, $P < 0.05$). There was no significant effect of the AMPH challenge or AMPH pretreatment on DA or 5–HT concentrations in the hypothalamus (F's < 1.0)

in locomotor activity when the lights first went out, but this was not sustained, and during the middle and end of the lights-off period locomotor activity was markedly depressed in AMPH-pretreated rats (Fig. 6A, B). By 4 weeks later group differences in nocturnal locomotor activity were no longer apparent (Fig. 6C).

The behavioral effects of the 2.6 mg/kg AMPH challenge were similar to experiment 1. As expected, the AMPH challenge produced significantly greater stereotyped behavior 28 days after the discontinuation of pretreatment than after 3 days; although in contrast to experiment 1 animals withdrawn for 3 days had significantly higher stereotypy ratings than controls. As in experiment 1, however, only the 28-day group showed a significant decrease in "stereotypy phase" locomotor activity (data not shown).

Withdrawal from AMPH: Effects on the postmortem tissue concentrations of monoamines and monoamine metabolites

Table 1 shows the average concentrations (ng/mg) of monoamines and their metabolites in control animals

"challenged" with saline (3, 7 and 28 day groups pooled). Figure 7 shows the average postmortem tissue concentrations of monoamines and their metabolites in AMPH-pretreated animals "challenged" with saline 3, 7 or 28 days after the cessation of pretreatment. These latter values are expressed as a per cent of the average levels in saline "challenged" control animals; i.e., as a percent of the values in Table 1. The only statistically significant changes in the basal postmortem tissue levels of these compounds were: (1) an increase in the ratio of HVA/DA in the caudate-putamen of AMPH-pretreated rats withdrawn for 3 or 28 days (Fig. 7A); and (2) a decrease in hypothalamic NE concentrations 3 and 7 days after the discontinuation of pretreatment. By 28 days hypothalamic NE concentrations had returned to normal (Fig. 7D). Based on predictions from previous studies involving stress-induced behavioral depression (Weiss et al. 1980), we also tested for a correlation between NE concentrations and locomotor activity. As predicted, there was a significant positive correlation between hypothalamic NE concentrations and late night locomotor activity ($r = +0.47$, $F = 10.6$, $P < 0.003$). In contrast, nucleus accumbens NE concentrations were negatively correlated with late night locomotor activity ($r = -0.30$, $F = 3.9$, $P = 0.056$).

Figure 8 shows the effect of an AMPH challenge on the average postmortem tissue concentrations of monoamines in AMPH-naive control animals, and AMPH-pretreated rats withdrawn for 3, 7 or 28 days. The data are again expressed as a percent of control animals "challenged" with saline. Thus, comparison of control animals "challenged" with saline (group C in Fig. 8) and control animals challenged with AMPH (group 0 in Fig. 8 – for zero withdrawal) illustrates the effect of an AMPH challenge in AMPH-naive animals. This can be compared with the effects of an AMPH challenge in AMPH-pretreated rats that received their last pretreatment injection 3, 7 or 28 days earlier (groups 3, 7 and 28 in Fig. 8). In the caudate-putamen (Fig. 8A) there was no effect of AMPH pretreatment or the AMPH challenge on NE concentrations. The AMPH challenge did produce a significant increase in caudate-putamen DA concentrations in AMPH-naive control animals (group 0), and this was significantly attenuated in AMPH-pretreated animals 3 and 7 days after the discontinuation of drug treatment, but not 28 days later. In fact, the 3 and 7 day groups did not differ significantly from the saline "challenge" control group (group C in Fig. 8A). The AMPH challenge also produced a large decrease in caudate-putamen DOPAC concentrations in all groups, and this effect was significantly attenuated in AMPH-pretreated rats withdrawn for only 3 days (Fig. 8A).

The AMPH challenge elevated NE concentrations in the nucleus accumbens in all groups, except the 7-day group, but there was no statistically significant effect of AMPH pretreatment (Fig. 8B). As in the caudate-putamen, however, DA concentrations in the nucleus accumbens were elevated to a lesser extent in AMPH-pretreated rats withdrawn for 3 days than in the control animals challenged with AMPH, and this effect was no longer evident at 28 days. The AMPH challenge decreased nucleus accumbens DOPAC concentrations to the same extent in all groups.

In the frontal cortex there was no significant effect of AMPH pretreatment or the AMPH challenge on the concentrations of NE, DA or 5–HT (Fig. 8C). In the hypothalamus (Fig. 8D) the AMPH challenge did not affect NE concentrations in AMPH-naive control rats, but in AMPH-pretreated rats NE concentrations were significantly decreased 3 and 7, but not 28 days, after the discontinuation of pretreatment. There was no significant effect of AMPH pretreatment or the AMPH challenge on DA or 5–HT concentrations in the hypothalamus.

Discussion

The discontinuation of chronic escalating dose AMPH treatment resulted in both transient and persistent changes in brain and behavior. Spontaneous nocturnal locomotor activity was transiently depressed during AMPH withdrawal, especially towards the end of the lights-off period. This behavioral depression was most pronounced during the first 2 days of withdrawal, was still evident up to 1 week later, but had dissipated within 4 weeks. Catecholamine concentrations in some structures were altered in association with nocturnal hypoactivity. For example, the basal postmortem tissue concentration of NE was significantly reduced in the hypothalamus 3 and 7 days after the cessation of AMPH pretreatment, but returned to control levels by 4 weeks. Transient changes in caudate-putamen and nucleus accumbens DA concentrations were only evident after a challenge injection of AMPH. In control animals an AMPH challenge increased the postmortem tissue concentration of DA in the caudate-putamen and nucleus accumbens, but this effect was significantly attenuated in AMPH-pretreated animals between 3 and 7 days of withdrawal. Four weeks later an AMPH challenge increased DA concentrations to the same extent in AMPH-pretreated and control animals. In contrast, there was no effect of AMPH withdrawal on 5–HT concentrations in any of the structures studied.

Persistent behavioral changes produced by past experience with AMPH were not seen in *spontaneous* locomotor activity, but became apparent when animals received a subsequent challenge injection of AMPH. Pretreatment with escalating doses of AMPH produced a marked behavioral hypersensitivity to a challenge injection of AMPH. With the challenge dose of AMPH used here behavioral sensitization was characterized by more intense AMPH-induced stereotyped behavior (stereotyped sniffing and stereotyped head and limb movements) in AMPH-pretreated animals than in saline-pretreated controls. Interestingly, for the first week of withdrawal, during which time spontaneous locomotor activity was depressed, there was no difference between AMPH pretreated and control animals in their locomotor response to an AMPH challenge, and focused stereotypy was not evident in either group. By 2 weeks, however, behavioral sensitization was fully developed

and AMPH-pretreated animals showed a much larger response to the AMPH challenge than controls – and this hypersensitivity to AMPH persisted undiminished for at least 1 year. The only persistent change in brain monoamine or monoamine metabolite concentrations detected here was in the ratio of HVA to DA in caudate-putamen, which was significantly elevated in AMPH-pretreated rats 3 and 28 days after the discontinuation of AMPH pretreatment (Robinson and Camp 1987; also see Camp and Robinson 1988).

Withdrawal from AMPH – transient behavioral depression

There have been relatively few studies on the behavioral and neurochemical consequences of AMPH withdrawal. Behavioral depression has been reported after the discontinuation of continuous access to AMPH in drinking water (Herman et al. 1971; Tonge 1974; Lynch et al. 1977; Lynch and Leonard 1978), but in these studies it is difficult to ascertain exactly what dose the animal received, whether it was neurotoxic, or under what conditions behavioral depression was manifest. Nocturnal hypoactivity has also been reported following intermittent injections of a constant low dose of AMPH (Segal and Mandell 1974), but this effect was not as pronounced or persistent as reported here. Nevertheless, one consistent characteristic of the AMPH withdrawal syndrome appears to be a transient behavioral depression that lasts for varying lengths of time, depending on the nature of the pretreatment regimen (Utena 1966). Other features of the AMPH withdrawal syndrome that may be related to behavioral depression include changes in motivational and affective state. For example, the discontinuation of chronic treatment with AMPH or cocaine results in deficits in self-stimulation reward (Leith and Barrett 1976; Simpson and Annau 1977; Kokkinidis and Zacharko 1980; Cassens et al. 1981; Kokkinidis et al. 1986), operant behavior reinforced by a sweetened drinking solution (Carroll and Lac 1987) and reactivity to novel stimuli (Schreiber et al. 1976).

The behavioral depression described here was not a simple disturbance in motor function, because the animals were fully capable of generating high levels of locomotor activity (also see Kokkinidis et al. 1986). AMPH-pretreated animals showed a normal large increase in locomotor activity when the lights first went out, but as the night progressed they did not sustain as high a level of activity as controls, and did not show a normal increase in locomotion during the last hour and a half of the lights-off period. It has been suggested that the initial increase in locomotor activity seen just after the lights go off, and the early morning increase in activity seen just before the lights go on, are mediated by two independent circadian oscillators (Pittendrigh and Daan 1976). It is interesting to speculate, therefore, that one oscillator may be more susceptible to disruption by AMPH pretreatment than the other, thus accounting for the differential effect of AMPH pretreatment on early versus late night locomotor activity. Indeed, studies in which animals were given continuous access to AMPH in their drinking water suggest that AMPH can have different effects on these two oscillators (Honma et al. 1986), but it is difficult to compare studies using such different treatment regimens.

The inability of AMPH-pretreated animals to sustain high levels of locomotor activity throughout the night is reminiscent of the behavioral depression produced by exposure to uncontrollable stress. There are a number of different chronic stress paradigms that have been used to model clinical depression, and all of these produce behavioral depression in a variety of tasks (Weiss and Simson 1986; Zacharko and Anisman 1989). Similar to the behavioral depression reported here, stress-induced behavioral depression is not characterized by an inability to initiate behavior, but to sustain it. In reviewing studies on stress-induced depression Anisman (1984) has noted that "when confronted with a strong stimulus animals are able to *initiate* an active response, regardless of their stress history. However, animals that previously had been exposed to uncontrollable stress encountered great difficulty in *sustaining* active responses" (p 411). The behavioral depression produced by uncontrollable stress is short-lived (24–72 h) relative to that reported here, but the apparent similarity between the two phenomena, and the interchangeability of AMPH and stress in producing sensitization (Antelman et al. 1980), suggest they could be the result of related neurochemical adaptations (see below).

One of the most consistent neurochemical consequences of uncontrollable stress, and one that is strongly correlated with stress-induced behavioral depression, is a transient depletion of brain NE. For example, the magnitude of stress-induced behavioral depression is positively correlated with a decline in the postmortem tissue concentrations of NE in the hypothalamus (Weiss et al. 1980), which receives most of its NE input from the lateral tegmental NE system. The strongest correlation, however, is with locus coeruleus NE concentrations (Weiss and Simson 1986). Unfortunately, locus coeruleus NE was not measured here, but as in studies on stress-induced behavioral depression, hypothalamic NE was depleted during AMPH withdrawal, and there was a significant positive correlation between nocturnal locomotor activity and hypothalamic NE concentrations. A decrease in whole brain and regional NE concentrations following the discontinuation of chronic AMPH treatment has been reported before (Herman et al. 1971; Tonge 1974; Short and Shuster 1976; Lynch et al. 1977; Segal et al. 1980). This is the first report, however, to show a clear relationship between the time course of post-AMPH withdrawal behavioral depression and the time course of a depletion in hypothalamic NE concentrations in animals given doses of AMPH known to be non-neurotoxic.

Reports that antidepressant drugs alleviate symptoms of both stress-induced behavioral depression and post-AMPH withdrawal behavioral depression are consistent with the idea that these are related phenomena (Seltzer and Tonge 1975; Lynch and Leonard 1978; Kokkinidis and Zacharko 1980; Zacharko et al. 1984;

Zacharko and Anisman 1989). On the other hand, the neural adaptations to chronic stress and chronic AMPH treatment may be very different. For example, it has been hypothesized that following uncontrollable stress the depletion of NE is indicative of *increased* NE neurotransmission in NE terminal regions (Weiss and Simson 1986), and similar changes in NE neurotransmission are associated with withdrawal from opiates and benzodiazepines (Aghajanian 1978; Redmond and Huang 1982; Roth et al. 1982; Grant et al. 1985). In contrast, the limited evidence available suggests NE "turnover" in terminal regions is *decreased* during post-AMPH withdrawal depression. Not only are the postmortem tissue concentrations of NE reduced in NE terminal regions, but so is the concentration of the NE metabolite, MHPG (Cassens et al. 1979). Furthermore, urinary MHPG is decreased during AMPH withdrawal in humans (Schildkraut et al. 1971; Watson et al. 1972). One exception to the idea that NE "turnover" is decreased during AMPH withdrawal is a report of elevated MHPG in the cerebellum up to 10 days after the discontinuation of intermittant low dose AMPH treatment in rats (Sorensen et al. 1985).

This brief discussion emphasizes that the literature on changes in NE neurotransmission during withdrawal from AMPH (or stress, or opiates) is incomplete, sometimes contradictory, and therefore confusing. It should be kept in mind, however, that in most studies of these phenomena only neurotransmitter and/or neurotransmitter metabolite content has been measured in postmortem tissue, and at only one point in time. The postmortem tissue concentrations of monoamines were measured here as a first step in relating changes in brain neurochemistry to the dynamic changes in behavior seen over time during AMPH withdrawal, and in anticipation of subsequent in vivo neurochemical experiments utilizing microdialysis. Information about the postmortem tissue concentrations of neurotransmitters is required to interpret changes in the extracellular concentrations of neurotransmitters and neurotransmitter metabolites. However, by themselves, postmortem tissue measures are not only insensitive indicators of neurotransmission, but they can sometimes be misleading (Commissiong 1985). Thus, more direct in vivo measures of neurotransmitter dynamics will be required to determine if and how NE neurotransmission is altered during AMPH withdrawal.

It is also important to note that other neurotransmitter systems are probably altered during AMPH withdrawal. For example, the present study provides, to our knowledge, the first neurochemical evidence of a relationship between the time course of post-AMPH withdrawal depression and alterations in mesotelencephalic DA systems. The ability of an AMPH challenge to alter DA concentrations in the caudate-putamen and nucleus accumbens was attenuated in AMPH-pretreated animals 3–7 days after the discontinuation of pretreatment, but not 28 days later. Although more reliable measures of DA dynamics will be required to determine the exact nature of the alteration in DA neurotransmission, the evidence provided here suggests that mesotelencephalic DA systems are relatively unresponsive during AMPH withdrawal. Given the proposed role of nucleus accumbens DA in motivated behavior (Wise and Rompre 1989; cf. Berridge et al. 1989), it seems reasonable to suggest that decreased responsivity in DA systems may contribute to the marked anhedonia reported in humans during AMPH withdrawal (Gawin and Ellinwood 1988), and the attenuation in electrical brain self-stimulation seen in animals (Leith and Barrett 1976; Cassens et al. 1981; Kokkinidis et al. 1986).

Withdrawal from AMPH – persistent behavioral sensitization

Persistent changes in behavior as a result of past experience with AMPH were not apparent in spontaneous locomotor activity patterns, but as expected, when animals received a challenge injection of AMPH it was obvious they had been sensitized (Robinson and Becker 1986). Of particular interest in the present study is the finding that behavioral sensitization, indicated by the emergence of focused stereotyped behavior, persisted *undiminished* for at least a year after the discontinuation of AMPH pretreatment. This is the strongest evidence to date to suggest that non-neurotoxic doses of AMPH may change the neural systems that mediate the psychomotor stimulant effects of this drug for the life of the animal (Robinson and Becker 1986).

It is unlikely that behavioral sensitization is due to changes in AMPH disposition (McCown and Barrett 1980; Robinson and Becker 1986), and the apparently permanent sensitization-related changes in behavior were not due to AMPH neurotoxicity because neither DA or 5-HT were depleted (Seiden and Ricaurte 1987). There is, however, considerable evidence in support of the hypothesis that presynaptic changes in DA systems contribute to AMPH sensitization (Robinson and Becker 1986 for review). For example, both in vitro superfusion/incubation studies (Robinson and Becker 1982; Robinson et al. 1982; Kolta et al. 1985; Wilcox et al. 1986; Castañeda et al. 1988; Yamada et al. 1988; Kolta et al. 1989; Robinson 1990) and in vivo microdialysis studies (Robinson et al. 1988; Kazahaya et al. 1989) have shown that stimulated DA release is enhanced in the striatum and nucleus accumbens of AMPH-sensitized animals for up to 3 months after the cessation of repeated AMPH treatment. Whether DA release is still enhanced 6 months to a year after the discontinuation of pretreatment is unknown. Nevertheless, no other neural correlate of behavioral sensitization has been shown to account for as many features of the behavioral phenomenon (Robinson 1988). The obvious challenge for researchers who propose that other neural adaptations may underlie behavioral sensitization to AMPH is to establish, for example, whether they persist for as long as the behavioral phenomenon – which this study suggests may be for the life of the animal.

Another interesting finding was the length of time following the discontinuation of AMPH pretreatment that an AMPH challenge failed to produce a sensitized behavioral response. A fully sensitized response to the

challenge injection of AMPH was not evident until 2 weeks after the discontinuation of pretreatment. This is consistent with reports that the effects of prior AMPH treatment tend to "grow" in time during withdrawal (Hitzemann et al. 1977; Kolta et al. 1985; Antelman 1988 for review), and that injections widely spaced in time produce more robust sensitization than injections given close together in time (Post 1980). One explanation for the gradual emergence of sensitization is that the neural changes underlying sensitization continue to develop for some time following the cessation of pretreatment. This idea is supported by Kolta et al. (1985), who showed that AMPH-stimulated DA release from striatal tissue was not significantly enhanced 3 days after the discontinuation of AMPH pretreatment, but was enhanced 15–30 days later. An alternative explanation, however, is that the transient neurochemical changes underlying post-AMPH withdrawal depression can "mask" or suppress those responsible for sensitization, perhaps by a diaschisis-like process. Evidence of sensitization may become apparent, therefore, only as these more transient effects dissipate. Whichever the case, studies on the neural correlates of sensitization that focus on transitional periods of time, early during withdrawal, may reflect behavioral depression rather than sensitization; or some complex interaction of the two. In studying the neural basis of behavioral sensitization it would seem most prudent to use animals that have been withdrawn long enough for behavioral depression to dissipate. Otherwise it will be very difficult to dissociate neural changes associated with transient post-AMPH withdrawal depression from those associated with persistent behavioral sensitization.

In summary, following the discontinuation of treatment with escalating doses of AMPH rats showed a decrease in spontaneous nocturnal locomotor activity that lasted at least a week following the last injection of AMPH, but dissipated 2–4 weeks later. Post-AMPH withdrawal behavioral depression in rats may represent, therefore, an animal analogue of the "distress syndrome" seen in addicts during withdrawal from chronic psychomotor stimulant drug use (Gawin and Ellinwood 1988; Kokkinidis 1988). AMPH withdrawal in humans is also characterized by symptoms of depression and alterations in catecholamine function. The transient changes in brain and behavior described here were robust, lasted at least 1 week, and were easily quantified. This paradigm may prove valuable, therefore, in relating neural adaptations provoked by AMPH withdrawal to behavioral depression. In addition to these transient effects, AMPH also had very persistent, if not permanent, effects on the responsiveness to a subsequent AMPH challenge. Thus, this paradigm allows one to study not only the initial "distress syndrome" but also the persistent consequences of past AMPH use. In developing potential therapeutic interventions for AMPH withdrawal it may be especially important to consider the interaction between post-AMPH withdrawal depression and AMPH sensitization. For example, the antidepressant drug desipramine has been reported to facilitate cocaine abstinence when given early during withdrawal (Gawin et al. 1989), but to precipitate relapse when given after 1–6 months of abstinence (Weiss 1988). This latter effect may be related to the sensitizing properties of desipramine itself (Fibiger and Phillips 1981; Maj et al. 1987), and raises the possibility that some treatments which are effective in alleviating transient depressive symptoms may later exacerbate AMPH craving and/or AMPH sensitization. It will be important to delineate potential interactions of this kind in evaluating new therapeutic approaches to AMPH abuse.

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