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Behavioral Assessment of High-Dose Amphetamine Withdrawal: Importance of Training and Testing Conditions

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SCHINDLER, C. W., A. M. PERSICO, G. R. UHL AND S. R. GOLDBERG. *Behavioral assessment of high-dose amphetamine withdrawal: Importance of training and testing conditions.* PHARMACOL BIOCHEM BEHAV 49(1) 41-46, 1994.—Chronic *d*-amphetamine-treated rats were given twice daily injections at a dose of 7.5 mg/kg for 2 weeks. Acute amphetamine and saline groups of rats were given saline treatments during this time, except that for the acute group the final injection was 7.5 mg/kg *d*-amphetamine. Acute and chronic amphetamine groups habituated to the locomotor activity testing apparatus showed increases in both distance traveled and repetitive movement time that lasted up to 6 h following the final injection. When animals were not habituated to the activity test apparatus, however, a significant decrease in repetitive movement time was noted for the chronic amphetamine group 24–54 h following the final amphetamine injection; no differences were observed for distance traveled when the locomotor activity apparatus was novel. Swim test immobility time was assessed twice following the last injection, with the second test following the first by approximately 24 h. During the first test, decreases in immobility were observed for both chronic and acute amphetamine groups, 6–12 h following the last injection. However, during the second test, decreases in immobility time were observed only for the chronic amphetamine groups 36–72 h following the final injection. These results indicate that 24 to 72 h after the end of the chronic amphetamine regimen a withdrawal effect was observed for both repetitive movement time in the locomotor activity test and immobility time in the swim test. The withdrawal effect was observed only for the locomotor activity groups for whom the test apparatus was novel, and only during the second test of immobility time for the swim test groups. Thus, the method of behavioral assessment can be critical for the demonstration of a high-dose amphetamine withdrawal effect.

Amphetamine Withdrawal Locomotor activity Swim test Rats

IN HUMANS, the abrupt discontinuation of long-term amphetamine use leads to a withdrawal syndrome that is characterized by a number of signs and symptoms, including clinical depression [e.g., (4,7,14,18)]. Withdrawal effects have proven to be more difficult to assess in animal models of amphetamine withdrawal. Although consistent reductions in intracranial self-stimulation have been noted following withdrawal from high-dose amphetamine treatment (5,6,8,16), the utility of this procedure is limited in that it requires surgery and extensive training prior to testing. In contrast, inconsistent effects have been observed with procedures that require little training, such as locomotor activity. For example, although Herman et al. (2) reported inhibition in locomotor activity following long-term amphetamine treatment, Kokkinidis et al. (5) reported that amphetamine withdrawal had no effect on locomotor activity.

The primary purpose of the present experiment was to investigate the role of behavioral test conditions and, in particular, environmental novelty, in the assessment of amphetamine withdrawal using automated measurements of locomotor activity. Novelty was manipulated by either exposing animals to the test apparatus throughout the chronic treatment phase, or by exposing the animals to the test apparatus only during a single test session during the period of drug withdrawal. The potential role of novelty in assessing the behavioral effects of amphetamine is suggested by findings that amphetamine can

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have different effects on exploration when assessed in novel or nonnovel environments (1), and that diminished reactions to novel stimuli are observed both with acute amphetamine treatment (3) and during amphetamine withdrawal (15).

In order to independently confirm the observation of amphetamine withdrawal, separate groups of rats were also given a forced-swim test (13). For this test, immobility time, or time spent not trying to escape the water, is the dependent measure. Immobility time has been purported to assess behavioral despair. Previous evidence does suggest that this test is sensitive to amphetamine withdrawal (5). Antidepressant treatments can decrease immobility time (12) as well as reverse the depression of intracranial self-stimulation behavior produced by amphetamine withdrawal (6).

METHOD

Subjects

The subjects were 233 naive, 350 to 450 g, male Sprague–Dawley rats (Charles River Laboratories). They were housed three to a cage in a temperature- and humidity-controlled room that was on a 12L : 12D cycle (lights on at 0700 h). The animals had free access to food and water throughout the experiment.

Apparatus

For locomotor activity testing, the animals were placed in a standard sized (47 × 24 × 20 cm) plastic rat housing tub that was placed inside an infrared activity monitor (Opto-Varimex, Columbus Instruments, Columbus OH). Animal movements were analyzed with supplied software (Auto-Track, Columbus Instruments), which tabulated distance traveled in cm and repetitive movement time in seconds. Repetitive movement was defined as the repeated make and break of the same photocell beam, and is a putative measure of stereotypy. Each activity monitor was placed inside a larger chamber (model EPC-010, BRS/LVE, Beltsville MD) that provided sound attenuation.

Procedures

Two different procedures were used to assess the effects of amphetamine withdrawal on locomotor activity. For the first habituated group, 24 rats were given an IP injection twice per day for 14 days. The first injection was given at approximately 0700 h and the second at approximately 1800 h. After each injection the animals were placed in the activity monitors for a period of 30 min. On the 15th day, the animals received the morning injection and were placed in the activity monitor for 30 min (time point 0). They were again placed in the activity monitor for 30 min 1, 6, 12, 24, 36, 54, and 168 (1 week) h following the last injection. No injections preceded these sessions. Of these animals, 12 were given 14 days of twice-daily 7.5 mg/kg *d*-amphetamine treatment and tested on day 15 with 7.5 mg/kg *d*-amphetamine (chronic group), 6 were given 14 days of twice-daily saline treatment and tested with 7.5 mg/kg *d*-amphetamine on day 15 (acute group), and 6 were trained and tested with saline (saline group). Withdrawal from similar treatment schedules has been show to produce a significant decrease in intracranial self-stimulation behavior (6) and alterations in several endocrine, immune, and neurochemical properties (17).

For the second novelty procedure, animals were given all their injections in the home cage and were only exposed to the activity chamber once during testing. Fifty-six animals were given 14 days of twice daily 7.5 mg/kg *d*-amphetamine and given 7.5 mg/kg *d*-amphetamine as the final injection (chronic group), 60 animals were given 14 days of twice-daily saline injections and 7.5 mg/kg *d*-amphetamine as the final injection (acute group), and 58 animals were given saline throughout (saline group). Different groups of animals were tested in activity monitors for 30 min at various time points following the final injection. Each group was only tested once. There were five to six subjects per test time point, except for the 24- and 54-h points where five to six additional animals were used to replicate the observed effects. Nine additional groups of animals (n = 5–6) were tested subsequently at the 30-, 72-, and 120-h time point as a further replication.

For the swim test, animals were given *d*-amphetamine treatments identical to those described for the novelty locomotor activity groups above. Following the final injection of *d*-amphetamine or saline, each of the animals was tested twice. For the first test, the rats were placed in a 34 × 23 × 43 cm plastic container filled to a depth of 25 cm with 25°C tap water. They remained in the water for a total of 15 min. During the initial 5 min, immobility time was recorded for each rat. A rat was judged as immobile whenever it floated passively in the water and made no further movements other that those required to keep its head above water (13). The second test was conducted in a similar manner, except that the rats remained in the water for only 5 min, during which time immobility was recorded. The group first tested at 6 h received a second test 18 h later. The second test followed the first by 24 h for all other groups. The times for the first test were 6 h (chronic n = 5, acute n = 6, saline n = 6), 12 h (chronic n = 6, acute n = 5, saline n = 6), 30 h (chronic n = 10, acute n = 8, saline n = 12), 48 h (chronic n = 6, acute n = 5, saline n = 6), and 168 h (1 week) (chronic n = 6, acute n = 6, saline n = 5) following the final injection.

Data Analysis

The data were tabulated across the entire 30-min test session for distance traveled and repetitive movement time. When expressed as a percent of control, the time point for the appropriate saline group was used as the control. For the swim test, difference scores were also calculated for immobility times in the first and second test. Data were subjected to an analysis of variance with follow-up contrasts to determine individual effects (19).

Drugs

d-Amphetamine sulfate (Sigma Chemical Co., St. Louis, MO) was dissolved in sterile saline and injected IP in a volume of 1 ml/kg. Doses are expressed as the salt.

RESULTS

In general, the rats tolerated the chronic amphetamine treatment with no overt sign of toxicity. The clearest sign of amphetamine treatment was the appearance of intense sniffing stereotyped behavior that was observed beginning 1–2 days after the start of treatment. Although the saline-treated animals gained approximately 50–75 g over the course of treatment, the chronic amphetamine rats tended to maintain a steady weight.

Previous research has also shown that differences in locomotor activity are observed for the novelty and habituated treatment procedures for animals treated chronically with sa-

line (11). Habituated animals, which have been placed in the chambers during the chronic treatment phase, showed lower levels of activity for both distance traveled and repetitive movement time than the novelty animals exposed to the chamber for the first time during the test. Further, there was also a tendency for activity levels to be depressed for 1–6 h following the final injection of the chronic saline regimen and to then increase. As both the training and test conditions influenced the level of activity for the saline groups, the presentation of results for the drug tests were normalized to the saline conditions.

During the habituation phase, locomotor activity was assessed for 30 min following only the morning injection. For both the amphetamine and saline groups, both distance traveled and repetitive movement time decreased over training days. Repetitive movement time for the amphetamine group stabilized at a level clearly above that for saline, although distance traveled was similar for both the amphetamine and saline groups. Figure 1 presents the test results for the habituated animals tested with 7.5 mg/kg presented as a percent difference from the saline control subjects. No differences were evident between the acute and chronic treatment groups for either distance traveled or repetitive movement time ($p > 0.6$), although both groups differed from the saline control group ($p < 0.05$). Small increases in activity were noted 0–1 h following the final injection for both the acute and chronic groups, with increases in distance traveled being larger for the acute amphetamine group. An increase in both measures for both groups was seen at 6 h, although this increase primarily reflected the decrease in activity observed for the saline group (11).

Although the habituated animals showed no evidence of a withdrawal effect, such an effect was noted for the novelty animals as reflected in a clear difference in repetitive movement time between the acute and chronic groups ($p < 0.001$). For the chronic amphetamine-treated animals, suppressed repetitive movement time was noted beginning 12–24 h after the last amphetamine injection and persisted until at least 54 h following the last injection (Fig. 2). To further substantiate this effect, additional animals were tested 30, 72, and 120 h following the final injection. For these replication groups, normalized repetitive movement time was depressed at 30 h, but had recovered to near normal levels by 72 h. No difference between the acute and chronic groups were noted for distance traveled. The acute amphetamine group did not differ from the saline controls ($p > 0.4$) for either measure.

Figure 3 (top panel) shows immobility scores for the first swim test. *d*-Amphetamine lowered immobility times in both the chronic and acute amphetamine groups. This effect was apparent at both the 6-h and 12-h time points. The acute and chronic amphetamine groups did not differ for this test ($p > 0.08$). For the second test (Fig. 3, bottom panel), there was a difference between the chronic amphetamine group and the other two groups ($p < 0.001$). Immobility times for both the saline and acute amphetamine groups increased from the first to the second test by approximately 50 s. Immobility scores for the chronic amphetamine groups at the 36-, 54-, and 72-h time points remained near the first test level. These effects were also reflected in the immobility time difference scores. A significant effect was only observed at the 54-hr time point, however (data not shown).

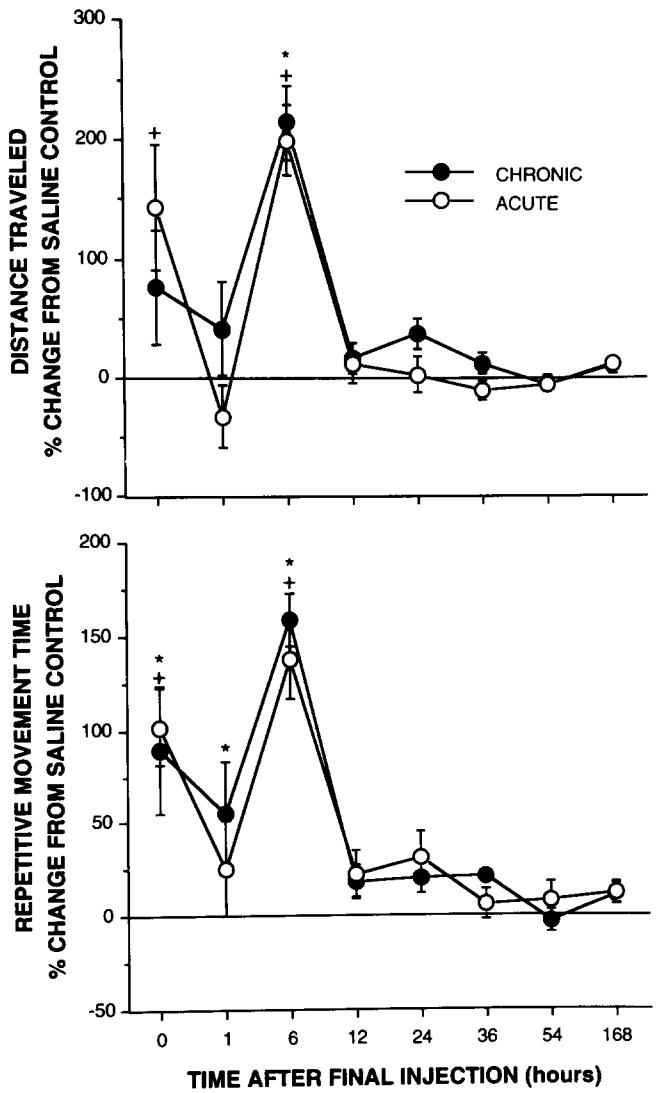

FIG. 1. Locomotor activity during amphetamine withdrawal for the habituated groups. The rats were treated for 2 weeks with either twice-daily IP injections of 7.5 mg/kg *d*-amphetamine (CHRONIC) or twice-daily IP injections of saline (groups ACUTE and SALINE). For the final injection, group ACUTE received 7.5 mg/kg *d*-amphetamine. Following each injection, the rats were placed in the locomotor activity chamber for 30 min. Following the final injection, locomotor activity was assessed for 30 min at each of the time points indicated. The top panel shows the measure of distance traveled and the bottom panel shows repetitive movement time, error bars are ±1 SE. All measures are expressed as a percent difference from the saline control values. Times are in hours. Note that the x-axis is not linear. $^{*}p < 0.05$, CHRONIC vs. SALINE; $^{+}p < 0.05$, ACUTE vs. SALINE.

DISCUSSION

The present study shows an amphetamine withdrawal effect on locomotor activity that is dependent upon both treatment and test conditions. For those animals habituated to the locomotor testing apparatus, the results were remarkable for the lack of withdrawal-induced differences between acute and chronic treatment condition on either distance traveled or repetitive movement time. Activity was increased over saline controls for both the chronic and acute groups of habituated animals, but these groups differed from each other at no time point. In contrast, evidence of a withdrawal effect in repetitive movement time was observed for the novelty groups. Repeti-

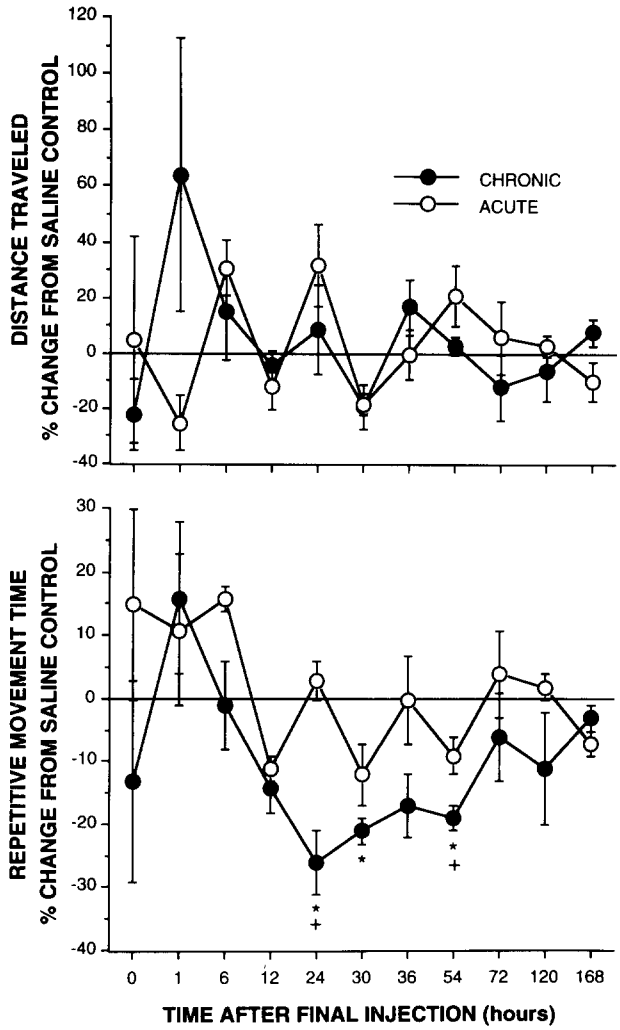

FIG. 2. Locomotor activity during amphetamine withdrawal for the novelty groups. The rats were treated for 2 weeks with either twice-daily injections of 7.5 mg/kg *d*-amphetamine (CHRONIC) or twice-daily IP injections of saline (groups ACUTE and SALINE). For the final injection, group ACUTE received 7.5 mg/kg *d*-amphetamine. Following the final injection, locomotor activity was assessed for 30 min in different groups of rats at each of the time points indicated. The rats were placed in the locomotor activity chamber only once. The top panel shows the measure of distance traveled and the bottom panel shows repetitive movement time. All measures are expressed as a percent difference from the saline control values, error bars are ± 1 SE. Times are in hours. Note that the x-axis is not linear. $^{*}p < 0.05$, CHRONIC vs. SALINE; $^{+}p < 0.05$ CHRONIC vs. ACUTE.

tive movements were clearly suppressed 24 h following the final amphetamine injection for the chronic treatment group and continued to be suppressed until at least 54 h. No suppression of repetitive movements was seen with the acute treatment group. The current finding was reproducible in subsequent tests at intermediate time points and observed with automated recording equipment not subject to observer bias or interlaboratory differences in observation techniques.

Although evidence of withdrawal effects from amphetamine have been noted previously [e.g., (2)], the present results point to factors that may account for some of the inconsistencies in the literature. First, no evidence of withdrawal was noted for the habituated animals. This may relate to the lower baseline level of activity noted in habituated animals, making it difficult to observe further decreases. If this were the case, increases in activity might be more apparent with the habituated response, as was observed (compare Figs. 1 and 2). However, the novelty test would also be expected to be more stressful to the animal, which might also have contributed to the differences observed between the novel and habituated tests. Second, the specific measure of activity used can be a critical factor in the observation of withdrawal. In the present

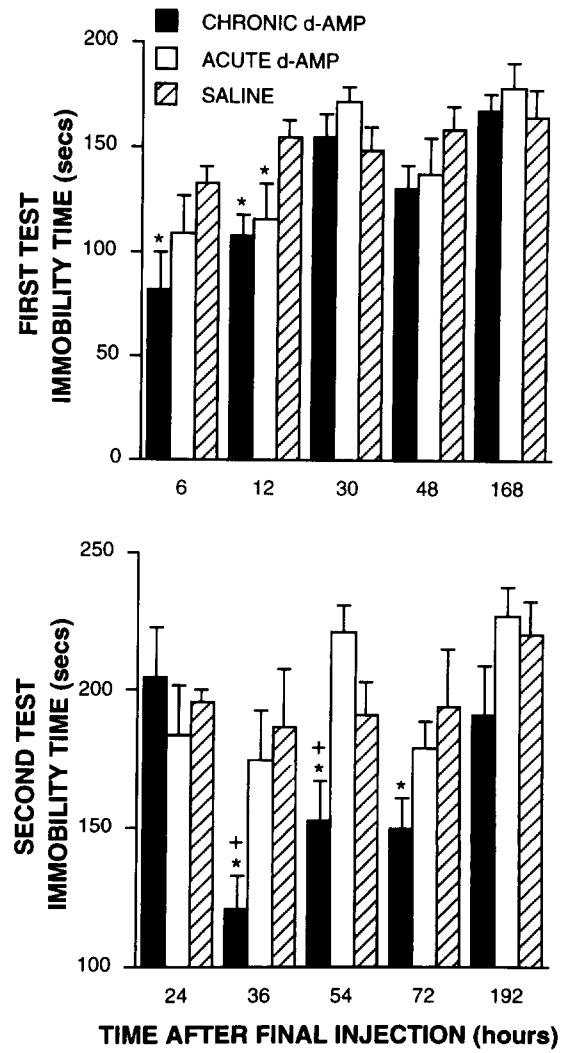

FIG. 3. Time spent immobile (immobility time) in seconds during swim tests conducted 6 to 192 h after a final injection of 7.5 mg/kg *d*-amphetamine (chronic and acute groups) or saline. The top panel shows the results of the first test which occurred 6 to 168 h after the final injection and where immobility time was recorded for the initial 5 min of a 15-min swim test. The bottom panel shows immobility time for the second 5-min test, which followed the first test by 24 h, except for the 24 h group where the first test was given 6 h following the final injection. Error bars are 1 SE. $^{*}p < 0.05$ vs. SALINE; $^{+}p < 0.05$ CHRONIC vs. ACUTE.

study, no evidence of withdrawal was noted for the distance traveled measure. As distance traveled represents a measure of gross movement activity and ambulation, this result confirms previous observations that no withdrawal effects are observed for these kinds of measures [e.g., (5)]. The finding of a withdrawal effect for repetitive movement time suggests that withdrawal may be more notable with stereotypy than with ambulation. However, the exact relationship between the repetitive movement time measure and stereotypy is not clear. Although certain types of stereotypic movement may well produce increases in repetitive movement time, this may not be the case for all types of stereotypy.

The results for the swim test confirm many of the findings of withdrawal for repetitive locomotor activity. Evidence of withdrawal for the chronic group was noted in the second test, beginning 36 h following the last injection and lasting until at least 72 h following the last injection. Although an effect was not noted until the second test, it could well be that the process responsible for that effect was occurring closer in time to the first test. If the increase in immobility time from the first to the second test resulted from the learning process of habituation, then these results may stem from a disruption of the memory processes taking place immediately following the first test. To affect the second test, this withdrawal-related disruption could have actually occurred anywhere from 12 to 72 h following the final injection, a time course that overlaps that for the locomotor activity effect. The present swim test results are not in agreement with those of Kokkinidis et al. (5), who found an increase in immobility time during *d*-amphetamine withdrawal in the second swim test. However, there are a number of differences between the two studies that could account for this difference. For example, Kokkinidis et al. used mice as subjects and the second test followed the first by only 30 min.

The onset of alterations in novelty locomotor responses and swim test immobility time described above overlaps with findings of decreased transcription factor gene expression and dopamine levels in the prefrontal cortex and dopamine levels in the striatum (11) in animals treated with the same injection regimens. The behavioral signs of withdrawal, though, last at least 12–24 h longer than alterations in neurotransmitter and transcription factor mRNA levels, which return to baseline by 54 h (11). Similar delays between normalization of neurochemical parameters and recovery of normal locomotor responses have been recorded following chronic injection stress (11). Recovery of normal behavioral responses during amphetamine withdrawal may, therefore, require a series of biochemical events and may not simply mirror recovery of normal neurotransmitter function. Further evidence of this complex cascade is provided by tyrosine hydroxylase, whose mRNA level increases after the onset of behavioral and neurochemical alterations and returns to baseline well after recovery of normal behavioral responses (9).

Although evidence of behavioral signs of withdrawal after chronic amphetamine treatment was noted in the present study, the results are also remarkable in that no evidence of either tolerance or sensitization were noted with locomotor activity. Previous research from our laboratory (10) has noted tolerance using biochemical measures with the same injection regimen employed in the present study. However, evidence of tolerance was noted for behavior only at a lower testing dose. That is, when the final injection for both the chronic and acute groups was 2.0 mg/kg amphetamine, tolerance to the locomotor activating effects of amphetamine were noted. Sensitization is typically noted when doses of amphetamine lower than 7.5. mg/kg are given repeatedly [cf., (5)]; preliminary evidence from our laboratory confirms this finding (C. Schindler and A. Persico, unpublished observations).

Overall, the results of the present study show that the ability to observe tolerance or withdrawal effects after long-term, high-dose amphetamine treatment can depend on both the treatment and test conditions. The results for both repetitive movement time and the swim test suggest that the time course for withdrawal after the chronic *d*-amphetamine regimen used here occurs between 12 and 72 h following the final amphetamine injection.

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